Nutrition and Health
Series Editor: Adrianne Bendich

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Richard J. Deckelbaum *Editors*

Preventive Nutrition

The Comprehensive Guide for Health Professionals

Fifth Edition

Humana Press

Nutrition and Health

Adrianne Bendich, Ph.D., FASN, FACN, Series Editor

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Nutrition and Health
ISBN 978-3-319-22430-5 ISBN 978-3-319-22431-2 (eBook)
DOI 10.1007/978-3-319-22431-2

Library of Congress Control Number: 2015958761

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Humana Press is a brand of Springer
Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

A.B. dedicates this book to my dearest D.
R.J.D. dedicates this volume to his children, Leona, Michael, Dan, and Ariel, who continue to lead him in healthy nutrition and active lifestyles.

Preface

The overriding objective of *Preventive Nutrition: the Comprehensive Guide for Health Professionals* is to provide our peers, graduate and medical students, and knowledgeable professionals interested in nutrition with relevant, data-driven reviews of the most objective, up-to-date evaluations of the role of nutrition in health and disease prevention. For the past 20 years, we have developed five volumes on this topic and have provided 160 chapters contributed by over 200 of the most respected research- and practice-oriented nutritional professionals, many of whom have provided their chapter for each of our volumes. The volumes contain hundreds of tables and figures and over 100,000 relevant references. Because our aim was to make this the "go-to" volume for our readers, we have included lists of relevant books and websites.

Of course, in the past 20 years, the field of communication has changed dramatically, and we feel fortunate that Humana Press was sold to Springer because Springer is the leader in electronic publishing. Although fewer people and even fewer libraries purchase hard copies of technical books such as ours, they are downloading books and chapters within books, and this is happening around the world. We are very pleased to note that more than 200,000 chapter and book downloads of *Preventive Nutrition* volumes have been made since this format became available. It is this great interest in the information in our volumes that has propelled us to develop the fifth edition so that our readers have the most current information available in the form of expert reviews.

Each of the volumes has contained new chapters that broaden the basics and add to our perspectives of where clinically based nutrition is headed. The fifth edition includes many new chapters and is the largest of the volumes. Twelve new chapters review the regulatory environment for foods and dietary supplements and how these differ between countries; the consequences of national tariff structures on access to nutritious foods; the development of global nutrient density metrics; diet-gene interactions; nutritional aspects of stroke prevention and care; fiber and Type 2 diabetes; bone health, obesity and diabetes, and the roles of sugars and nonnutritive sweeteners; nutritional positives and negatives of bariatric surgery; sodium consumption in Southeast Asia and health consequences; food security in developing nations; preventive nutrition and the food industry and in the supplement industry; and the role of preventive nutrition in clinical practice.

Within the updated chapters, we have many new evidence-based data that assure the economic benefits of disease prevention as well as the public health benefits to nations that invest in the improvement of their population's diet: research that indicates that all new drugs need to be tested for their nutritional consequences and continued research that adds to the totality of the evidence of the role of nutrients and other dietary components in reducing the risk of cancers, cardiovascular and cerebrovascular diseases, and diabetes and obesity. We learn about folic acid supplementation during the periconceptional period not only with reducing neural tube birth defects but also to significant reductions in major congenital heart defects, associations between dietary carbohydrates and risk of age-related macular degeneration, effects of proton pump inhibitors on increasing the risk of bone fractures, and the role of chronic inflammation associated with obesity on health outcomes in

low-income nations in Africa as well as Latin America and South America. Thus, our authors have again provided readers with the most relevant research from around the world that can impact the health of their patient population as well as their healthy clients. The chapters also serve as critical reviews of nutrition research in areas of great importance to physicians, nutritionists, dieticians, dentists, nurses, pharmacists, graduate students, medical students, allied health professionals and health educators, and policy makers who are interested in the potential for preventive nutrition strategies to reduce the risk of chronic diseases.

We are sincerely grateful to all of our authors who have developed such excellent chapters for our volumes. The best part of being the volume editors is getting to read the chapters and learning so much from the experts! We are especially saddened by the passing of Dr. Andrew Czeizel who contributed a chapter to each of the five Editions on the topic of nutrition's role in preventing serious birth defects. We acknowledge and thank our excellent colleagues at Springer/Humana Press including Joanna Perey, Martine Chevry, and Richard Lansing, the production staff, and others who have worked so hard to prepare this volume for publication. We also thank our staffs and our families for providing their support as we spend many hours and days working on these volumes.

Morristown, NJ, USA Adrianne Bendich, Ph.D., FASN, FACN
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Series Editor Page

The great success of the *Nutrition and Health* series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers and clinicians in their respective fields; (3) extensive, up-to-date fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and the consequences; (7) virtually no overlap of information between chapters, but targeted, interchapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patient as well as health professional questions which are based upon the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter and in the choice of chapter authors. The international perspective, especially with regard to public health initiatives, is emphasized where appropriate. The editors, whose trainings are both research and practice oriented, have the opportunity to develop a primary objective for their book: to define the scope and focus and then invite the leading authorities from around the world to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fifth Edition, edited by me and Richard Deckelbaum, M.D., is a very special addition to the *Nutrition and Health* series and fully exemplifies the series' goals. The first volume was published in 1997, and subsequent editions were published in 2001, 2005, and 2010 (the table of contents for each of these volumes is included in the Appendices of this volume). Each volume was given excellent evidence-based reviews by health professionals in both public health and clinical nutrition professional communities, and thus a fifth edition that could maintain the timeliness of the volume was warranted.

Over the past 6 years, there have been major advances in the realization that diet, foods, nutrients, bioactive factors, and microbes that inhabit our digestive tract affect our overall health. Moreover, treatment of acute and chronic diseases that may require multiple medications and advanced treatments with devices such as radiation and/or surgery has been shown to affect appetite, digestive tract functions, and overall nutritional status. Likewise, research on the nutritional requirements of geriatric patients, pregnant women, adolescents, children growing up in countries in economic transition, and those patients and clients with chronic disease conditions has expanded. At the same time, major adverse health risks including obesity and diabetes continue to increase in these already at-risk populations. Moreover, the fields of nutrigenomics, metabolomics, and other nutrition-related omics have added to our knowledge of the key role of nutrition in every aspect of our genetics and vice versa. As the science has been expanding, there has also been a parallel growth in the awareness that government lawmaking and regulations can have global impact on our food and dietary supplement choices.

This updated text, containing 40 chapters, integrates clinical practice and the underlying science. The authors of five chapters are also editors of volumes that are part of the *Nutrition and Health* series and, along with the other authors, are the leading global experts in their fields. There are 14 new subject areas that have been added to the fifth edition. We are very fortunate to have many authors who have agreed to update their excellent chapters, and six of our authors have contributed a chapter to each of the five editions of *Preventive Nutrition*: Walter Willett, Robert Heaney, John Bogden and Donald Louria, Andrew Czeizel, and Theresa Scholl. The current volume is relevant for practicing as well as research-centered healthcare professionals as there are in-depth discussions of the basic assessment tools and demographics of the different diseases and disease conditions that affect certain populations preferentially, such as age-related macular degeneration and alterations in muscle metabolism that are often adversely affected by the aging process. There are also clear, concise recommendations about dietary intakes and use of drugs and supplements. The current regulations that govern the production and labeling of foods in different countries and efforts to globalize the rules are reviewed as is the potential impact of trade regulations. Thus, the volume provides a broad base of knowledge concerning the physiology and pathology associated with nutritionally relevant interventions as well as the public health aspects of food policies that can enhance the potential for a more healthful life.

Preventive Nutrition serves a dual purpose of providing in-depth focus on the nutritional aspects of reducing the risk of cancer, cardiovascular disease, and obesity and diabetes as well as examining the global issues that affect clinical nutritional care throughout the world. The volume includes reviews that cover the time frames from prepregnancy care and birth to elder care throughout the last 30 or more years of the lifespan. The book is organized as a stand-alone resource text that provides the basics of nutritional assessment of the patient and interventions in healthy individuals and patients and reflects upon the necessity of medical nutrition support as a keystone for disease management. The volume includes extensive, in-depth chapters covering the most important aspects of the complex interactions between diet, obesity, cardiovascular disease, diabetes, and loss of cognitive functions and the impact of such a loss of certain mental functions on nutritional status.

Global Issues

The volume is organized into seven relevant parts. The seven introductory chapters in the first part, entitled "Global Issues," provide readers with an historic perspective of the concept of preventive nutrition and its public health benefits. There are chapters that review the history of preventive nutrition and the economic consequences of disease prevention; another chapter examines the public health benefits of preventive nutrition strategies, and the interactions between science and regulations are reviewed in the third chapter. There is a new chapter that looks at the flow of foods globally and other chapters on nutrient density, drug–nutrient interactions, and the new area of nutrigenomics. There are four new chapters in the first part that address regulatory aspects of foods and dietary supplements, the growing importance of global food companies and their influence on trade agreements that determine the types and quantities of foods that are imported into a country, and the value of determining the nutrient density of foods and the effects of genetics on nutritional status.

The first chapter, by Jeffery Blumberg, who contributed the first chapter to the first edition of *Preventive Nutrition* in 1997, provides a broad basis for understanding the importance of preventive nutrition strategies for reducing the risk of the major diet-related diseases that are also the major chronic, noninfectious diseases affecting populations around the world. The major diet-associated chronic diseases reviewed include, but are not limited to, obesity, diabetes, cardiovascular disease, and cancer. The chapter includes an overview of the commitment of preventive nutrition strategies at the global level with the World Health Organization and other global health-related organizations

reaffirming the right of all citizens to have access to safe, sufficient, and nutritious food. The authors inform us that in 2014, the Second International Conference on Nutrition acknowledged that malnutrition, including undernutrition, micronutrient deficiencies, overweight, and obesity, is widespread and affects not only the health and well-being of individuals but restricts the attainment of human potential and poses a significant burden of adverse social and economic consequences to individuals, families, communities, and nations. The economic value of instituting preventive nutrition strategies is documented, and the behavioral strategies currently considered of greatest potential are reviewed. Culture, economic status, and access to safe, fresh foods are critical issues as implementation of behavioral changes can only be successful in the long term if these are supported in the home, community, school, and other social environments. The importance of government interventions, such as food fortification with essential vitamins and minerals, is discussed as is the development of guidance tools such as dietary guidelines. The next chapter by Walter Willett, who has contributed a chapter to each of the five volumes of *Preventive Nutrition*, continues to examine the totality of the evidence that clearly indicates that certain dietary components can greatly impact health and reduce the risk of several chronic diseases. The chapter provides over 200 critical references and guidelines for dietary intakes and health behaviors based upon the most consistent data that link certain macro- and micronutrients as well as fiber to lower risks of cardiovascular disease, diabetes, obesity, and other major health concerns of the adult and aging populations. Eight key recommendations for implementing preventive nutrition strategies are also included.

The third chapter provides an historic perspective of the development of the concept of functional foods and its outgrowth into dietary supplements. It is quite timely that we include this new, comprehensive chapter that examines the regulatory environment subsequent to and the changes following the enactment of the Dietary Supplement Health and Education Act (DSHEA) of 1994. The US regulations are compared with the Japanese standards for Foods for Specified Health Uses (FOSHU) and the growth of the dietary supplement market in the United States and globally following the enactment of DSHEA. Chapter 3 contains seven informative tables including a description of currently permitted health claims, criteria for good manufacturing practices for dietary supplements, and lists of contaminants documented by FDA that were recently found in marketed supplements. Given the fact that more than 85,000 different dietary supplements are currently marketed in the Unites States and around the world, it is helpful to know that so few have been found to be tainted and also to better understand the value of the regulations in maintaining the safety of dietary supplements and accuracy of their claims. Of equal importance is the next chapter, Chap. 4, which reviews the changes in the mechanisms for determining the contents and nutritive value of foods that are traded around the world. We learn that there is an increased use of regional trade agreements that may favor less nutrient-dense foods than would be recommended by regional nutritionists and public health advocates. This new chapter informs us of another layer of complexity in the development of preventive nutrition strategies to improve health.

Chapter 5 examines the growing global interest in determining the nutrient density of different foods and describes the use of nutrient density in developing a rating system for foods that is called nutrient profiling. This new chapter explains that nutrient density is based upon a comparison of the content of nutrients that are at usually lower than recommended intakes versus the total caloric content of the food. Once the nutrient density of foods is calculated, these can be compared to one another using a common denominator such as weight, cost, and energy used to produce the food, and this is the basis of nutrient profiling. Globally, the rating systems have been used to identify foods that are affordable, sustainable, and nutrient-rich. Nutrient profiling has also resulted in development of new food labels and reformulation of many food industry products as a positive marketing tool for consumers.

Chapter 6 examines the growing importance of drug-nutrient interactions especially in light of the increase in the aging population who often takes several drugs in response to chronic diseases that can independently affect nutritional status. The chapter includes eight important tables, more than 150 relevant references, and discussions of drugs that can directly influence food intake through effects on

the central nervous system or the gastrointestinal (GI) tract including anorexia, nausea, vomiting, diarrhea, constipation, nutrient malabsorption, taste disturbances, liver enzyme activities, and endocrine functions including satiety and insulin secretion as examples. The chapter includes detailed tables that list drugs and their effects on the nutritional status, GI tract, and cognitive functions. Of importance, several well-known drugs can adversely impact individual nutrient levels and/or overall nutritional status.

The last chapter in this section reviews the new topic of nutrient-gene interactions. Chapter 7 discusses the various influences that genes have on an individual's response to diet patterns and individual nutrients and focuses on the common haptoglobin genetic polymorphism as an example. The genetic terminology used is defined, and examples are provided. The two-way interactions of nutrition on genes and genes on nutritional status are examined. Diet-gene interactions play a significant role in the between-person variability of nutritional status in response to intake of specific nutrients and can obviously be a critical factor in clinical research. The chapter includes a comprehensive discussion of the haptoglobin gene polymorphisms, their effects on macro- and micronutrient metabolism, and the potentially adverse effects in individuals with diabetes and certain other chronic diseases. There is an in-depth discussion of the variability of the antioxidant functions of the haptoglobin polymorphisms that can affect the serum concentrations of the essential nutrients, vitamins C and E, with the consequence of reduced serum vitamin concentrations. The importance of adding as many genetic phenotypes to the genome-wide association study results is stressed.

Cancer Prevention

The second part of the volume, containing five chapters (Chaps. 8–12), describes the fundamental role of nutrients, dietary supplements, and dietary components in the reduction of cancer risk in the most common cancers. The first chapter in this section reviews the risk factors for lung cancer, the number one cancer killer of both men and women in the United States and around the world. Chapter 8 begins by acknowledging the very significant effects of tobacco smoking on lung cancer risk. Modes of smoking examined include hookas and electronic cigarettes and secondhand smoke. Additional links to environmental exposures in the workplace and/or home environment are discussed. A family history of lung cancer is also associated with increased risk. The difficulty of parsing out the potential role of diet and dietary factors in light of the very large effect of smoking is presented, and the chapter concludes that higher than average fruit consumption appears to have the most consistent evidence of lowering the risk of lung cancer even in smokers. The relationship between lung cancer and vegetables, micronutrients, phytochemicals, fat, body mass index, beverages, and meat intake is also included. Chapters 9 and 10 examine the role of diet and dietary components in the development of cancers in female and male reproductive organs, respectively. Chapter 9 is authored by Donato Romagnolo who coedited the *Bioactive Compounds and Cancer* volume with the late John Milner which was published in the *Nutrition and Health* series in 2010. Chapter 9 stresses the importance of changes to nuclear factors other than DNA mutations, termed epigenetic changes that have been shown to affect endocrine cancer risk in women. Examples discussed include alterations to histones, chromatin, and DNA methylation. The authors summarize recent findings related to epigenetic silencing of tumor suppressor genes in endocrine tissues that contribute to the development of breast, uterine, and ovarian cancers. Nutrients discussed include folic acid, vitamin D3 and vitamin C, and the impact on enzymes associated with the adverse effects of mutations. Data are reviewed that link certain bioactive components of the diet including genistein, resveratrol, and epigallocatechin gallate and certain dietary patterns, such as the Mediterranean diet, to maintenance of tumor suppressor levels. Therefore, these dietary factors may be found to help reduce the risk of endocrine tumors in women. Chapter 10 reviews the associations between certain dietary factors and risk of prostate cancer and

concentrates on data collected following the development of the prostate-specific antigen diagnostic assay. Survey data are examined with the goal of identifying risk factors for potentially lethal versus indolent disease. Increased risk of lethal disease is associated with above average height and obesity, whereas protective dietary factors include coffee, lycopene from tomato sources, and fish intake. The mixed findings for vitamin E and selenium and dairy, calcium, and vitamin D are summarized.

Chapter 11 provides an extensive and in-depth review of the published cohort and randomized, placebo-controlled studies (RCT) of the use of dietary supplements including multivitamins, beta carotene, vitamin E, selenium, vitamin C, and calcium (with or without concomitant vitamin D supplementation) and the risk of cancer occurrence and mortality. The chapter includes more than 200 references and comprehensive tables that consistently point to the lack of evidence of benefit of the use of these supplements for cancer risk reduction. Of great importance, the authors have analyzed the many reasons for the numerous null effect findings and indicate that further research is needed. The final chapter in this section, Chap. 12, discusses the importance of the nonnutritive components of foods and their mechanisms of action that may be of value in cancer prevention. This comprehensive chapter, containing over 250 relevant references, reviews the studies that suggest that intakes of some specific fruits and vegetables containing nonnutritive bioactive components are inversely associated with the risk of several cancers. There are discussions of carotenoids, polyphenols, indoles, and isothiocyanates and their in vitro effects on cellular processes including proliferation, apoptosis, differentiation, cellular and hormonal signaling, cell-cycle regulation, invasive potential, and induction/inhibition of detoxification/bioactivation enzymes. The chapter also includes an in-depth review of the survey data and clinical studies that have examined the association of dietary bioactive intake and cancer risks.

Cardiovascular and Cerebrovascular Disease Prevention

The third section contains five clinically oriented chapters covering both cardiovascular and cerebrovascular diseases and the importance of diet in both primary and secondary prevention. The first chapter in this section includes a new topic and author and reviews the area of diet quality and the potential for overall changes in diet to reduce the risk of cardiovascular disease (CVD). Chapter 14 examines the data from nutritional epidemiology studies that look at overall dietary patterns that reflect the complex and multidimensional nature of diets consumed in different populations and the cumulative effects of dietary preferences on CVD risks. The two major research tools for these studies are reviewed in detail: a priori generation of predefined dietary scores or indexes based on dietary recommendations or guidelines and a posteriori derived data based on factor analyses including principal component analysis or cluster analysis. Studies using the Mediterranean diet score and the Dietary Approaches to Stop Hypertension (DASH) diet score, the Healthy Eating Index, as well as the Healthy Diet Indicator are reviewed. Diets identified with factor analysis and discussed include variations of the prudent diet, the Western diet, the Mediterranean-type diet, and certain other patterns identified in relevant studies. The majority of investigations find a significant association between a "healthy diet" pattern and reduced CVD risk.

The next two chapters, written by new authors to the *Preventive Nutrition* volumes, review the more traditional preventive cardiology nutrition recommendations concerning omega-3 and omega-6 fatty acids and the substitution of non-hydrogenated unsaturated fats for saturated and *trans* fats. Chapter 13 reviews the importance of dietary intakes of both n-6 and n-3 classes of fatty acids. The chapter reviews the chemistry, food sources, supplement sources, and the major intervention and survey studies that consistently show that compared to saturated fats, consumption of the two fatty acid classes is cardioprotective. The included tables and figures aid the reader in understanding the complexities of the fatty acid's mechanisms of action as well as enumerating dietary sources. Chapter 15

reviews the historical developments in the appearance of industrially produced *trans* fats. There is an in-depth review of the chemistry, production, and observation/intervention clinical studies that consistently find adverse cardiovascular effects associated with high intakes of industrially produced *trans* fats. There is also an extensive review of the beneficial cardiovascular effects seen with intakes of ruminant *trans* fats. Comprehensive tables and figures include a listing of countries, states, and cities that either voluntarily or via regulations ban the use of industrial *trans* fats in their food products.

Chapter 16 is a new chapter and emphasizes the major risk factors for stroke that have a link to diet. Dr. Corrigan, who is one of the chapter authors, coedited a volume entitled *Handbook of Clinical Nutrition and Stroke* in 2013 for the *Nutrition and Health* series. The modifiable or preventable components of stroke risk include hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation, smoking, physical inactivity, obesity, drug abuse, alcohol use, and high saturated fat-containing diets. The authors examine the links between each of these and how dietary changes can significantly reduce the risk of strokes. For example, hypertension can often be significantly reduced when excess body weight, salt, and alcohol intakes are reduced. Data also suggest that following the adoption of mandatory folic acid fortification, stroke mortality decreased at a greater rate in the United States and Canada than in countries that did not institute folic acid fortification strategies, suggesting a positive effect of fortification on cerebrovascular health. The last chapter in this section reviews these data and brings us up to date on the mechanisms by which the B vitamins, folic acid, and vitamin B12 influence the potential for vascular cognitive impairment in the brain. This chapter is especially timely as the number of elderly in the United States and globally continues to increase and the incidence of age-related neurological disorders, such as Alzheimer's disease and vascular dementia, is also increasing. Chapter 17 reviews the relationship between folic acid, vitamin B12, and vitamin B6 and the formation of homocysteine, a nonessential amino acid; elevated blood levels of homocysteine are considered risk factors for both cardio- and cerebrovascular disease. Moreover, elevated blood homocysteine levels are linked to inadequate intakes of these B vitamins. The chapter includes a comprehensive review of the recent survey and intervention studies.

Diabetes and Obesity

The last 6 years have seen an explosion of research in the fields of obesity and diabetes, and we have included six new areas of clinical significance and two timely updates in this part of the volume. The eight chapters in this part begin with an overview of childhood obesity that is followed by a chapter that updates us on the latest findings from the dietary intervention component of the Women's Health Initiative (WHI), a new chapter on fiber and type 2 diabetes, a related chapter on the effects of the metabolic syndrome as well as diabetes in the aging population, another new chapter that crosses the fields of orthopedics and obesity as well as diabetes, and a third new chapter that explores the nutritional consequences of bariatric surgery. A controversial source of calories is added sugars, and there is a new chapter by Dr. Rippe who edited a volume entitled *Fructose, High Fructose Corn Syrup, Sucrose and Health* published in 2014 in the *Nutrition and Health* series. The last chapter in this part is a new chapter that explores the role of low calorie sweeteners (LCS) in weight management.

Chapter 18 comprehensively reviews the current status of childhood obesity, the genetic components, and the environmental factors that have been identified as key issues in this global epidemic. New areas including the microbiome and maternal factors including breast feeding are also reviewed. Potential interventions for reducing the medical risks associated with childhood obesity are included.

The fourth edition of *Preventive Nutrition* included a critical review of the findings from the Women's Health Initiative (WHI). This volume includes an updated report from the WHI and includes ten figures and tables that provide a comprehensive review of the data from the largest intervention study ever undertaken in postmenopausal women. Chapter 19 includes a detailed review of the diet

modification (DM) arm that attempted to reduce fat calories to 20 % of intake as well as an analysis of the findings from the calcium and vitamin D arm of the study. Relevant findings from the observational study are also included. Following the closure of the intervention part of the WHI, observations have continued and will continue until 2020. Data from the post-intervention study are reviewed. Even though the overall data from the DM study did not find that the low-fat diet reduced the risk of breast cancer or colorectal cancer and was not associated with reduced risk of cardiovascular disease, obesity, or diabetes, there were many significant findings in subgroup analyses, and many lessons were learned about clinical trial design. In the calcium and vitamin D supplementation arm, there were also significant benefits associated with the intervention in subgroup analyses. As an example, in women who were not taking calcium or vitamin D at baseline, there was a 35 % reduction in hip fracture risk if these women were randomized to calcium plus vitamin D compared to placebo. The chapter also includes a list of relevant publications from the WHI study.

Chapter 20 highlights the newest data showing the strong effects of high-fiber diets on reducing the risk of diabetes. The chapter, containing more than 200 relevant references and more than a dozen tables and figures, reviews the survey and randomized control studies that have shown that healthy, higher-fiber diets (about 30 g/day) lower diabetes risk compared to low-fiber, Western diets. Potential mechanisms for fiber's lowering diabetes risk are reviewed, and the fiber content of good sources of fiber in common foods is tabulated.

Chapters 21 and 22 concentrate on the effects of diabetes in the aging population and remind us that almost half of the population that is 75+ is classified as having prediabetes. Diabetes in the aging population has particularly serious consequences including loss of vision and hypoglycemic-related falls that may result in hip and other fractures, decreased cognition, increased rates of depression, and worsening cardiovascular disease that could result in loss of limbs. Chapter 21 examines the potential for secondary and tertiary nutrition prevention of diabetes morbidities and reviews the importance of treating prediabetes in senior patients. The chapter includes in-depth analyses of both the physical and mental consequences of diabetes and the treatment effects of diabetes management. The seven tables and almost 100 relevant references enhance our understanding of the complexities surrounding glucose control in the aging population. Chapter 22 highlights the latest findings that both diabetes and obesity increase the risk of fractures in older patients. These data are particularly important as currently, diabetes is not considered as a risk factor for inclusion in the assessment of age-associated fracture risk. The new data indicate that the increased risk of hip fracture in older patients with type 2 diabetes occurs independent of the higher bone mineral density seen in these patients. Factors that may increase fracture risk include the use of certain diabetic drugs that are known to increase risk, long-term loss of glucose control, reduced bone formation and increased bone degradation at the cellular level, decreased bone strength even when there is increased bone mineral density, and abnormal bone matrix and bone quality. In addition to the potential for obese individuals to have a greater risk of diabetes, nondiabetic obese older individuals have a greater risk of fractures than those age-matched individuals who are not obese. Obesity can cause changes in the vertebrae, reduce direct sight of one's feet, and increase risk of falls; visceral obesity is linked to lower bone mineral density.

Chapter 23, also a new topic, examines the effects of bariatric surgery on nutritional status. There are reviews and illustrations of the four most commonly used surgical procedures and their consequences to the gastrointestinal tract that often result in nutritional deficiencies that may only become apparent after months or years postsurgery. Both the direct effects of removal of gastric tissues that result in malabsorption of nutrients and the effects of banding or restrictive absorption are reviewed. Some surgeries involve both the stomach and small intestine and can affect certain functions of these organs; bile secretion and the microbiome can also be affected. Although bariatric surgery has great benefits on weight loss and reduction in diabetic symptoms, there are certain consistent nutritional adverse effects including the potential for severe thiamin deficiency resulting in beriberi, loss of lean body mass and bone mass, dumping syndrome, fat-soluble micronutrient deficiencies, and other essential nutrient deficiencies that are reviewed in the chapter.

The next two chapters examine the roles of added sugars and sugar substitutes in weight management. Chapter 24 focuses on fructose-containing sugars including sucrose, high fructose corn syrup, and fructose. Sucrose and high fructose corn syrup are the predominant added sugars in the human diet. Honey, fruit juice concentrates, and agave nectar are also fructose-containing sources that may be consumed by certain population groups such as vegetarians and young children. There is an historical overview of sugar incorporation into foods from the use of honey up to the commercial development of high fructose corn syrup in 1984. The chapter includes a comprehensive discussion of the chemistry, metabolism, and physiology of sugar utilization, as well as a review of the manufacture of sugars and its many functions within commercially produced foods. There is an in-depth review of the intervention studies to determine the potential effects of added sugars on weight gain and/or obesity and/or nonalcoholic liver disease and the totality of the evidence points to a lack of effect.

The last chapter in this section contains a detailed review of the history of the use of artificial and other LCS, the regulatory framework, and an objective review of the clinical research, both RCT and observational studies involving these products. Chapter 25 concentrates on LCS that are permitted for use in the United States and their use as aids in weight loss as well as weight management. The first to be used was saccharin that was discovered in 1879, followed by aspartame that was discovered in 1956. Acesulfame potassium (Ace-K), discovered in 1967; sucralose, discovered in 1989; and later-discovered LCS including neotame, advantame, steviol glycosides – derived from the Stevia plant and Luo Han Guo fruit extracts – and a group of LCS known as sugar alcohols or polyols are included in this comprehensive chapter.

Prevention of Major Disabilities: Geriatrics

Three significant health risks associated with aging are osteoporosis and hip and other bone fractures, age-related eye diseases resulting in blindness, and depressed immune responses that increase risk of cancer, influenza, pneumonia, and other infectious diseases. Chapter 26 is coauthored by Robert Heaney who has contributed the chapter on the topic of diet and osteoporosis prevention for all five editions and is a coeditor of the series volume entitled *Calcium in Human Health* published in 2006. This expanded chapter includes not only data on the importance of adequate calcium intake for fracture prevention but also the critical importance of adequate protein and calcium/vitamin D following a fracture. In addition to an in-depth discussion of calcium requirements, there are reviews of other nutrients considered to be of value for optimal bone health. The importance of dietary supplements under certain circumstances is also considered.

Age-related macular degeneration (AMD) is the primary cause of blindness in adults over 65 years, and risk increases with further aging. Major risk factors include smoking and obesity. Chapter 27 includes 100 references and a detailed description of the stages of AMD and reviews the clinical and laboratory studies that indicate the potential for weight loss, lowering the glycemic load of the diet, as well as supplementation with certain nutrients to reduce the risk of AMD onset or progression of the disease. Data from the Age-Related Eye Disease Study (AREDS 1 and 2) and other clinical studies are reviewed, and these indicate that higher than currently recommended intakes of vitamins C and E, zinc, and the carotenoid lutein reduced the risk of AMD progression in certain circumstances. There is also a comprehensive review of the association between the intakes of fat types, cholesterol, and relevant dietary carotenoids and AMD risk.

The last chapter in this section examines the changes in immune responses as we age. John Bogden served as coeditor of the volume entitled *Clinical Nutrition of the Essential Trace Elements and Minerals* which was the second volume published in the *Nutrition and Health* series in 2000, and he and Donald Louria have provided this chapter for each of our volumes. Chapter 28 reviews the primary changes that are due to the age-dependent intrinsic decline in immunity as well as the secondary

changes that are the result of environmental factors such as immunosuppressive effects of certain prescription and nonprescription drugs, obesity, and other dietary factors. This immunosuppression is a major risk factor for increased fatalities due to pneumococcal pneumonia in older people and increased incidence of diseases such as urinary tract infections and varicella zoster shingles. Influenza sepsis is also more frequent and more severe in older people that may be related to concomitant changes in the urinary tract, respiratory tract, and nervous system that result in greater infectious disease morbidity and mortality in older people. The chapter reviews the effects of dietary and multi-/single nutrient deficiencies on immune functions and also includes an in-depth analysis of the RCT and observational studies involving single micronutrients and multiple micronutrients in the aging population.

Prevention of Major Disabilities: Adults and Children

The sixth part of the volume includes three chapters that examine conditions/diseases that affect nutritional status in adults followed by three chapters that review maternal nutritional choices and their potential consequences to their children. Chapter 29 reviews the numerous new findings that link the chronic, long-term use of proton pump inhibitors (PPIs) with significant increases in risk of bone fractures and the potential mechanisms for this effect that appears to include certain nutrients. Of importance are the data (details presented in comprehensive tables) that indicate that bisphosphonates, the most commonly used class of drugs for treatment of osteoporosis, have reduced efficacy when taken with PPIs. There is a comprehensive review of the importance of gastric acid secretions, the effects of *Helicobacter pylori* infection on gastric secretion as well as the consequences of its therapy on nutritional status, gastroesophageal reflux disease (GERD), nonsteroidal antiinflammatory drugs (NSAIDS) and ulcers, and the nutritional consequences. Nutrients reviewed include calcium and vitamin D, vitamin B12, iron, and magnesium.

Three chapters examine the critical importance of maternal nutrition very early in pregnancy and have been updated by authors who have contributed to many of the volumes of *Preventive Nutrition*. Chapter 30 reminds us that even in 2015 nearly 6.3 million children under 5 years of age die unnecessarily in low- and middle-income countries. Undernutrition is the direct cause of almost half of these deaths that often involve infectious diseases. Vitamin A and zinc deficiencies in particular have been estimated to account for one million deaths per year or 9 % of the global childhood burden of disease. Iron deficiency is a significant risk factor for maternal mortality at a rate of 115,000 deaths per year. The chapter concentrates on the effects of maternal and infant malnutrition on immune function and the resulting increased risk of infections as well as chronic inflammation seen in malnourished obese mothers and their children. There are in-depth discussions of the immune and inflammatory effects of deficiencies of vitamins A, B vitamins, C, D, E, and K as well as zinc, iron, selenium, and iodine that are supported by 350 relevant citations. The varied approaches to rectify these nutritional deficiencies are also reviewed.

Chapter 31 updates us on the current global rates of human immunodeficiency viral (HIV) infections in adults including pregnant and lactating women and includes almost 200 references and ten informative figures and tables. HIV infection is not only a viral infection, but is also a nutritionally progressive disorder with major metabolic changes in nutrient utilization as the balance of viral replication and immune and inflammatory responses changes over time. HIV treatments also have certain adverse metabolic effects. Additionally, in countries where food insecurity is a critical issue, such as seen in sub-Saharan Africa, inadequate protein and essential nutrient intakes decrease immune responses that are further worsened by HIV. The chapter includes a broad analysis of the implications of poor nutrition in the patient infected with HIV and the consequent risks to community and global health. With regard to women and their children, it is important to note that women make up about half of people infected with HIV globally. In 2013, 3.2 million children were living with HIV

worldwide. Most childhood infections occurred through pregnancy, birth, or breastfeeding. The effects on families and communities are enormous, with approximately 17.8 million children worldwide being orphaned due to AIDS, most of whom were from sub-Saharan Africa. Pediatric HIV infection can be contracted through exposure to the virus in breast milk. If an HIV-infected mother is given antiretroviral therapy, mother-to-child-transmission of HIV is approximately 5 % prenatally and up to 45 % after 2 years of breastfeeding. Thus, short-term breastfeeding is currently recommended. Unfortunately, bottle-feeding is often unaffordable and culturally unacceptable.

Chapter 32 continues the exploration of the databases developed in Hungary that include both survey and randomized controlled intervention data that was the original data to prove the power of periconceptional use of a folic acid-containing prenatal vitamin/mineral supplement to significantly reduce the occurrence of neural tube defects. We are fortunate to have a chapter by Andrew Czeizel in each of the five volumes of *Preventive Nutrition*. This updated chapter demonstrates the importance of relatively high doses of folic acid supplementation in the first trimester and continuing through pregnancy to significantly reduce the risk of major cardiovascular birth defects. The chapter includes detailed tables and informative figures that help the reader visualize these defects as well as extensive descriptions of the methodology used to evaluate these birth defects. We were very saddened to learn of Dr. Czeizel's recent passing. Chapter 33, updated by Theresa Scholl who has also provided a chapter for each of our volumes, reviews the significant issues that increase the risk for preterm delivery and draws from the author's long-term study of teen pregnancies in Camden, NJ. We are reminded that preterm delivery (<37 weeks of gestation) is an important public health issue as well a potentially devastating outcome for parents and the preterm infant. Consequences of preterm birth, often including low birth weight, are the leading cause of neonatal mortality and rank second only to birth defects as the leading cause of death during the first year of life. The chapter sensitizes us to the complexities associated with the many potential causes of preterm birth which may or may not be linked to nutritional status and thus the difficulties of finding a single remedy. Nevertheless, numerous essential micronutrient deficiencies have been linked to increased risk of preterm birth. Also, the recent findings linking maternal obesity as well as maternal fasting and/or severe underweight with increased risk are reviewed and referenced in detail. Chapter 34 describes the data that suggests a strong link between maternal starvation early in pregnancy and a significant increased risk of the development of schizophrenia in their offspring as these children approach adulthood. The chapter reviews the findings from two Chinese famine studies and notes the similarities with the earlier Dutch famine study. In all three population groups, there was well-documented prenatal exposure to famine that increased the risk of schizophrenia and, in some cases, other forms of major mental illness in later life. The specific risk factors, nutrient deficiencies, and mechanisms involved remain unclear; however, biological pathways that link prenatal nutritional adversity to brain functions are beginning to be elucidated.

Nutrition Transitions Around the World

The six chapters in this section examine the role of nutritional factors, economic status, and regulatory issues on the potential for improving the health of the global population. Many of the chapters include new areas of interest. The first two chapters examine the effects of ethnic food habits on chronic disease risks. Chapter 35 reviews data from Latin America and South America and examines the major changes resulting from urbanization. Throughout Latin America and South America, there has been a significant change in the demographics from rural populations suffering from semi- and overt starvation accompanied by infectious disease morbidity and mortality in young children to 75 % of the Latin Americans living in urban environments with chronic diseases associated with increased body weight as the major health concern. The chapter contains relevant figures that depict the changes in food

patterns, food preferences, socioeconomic changes, and the consequences of increased availability of low-cost, high-calorie, refined foods. There are numerous examples of the significant increase in rates of obesity in young children and teens throughout the Latin American and South American countries. Chapter 36 examines the role of salt intake on the risk of hypertension in Southeast Asians from Indonesia, Malaysia, Philippines, Singapore, Thailand, and Vietnam. The chapter includes reviews of ethnic variations in body fat; excess salt intake from traditional salted fermented foods and condiments; and increased urbanization and economic development that further contribute to increased levels of salt consumption in the region. The in-depth literature review and detailed tables indicate that further studies in the individual nations are required to accurately assess adult sodium status; however, it is well accepted that sodium intakes are higher than recommended, and suggestions for reduction and substitutions are included.

Food security is a growing area of research especially as the world becomes more urbanized and individuals do not grow their own crops or raise their own farm animals. This new chapter, Chap. 37, examines the effects of this nutrition transition to more calorie-dense, nutrient-poor diets in low-income countries. National surveys indicate that childhood stunting remains a critical issue and that their mothers may be overweight or obese, but remain iron-deficient, as an example. The chapter reviews the nutrition-sensitive food security programs and policies that have the potential to beneficially impact nutrition outcomes in low-income countries in the face of rapidly transitioning food and lifestyle environments. Initiatives discussed include biofortification of staple foods and implementation of food safety programs and education programs in emerging urban areas.

A major focus of the fifth edition is the presentation of a wide range of perspectives on the importance of preventive nutrition strategies and ways to reach the goals outlined by chapter authors. Chapters 38 and 39 are authored by well-recognized scientists who work in industry settings. Chapter 38 examines the role of food fortification in the improvements seen in global health and reviews data from both developing and developed nations. We are reminded that fewer and fewer people are producing the world's food and as populations move from rural to urban environments, there is a greater risk of food insecurity and hidden hunger (lower than recommended intakes of essential nutrients while consuming adequate or above-adequate calories) especially among the poorest populations. The chapter posits the question of the ability of nutritionists to stay ahead of the rapidly changing foods produced and the value of determining their nutrient content rather than determining the nutritional status of the population to determine the focus for improving nutrient deficits. Chapter 39 reviews the regulation of dietary supplements in the United States and comparable products in Canada, China, Japan, and the EU and then considers the evidence that supplementation has a role to play in human health. The term dietary supplement is defined for each country, and the approved health claims for each country are explained with the aid of ten excellent tables. The chapter concludes with a summary of the clinical studies involving supplements and their findings.

The final chapter in the volume, Chap. 40, takes a new look at the economic burden of unhealthy eating habits in developed nations such as the United States and the potential for clinical nutritionists to positively impact the dietary intakes of clients and patients. The chapter includes nine informative tables that outline the food sources of essential nutrients. There is a comprehensive discussion of the five primary topic areas that are addressed in the 2015 Dietary Guidelines: food environment, physical activity environment, agriculture/aquaculture sustainability, food systems, and food safety. Also included are reviews of the clinical data that demonstrate the effectiveness of dietary management of chronic diseases such as cardiovascular disease, obesity, osteoporosis, and diabetes and discussions of how the data can be implemented into patient diagnosis and care, monitoring, and evaluation.

The above description of the volume's 40 chapters attests to the depth of information provided by more than 70 well-recognized and respected chapter authors. Each chapter includes complete definitions of terms with the abbreviations fully defined for the reader and consistent use of terms between chapters. The volume includes over 200 detailed tables and informative figures; an extensive, detailed index; and more than 4600 up-to-date references that provide the reader with excellent sources of worthwhile information.

In conclusion, *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fifth Edition*, edited by Adrianne Bendich, Ph.D., and Richard Deckelbaum, M.D., provides health professionals in many areas of research and practice with the most up-to-date, well-referenced volume on the importance of maintaining the nutritional status of healthy clients as well as patients regardless of their age or the cause of their illness. The volume contains excellent chapters that identify global issues that affect populations around the world, chapters that carefully document the critical value of preventive nutrition strategies as well as medical nutrition evaluation, treatment support, and management of patients with many serious diseases including cancer, cardiovascular and cerebrovascular diseases, diabetes, and obesity. Specific chapters examine the major disabilities seen in the geriatric populations and in adults, with an emphasis on women of child-bearing potential and children. There are also timely chapters that examine the nutritional transitions that are affecting developing nations. This volume serves the reader as the benchmark in the complex area of interrelationships between nutrients, diet and food patterns, and the use of nutritional and/or nonnutritional supplements. Moreover, the physiological, pharmacological, and pathological interactions between nutrition, health, and disease are clearly delineated so that students as well as practitioners can better understand the complexities of these interactions. Unique chapters examine changes in critical organ systems such as the eye, bone, brain, and immune system and provide guidance concerning potential benefits from clinically relevant nutritional interventions. We applaud all of our authors for their efforts in helping us to develop the most authoritative resource in the field to date, and we hope that this excellent text serves as a critical addition to the *Nutrition and Health* series.

Adrianne Bendich, Ph.D., F.A.S.N., F.A.C.N.
Series Editor

About the Editors

Dr. Adrianne Bendich, Ph.D., F.A.S.N., F.A.C.N., has served as the "Nutrition and Health" Series Editor for 20 years and has provided leadership and guidance to more than 200 editors that have developed the 70+ well-respected and highly recommended volumes in the series.

In addition to **"Preventive Nutrition: The Comprehensive Guide For Health Professionals, Fifth Edition," edited by Adrianne Bendich, Ph.D. and Richard J. Deckelbaum, M.D.**, major new editions in 2012–2016 include:

1. **Glutamine in Clinical Nutrition**, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2015
2. **Nutrition and Bone Health, Second Edition**, edited by Michael F. Holick and Jeri W. Nieves, 2015
3. **Branched Chain Amino Acids in Clinical Nutrition, Volume 2**, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2015
4. **Branched Chain Amino Acids in Clinical Nutrition, Volume 1**, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2015
5. **Fructose, High Fructose Corn Syrup, Sucrose and Health**, edited by James M. Rippe, 2014
6. **Handbook of Clinical Nutrition and Aging, Third Edition**, edited by Connie Watkins Bales, Julie L. Locher, and Edward Saltzman, 2014
7. **Nutrition and Pediatric Pulmonary Disease**, edited by Dr. Youngran Chung and Dr. Robert Dumont, 2014
8. **Integrative Weight Management** edited by Dr. Gerald E. Mullin, Dr. Lawrence J. Cheskin, and Dr. Laura E. Matarese, 2014
9. **Nutrition in Kidney Disease, Second Edition**, edited by Dr. Laura D. Byham-Gray, Dr. Jerrilynn D. Burrowes, and Dr. Glenn M. Chertow, 2014
10. **Handbook of Food Fortification and Health, Volume I**, edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013

11. **Handbook of Food Fortification and Health, Volume II**, edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013
12. **Diet Quality: An Evidence-Based Approach, Volume I**, edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013
13. **Diet Quality: An Evidence-Based Approach, Volume II**, edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013
14. **The Handbook of Clinical Nutrition and Stroke**, edited by Mandy L. Corrigan, M.P.H., R.D. Arlene A. Escuro, M.S., R.D., and Donald F. Kirby, M.D., F.A.C.P., F.A.C.N., F.A.C.G., 2013
15. **Nutrition in Infancy, Volume I**, edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013
16. **Nutrition in Infancy, Volume II**, edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013
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22. **Alcohol, Nutrition and Health Consequences**, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
23. **Nutritional Health, Strategies for Disease Prevention, Third Edition**, edited by Norman J. Temple, Ted Wilson, and David R. Jacobs, Jr., 2012
24. **Chocolate in Health and Nutrition**, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
25. **Iron Physiology and Pathophysiology in Humans**, edited by Dr. Gregory J. Anderson and Dr. Gordon D. McLaren, 2012

Earlier books included **Vitamin D, Second Edition** edited by Dr. Michael Holick; **Dietary Components and Immune Function** edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi and Dr. Victor R. Preedy; **Bioactive Compounds and Cancer** edited by Dr. John A. Milner and Dr. Donato F. Romagnolo; **Modern Dietary Fat Intakes in Disease Promotion** edited by Dr. Fabien De Meester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; **Iron Deficiency and Overload** edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; **Nutrition Guide for Physician**s edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; **Nutrition and Metabolism** edited by Dr. Christos Mantzoros and **Fluid and Electrolytes in Pediatrics** edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include **Handbook of Drug-Nutrient Interactions** edited by Dr. Joseph Boullata and Dr. Vincent Armenti; **Probiotics in Pediatric Medicine** edited by Dr. Sonia Michail and Dr. Philip Sherman; **Handbook of Nutrition and Pregnancy** edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch, and Dr. Elliot Philipson; **Nutrition and Rheumatic Disease** edited by Dr. Laura Coleman; **Nutrition and Kidney Disease** edited by Dr. Laura Byham-Grey, Dr. Jerrilynn Burrowes, and Dr. Glenn Chertow; **Nutrition and Health in Developing Countries** edited by Dr. Richard Semba and Dr. Martin Bloem; **Calcium in Human Health** edited by Dr. Robert Heaney and Dr. Connie Weaver and **Nutrition and Bone Health** edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich is President of Consultants in Consumer Healthcare LLC and is the editor of ten books including **Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fifth Edition** coedited with Dr. Richard Deckelbaum (www.springer.com/series/7659). Dr. Bendich serves on the Editorial Boards of the Journal of Nutrition in Gerontology and Geriatrics, and Antioxidants, and has served as Associate Editor for "Nutrition" the International Journal, served on the Editorial Board of the Journal of Women's Health and Gender-Based Medicine, and served on the Board of Directors of the American College of Nutrition.

Dr. Bendich was Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare and provided medical leadership for many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid-containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has coauthored over 100 major clinical research studies in the area of preventive nutrition. She is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich received the Roche Research Award, is a *Tribute to Women and Industry* Awardee, and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences. Dr. Bendich was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. In 2012, she was recognized for her contributions to the field of clinical nutrition by the American Society for Nutrition and was elected a Fellow of ASN. Dr. Bendich is Adjunct Professor at Rutgers University. She is listed in Who's Who in American Women.

Richard J. Deckelbaum, M.D., C.M., F.R.C.P.(C), received his education at McGill University in Montreal, Canada. He now directs the Institute of Human Nutrition at Columbia University where he holds professorships in nutrition, pediatrics, and epidemiology. In addition to his ongoing basic research in cell biology of lipids, cardiovascular diseases, and issues of human nutrition, he has been active in translating basic science findings to practical application in different populations. Dr. Deckelbaum has published over 350 research and other publications. He has chaired task forces for the American Heart Association, the European Atherosclerosis Society, the WHO, the Institute of Medicine, and the March of Dimes and has led and/or served on advisory committees of the National Institutes of Health, the FDA, RAND Corporation, and of the US National Academies of Science, as well as the US Dietary Guidelines Committee. Dr. Deckelbaum has directed novel "econutrition" task forces and activities integrating health, nutrition, ecology, and agriculture.

Early in his career, he was a physician in Zambia and afterwards helped establish the first children's hospital in the West Bank of the Jordan and then continued to organize research and health programs among Egyptian, Palestinian, and Israeli populations. He cofounded the Medical School for International Health (MSIH), a "novel" medical school at Ben-Gurion University of the Negev in Israel, in affiliation with Columbia University Medical Center. MSIH aims to help build the international health work force through inoculating global health skills into medical education. Dr. Deckelbaum was President of the Global Health Education Consortium (GHEC) and a Board Member of the Consortium of Universities for Global Health (CUGH). In addition to other awards and honors, he has received lifetime achievement awards by GHEC and by McGill University. He served on the Food and Nutrition Board of the National Academies of Science and is a Senior Fellow of the Synergos Institute. He participates in planning and coordinating nutrition education, policy, and research programs in the Mideast, Asia, and Africa. Dr. Deckelbaum promotes and is active in projects related to health and science as a bridge between different populations nationally and internationally.

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Part I
Global Issues

Chapter 1
Preventive Nutrition: From Public to Personal Recommendations and Approaches to Behavior Change

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Key Points

- National dietary guidelines for general audiences are increasingly focused on a holistic view of nutrition with an emphasis on whole foods and dietary patterns in the context of long-term environmental sustainability.
- Personally tailored nutrition guidance is emerging via assessment of genetic and biochemical profiles directed to individualized dietary recommendations for health promotion and disease prevention.
- The Socioecological Model, Health Belief Model, Theory of Reasoned Action and Planned Behavior, Transtheoretical Model, and Social Cognitive Theory are frameworks that help to explain and predict dietary behavior at the individual level.
- Motivational interviewing is an effective behavioral strategy at the individual level within a health care setting for promoting dietary behavior change.
- Smartphone applications may be useful tools to promote dietary behavior change and assist with self-monitoring of dietary behaviors.
- Culture strongly influences dietary preferences and health beliefs also influence dietary behaviors.
- Health care professionals can play broad but nontraditional roles in support of their patients by addressing the upstream root causes of diseases and conditions related to diet.
- Policies at global, national, and local levels can shape dietary behaviors by influencing food availability and price, dietary guidelines, nutrition information, nutrition standards, and support for nutrition programs.

Keywords Behavior change • Community-level change • Dietary acculturation • Dietary guidance • Dietary supplementation • Fat tax • Food desert • Food security • Food swamp • Health belief model • Motivational interviewing • MyPlate • Nutrition security • Preventive nutrition • Personalized nutrition • Soda tax • Smartphones • Social Cognitive Theory • Socioecological Model • Sustainability • Theory of Reasoned Action and Planned Behavior • Transtheoretical Model • Upstream doctors

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_1

Introduction

The first International Conference on Nutrition in 1992 and subsequent World Food Summits and related action plans organized by the Food and Agriculture Organization of the United Nations and the World Health Organization have repeatedly reaffirmed their commitment of the right of everyone to have access to safe, sufficient, and nutritious food. In 2014, the Second International Conference on Nutrition acknowledged that malnutrition—including undernutrition, micronutrient deficiencies, overweight, and obesity—is widespread and not only affects health and well-being of people but also restricts the attainment of human potential and pose a high burden of adverse social and economic consequences to individuals, families, communities, and nations [1]. The root causes of factors leading to malnutrition are complex and multidimensional and involve cultural, economic, environmental, political, and social factors. Nonetheless, we clearly face a conundrum in the expansive body of scientific knowledge accumulated during the last 40 years about how individual nutrients and dietary patterns affect human metabolism and health and the concurrent growing prevalence around the world of diet-related, noncommunicable chronic diseases like obesity, type 2 diabetes, cardiovascular disease, cancer, and osteoporosis [2, 3]. Implementing practices and policies that promote health at both public and personal levels requires new ways of utilizing the reductionist and holistic paradigms of preventive nutrition and approaches that help support behavioral changes within communities and in individuals.

Preventive Nutrition and Public Health

About 80 % of all chronic disease and associated premature death around the globe is likely preventable with the uncontroversial knowledge we now possess. Among adults who do not smoke and avoid excessive consumption of alcohol, diet influences long-term health prospects more than any other environmental factor. Over the last 30 years, the more affluent countries have made substantial progress in improving life expectancy at birth and overall healthy life expectancy (i.e., the number of years that a person at a given age can expect to live in good health, taking into account mortality and disability). However, the prevalence of morbidity and chronic disability now accounts for about half of the health burden in these countries. In the USA, about half of all adults have one or more preventable chronic diseases directly related to poor quality dietary patterns and physical inactivity, including cardiovascular disease, hypertension, type 2 diabetes, and diet-related cancers. More than two-thirds of adults and nearly one-third of children and adolescents are overweight or obese.

Although the USA spends the most per capita on health care across all countries, it lags behind other high-income countries for life expectancy and other health outcomes [4]. Among the 34 countries in the Organisation for Economic Co-operation and Development, the US rank for healthy life expectancy fell from fourteenth to twenty-sixth between 1990 and 2010 [2]. Other than alcohol use and physical inactivity, the leading factors related to disability-adjusted life years are related to diet, including high body mass index, blood pressure, and fasting glucose. Among the initiatives to address the determinants of this trend have been a number of calls to modify the food supply [5–7]. The notion that healthy life years can be increased through preventive nutrition is supported by findings from the prospective European Prospective Investigation into Cancer and Nutrition study where adhering to four factors at baseline (two related to nutrition)—eating a healthful diet (high intake of fruit, vegetables, and whole grains with low meat consumption) and having a body mass index less than 30 kg/m^3 as well as performing over 3.5 h/week of physical activity and never smoking—was associated with a 78 % reduction in the risk of developing a major chronic disease and a 93 % reduction in the risk of diabetes; a risk reduction of 50 % was achieved by adhering to just one of these variables [8]. Nonetheless, most health care systems continue to be focused on disease treatment rather than prevention.

Dietary Guidelines: A Holistic Approach

Dietary guidelines for general audiences are a public policy of preventive nutrition and have been increasingly focused more on a holistic view of nutrition with an emphasis on whole foods and dietary patterns rather than on specific macronutrients and micronutrients. This is a sound approach as the evidence for diet–health relationships is more consistent for dietary patterns than for single foods or ingredients. In part, this is because a broader, holistic perspective of preventive nutrition includes the interactions between foods and food components where synergistic interactions appear to occur [9–11]. Nonetheless, challenges exist in summarizing these data due to the varying definitions of food groupings found in the literature. For example, "total meat" has been defined as a variety of different combinations of categories including meat (red, processed, or other), sausage, poultry, fish, and eggs. The definition of vegetables does not distinguish between categories and also often excludes potatoes but when included, information is rarely provided about their preparation, e.g., baked, boiled, or fried.

Despite such limitations, there is a broad consensus that healthy dietary patterns are higher in fruits and vegetables, whole grains, seafood, legumes, and nuts and lower in sugar-sweetened foods and beverages, refined grains, red and processed meat [12]. These broad recommendations are commonly conveyed to the general population using easy-to-follow visual aids such as the United States Department of Agriculture (USDA) Good Guide Pyramid and its more recent iteration as MyPlate [13]. However, it is important to recognize that the relative strength and consistency of the evidence supporting links to health outcomes varies between these groupings of foods. For example, the 2015 U.S. Dietary Guidelines Advisory Committee found consumption of vegetables and fruit were the only characteristics of the diet that were uniformly identified in every conclusion statement across all health outcomes [12]. Whole grains were identified slightly less consistently in this regard, but were also included in all the conclusion statements with moderate to strong evidence. Among food groups with limited evidence, grains were not as consistently defined nor identified as a key characteristic necessary to health promotion. Other food groups that were identified as beneficial albeit for a limited number of health outcomes included low- or non-fat dairy, seafood, legumes, nuts, and alcohol. Based on moderate to strong evidence, food groups with detrimental impacts on health outcomes at higher intakes included red and processed meats, sugar-sweetened foods and beverages, and refined grains. Some controversy remains about recommendations concerning the potential for untoward outcomes associated with saturated fat intake from some whole foods like dairy products [14–16]. Following guidelines for a healthful dietary pattern appear associated not only with a reduced risk of prevalent disorders like cardiovascular disease and obesity but also can have benefits across a broad array of chronic conditions and can thus be promoted to entire populations. It is important also to recognize that these dietary patterns can be achieved in a variety of ways but need to be tailored to sociocultural preferences so as to be more likely to be adopted by diverse population groups.

This holistic approach to improve nutritional recommendations by considering broad dietary patterns is considered a holistic or top-down approach that presumes the complex relations underlying diet–health relationships cannot be modeled on the basis of a linear cause–effect relation between a single nutrient and its physiologic or metabolic effect [17, 18]. Thus, it would appear that preventive nutrition must be considered first from a holistic perspective with reductionist approaches considered thereafter to provide a biological plausibility for a particular recommendation [19]. However, historically, nutritional recommendations have been developed from reductionist or bottom-up approaches from specific considerations of foods as a source of critical ingredients, e.g., dairy for calcium and citrus fruits for vitamin C.

Holistic recommendations at the food level are able to incorporate not only the nutrient profile of each food group but also the complex impact of food forms and matrices. Examples of these complexities include compound interactions within a food matrix, physical structures of compactness and

particle size, and other physiochemical properties like hygroscopicity. These properties are largely determined at agronomic, postharvest, and processing steps but can have direct effects on nutrient bioaccessibility as well as physiological responses such as glycemia and satiety [20, 21]. Recommendations for food diversity are a part of all dietary guidelines but it is worth noting supportive evidence from human studies demonstrating that a larger botanical diversity in the diet is associated with a greater reduction in oxidative stress [22] and higher cognitive performance [23].

Another aspect of a holistic perspective on preventive nutrition is sustainability and the need to ensure access to sufficient, nutritious, and safe food today and for future generations [24, 25]. Diets higher in plant-based foods are associated not only with better health outcomes but also with less environmental impact in terms of increased greenhouse gas emissions and use of land, water, and energy. However, seafood is an important component of most healthful dietary patterns and there are concerns about the sustainability of current efforts to meet global demands. The 2015 Dietary Guidelines Advisory Committee concluded that "linking health, dietary guidance, and the environment will promote human health and the sustainability of natural resources and ensure current and long-term food security" [12]. However, challenges to food security (i.e., ensuring that sufficient food is available) as well as nutrition security (i.e., ensuring that food quality meets human nutrient needs) are self-evident when resources such as fertile land and fresh water are diminishing and changes in temperature and atmospheric carbon dioxide can reduce both crop yields and the nutrient quality of important plant foods [26, 27]. Because these problems are complex, solutions must be multi-pronged and directed to achieving greater production of nutritious foods with fewer resource inputs, improved food stability for storage and distribution, reduced food loss and waste, broader use of natural foodstuffs, and the development of novel foods using new technologies. Approaches to these challenges include genetically engineering plants to improve sustainability, yield, and nutrition [28]; developing processing methods to safely enhance the preservation, storage, nutrient content, and transportation of food [29]; creating approaches to reduce loss and waste throughout the food supply chain [30]; and exploring the potential value of uncommon foods such as insects and novel food production such as bioprinting.

Preventive Nutrition and Personal Health

Dietary guidelines represent a science-based government policy to promote public health via a safe, affordable, and sustainable food supply. As national policies, these guidelines are intended to influence standards and initiatives across the public and private sectors, including business, education, and health care as well as the food industry and retailers. However, with respect to achieving the adherence of individuals to promulgated guidelines such as the Dietary Guidelines for Americans, the goals appear to have been more aspirational than actionable. Indeed, since the introduction of the Dietary Guidelines for Americans in **1980**, obesity rates in the USA have more than doubled in children and adults, with marked disparities between ethnic and racial groups noted [**31**, **32**]. In **2013**, median intakes of fruits and vegetables among adults was **1.1** and **1.6** servings daily, with the percentage of adults who report consuming fruits and vegetables less than one time daily at **37.7** and **22.6**, respectively [**33**]. These values have changed little over the last few decades. Thus, it is unsurprising that many Americans fall short of meeting the Estimated Average Requirement or Adequate Intake for several essential nutrients including vitamins A, C, D, E, K, folate, and choline plus the minerals calcium, magnesium, and potassium as well as fiber. Iron is noted as a shortfall nutrient for adolescents and premenopausal women. Two nutrients, sodium and saturated fat, are overconsumed in the USA relative to the Tolerable Upper Intake Level.

Dietary Supplementation

Despite identifying shortfalls in ten essential nutrients and fiber, only three nutrients (vitamin D, calcium, and potassium) and fiber are classified as "nutrients of public health concern." The rationale for de-emphasizing the documented shortfall of the other micronutrients is based on a lesser degree of evidence being available associating their underconsumption with adverse chronic outcomes. However, such consideration appears to dismiss the role these vitamins and minerals play via their essentiality in supporting biochemical and physiological pathways necessary to cellular function and health in those individuals with poor status of these nutrients. Historically, the recognition of major shortfall nutrients has been successfully, but only partly, addressed by the mandatory fortification of staple foods—including the addition of iodine to table salt, folic acid to refined flour, and vitamin D to fluid milk—and, to replace the nutrients lost during the refinement of flour, enrichment with iron, folic acid, riboflavin, and thiamine. Voluntary fortification with nutrients and bioactives is increasingly common today with examples such as fruit juices fortified with calcium and vitamin D, breads with added omega-3 fatty acids, and vegetable oil spreads formulated with plant sterols. Nonetheless, as shortfalls in so many nutrients continue to be prevalent and recognized by consumers, personal choices (with or without the advice of health care professionals) to increase intake of nutrients via dietary supplements is common in the USA with about 50 % of adults using them [34]. While enrichment and/or fortification contribute significantly to intakes of vitamins A, C, D, folate, thiamin, and iron, dietary supplements further reduce the percentage of the population consuming less than the Estimated Average Requirement for all nutrients [34–36]. Dietary supplements have long been an important source of vitamin E intake with results from the U.S. National Health and Nutrition Examination Surveys showing that 64 % of the mean daily intake of vitamin E among adults over 19 years was obtained from supplements [37].

Suggesting an efficacy greater than simply filling nutrient shortfalls, in evaluating a nationally representative sample of American adults, Bailey et al. [38] reported the use of multivitamin/multimineral supplements was associated with a reduced risk of cardiovascular disease mortality among women, but not men. Applying the results of randomized clinical trials demonstrating the efficacy of selected dietary supplements, Shanahan and de Lorimier [39] conducted a cost-benefit scenario analysis of their use by specific populations via calculating the avoided hospital utilization costs given 100 % use of a supplement regimen at preventive intake levels and subtracting the cost of utilizing the supplement. Their model predicted an average of 137,210 avoided cardiovascular events per year from 2013 to 2020 if all US adults over the age of 55 diagnosed with coronary heart disease were to use omega-3 dietary supplements at a preventive intake level of 1000 mg and provide an annual total hospital utilization cost avoidance of $2.06 billion. An independent analysis of the same population estimated an average of 101,028 avoided cardiovascular events per year from 2013 to 2020 could result from the use the folic acid, B6, and B12 at protective intakes averaging 1.7 mg, 29 mg, and 0.5 mg, respectively, to yield an annual cost saving of $1.52 billion. Estimating the total health care expenditures for managing and treating osteoporosis-attributed bone fractures among all US women over the age of 55 with osteoporosis, the use of daily supplement of calcium and vitamin D at about 1000 mg and 700 IU, respectively, would avoid an average of 151,053 osteoporosis-attributed bone fractures per year from 2013 to 2020 would result in an annual savings of $1.87 billion.

Personalized Nutrition: A Reductionist Approach

While dietary guidance, food fortification and enrichment, and self-selected dietary supplementation represent usual approaches to preventive nutrition, substantial attention is now being directed not only to continuing and improving empirically based nutrition but also to individually based nutrition or personal

nutrition. In contrast to the holistic concept of nutrition with its traditional one-size-fits-all recommendations, personally tailored nutrition might be considered a reductionist or bottom-up approach that works to combine dietary intake data, nutrigenomics, biomarker analyses, and life-style information to identify genetic or epigenetic linkages via particular biochemical and physiological pathways to food and nutrient needs specific to an individual. While the holistic approach is based upon our ability to predict the average response to a dietary intervention for large groups of individuals, the specific response for each individual in the group can vary markedly. This is the focus of personalized nutrition, an approach that attempts to identify the underlying relationship between our diet and genes (in both the human genome and microbiome) [40–43]. This approach is being developed through government efforts, e.g., the European Union's FP7 project titled Food4Me: "Personalised nutrition: an integrated analysis of opportunities and challenges," as well as by private companies for commercial application.

It is clear that different people can have different metabolic responses to the same or similar foods. Via nutrigenomics, personalized nutrition can focus on biochemical pathways—such as antioxidant defenses, one carbon metabolism, glucoregulation, carbohydrate and lipid metabolism, and inflammation—to identify those where physiological mechanisms may be less functional or impaired. For example, the association between cardiovascular disease and dyslipidemia and abdominal obesity is so well established that prevention of the disease has been focused almost exclusively on the normalization of lipid profiles and weight reduction via diet, physical activity, and medication. However, successful modification of these risk factors has yielded at best a 30–40 % reduction in morbidity and mortality. Numerous studies of families and relatives have long suggested a strong genetic predisposition to cardiovascular disease but it was with the sequencing of the human genome that strong associations were identified of specific variants of select target genes and the risk for cardiovascular disease [44]. Most of the early candidate genes belonged to the APO family which play a role in lipoprotein metabolism and lipid transport with functional polymorphisms at the *APOA4* and *APOE* loci contributing significantly to the variability of lipoprotein responses to dietary fat and cholesterol [45]. More recent advances have revealed that some of the individual variation in these responses are mediated by differential regulation by micro-RNAs [46], the peroxisome proliferator activated receptor (*PPAR*) family of transcription factors [47], cytokine-mediated inflammatory pathways [48], folate-mediated one-carbon metabolism [49], and clock circadian regulator genes [50]. Importantly, many of these studies have been conducted with relatively small samples sizes and, thus, low statistical power and inadequate replication. However, work with larger cohorts combined from different populations are now showing more consistent results with application not only to specific nutrients but also to whole foods and dietary patterns [51, 52]. Corella and Ordovás [53] have described the translational goals of this research as stimulating the broad use of genetic profiles to guide clinicians in the classification of their patients according to their disease rather than on their symptoms and to begin using genetic profiles to implement targeted recommendations and therapies aimed to improve prevention.

While the concept of personalized nutrition continues to emerge toward broader use, it is important to recognize that it is already being applied in clinical practice and in the commercial world of direct-to-consumer personal genetic tests [54]. The question of whether there is a sufficiently strong scientific and ethical basis for the application of genetic-based personalized nutrition has been raised in many reports [55]. Consumer interest in this area suggests that direct access to personal genetic information may motivate individuals to adopt diet and other lifestyle behavioral changes directed to promoting healthy aging and reducing the risk of disease, but the available evidence supporting this notion is very limited [56–59]. In a cohort of young adults already participating in a nutrigenomics study, Nielsen and El-Sohemy [60] conducted a randomized clinical trial comparing the effects of providing genotype-based dietary advice with general recommendations on behavioral outcomes and found most proffered information to be understandable and useful; a minority of the participants expressed unease about learning their genetic information. However, a Cochrane review [61] concludes that the current evidence does not support DNA-based test results as being effective in motivating people to change their behavior. While more research is undoubtedly necessary to substantiate the validity of individualized assessments of gene–diet relationships and their ability to direct behavioral

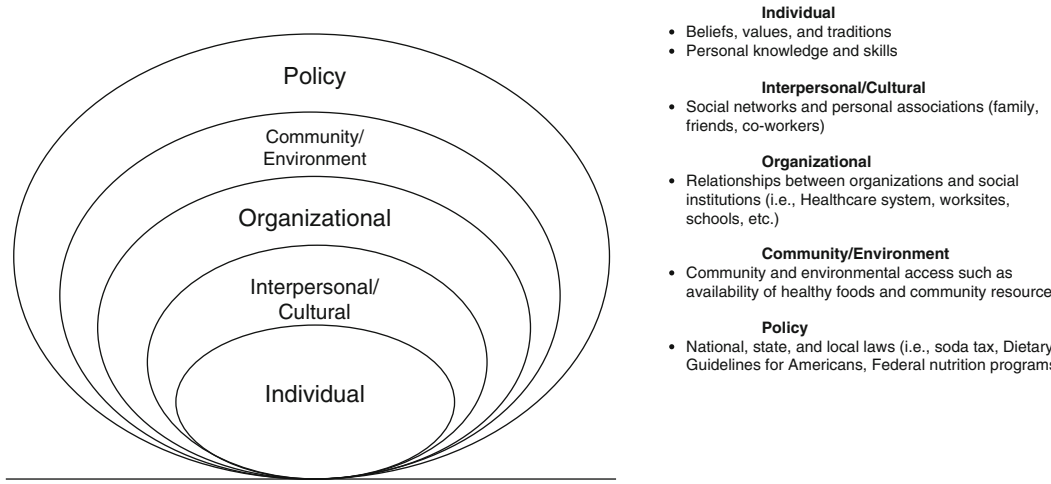

Fig. 1.1 Levels of influence: socioecological model. Adapted from http://www.cdc.gov/cancer/crccp/sem.htm

change, personalized nutrition should be evaluated in the context of the available evidence for generally accepted standards of dietary advice (such as the Dietary Guidelines for Americans) which are recognized as being limited, not particularly effective in modifying behavior in a diverse population, and in need of further research and substantiation. Nonetheless, personalized nutritional assessments are unlikely to be reimbursed by insurance programs or public health services at this time, so the associated expense will substantially limit a broad adoption of this approach.

The holistic approach to preventive nutrition via dietary guidelines that consider dietary patterns and integrate physical activity into an overall healthy lifestyle are essential to public health policy. The reductionist approach of personalized nutrition focuses more narrowly, assessing particularly individual traits through nutrigenomics and related biochemical pathways to identify nutrients, foods, and/or dietary patterns specific to directing them toward better physiological function and health. Importantly, both approaches are useful and can inform and help substantiate each other. Both approaches strive to provide information on which people as individuals or communities can make positive changes in their food choices and diet to improve health outcomes. However, this goal ultimately is more closely tied to behavioral science than nutrition and biology and requires a full integration of biological, economic, and social aspects of diet and nutrition, a "society-behavior-biology nexus," to promote healthy lifestyle at individual and population levels [62].

Health promotion efforts to improve dietary intake historically were developed with an emphasis on the individual, using principles from health psychology [63]. Although an ecological perspective has long been fundamental to the field of public health [64, 65], it is only relatively recently that the role of environmental factors in influencing dietary behavior and behavior change has gained prominence. The Socioecological Model, with its multiple spheres of influence of individual-interpersonal-organizational-community (Fig. 1.1) [66], has gained increasing endorsement as a framework for understanding and changing dietary behaviors [67–70].

Individual-Level Behavior Change

Research findings generally suggest that individual-level interventions, including nutrition counseling, are more effective when based on behavioral theory [65, 71–73] although some reviews have found little benefit from theory use [74]. Psychological theories attempt to understand relationships between

the variables in a situation, providing a way to explain and predict how people will behave given a certain set of characteristics and circumstances. Initial theoretical models on which dietary interventions were developed were simplistic and were based mainly on knowledge, attitudes, and beliefs [63].

Behavioral theories have evolved and the ones used currently are more complex, specifying both an increased number of constructs and hypothesized mediation pathways among the constructs. Common ones include the Health Belief Model, the Theory of Reasoned Action and Planned Behavior, the Transtheoretical Model, and Social Cognitive Theory [65, 75]. The Health Belief Model comprises the constructs of perceived susceptibility and perceived severity, which combine to produce perceived threat, as well as perceived benefits, perceived barriers, cues to action, and self-efficacy. The Theory of Reasoned Action posits that behavior is predicted most proximally by intention, which itself is predicted by attitude and subjective norm. Attitude in turn is predicted by behavioral beliefs and outcome evaluations, while subjective norm is predicted by normative beliefs and motivation to comply. In recognition of the important role of barriers, the theory was later updated to include perceived behavioral control, comprised of control beliefs and perceived power. The amended model is known as the Theory of Planned Behavior. In the 1980s the Transtheoretical Model was developed in relation to smoking behaviors and was later applied to dietary change. This model examines readiness to change, and includes the stages of change: precontemplation (no intention to change), contemplation (considering change), preparation (planning for change), action (adopted behavior change), and maintenance (continued practice of new behavior over time). In Social Cognitive Theory, a key element is a reciprocal relationship between personal factors, the environment, and behavior. Key constructs are outcome expectations, self-efficacy, facilitation, incentive motivation, and self-regulation. Theory-based strategies for which there is strong evidence of effectiveness are self-monitoring, goal-setting, problem solving, and social support [72, 76]. However, despite evidence that theory and theory-based strategies produce significant effects when used in dietary change interventions, major critiques are that these effects are modest and that evidence for sustainability is limited [77].

In part because of these critiques, in the current era there has been a reconsideration of noncognitive influences on behavior. Dual-process models implicate both automatic (emotional, habitual) and reflective (cognitive) processes in behavior change. According to these models, strategies that target the automatic system provide motivation for change, while those that target the reflective system provide clear direction for doing so; change is most effective when both are involved. There has been a growth in recent years of reports describing how both reflective and automatic factors may be considered in dietary behavior change, e.g., by creating and changing habits and by adjusting for issues related to willpower [78–82]. Behavioral economics, which has been posited to act on the automatic system in addition to correcting for biases in the reflective system [83–85] is also a source of recent interest. There is growing evidence for the effectiveness of "choice architecture" strategies in which the healthier dietary choices are made the easier, default choices as used in both homes [86, 87] and schools [88, 89]. There is mixed evidence for financial rewards, another aspect of behavioral economics, as a strategy for facilitating dietary change [72, 90].

A behavioral strategy at the individual level that is increasingly being used within the health care setting is motivational interviewing (MI). MI is a "collaborative conversation style for strengthening a person's own motivation and commitment to change" [91]. Rather than simply telling a patient what she should do (traditional advice-giving), a practitioner using MI techniques guides the patient toward a commitment to change in a way that builds on their own values, desires, strengths, and resources. This counseling technique is closely aligned with Self-Determination Theory [92], which distinguishes among types of motivation, on a continuum from extrinsic (coming from outside of oneself) to intrinsic. Internalization of extrinsically motivated behaviors is facilitated by promoting autonomy, competence, and relatedness; MI provides specific techniques for fostering these [93]. MI also provides a way to operationalize elements of other behavioral theories, such as the Transtheoretical Model. It is a way to help clients in the contemplation stage explore and move past their ambivalence.

A growing body of evidence demonstrates the effectiveness of brief MI in making healthful changes related to diet and weight loss [72, 94, 95]. There are also a number of challenges to utilizing MI. It can require extensive training to learn MI techniques. However, within a primary care practice, different members of the team can take the lead on conducting preventive sessions; increasingly, these professionals are being introduced to MI concepts within their training. Further, MI does not seem to require more time (per visit or number of visits) than other types of patient counseling [96].

Another approach to behavior change at the individual level, and an area in which health professionals may play a key role, is the use of technology. As much of the globe is digitally connected, technological solutions to dietary behavior change hold great promise. In a review of interventions to increase adult fruit and vegetable intake conducted in seven countries, face-to-face counseling was found to produce consistently positive results, but technological channels for intervention were also reasonably effective, even in some developing countries [97].

Smartphones are increasingly used worldwide and represent a promising technological channel for dietary behavior change. A survey by the Pew Research Center found that 58 % of people in the USA own a smartphone, while a median of 24 % in emerging and developing countries own one [98]. Smartphones provide a means to access information and tools, including applications (apps). Apps may improve dietary intake and adherence to specific diets since they provide a way to conduct health promotion that is convenient and accessible, and that can provide information and feedback at the location and time that dietary decisions are made. There is also an ability to tailor and customize apps to increase the appeal and relevance to individuals. Smartphones and apps also represent a way to reach large numbers of people. Worldwide, relatively few products and platforms are used [99], which increases the potential for widespread dissemination and use of apps and other tools. Once someone owns a smartphone, the apps are inexpensive and so have the potential for reaching underserved populations; e.g., Ball et al. [100] found a smartphone approach to be appealing and feasible for socioeconomically disadvantage women in Australia.

The most common behavioral strategy used in apps is self-monitoring [101, 102], itself a key construct from behavioral theory. Other common strategies are goal-setting and progress tracking, feedback, and support from coaches [103]. Self-monitoring with feedback messages seems to be effective for behavior change [104, 105]. Apps may also provide social support and accountability when they are linked to social media. However, there are a number of concerns related to the use of smartphone apps and similar technologies for dietary behavior change. While many utilize theory-based strategies of self-monitoring and goal-setting, few are clearly based on behavioral theory [101, 106]. Most developers have largely ignored the body of evidence regarding the use of behavioral theory for health promotion, likely because they lack meaningful exposure to this field. However, the fact that certain constructs from behavioral theory are represented in some apps suggests that it should be possible to integrate others [106, 107]. There is also speculation that current theories may not be adequate in an environment that is so highly interactive and dynamic, offering immediate adjustments in response to user input [106]. This is supported by mixed evidence on the effectiveness of apps that use self-monitoring. Some studies suggest that rates of self-monitoring using an app are similarly low compared to paper methods [108]; Helander et al. [109] tested an app which required photographs of consumed food to be uploaded so crowdsourced feedback could be received and found that less than 3 % of users employed it actively over time. However, other evidence suggests that apps are capable of significantly increasing adherence to self-monitoring of dietary intake [110–112]. Even among users who do adhere to self-monitoring, there may be issues of selective input, especially when there is some form of accountability [109]. In general, attenuation to the monitoring over time may be expected.

There is a concern regarding this technology that little information is available on how data are stored and used by companies that create the apps, presenting numerous issues where privacy may be compromised. In addition, there is a concern that the quality of the content may be suboptimal [99, 113]. While some apps have been formally evaluated for effectiveness [110, 114] many others have not [102]. While apps have the potential for reaching underserved populations, there still may be

issues of usability, accessibility, compatibility, and cost that could serve to exacerbate rather than improve health disparities related to diet [99].

Smartphones provide new ways for health care professionals to engage with patients. Health care professionals may help people set goals within the app and hold them accountable for progress. There is some evidence that it is best to use apps in conjunction with dietary counseling. For example, Wharton et al. [110] found that when users received feedback on calories consumed but not diet quality, they ate smaller quantities of low-nutritional-quality foods rather than improving their diet quality. This suggests the importance of nutritional counseling to place feedback from the app into context. Health professionals can help clients identify the type of tool that will be most feasible, compatible, and effective for them, and then provide information on the best ones in that category. They can encourage adherence, help users problem-solve, and contextualize the feedback provided by the app. They can help vet content and discuss and correct inaccuracies [115].

Health care professionals clearly have an important role to play in effecting dietary behavior change. However, there still exists little practical guidance on the best interventions for dietary adherence. In their Cochrane review, Desroches et al. [18] suggest that effective strategies in this setting include telephone follow-up, the use of educational video, behavioral contracts, and nutritional tools (menus and portion size examples) or the use of combinations of these strategies. Studies of interventions for primary health care settings have been conducted in developed countries; however, there is a major gap in our knowledge with respect to information from emerging and developing countries [116].

Cultural Influences on Dietary Behaviors

Culture plays a major role in diet and lifestyle behaviors of people in the USA and worldwide by shaping a group's norms, beliefs, customs, and values. As indicated in Fig. 1.1, the individual level is the base layer that influences diet and lifestyle behaviors and the interpersonal/cultural level relates to one's social networks and the culture of these groups, which likewise influences food behaviors. Although less influential today due to globalization, historically available flora and fauna shaped cultural diets around the world. Since the industrial revolution, the advent of processed foods, and modern-day trade agreements, diet and food patterns around the world have evolved. The Western diet and culture, which has widespread impacts and influence globally, is often described as being high in red meat, refined grains, fried foods, sweets, and sugar-sweetened beverages [117]. The American food culture in particular is a contributing factor to obesity and other diet-related diseases such as heart disease and diabetes around the world.

Although the Western diet is common in the USA, racial/ethnic heterogeneity lends itself to multicultural cuisines and diets. By 2044, for example, the USA will continue to be a melting pot, as it is estimated that the US population will become a majority minority nation, with 50.3 % of Americans being from a minority ethnic/racial group [118]. Although not exclusive, an individual's race/ethnicity plays a role in shaping their cultural preferences and therefore their diet and lifestyle preferences. For example, Chinese-Americans that are less acculturated to the Western diet may be more inclined to consume a diet higher in fresh vegetables, white rice, seafood, and soybeans and tofu [119]. Recent Hispanic immigrants from Central American countries such as Guatemala, Honduras, and El Salvador may hold the cultural norms of consuming corn, beans, rice, plantains, and herbs such as cilantro and chilies, while Hispanic immigrant from the Caribbean may be more inclined to consume foods such as sofrito seasoned beans and stews, plantains, rice, and yucca [120].

Cultural beliefs may also influence perceptions of ideal body shape, which consequently would impact the likelihood and motivation to change one's behaviors to lose weight. For example, research

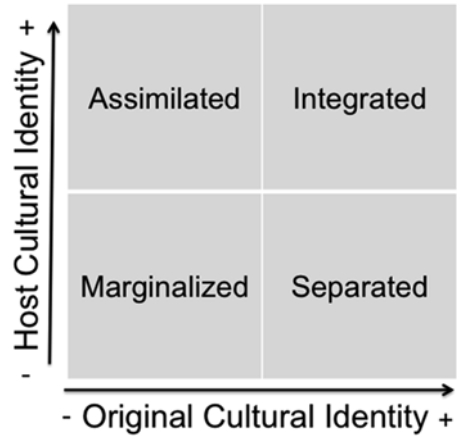

Fig. 1.2 Berry's acculturation model. Adapted from Berry [125]

consistently reveals that African American women generally underestimate their weight and prefer larger body sizes than other racial/ethnic groups [121–123]. In a survey of college-age students, Yates et al. [123] examined the relationship between BMI and body/self-dissatisfaction and found that African-American women had the highest BMI of any female group, yet were reasonably satisfied with their bodies.

Whether or not an individual adheres to the dietary cultural beliefs and practices of their respective racial/ethnic group will depend on their level of acculturation to Western practices and diet. As shown in Fig. 1.2, Berry's theory of acculturation describes four different subgroups based on varying levels of acculturation, in which the marginalized, separated, and integrated (bicultural) would be more inclined to consume their culture's traditional diet [124, 125]. Dietary acculturation is described as the multidimensional process to which a minority group adopts the eating pattern of the host country [126]. In this process, a person does not appear to move linearly from a more traditional diet to a more acculturated diet. Instead, for example, migrant groups find new ways to consume traditional foods, exclude other foods, and introduce new foods [119, 126–130]. Additionally, dietary acculturation is influenced by a variety of factors including diet and disease-related attitudes and beliefs, taste preferences, values ascribed to traditional eating patterns, availability and cost of traditional foods, and time constraints in the host country [126].

Although there is increased migration across the globe and globalization of the food market with similar influences of acculturation in each respective context, diets vary considerably across the world due to cultural influences and historical food availability. The Mediterranean diet, a dietary pattern that has grown in popularity due to its health benefits, is characterized by abundant plant foods such as fruit, vegetables, potatoes, beans, nuts, and seeds, olive oil as the main source of fat, dairy products, fish and poultry consumed in small amounts and with limited consumption of eggs (0–4/week) and red meat, and low-to-moderate amounts of red wine [131]. Culture also influences the acceptance of different foods; e.g., in Sardinia, a cultural delicacy includes Casu Marzu, a cheese derived from insect larvae, which would be considered taboo in many other places. Haggis, a savory Scottish pudding mix of sheep innards including sheep lungs tied up in sheep stomach has been banned in the USA since 1971 due to a government prohibition of the sale of sheep lung [132]. As culture plays an essential role in determining food choices, preferences, and ideals, considering cultural beliefs and dietary preferences is essential in the development of effective nutrition and lifestyle interventions.

Community-Level Change

A community-level approach to behavior change has emerged with the greater appreciation of the limitations of individual-level efforts and a growing sense that the "toxic food environment" plays a major role in the current obesity epidemic [133]. Community-level approaches to dietary behavior change may be more effective than individual-level strategies alone since they can account for the range of social and physical contexts that help shape behavior [134]. Also, the burden for change can be dispersed among sectors, with modest, low cost, and replicable changes made in multiple settings within a community allowing for the potential to reach a larger proportion of the population [135]. Sustainability appears more likely when there is a community ownership that translates into institutional and cultural changes. A community-based approach, in addition to being consistent with the Socioecological Model, is currently being considered in terms of systems theory in which the dynamic interplay within and among levels is recognized [136, 137].

There is an increasing acknowledgement of the community as an important object of intervention and research [138]. The feasibility and effectiveness of a multilevel, multi-setting strategy has now been demonstrated for childhood obesity prevention studies in multiple countries [139–148]. While there appears to be a moderate degree of evidence for effectiveness when a school component is included, more equivocal results are found when adults are the target population [149]. Importantly, in 2009, the World Health Organization reported that of 65 interventions conducted in community settings, only three were conducted in emerging or developing countries [116].

Despite some success in working at the community level, there are a number of challenges, especially as the field has moved to systems-based thinking, related to feasibility in implementation. For example, the Healthy Towns program in England was designed to work with communities to create environmental change to support healthy food choices [150]. However, there was a disconnect between how the project was theorized and how it was implemented; in the nine towns included, the intervention involved multiple sectors in the communities but failed to take a systems approach in promoting dynamic interactions among them. In terms of evaluation, identifying and working with an entire community renders randomized, controlled trials infeasible. There are also issues related to choice of a control group, contamination, and causal attribution. It is difficult to measure and model statistically the effectiveness of interventions designed to promote complex, dynamic interactions among multiple community sectors and among the multiple layers of the Socioecological Model. Given the dynamic nature of the theorized changes, intervention strategies and expected outcomes may shift, indicating the need for new research methods for planning and evaluation [138].

Issues of Access

Increasing consideration regarding the availability of fresh, healthy, and affordable foods within certain communities is reflected in the popular term "food desert." According to the United States Department of Agriculture's Economic Research Service, an estimated 23.5 million people in the USA live in a food desert [151]. These communities have limited access to supermarkets and grocery stores and instead have a high density of fast-food restaurants and convenience stores. In addition, the term "food swamps" has been coined to describe communities with an overabundance of fast-food restaurants [152]. Living in such neighborhoods is associated with poor diets, higher levels of obesity, diabetes, and heart disease. Moore et al. [153] examined the association between diet quality and local food environment found that Americans with no supermarkets near their homes were 25–46 % less likely to have a healthy diet, according to the Alternative Healthy Eating Index, compared to those with the most stores. In Leeds, England, Wrigley et al. [154] found improvements in fruit and vegetable consumption after the introduction of a supermarket in a deprived community. In contrast, in

Glasgow, Scotland, Cummins et al. [155] found little impact on fruit and vegetable consumption once a grocery store was introduced, underscoring the apparent influence of other factors.

In the USA, racial segregation has been shown to be associated with the presence or lack of supermarket access [156–159]. Bower et al. [159] reported that economically depressed black neighborhoods face the most limited access to quality food and found racial composition and poverty were independently associated with food store availability, although this effect was not observed in rural areas. Similarly, Morland et al. [157] explored the distribution of supermarkets in Mississippi, North Carolina, Maryland, and Minnesota and found 4 times more supermarkets located in white neighborhoods compared to black neighborhoods.

These environmental conditions, coupled with genetic and socioeconomic factors, contribute significantly to the racial/ethnic disparities seen in the USA. Blacks are twice as likely to experience a stroke and 3 times more likely to die from heart disease than their non-Hispanic white counterparts [160]. American Indian, Alaska Native, African American, Hispanic/Latino, and Asian/Pacific Islander adults are about twice as likely to be diagnosed with type 2 diabetes compared to their white counterparts [161]. These statistics and their underlying causes suggest need to prioritize minority groups and communities for interventions.

The Role of Health Professionals

Beyond the influence of health professionals at the individual level, a growing trend in clinical care is not only to treat symptoms, but also to consider the social and environmental conditions that contribute to disease [162, 163]. This approach is particularly important in working with underserved communities that are at higher risk for disparities in health outcomes. "Upstream doctors" are taking a broad and innovative approach to improve the standard of care in the clinical setting by focusing on the root causes of disease and assisting with mobilizing systems to optimize the resources necessary to meet their patients' needs. Rishi Manchanda [163, 164] notes that "where health begins is not in the four walls of a doctor's office, but instead where we live, where we work, where we eat, sleep, learn and play, where we spend the majority of our lives." Of course, an upstream approach can be employed by any professionals capable of using this approach to create solutions within the clinical system and/or other sectors such as public health, social work, and law.

Upstream physicians are moving beyond the individual level of the Socioecological Model (Fig. 1.1) to target community and organizational structures and improve their patients' health. Beyond the traditional standard of care, upstream clinical care integrates issues about patients' transportation, working and living conditions, access to affordable and healthy foods, and availability of various community resources. Although most of the literature regarding the upstream approach has been applied in the USA, this more holistic and comprehensive approach to health care could be applied internationally.

Policy-Level Initiatives

Policy initiatives at the local, state, national, and global levels contribute to dietary behaviors by influencing factors such as laws, national dietary guidance, regulations for federal nutrition programs, taxes on selected foods such as sodas, and on the global scale, funding allocation for nongovernmental organizations and research initiatives. Despite the imperative presented by diet-related diseases, the USA is slower than some other countries in enacting regulatory solutions to dietary behaviors, in part due to consumer resistance to "nanny state" interventions, the notion of reliance instead on personal responsibility, and pressures from the food and beverage industry via marketing and lobbying [165].

Policy Initiatives in the USA

National, policy-level initiatives range from direct food assistance and dietary guidance to standardization of how nutrition information is provided for the consumer. National food assistance programs include the National School Lunch and Breakfast Programs (NSLP and SBP), Supplemental Nutrition Assistance Program (SNAP), and the Women Infant and Children (WIC) program for which funding is designated by Congress. The dietary recommendations for these national initiatives are informed by the Dietary Guidelines for Americans which, by Congressional mandate, are released every 5 years. These guidelines are developed by an advisory committee of independent, nongovernment affiliated researchers and consist of key dietary recommendations for the general population to help people choose a healthy diet. Other important national policy initiatives include the requirement of calorie labeling on menus and menu boards in chain restaurants, retail food establishments, and vending machines with 20 or more locations, which was a provision under the 2010 Patient Protection and Affordable Care Act. Other federally mandated initiatives include the implementation of the Nutrition Facts Panel label stemming from the Nutrition Labeling and Education Act of 1990. Currently, the label is under revisions by the Food and Drug Administration (FDA). In 2014, the FDA revoked *trans-fat* as a Generally Recognized as Safe (GRAS) food additive, essentially banning food manufacturers from using this ingredient [166].

In addition to these national policies, local governments are also playing a role in shaping the food and diet landscape. For example, in 2014, Berkeley, California, passed the first soda tax in the country, at 1-cent-per-ounce of soda [167]. In an arguably more radical initiative of 2011, Los Angeles, California, banned the development of new fast-food restaurants in South Los Angeles [168]. Action by individual cities can help develop suitable evidence and set a precedent for national regulation; e.g., New York, New York and Philadelphia, Pennsylvania required chain restaurants to post calories prior to the development of national legislation. As noted above, some of the efforts at mandatory regulations have been thwarted by consumer opinion, lobbyist efforts, and city court systems. For example, New York City proposed a limit on soft drinks to no more than 16 oz but the New York Court of Appeals found such policies exceeded the reasonable scope of authority of the New York City Board of Health [169]. California Proposition 37, a ballot initiative to label foods containing ingredients derived from genetically modified organisms (GMOs), was rejected by voters in November 2012 [170].

With ever more local and national level initiatives, there is an increased need for evaluation of such policies. While evaluation of federal nutrition assistance programs is comparatively robust, evaluation of the effectiveness of other policies, such as calorie labeling and mandatory taxes, has not been extensive. Important questions for evaluation include addressing whether calorie labeling has the same effect on the health-conscious consumer versus those who are food insecure, whether revisions to the Nutrition Facts Panel influence food purchases of those with lower levels of health literacy, etc. Some studies have shown that calories on menus have no effect on food purchases, while others suggest a decrease in total caloric intake [171–173]. These conflicting results highlight the need for more long-term studies on the effects of such policy changes.

International Initiatives

There are a number of policy-level strategies being implemented in nations worldwide. For example, in 2014, Mexico implemented a restriction on food marketing to children on television and in movie theaters. In Denmark, a tax on foods containing more than 2.3 % calories from saturated fat was implemented but the legislation was eventually repealed when consumers responded by purchasing more grocery items internationally to avoid this “fat tax” [174].

Global efforts by the United Nations, the World Health Organization, and the Food and Agriculture Organization can impact diets internationally. In 2000, leaders of nearly 200 countries met at the United Nations and endorsed Millennium Development Goals to be reached by 2015. These measurable goals—including the eradication of extreme poverty and hunger to reduce child mortality and creating global partnerships to promote environmental sustainability—have been playing a role in global policy related to nutrition and dietary behavior. Particularly, these goals have influenced funding allocations for hunger relief programs within nongovernmental organizations as well as research priorities. Additionally, multinational and bilateral free trade agreements, such as the North America Free Trade Agreement, the Central America Free Trade Agreement, and the Asia-Pacific Free Trade Agreement, can have an impact on availability of food and dietary choices by influencing food quality, availability, and prices within a country.

Conclusion

There is a broad consensus that dietary patterns which promote health and reduce the risk of chronic disease are higher in fruits and vegetables, whole grains, seafood, legumes, and nuts and lower in sugar-sweetened foods and beverages, refined grains, red and processed meat. Nonetheless, nutrition is not at the core of healthcare and most systems for public health are focused on disease treatment rather than prevention. Holistic approaches to public health via preventive nutrition can be complemented by assessing individual traits through nutrigenomics and related biochemical pathways to promote physiological function and wellness. Importantly, achieving prevention requires the integration of biological, economic, and social aspects of diet and nutrition. Behavioral models that incorporate the multiple spheres of influence of individual, interpersonal, economic, organizational, cultural, and community elements are a critical part of preventive nutrition. At the individual level, the use of motivational interviewing and self-monitoring by various technologies have demonstrated effectiveness but may work best in the context of larger community strategies in which healthier dietary choices are made the easier choices. Further, policy initiatives at the state and national levels contribute to dietary behaviors by influencing factors such as laws, national dietary guidance, regulations for federal nutrition programs, and taxes on selected foods. On the global scale, funding allocation for non-governmental organizations and research initiatives are essential both to evaluating preventive nutrition efforts across countries and cultures and translating these results into ever more effective programs.

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Chapter 2
Public Health Benefits of Preventive Nutrition: Global Perspective

Walter C. Willett

Key Points

- Staying lean and physically active throughout adult life has major health benefits.
- Diets low in the percentage of energy from fat have not been associated with lower risks of heart disease, cancer, or better long-term weight control.
- Avoiding industrially produced trans fat, keeping saturated fat low, and emphasizing unsaturated fats will minimize risks of heart disease and type 2 diabetes.
- Consuming grains in their original high fiber/whole grain form is likely to reduce risk of type 2 diabetes and heart disease. Consumption of sugary beverages increases risk of type 2 diabetes and probably heart disease.
- High intake of fruits and vegetables will help prevent risks of cardiovascular disease, but the benefits for cancer reduction appear modest.
- High consumption of alcohol and alcoholism have many adverse health and social consequences, and intakes as low as one drink per day or less are associated with greater risks of breast cancer. In contrast, moderate consumption of alcohol reduces risks of coronary heart disease and type 2 diabetes.

Keywords Diet • Nutrition • Health • Disease • Prevention

Introduction

Until very recently, most populations had no choice but to consume foods that were produced locally, and availability was often extremely seasonal. This resulted in diets that were highly variable across the globe; for example, in some Arctic climates almost no carbohydrates, fruits, or vegetables were consumed and diets consisted mainly of fat and protein from animal sources. In other regions, populations subsisted on primarily vegetarian diets with the large majority of calories from carbohydrate sources. The fact that humans could survive and reproduce with such varied dietary patterns is a testimony to the adaptability of human biology. Yet, disease rates and overall mortality varied dramatically among these various population and formal studies of these relationships provided early clues about the importance of diet in human health and disease; these "ecological" studies are described in more detail below.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_2

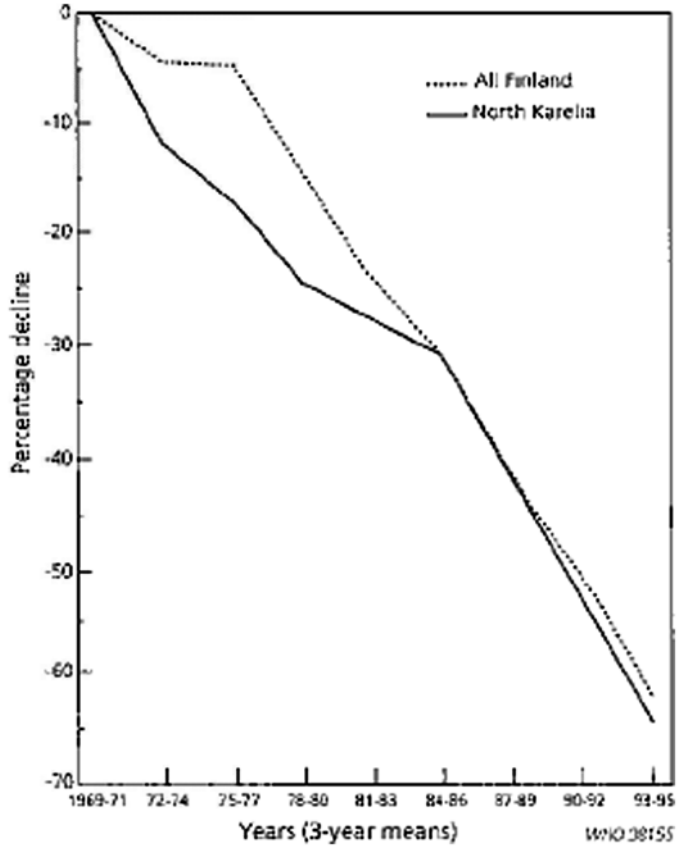

Fig. 2.1 Percentage decline in age-adjusted coronary mortality of 35–64-year-old males in Finland

In the last few decades, enormous changes have occurred in the diets of most populations. These changes were due to combination of increased wealth of some groups, new processing and preservation technologies, and greatly expanded transportation infrastructures. Collectively, these changes have allowed foods to be transported across and among continents and to be available virtually the whole year. At first, these changes globally were described as the "westernization" of diets because of increases in meat, dairy products, and processed foods. However, many of the more recent changes are not necessarily toward the diets of western countries, but instead emphasize refined starches, sugar and sugary beverages, and partially hydrogenated vegetable fats. These patterns, which have been described as "industrial diets" are usually the cheapest source of calories and they have permeated poor populations of both rural and urban countries around the world.

The recent changes in diets, along with changes in physical activity and tobacco use, have profoundly affected rates of disease, sometimes positively but often adversely. On the positive side, we have seen dramatic declines in rates of coronary heart disease (CHD) in many western populations, where this has been the leading cause of death. For example, in Finland, which at one time had the highest rates of CHD, mortality from this cause has declined by more than 80 % (see Fig. 2.1) [1]. On the other hand, in Japan, formerly a country with very low rates of colon cancer, rates of this malignancy have increased greatly and now have surpassed those of the USA [2]. Most importantly, at present an epidemic of obesity, cardiovascular disease, and diabetes has affected almost all the world's populations, rich and poor [3]. This epidemic, which could reverse important gains in life expectancy [4] is likely to be the greatest challenge to public health in this century, unless an unforeseen problem emerges.

In this brief overview, I will address the components of diet and nutrition that have well-documented relationships to human health and disease. The focus is on the prevention of major illness in adults, and most of the evidence is based on studies of diet during midlife and later. A fundamental conclusion is that the vast majority of deaths due to coronary heart disease, stroke, diabetes, and some important cancers are preventable by healthy diets in combination with regular physical activity and avoidance of tobacco [5]. The relation of diet during pregnancy, infancy, and early childhood to childhood mortality, unfortunately still a major issue in many poor countries, has been extensively studied and is addressed in other chapters of this volume. This study builds on earlier reviews [6], emphasizing newer evidence and understanding.

Sources of Evidence

Traditionally, animal experiments and small human metabolic studies formed the basis of dietary recommendations. Inevitably, the study of chronic disease in humans has required epidemiologic approaches. Initially, investigations compared dietary intakes and disease rates among populations in various countries, which were termed ecological studies. These analyses highlighted the large differences in disease rates worldwide and provided many hypotheses; however, such studies are limited because many other factors besides diet vary across cultures and the data are inherently aggregated. The next generation of studies was primarily case–control investigations, which mainly examined dietary factors retrospectively in relation to risk of cancer and other diseases. Not surprisingly, such studies have often been misleading due to biased recall of past diet and other artifacts. Now, large prospective studies of many thousands of persons are providing data based on both biochemical indicators of diet and dietary questionnaires that have been rigorously validated [7]. Prospective studies are less subject to biases resulting from the retrospective reporting of dietary intakes or the effects of disease on biochemical indicators. Micronutrient supplements can potentially be evaluated in randomized trials; however, trials of dietary interventions may often be unfeasible due to difficulties in maintaining compliance for the necessary long periods, which could be decades. Recent advances in molecular biology have yet to contribute substantially to dietary recommendations, but in the future these approaches may provide useful intermediary end points, allow the study of gene-diet interactions, and enhance our understanding of the mechanisms by which dietary factors influence disease. Ultimately, our knowledge is best based on a synthesis of epidemiologic, intervention, animal, and mechanistic studies.

Specific Dietary Components

Dietary Fat

Until recently, reviews and dietary guidelines have consistently emphasized reducing total fat intake, usually to 30 % of energy or less [8–10], to prevent coronary heart disease (CHD). The classical diet-heart hypothesis has rested heavily on the repeated observation that serum total cholesterol levels predict CHD risk; serum cholesterol has thus functioned as a surrogate marker of risk in hundreds of metabolic studies. These studies, summarized as equations by Keys [11] and Hegsted [12], indicated that, compared to carbohydrates, saturated fats and dietary cholesterol increase, and polyunsaturated fat decreases, serum cholesterol, whereas monounsaturated fat has no influence. These widely used equations, while valid for total cholesterol, have become less relevant as surrogate variables for CHD risk with the

recognition that the high-density lipoprotein cholesterol fraction (HDL) is strongly and inversely related to CHD risk, and that the ratio of total cholesterol to HDL is a better predictor [13, 14].

Substitution of carbohydrate for saturated fat (the basis of the American Heart Association diets during most of the last 30 years) tends to reduce HDL as well as total and low-density lipoprotein (LDL) cholesterol; thus, the ratio does not change appreciably [15]. In contrast, substituting poly- or monounsaturated fat for saturated fat reduces LDL without affecting HDL, thus providing an improved ratio [15]. In addition, monounsaturated fats, compared to carbohydrate, reduce blood sugar and triglycerides in adult onset diabetics [16]. Questions have been raised as to whether the reductions in HDL resulting from a high-carbohydrate diet have the same adverse effect as reductions caused by other factors [17], and some drugs that raise HDL have failed to reduce risk of CHD. Although the causal role of HDL is difficult to address directly, other factors that influence HDL levels, including alcohol, estrogens, obesity, smoking, exercise, and some medications, usually affect CHD risk in the predicted direction [18, 19].

The use of the usual cholesterol prediction equations has been further complicated by the recognition that different saturated fats vary in their influence on LDL levels: 18:0, stearic acid (the main fat in chocolate and a major saturated fat in beef fat), has little effect; 16:0, palmitic acid (the main fat in palm oil also found in beef fat), modestly increases LDL, and 14:0, myristic acid (the main saturated fat in butter and other dairy fats), most strongly increases LDL [20, 21]. However, this usually does not have practical importance in usual diets because intakes of the various saturated fats are strongly correlated with each other. However, stearic acid produced by the complete hydrogenation of vegetable oils is sometimes considered as a replacement for trans-fatty acids (see below). The assumption that stearic acid is "neutral" is not warranted as long-term studies are limited; the available evidence suggests that it may be more strongly related to risk of cardiovascular disease than other saturated fats [22, 23], and in a controlled feeding study high amounts of stearic acid (as in interesterified fat) had adverse effects on glucose regulation [24].

The optimal amount of polyunsaturated fat intake in the diet remains uncertain. The earlier metabolic studies predicting total serum cholesterol [11, 12] suggested that intakes should be maximized, and the American Heart Association has recommended intakes of 10 % of energy (compared to US averages of about 3 % in the 1950s and 6 % at present). Concerns have arisen from animal studies in which omega-6 polyunsaturated fat (typically as corn oil) has promoted tumor growth [25], and the possibility that high intakes of omega-6 relative to omega-3 fatty acids might promote coronary thrombosis [26, 27]. However, as described below, available evidence from human studies has not supported these concerns at levels of omega-6 fatty acid intake up to about 10 % of calories.

The relation to CHD incidence has been examined in many epidemiologic studies. In Keys' pioneering ecological study of diets and CHD in seven countries [28, 29] total fat intake had little association with population rates of CHD; indeed, the lowest rate was in Crete, which had the highest fat intake due to the large consumption of olive oil. Saturated fat intake, however, was positively related to CHD in Keys' study. In contrast to international comparisons, little relationship has been seen with saturated fat intake in many prospective studies of individuals [30–33] when compared to a similar intake of energy from carbohydrate or all other calories (which are primarily sugar and refined starch in most diets). Some studies, however, tend to support a modest association between dietary cholesterol and CHD risk [34], and inverse associations have been seen with polyunsaturated fat [30, 32, 35]. Similarly, dietary intervention trials have generally shown little effect on CHD incidence when carbohydrate replaces saturated fat, but replacing saturated fat with polyunsaturated fat has been associated with lower incidence of CHD [36–39]. In the Women's Health Initiative (WHI), by far the largest trial to examine fat intake and incidence of CHD [40], 48,000 women were randomized to a low-fat diet or their normal diet. No effect was seen, which is consistent with epidemiologic studies because the type of fat was not modified, but any conclusion from this study must be tempered because the compliance with the low-fat diet was poor [41]. In contrast, to the WHI, a reduction in

cardiovascular disease was observed in the Spanish PREDIMED study among those randomized to a Mediterranean diet with added nuts or olive oil when compared to those assigned to a low fat diet [42]. Although the study focused on increasing plant sources of monounsaturated fat, it is difficult to ascribe the benefits entirely to monounsaturated fats because this change was embedded in an overall Mediterranean diet.

Much confusion was generated by a recent meta-analysis of published papers concluding that saturated fat had no relation to risk of CHD, and that replacement of saturated fat with polyunsaturated fat is unsupported by evidence [43]. However, this meta-analysis was seriously flawed in many ways, including gross errors in data extraction, omission of important studies, and failure to cite previous analyses based on individual level data (see online comments regarding Chowdhury et al.). Most importantly, this analysis did not distinguish between substitution of carbohydrate for saturated fat versus substitution of polyunsaturated fat for saturated fat. As shown in a more complete and detailed analysis, the latter substitution is supported by prospective studies, randomized trials, and beneficial effects on CHD risk factors [35]. As shown in a more complete and detailed analysis, the latter substitution is supported by prospective studies, randomized trials, and beneficial effects on CHD risk factors [35]. Thus, the effect of saturated fat depends on the macronutrient to which it is compared (see Fig. 2.2).

Trans-fatty acids are formed by the partial hydrogenation of liquid vegetable oils in the production of margarine and vegetable shortening and can account for as much as 40 % of these products. Even higher levels of trans fats are found in "vegetable ghee," which is widely used in the middle east and south Asia [44]. In the USA intake of trans-fatty acids from partially hydrogenated vegetable fats (which increased from nothing in 1900 to a peak of about 5.5 % of total fat by about the 1960s) has closely paralleled the epidemic of CHD during this century, in contrast to intake of animal fat, which has steadily declined over this period [45]. Trans-fatty acids increase LDL and decrease HDL [45–51], raise Lp(a), another lipid fraction implicated in CHD etiology [49, 52], and increase C-reactive protein and other inflammatory markers [53]. Positive associations between intake of trans-fatty acids and CHD have been seen among regions in the Seven Countries Study [54]. In the most detailed prospective study, trans-fatty acid intake was strongly associated with risk of CHD [32] and, as predicted by metabolic studies, this association was stronger than for saturated fat. The association between trans-fatty acid intake and risk of CHD has been confirmed in other prospective studies; in a meta-analysis, a 2 % energy increase in trans fat intake was associated with a 23 % increase in risk of CHD [51]. Higher intake of trans fat has also been associated with risk of type 2 diabetes [55], gall stones [56], dementia [57], weight gain [58], and an adverse effect on insulin resistance has been shown in a long-term study in monkeys [59].

Since 2005 the U.S. Food and Drug Administration has required that food labels include the trans fat content (see chapter by Mensink). Denmark has banned the sale of industrially produced trans fat, which was hardly noticed by consumers. In the USA the use of trans fats in restaurants has been banned by many cities, Puerto Rico, and the state of California. This has caused manufacturers to reformulate their products, and intake has decline by as much as 75 % [60], blood lipids have improved in national surveys [61], and cardiovascular disease is declining more rapidly in cities that first banned trans fats [62].

High intake of omega-3 fatty acids from fish reduces platelet aggregability and prolongs bleeding time [27], slightly reduces blood pressure [63], decreases serum triglycerides, but increases LDL cholesterol [64]. Fish consumption was associated with a greatly reduced risk of myocardial infarction (MI) in one prospective study [65] and in a randomized trial among postinfarction patients [66]. Subsequent data have been less supportive of a major effect of fish consumption on overall risk of CHD [67–69], but the benefits of omega-3 fatty acids appear to be primarily in prevention of fatal arrhythmias that can complicate CHD, rather than in prevention of infarction [70–72]. The amount of omega-3 fatty acids needed to prevent arrhythmia is remarkably modest—on the order of 1 g per day

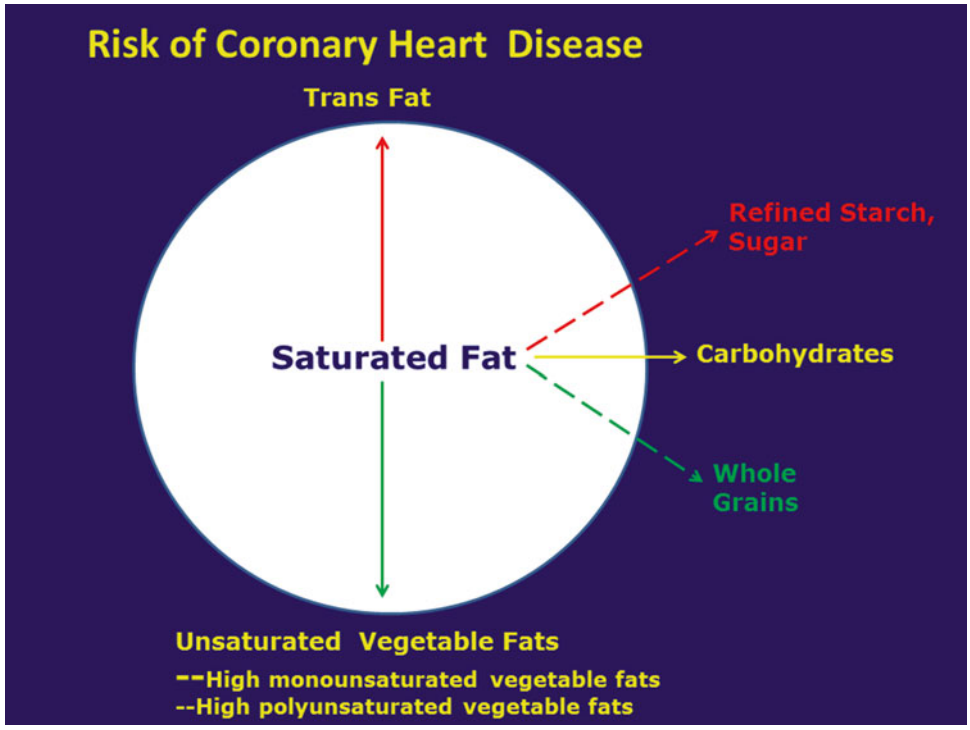

Fig. 2.2 Relation between saturated fat and risk of CHD depends on the macronutrient to which it is compared (Copyright, 2015, Walter Willett)

or perhaps even less [72], and observational studies suggest that further increases are likely to have at most a small effect. Thus, adding supplements of omega-3 fatty acids to the diets of populations whose intakes are already high will likely have little effect. This is a likely explanation for some recent studies in which supplementation did not reduce serious cardiac arrhythmias [73]. Some plant oils, including soybean, rapeseed (canola), and flaxseed, also contain substantial amounts of the 18-carbon omega-3 fatty acid, alpha-linolenic acid (ALA). Because vast regions of the world consume little omega-3 fatty acids from any source, and the global supply of fish is limited, whether ALA can provide similar benefits as the longer-chain fish oils is a crucial public health issue. More data are needed, but available evidence suggests that higher intakes of ALA can prevent both fatal and nonfatal CHD [70, 74]; in eastern Europe, increases in rapeseed oil have been associated with rapid declines in CHD mortality [75].

Dietary Fat and Cancer

Another major justification for reduction of dietary fat has been anticipated decreases in the risk of cancers of the breast, colon and rectum, and prostate [76, 77]. The primary evidence has been that countries with low fat intake, also the less affluent areas, have had low rates of these cancers [77, 78]. These correlations have been primarily with animal fat and meat intake, rather than with vegetable fat consumption.

The hypothesis that fat intake increases breast cancer risk has been supported by most animal models [79, 80], although no association was seen in a large study that did not use an inducing agent [81].

Moreover, much of the effect of dietary fat in the animal studies appears to be owing to an increase in total energy intake, and energy restriction profoundly decreases incidence [25, 79, 81]. Many large prospective studies have been published [82–88], and little or no association has been seen in all. In a pooled analysis of prospective studies including 351,821 women and 7329 cases of breast cancer, the relative risk for a 5 % of energy increment in total fat was 1.00 (95 % CI 0.98–1.03) [86]. In the Nurses' Health Study, no decrease in risk was seen with less than 20 % of energy from fat [89], and with 20 years of follow-up and multiple measures of diet, there was no hint of any positive association with intake of total or specific types of fat [87]. In the AARP cohort a weaker positive association (RR = 1.11) was found for women with the highest compared to the lowest intake of fat that was statistically significant due to the large number of cases [88]. A similar weak association was seen in the large EPIC cohort, which appeared to be specifically related to intake of saturated fat [90]. In the WHI trial of dietary fat reduction, only a slight and not statistically significant reduction in risk was seen [91], and even this slight difference could be due to the transient loss of weight in the intervention group. Thus, over a wide range of intake, dietary fat consumed by middle-aged women appears to have little or no influence on breast cancer risk. However, higher intake of animal fat, but not vegetable fat, by young adult women has been associated with a greater risk of breast cancer before menopause, suggesting that some components of animal foods rather than fat per se may increase risk [92]. *The relation of fat intake during childhood to risk of breast cancer has been minimally studied.*

As with breast cancer, prospective studies have not supported the hypothesized associations between dietary fat and risks of colorectal or prostate cancer [93]. *However, positive association between consumption of red meat, and particularly processed meat, and risk of colorectal cancer has been seen in many prospective studies* [93–96]. Also, consumption of red meat during adolescence or early adult life has been associated with a higher risk of breast cancer [35], although intake during midlife or later has not. These findings suggest that other components of red meat such as heat-induced carcinogens, the high content of heme iron, or nitroso compounds might be responsible for the elevated risk.

Although dietary fat does not appear to explain the high rates of breast, colon, and prostate cancer in Western countries, a massive body of evidence indicates that excessive body fat, the result of excessive energy intake in relation to physical activity, is an important risk factor for cancers of the endometrium, breast (after menopause), pancreas, colon, kidney, esophagus (adenocarcinoma), and some hematologic malignancies [93, 97, 98]. Excess body fat is now second only to smoking as a cause of cancer in the USA. This appears to be mediated through multiple mechanisms, including increases in circulating estrogen levels (breast and endometrial cancers), gastric reflux (esophageal cancer), insulin resistance (colon and pancreatic cancer), and possibly other pathways.

Dietary Fat and Body Fatness

In addition to being a major risk factor for cancer, overweight is an important cause of diabetes, cardiovascular disease, and other important diseases (see below), and short-term studies have suggested that reducing the fat content of the diet induces weight loss. However, population differences in weight do not appear to be due primarily to fat intake; in Europe, southern countries with relatively low fat intake have higher rates of obesity than Northern European countries [99]. Also, among 65 counties in China, no correlation was seen between body weight and fat intake, which varied from approx 6 to 30 % of energy [100]. Inconsistent associations have been observed in cross-sectional and prospective studies within countries, but such observations are particularly prone to distortion because subjects may alter their diets to modify their weight. In randomized trials of fat reduction, the optimal way to study this relationship, modest weight reductions are typically seen in the short term. However, in randomized

studies lasting a year or longer, reductions in fat from greater than 30 % of energy to 18–25 % of energy had minimal effects on overall long-term body weight [101]. Several recent randomized trials have compared very low fat, moderate fat, and low carbohydrate diets; weight loss over 1–2 years has been similar in all groups [102] or [103] on low fat/high carbohydrate diets. As predicted by shorter studies, cardiovascular risk factors have tended to be least desirable on low fat diets [103]. Very low fat intakes, less than 10 % of energy, in conjunction with a high volume of bulky food as consumed by some traditional societies, may induce weight loss [104], but long-term studies are needed. However, available evidence suggests that reductions in dietary fat over the ranges currently recommended will not have sustained benefits on body fatness, and that this is likely to have adverse metabolic effects.

What can we now say about dietary fat and health? In 1989, a major review concluded that dietary fat per se is not associated with risk of CHD [76]. This was generally ignored but subsequent studies have added further support for this conclusion and have also failed to support suggested major reductions in cancer and other risks. Both metabolic and epidemiological data strongly indicate that intake of partially hydrogenated vegetable fats should be minimized. Metabolic data and epidemiologic data suggest that saturated fat intake should be as low as reasonably feasible, but these data also suggest that the benefits will be minimal if carbohydrate rather than unsaturated fats replace the saturated fat. Definitive data are not available on the optimal intake of polyunsaturated fat, but intakes of up to at least 10 % of energy from linoleic acid (omega-6) have positive health benefits and no evidence of harm has been documented. Consumption of omega-3 fatty acids is essential and several servings of fish per week appear to provide adequate amounts for most healthy people. Whether ALA from plant sources can provide the same benefits as longer-chain fish oils is not fully resolved; this is a major global nutritional issue. Metabolic data as well as the experience of Southern European populations suggest that consuming a substantial proportion of energy as monounsaturated fat would be desirable. Although available evidence suggests that low total fat intakes have little benefit, consuming low amounts of red meat, especially processed meats, may decrease the incidence of colorectal cancer and possibly breast cancer.

Vegetables and Fruits

Recommendations to eat a generous amount of vegetables and fruits [76] are supported by epidemiologic studies of cardiovascular risk [105, 106]. Many early studies also suggested that high intake of these foods would greatly reduce the risk of a wide range of cancers [107, 108]. However, most of these studies were case–control investigations, and more recent cohort studies have tended to show much weaker—or no—relation between overall fruit and vegetable consumption and risks of common cancers, including those of the breast, lung, and large bowel [90, 93, 109, 110]. In a pooled analysis of large cohort studies, a modestly lower risk of estrogen receptor-negative (ER-negative) breast cancer was seen among women with greater intake of fruits and vegetables, although no relation was seen with overall risk of breast cancer [111]. This finding is supported by a pooled analysis in which prediagnostic plasma levels of beta-carotene and other carotenoids were inversely related to risk of breast cancer, again predominantly with ER-negative cases [112].

Plants contain numerous components that have potential anticancer activity [108]. Considerable evidence suggests that folic acid reduces risk of colorectal cancer [95, 113], but vitamin supplements and fortification are now greater sources in the USA than fruits and vegetables. Other chemical constituents of plants could reduce the formation of carcinogens, induce detoxifying enzymes, and block the effects of endogenous estrogens. Further details about the amounts of these substances in foods could permit more informative investigations as lumping fruits and vegetables all together has little biological rationale.

In contrast to the weakened evidence that high intake of fruits and vegetables reduces cancer incidence, evidence has been strengthened that greater consumption will reduce risk of cardiovascular

disease [105, 106]. High intake of fruits and vegetables reduces blood pressure [114], a major risk factor for cardiovascular disease, and potassium appears to be the primary explanation [115]. Evidence that elevated blood homocysteine is an independent risk factor for coronary heart and cerebrovascular disease [116–118], and that levels can be reduced by supplements of folic acid and vitamin B6 [119, 120] suggest one mechanism.

Some randomized trials of folate supplementation show a reduction in risk of stroke [121], although trials conducted in populations with high intakes of folate have not shown benefits of added supplement [122]. The evidence from randomized trials of folic acid in reduction of myocardial infarction has generally not supported the apparent benefit seen in epidemiologic studies [123]. However, this may be due to the existence of advanced coronary disease in most studies, the use of many drugs in these studies of ill patients, and the relatively short-term nature of these studies.

Suboptimal dietary folic acid, which is mainly obtained from fortified breakfast cereals, vegetables, and fruits, definitively increases risk of neural tube defects, the most common severe birth defect [124, 125] and may account for more than half of these cases. The effect of low folate intake may be particularly adverse among the approximately 10 % of the population who are genetically less efficient in utilizing the ingested form of this vitamin [126].

In both case–control [127] and prospective studies [128, 129], intake of dietary antioxidants, including the carotenoids lutein and zeaxanthin, and vitamin C has been inversely related to risk of cataracts. As cataract formation, which is increased by sunlight and cigarette smoking [130], involves the accumulation of oxidized and denatured proteins, this lesion may represent a convenient marker of long-term oxidative damage. High intake of lutein and zeaxanthin in the form of spinach has been associated with a decreased risk of advanced macular degeneration [131] and evidence for benefit in reducing progression of macular degeneration has been seen in a large randomized trial [132]. This is particularly notable because lutein and zeaxanthin are the carotenoids specifically concentrated in the macula, where they apparently play a protective role against photodamage [133].

Starches and Complex Carbohydrates

As protein varies only modestly across a wide range of human diets, a higher carbohydrate consumption is, in practice, the reciprocal of a low-fat diet. For reasons discussed under the topic of fat, a high-carbohydrate diet may have adverse metabolic consequences. In particular, such diets are associated with an increase in triglycerides and a reduction in HDL cholesterol [20]. These adverse responses are aggravated in the context of insulin resistance [134, 135], which to some degree is highly prevalent in western populations. Although Asian populations had been thought to be at lower risk for insulin resistance and type 2 diabetes, much evidence now indicates that these populations, and also Hispanic and African populations have a higher risk of type 2 diabetes, probably due to genetic susceptibility, compared to European populations, given the same diet, activity level, and BMI [136]. This has enormous implications because many of these populations have traditionally consumed large amounts of carbohydrate, which was well tolerated as long they were lean and active, which may become deleterious in the background of lower activity and even modest amounts of weight gain.

Several reasons exist to emphasize whole grains and other less refined complex carbohydrates as opposed to the highly refined products and sugar generally consumed in the USA. Adverse consequences of highly refined grains appear to result both from the rapid digestion and absorption of these foods, as well as from the loss of fiber and micronutrients in the milling process. The glycemic response after carbohydrate intake, which has been characterized by the glycemic index, is greater with highly refined foods as compared to less-refined, whole grains [137]. The greater glycemic response owing to highly refined carbohydrates is accompanied by increased plasma insulin levels

and appears to augment the other adverse metabolic changes due to carbohydrate consumption noted above [137] to a greater degree than with less refined foods. Diets with a high-glycemic index or glycemic load (the product of dietary glycemic index and total carbohydrate intake) appear to increase the risk of noninsulin-dependent diabetes [138] and possibly risk of CHD, particularly among women with greater insulin resistance [139].

Fiber intake, particularly from grain sources, has consistently been inversely related to risk of coronary heart disease and type 2 diabetes [31, 140–142]. Risk of MI appears to be reduced by higher intake of dietary fiber from grains to a greater degree than can be explained by the effect of fiber on blood lipids alone [143]. Anticipated reductions in colon cancer risk by diets high in grain fiber have been difficult to document epidemiologically [144–146], although an inverse relation has been seen in Europe [147]. However, reduced constipation and risk of colonic diverticular disease [130] are clear benefits of such diets. The role of soluble fiber, found in oat bran and some other plant foods, in lowering blood lipids has been hotly debated; current evidence suggests that a small effect may exist with large intakes [148, 149].

The importance of micronutrients in the prevention of many chronic conditions, discussed below, has reemphasized the problem of "empty calories" associated with diets high in sugar and highly refined carbohydrates. In the standard milling of white flour, as much as 60–90 % of vitamins B6 and E, folate, and other nutrients are lost [150]; this may be nutritionally critical for persons with otherwise marginal intakes. In the USA, thiamin, riboflavin, folate, and niacin are presently replaced by fortification, but other nutrients remain substantially reduced. Fortification of grains with folic acid has not been implemented in many countries despite clear benefits for reduction of neural tube defects and probably stroke (see above). One reason expressed for not doing so is the potential promotion of existing neoplasias, especially those of the colon [151]. This concern was heightened by an apparent transient pause in the decline in incidence of colon cancer in the USA and Canada, but this may also have been due to increased diagnosis due to screening by colonoscopy. Importantly, in the USA there has been no suggestion of any increase in colon cancer mortality after folic acid fortification (http://progressreport.cancer.gov/); instead a sharp decline has occurred.

Sugar in the form of soda and other beverages is of special concern because of the large amounts consumed by many populations, and because this appears to result in excess energy intake due to failure to suppress satiety [152]. Not surprisingly, daily consumption of sugary beverages is associated with increased risks of type 2 diabetes [153, 154].

Protein

Average protein consumption in the USA and other affluent countries substantially exceeds conventional requirements [76] and adequate intake can be maintained on most reasonable diets, including those without animal products. High intake of animal protein can increase urinary calcium loss [155], contribute to homocysteinemia [156], and has been hypothesized to increase risk of various cancers [157]; however, there is little evidence for the latter effect. Substituting protein for carbohydrate improves blood lipids and blood pressure [158]. Also, because protein from foods is not consumed in isolation, the effects of these foods will depend mainly on the quality of fat and carbohydrate that they contain [159]. In a series of analyses of major protein sources, replacement of red meat with poultry, fish, nuts, and legumes has been associated with lower risks of coronary heart disease [160], diabetes [161], stroke [162], and breast cancer [35].

Calcium, Vitamin D, and Dairy Products

Recommendations to maintain adequate calcium intake [76, 163] and to consume dairy products on a daily basis [164] derive primarily from the role of calcium in maintaining bone health. Calcium supplements in conjunction with vitamin D have reduced fracture incidence in older adults [165, 166], but in such studies benefits of calcium cannot be distinguished from those of vitamin D. In a meta-analysis of randomized trials, no reduction in overall fracture risk was seen with supplemental calcium alone [167], and in a meta-analysis of prospective studies calcium intake over about 500 mg/day was not associated with lower risk of fractures. Uncertainty remains regarding the optimal intake. In the USA intakes as high as 1200 mg/day have been recommended for postmenopausal women at risk of fractures [163], which are difficult to achieve without supplements, but in the UK 700 mg/day is considered adequate for those over 19 years of age (http://www.foodstandards.gov.uk/news/newsarchive/foodpromotionplans). However, many populations have low-fracture rates despite minimal or no dairy product consumption and low overall calcium intake by adults [168], and for this reason the WHO considers 500 mg/day to be adequate intake [6].

Milk and other dairy products may not be directly equivalent to calcium from supplements, as these foods contain a substantial amount of protein, which can enhance renal calcium losses [155] and milk contains many other nutrients and hormones. Several prospective studies have directly addressed the relation of dairy product consumption to fracture incidence; with the exception of one small study [169]; higher consumption of calcium or dairy products as an adult has not been associated with lower fracture incidence [170–172]. At best, the benefits of high calcium intake are minor compared with those from regular physical activity [173–176]. Low-calcium intake has been associated with risk of colon cancer in large prospective studies [177]; evidence from a randomized trial that calcium supplementation modestly reduces colon adenoma recurrence adds important evidence of causality to the epidemiologic findings [178].

Although calcium intakes can be increased by a high consumption of greens and certain other vegetables, greatly increased intakes would be required for most women to achieve the high calcium recommended levels by diet without regular use of milk and other dairy products. Calcium supplements are an inexpensive form of calcium without accompanying calories or saturated fat. Thus, dairy product consumption can be considered an optional rather than a necessary dietary component. Enthusiasm regarding high dairy consumption should also be tempered by the suggestion in many studies that this is associated with increased risks of prostate cancer [93, 179, 180] and possibly ovarian cancer [181]. Whether an increased risk is due to the calcium, lactose, or endogenous hormones in milk remains uncertain.

Until recently, the consequences of low vitamin D status were thought to be limited to rickets, osteoporosis, and fractures. However, almost every organ has been found to have vitamin D receptors and inadequate vitamin D status has also been associated with greater risks of infections [182], some cancers [183, 184], multiple sclerosis [185, 186], muscle weakness [187], coronary heart disease [188], and other conditions. The optimal intake and blood level of vitamin D (25 OH vitamin D) have been topics of major debate; the IOM has set 50 nmol/mL as an adequate intake based on bone indicators [163], but if other outcomes are considered, optimal levels appear to be in the range of 70–100 nmol/mL [182]; even if the lower level is used, a majority of US residents have suboptimal vitamin D status, and among persons with dark skin this may be as high as 90 %. The alternatives for increasing blood levels are primarily to increase sun exposure, which if not done carefully will increase risks of skin cancer, or to take supplements; the levels of vitamin D naturally present in fish or fortified milk can prevent rickets, but for most people it is difficult to reach optimal levels from these sources.

Salt and Processed Meats

Reduction of salt (sodium chloride) intake will decrease blood pressure. Law et al. [189] have concluded that a 3-g/day decrease would reduce the incidence of stroke by 22 % and of CHD by 16 %. Although the decrease in risk of cardiovascular disease achieved by reducing salt consumption is small for most individuals, the overall number of deaths potentially avoided is large, supporting policies to reduce consumption, particularly in processed foods and by institutions. In several case–control studies, the consumption of salty and pickled foods has been associated with stomach cancer [93].

Body Weight

Until recently, the issue of optimal body weight was controversial due to analyses that did not account for confounding influences of factors such as smoking (which is a strong cause of premature death and is also associated with low body weight) or the fact that many individuals, particularly at older ages, have low body weights because of chronic illness [190]. More detailed analyses indicate that middle-aged persons with a body mass index (BMI) even close to 25 kg/m^2 have a high prevalence of abnormal blood glucose, lipids, and blood pressure [191], and experience substantial increases in MI [192, 193], diabetes [194], hypertension [195], many cancers [97, 196], gallstones [197], and total mortality rates [98] compared to their leaner counterparts. Thus, the current guidelines based on a BMI range of 18–25 kg/m^2 are generally considered optimal, and the best health experience is achieved by avoiding increases in weight during adulthood [190]. As noted earlier, dietary fat composition over a wide range appears to have little relationship with weight maintenance; in contrast, low consumption of sugary beverages [152], trans fat [58], higher intake of dietary fiber [198], and overall diet quality such as a Mediterranean diet [103, 199] appear to be helpful for weight control. Regular physical activity and avoidance of extreme inactivity such as excessive television watching is crucial [200].

Alcohol

Many adverse influences of heavy alcohol consumption are well recognized, but moderate consumption has both beneficial and harmful effects, greatly complicating decisions for individuals (see Chap. 29). Overwhelming epidemiologic data indicate that moderate consumption reduces risk of MI [201–203], one to two drinks a day decrease risk by approx 30–40 %. Although it has been suggested that this effect may be a result of antioxidants in red wine [204], similar protective effects for equivalent amounts of alcohol have been seen for all types of alcoholic beverages [205, 206]. On the other hand, modest positive associations with risk of breast cancer incidence have been observed in dozens of studies with even one alcoholic drink per day [207, 208], possibly because alcohol increases endogenous estrogen levels [209, 210] and interferes with folate metabolism [211]. The overall effect of alcohol, as represented by total mortality, appears beneficial up to about two drinks per day in men [212]. Overall, a similar relation with total mortality is seen among women, but no net benefit was observed among those at low risk of coronary heart disease because of age less than 50 years or lack of coronary risk factors [213]. Furthermore, the risk of transition from moderate alcohol consumption to addiction and uncontrolled drinking has not been well quantified.

Vitamin Supplements

The most firmly established benefit of vitamin supplements, based on case–control, cohort, and randomized studies, is that folic acid supplements in the amounts contained in multiple vitamins can reduce the risks of neural tube defects by approximately 70 % [124, 214]. As noted above, correction of low folate levels can reduce the risk of stroke [121] and probably also the risks of coronary heart disease [123] and several cancers [215]. In a large randomized trial, a multiple vitamin/multimineral preparation modestly reduced total cancer incidence [216]; whether this was due to folate or other components is not known. The cardiovascular benefits of folate may be mediated in part through reductions of homocysteine, and in some populations correction of low levels of vitamin B-6 and B-12 as well as low folate may have similar benefits. Vitamin B-12 absorption declines with age, and supplements can prevent deficiency in older persons.

In prospective epidemiologic studies healthy men and women who consumed the highest amounts of vitamin E (mostly from supplements) had an approximately 40 % lower risk of MI compared to those having low vitamin E intakes [217, 218]. However, in randomized trials, mainly among patients with existing coronary heart disease, little benefit has been seen [219]. The apparent difference may relate to the study populations because persons with existing coronary disease were excluded from the epidemiologic studies, and they were typically on many drugs that could overlap in mechanisms with vitamin E. In a large trial among women without cardiovascular disease, a nonsignificant lower risk of coronary heart disease was seen with vitamin E supplementation, but vitamin E significantly reduced total cardiovascular mortality by 24 % [220]. The association between vitamin C and CHD risk has been inconsistent in prospective studies [218, 221]. Apart from a possible reduction in risk of cataracts [130], only limited evidence exists at present that high doses of vitamin C have substantial benefits.

Intake of preformed vitamin A (retinol) just above the RDA has been associated with excess risk of hip fracture in prospective studies [222, 223], possibly by competing with vitamin D at the receptor level, and elevated risks were seen for both use of multiple vitamins and specific supplements of vitamin A. In a more recent study, a modest positive association between vitamin A intake and risk of fractures was limited to those with low vitamin D intake, adding further evidence for an interaction with these vitamins [224]. The weaker association seen in this recent study may have resulted from reductions during the follow-up in the retinol content of breakfast cereals and multiple vitamins made in response to the evidence on fracture incidence. Serum levels of retinyl esters have not been associated with bone mineral density [225], but these findings are difficult to interpret because retinyl esters are highly variable and the degree to which a single measure represents long-term vitamin A intake is unclear.

In a randomized trial conducted in a region of China with low consumption of fruits and vegetables, a supplement containing beta-carotene, vitamin E, and selenium reduced incidence of stomach cancer [226].

Current evidence, although far from complete, suggests that supplements of folate and probably other vitamins, at the RDA level, contained in most nonprescription multivitamin preparations, have substantial benefits for at least an important, but unidentified, population subgroup, perhaps characterized by increased requirements or suboptimal diets. As intakes of many micronutrients appear marginal for many Americans [107, 227] the risks of using multivitamins low, and the cost of supplements is minimal (especially compared to that of fresh fruits and vegetables), the use of a daily or several-times-a-week multiple vitamin appears rational for the majority of Americans, given current knowledge. Multiple vitamins may have little benefit in someone consuming an optimal diet, but such persons are not common in the USA [228] and rare in low-income populations [229]. Further, inclusion of vitamin D, at doses of at least 1000 IU per day, will provide a critical nutrient that cannot be obtained in sufficient amounts by diet, although many people may require additional amounts as a separate supplement to reach adequate levels.

Vitamin E supplements do not benefit persons with established coronary heart disease, but for others at risk of CHD it can be rational to use these while waiting for further data. For other vitamins and minerals there is presently limited evidence of benefit of supplements over the RDA levels. Intake of vitamin A at levels above the RDA can potentially be harmful. In one study, intake of supplements containing more than 10,000 IU/d of preformed vitamin A was associated with risk of specific birth defects [230].

Conclusions and Recommendations

Any set of dietary or nutritional recommendations must be made with the qualification that information is currently incomplete, and some conclusions may be modified with new data. Most importantly, the common major diseases in the USA develop over many decades, and large-scale nutritional epidemiologic studies have only begun in the last 30 years; a full picture of the relation between diet and disease will require additional decades of careful investigation. Nevertheless, combining metabolic, clinical, and epidemiologic evidence, several general recommendations that are unlikely to change substantially can be made to those who are interested in consuming a healthy diet.

1. Stay lean and active throughout life. For most individuals, body weight should not increase by more than 5–10 lb after age 21. Because most of us work at sedentary jobs, weight control will usually require conscious regular daily exercise as well as some effort to avoid overconsumption of energy, which can be facilitated by a high-quality diet.
2. Trans-fatty acids from partially hydrogenated vegetable oils should be avoided completely. These unhealthy fats can be replaced with a combination of vegetable oils that include a mix of monounsaturated and polyunsaturated fats.
3. Grains should be consumed primarily in a minimally refined, whole grain form and intake of simple sugars, especially as beverages, should be low.
4. Vegetables and fruits should be consumed in abundance (5 servings/day is minimal) and include green leafy and orange vegetables daily.
5. Red meat should be consumed only occasionally and in low amounts if at all; nuts and legumes as well as poultry and fish in moderation are healthy alternatives.
6. The optimal consumption of dairy products and calcium intake is not clear, and dairy products should be considered as optional. High consumption of milk (e.g., more than 2 servings per day) is not likely to be beneficial for middle-aged and older adults, and may increase risk of prostate and ovarian cancer. Adequate calcium intake may be particularly important for growing children, adolescents, and lactating women; supplements should be considered if dietary sources are low.
7. Unless one is extremely careful about a healthy food selection at every meal, consuming a daily RDA-level(DV) multiple vitamin containing folic acid and at least 1000 IU of vitamin D provides a sensible nutritional safety net. Because menstrual losses of iron are often not adequately replaced by iron intake on the low-energy diets of women in a sedentary society, it makes sense for most premenopausal women to use a multiple vitamin/multimineral that also contains iron. Pending further data, the use of a vitamin E supplement at 400–800 IU/day is reasonable for most middle-aged and older healthy persons as available evidence suggests that this may reduce risk of cardiovascular disease. Personal physicians should be made aware of any nutritional supplements that are being consumed in the event of possible interactions with medications or diagnostic tests. Further, use of supplements should not be considered as an alternative to eating a healthy diet because foods contain a wide variety of additional factors that are likely to contribute to good health.
8. Finally, be adventuresome in eating! Unfortunately, most of us in the USA are heirs to the rather monotonous Northern European dietary tradition centered on the consumption of meat, dairy products, and potatoes. Contemporary food processing has added to the deleterious effects of this diet

by the removal of dietary fiber and micronutrients through over-refining of foods, and has profoundly and adversely altered the biological effects of vegetable oils through the process of partial hydrogenation. To further aggravate matters, the worst aspects of diet tend to be the most heavily marketed and promoted. Fortunately, healthy diets do not have to be invented or discovered through new technological advances. Existing foods together with the lessons of various cultural models of eating based primarily around minimally processed foods from plant sources provide a means of achieving a diet that is healthy as well as interesting and enjoyable.

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Chapter 3
Nutritional and Dietary Supplements: Code or Concern

Roger Clemens and Peter Pressman

Key Points

- Dietary supplements are regulated in the USA; amendments to and enhanced enforcement of these regulations may be warranted.
- Dietary supplements may be beneficial by filling nutrition gaps as noted by the 2010 Dietary Guidelines Advisory Committee, particularly in at-risk groups.
- Safety of dietary supplement ingredients and efficacious doses are imperatives.
- Health claims associated with dietary supplements, such as those directed to weight management, athletic performance, and cognitive functions deserve careful premarket review.

Keywords Dietary supplements • DSHEA • Quality • Regulations

Introduction

Surveys indicate more than 50 % of Americans regularly consume at least one dietary supplement in an effort to improve personal health and wellness and to fill nutrient gaps [1]. To improve the quality of these products and to provide consumers with more options in the nutrition and wellness continuum, the Dietary Supplement Health and Education Act (DSHEA) was enacted in 1994. Five years later, a landmark decision by the D.C. Court of Appeals opening the door for health claims on these products. USP-NF and NSF quality programs voluntarily engaged numerous dietary supplement manufacturers. Yet, these products are often recalled due to contaminants and adulterants, particularly those containing medications. Some organizations and investigators argue dietary supplements do not have any value in managing public health issues, yet the 2010 Dietary Guidelines for Americans noted some of these products are valuable among at-risk populations. The dietary supplements market is expected to experience at 4.54 % compound annual growth rate through 2016 [2]. Revenue from this market is expected to exceed $57 billion by 2021, with the greatest anticipated growth in China, India and Brazil, while the global functional foods category is expected to exceed $73 billion.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_3

United States Regulatory Environment

It has been 20 years since the USA formalized statutes that impact the nature, quality, and standards of dietary supplements within this market. Sponsored by Senator Orrin Hatch (R-UT), the Dietary Supplement and Health Education Act of 1994 (Public Law 103-417) signaled an new era of the Food and Drug Administration's efforts to provide consumers the choice to improving wellness and decreasing chronic disease through the use of dietary supplements.

Dietary supplements, as the name implies, were intended to supplement the diet with one or more specific ingredients, which included a vitamin, a mineral, an herb or other botanical, or an amino acid (FD&C Act, Section 201(ff)(1)). Dietary supplements were projected to increase total dietary intake of these substances provided in forms or combination of forms as a concentrate, metabolite, constituent, or extract.

Unlike food additives and other food ingredients, the onus dietary supplement safety falls upon the Food and Drug Administration. This safety burden is triggered if the product or its ingredient(s) presents a significant or unreasonable risk of illness or injury under specific conditions, contains a component for which there is insufficient information that assures the absence of a significant or unreasonable risk, deemed by the FDA that the product or ingredient poses an imminent public health hazard, or contains an ingredient that renders the product adulterated.

Dietary supplements are not drugs. Drugs, under US statutes, refer to articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals, and articles, other than food, intended to affect the structure or function of the body of man or other animals (FD&C Act, Section 201(g)). In addition, drugs are administered through several routes, such as transdermal, injection, sublingual, nasal spray, and orally. However, dietary supplements, like that of foods, are administered only via enteral means, such as ingestion in a capsule, tablet, liquid, powder, softgel, or similar forms. In addition, these products do not represent a meal or conventional food [3]. The administration of a proposed diet supplement via any other route is considered as a drug.

Interestingly, a dietary supplement may contain a drug substance as long as that substance was marketed as a dietary supplement or food prior to its approval as a drug (21 U.S. Code § 321(ff)(3). This statute was critical in the case of *Pharmanex v. Shalala* (Case 99-4087, U.S. Court of Appeals, 10th Circuit), in which the FDA prohibited the marketing of red yeast rice that contained mevinolin a component of Cholestin [4]. Mevinolin, which is chemically identical to lovastatin, was approved by the FDA in 1987, whereas Pharmanex marketed Cholestin 10 years later as a dietary supplement. In 1998, the FDA issued a decision that Cholestin did not meet the definition of a dietary supplement. The court supported the FDA's interpretation of the regulations in that a dietary supplement may not contain previously approved drug (21 U.S. Code § 321(ff)(3)(B)).

Approval and Safety

While under DHSEA, dietary supplement manufacturers are responsible for product safety prior to marketing, these same producers are not responsible for safety of ingredients or products marketed prior to implementation of the Act (October 15, 2994) (21 U.S. Code § 350b(c)). However, it is important to note that under DSHEA, these producers are not required to obtain FDA approval prior to marketing their products, and that product safety as presumed, unlike that of food additives and drugs. Even though DSHEA did not require registration of production facilities, such registration is required under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and the FDA Food Safety Modernization Act (FSMA) of 2011 [5, 6].

While there is a presumption of safety of these products, it is incumbent upon dietary supplement manufacturers to provide documentation that asserts "reasonable certainty that the substance(s) is not

harmful under the intended conditions of use." [7] Unlike ingredients in conventional foods, there is not a provision under DSHEA that stipulates dietary supplement ingredients must be Generally Recognized As Safe or meet the provisions of the Food Additive Amendments.

Functional Foods and DSHEA

There is a strong nutraceuticals or functional foods movement in the USA and beyond. This concept, initiated by Stephan DeFelice in the mid-70s and based on the regulatory foundation of orphan drugs, encourages medical discovery within the food and dietary supplement continuum. Functional foods which reside in the interface of nutrition and pharmaceuticals do not have a statutory definition in this country. However, the integration of functional foods with mainstream marketing the health benefits of specific foods or combinations of foods is founded in the Japanese market under FOSHU (Food for Specified Health Use). Such food products intended to promote improved health and health maintenance have an official claim based on their physiological effects [8]. The evidence of such claim approval is shown in Table 3.1. An overview of specific documentation is summarized by Yamada et al., 2008 [9]. The mandate documentation for health claims for foods and ingredients is extensive and reviewed within the Japanese Ministry of Health, Labor and Welfare [10], whereas the proliferation of health claims within the USA, particularly with respect to dietary supplements, does not require premarket approval and often based on minimal clinical or observational evidence despite a 2008 industry guidance from the Food and Drug Administration [11].

When the Dietary Supplement Health and Education Act (DSHEA) of 1994 was signed into law, the dietary supplement market was nearly $4 billion. Revenue from this market is expected to exceed $57 billion by 2021, with the greatest anticipated growth in China, India, and Brazil, while the global functional foods category is expected to exceed $73 billion (Nutrition Business Journal 2013) [12].

Prior to DHSEA, which is fundamentally a post-market program, the FDAs jurisdiction included therapeutic and health claims review of dietary supplements was restricted. Following the 1994 passage of this unique regulatory framework, the agency convened an array of advisory committees to address reported safety issues associated with dietary supplements. For example, in 1995 the FDA convened a working group to review public scientific publications regarding the safety of ephedra (aka ma huang), and to establish a safe dose of this herb commonly found in dietary supplements claimed to promote weight loss and enhance athletic performance. While the agency called for warning labels on ephedra-containing products, the FDA withdrew this effort since under DSHEA the FDA would need to execute research to prove the dietary supplement was not safe. After reviewing numerous reports on potential adverse events, including 155 deaths associated with ephedra-containing products and many years of litigations and debates, the U.S. Court of Appeals for the Tenth Circuit in Denver reversed the 2005 opinion of U.S. District Judge Tena Campbell that ruled in favor of Nutraceutical International, which claimed these products were safe based on a history of use. The Appeals court concluded that ephedra and its family of alkaloids posed an unreasonable risk of illness or injury to users, particularly those at

Table 3.1 Criteria for Health Claims Approval under FOSHU

• Effectiveness on the human body is clearly proven
• Absence of any safety issues (animal toxicity tests, confirmation of effects in the cases of excess intake, etc.)
• Use of nutritionally appropriate ingredients (e.g., no excessive use of salt)
• Guarantee of compatibility with product specifications by the time of consumption
• Established quality control methods, such as specifications of products and ingredients, processes, and methods of analysis

Source: http://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html

risk of heart disease and high blood pressure based on an FDA-commissioned meta-analysis conducted by the RAND Corporation, and denied a petition to rehear the case. The court's decision also extended the FDA's authority to ban any dietary supplement ingredient even if there is a very small risk of illness or injury [13].

Health Claims

Pearson v Shalala (1999)

Under the Nutrition, Labeling and Education Act of 1990 (NLEA), Public Law 101-535), the FDA permitted continuation of nutrient content claims used prior to October 25, 1989. This statute empowered the FDA to require nutrition labeling of foods under its regulatory jurisdiction. NLEA outlined specific nutrient labeling criteria and nutrient content claims. However, claims that characterized the relationship of a given nutrient to a disease or health-related condition required substantiation that the claim is truthful and not misleading under Section 403(r)(5) of the Federal Food, Drug and Cosmetic Act.

Neither NLEA nor DSHEA defined "substantiation." According to the Federal Trade Commission, this term typically refers to "competent and reliable scientific evidence" relative to the potential benefits and safety of the product. Within the characterization of this evidence, the agency recommended the meaning of the claim to include the relationship of the evidence to the claim, the quality of that evidence, and the totality of the evidence. Under DSHEA, the FDA only permitted claims if there was "significant scientific agreement" (SSA) among experts that support the adequacy of available evidence.

In 1998, Durk Pearson and Sandy Shaw sought SSA on four specific health claims for dietary supplements. These four claims suggested consumption of several dietary supplements would reduce the risk of certain diseases. (US Court of Appeals, District of Columbia Circuit, No. 98-5043, 98-5084). Those claims were:

1. "Consumption of antioxidant vitamins may reduce the risk of certain kinds of cancers."
2. "Consumption of fiber may reduce the risk of colorectal cancer."
3. "Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease."
4. "0.8 mg of folic acid in a dietary supplement is more effective in reducing the risk of neural tube defects than a lower amount in foods in common form."

In this landmark case, the U.S. Court of Appeals invalidated FDA regulations that prohibited certain health claims on foods. The court ruled that the FDA (1) violated the First Amendment by banning misleading health claims without considering the use of curative disclaimers and (2) violated the arbitrary and capricious standards of the Administrative Procedure Act (1946; Public Law 79-404) by failing to clarify the standard of "significant scientific agreement."

Claims for dietary supplements fall under two basic categories: Dietary guidance and nutrient content claims. Dietary guidances, while not specific product claims, are based on an evidence-based review system, such as the Dietary Guidelines for Americans [14]. Such a system provides guidance as to the strength and quality of that evidence, and its relevance to specific population groups. For example, encouraging the increased consumption of fruit and fiber is a dietary guidance.

Nutrient content claims include statements such as the product contains high dietary fiber or low sodium. These are consistent with NLEA (Public Law 101-53) permitted claims and as allowed under FDA's guidance document for health claims.

Health claims, on the other hand, refer to the relationship between a food, food component or dietary supplement ingredient and disease or health-related condition [15]. These relationships have

Table 3.2 Authorized health claims based on an authoritative statement by Federal Scientific Bodies

Approved claim	Claim requirement (required wording)
Whole grain foods and risk of heart disease and certain cancers	"Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers"
Whole grain foods with moderate fat content and risk of heart disease	"Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may help reduce the risk of heart disease"
Potassium and the risk of high blood pressure and stroke	"Diets containing foods that are a good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke"
Fluoridated water and reduced risk of dental carries	"Drinking fluoridated water may reduce the risk of [dental caries or tooth decay]"
Saturated fat, cholesterol, and trans fat, and reduced risk of heart disease	"Diets low in saturated fat and cholesterol, and as low as possible in trans fat, may reduce the risk of heart disease"
Substitution of saturated fat in the diet with unsaturated fatty acids and reduced risk of heart disease	"Replacing saturated fat with similar amounts of unsaturated fats may reduce the risk of heart disease. To achieve this benefit, total daily calories should not increase"

been reviewed and authorized by the FDA. In addition, the Food and Drug Administration Modernization Act (FDAMA) of 1997 provided an alternative means to health claims based on authoritative statements from the National Academy of Sciences or a scientific body within the US government, such as the Centers for Disease Control and Prevention (CDC) involved in public health and nutrition research. These types of claims, directed to the general population or specific population groups, such as the elderly or women of child-bearing age, are intended to encourage the maintenance of healthy dietary practices. Currently allowed health claims are shown in Tables 3.2 and 3.3.

Good Manufacturing Practices

The final rule (21CFR111) for cGMPs applied to dietary supplements was issued in 2007 [16]. The purpose of these cGMPs was to ensure the quality of dietary supplements through quality control procedures, the design and construction of production facilities, and testing of ingredients and finished products. These cGMPs are very similar to those required of pharmaceutical agent production and infant formula manufacturing. Unlike these cGMPs, it is incumbent that cGMPs for dietary supplements include requirements for valid analytical methods, full characterization of ingredients (including CAS numbers) regardless of they are innate or synthetic.

The new regulation required manufacturers to provide finished or commercial products that were free of contaminants and accurately labeled, as well as extensive management of all ingredient, production, and analytical records. Several critical elements in ensuring quality included ingredient verification (eliminate wrong ingredients), accuracy of ingredients as formulated, appropriate packaging (containers and closures), accurate labeling, and elimination of potential issues associated with natural toxins, bacteria, pesticides, glass, heavy metals and other substances or materials that may pose public health hazards. These and other components of the final rule for cGMP are shown in Table 3.4.

Adverse event reporting was stipulated under the Dietary Supplement and Nonprescription Drug Consumer Protection Act of 2007 and revised in 2013. Adverse events, as reported on Form FDA 3500A, must include a spectrum of personal data of those impacted, outcomes attributed to the adverse event, array of medical information and specific information germane to the dietary supplement (e.g., lot number, expiration date) plus manufacturer, packer or distributor contact information.

Table 3.3 Requirements for health claims made in labeling

Approved claim	Requirements for the food (including dietary supplements)	Claim requirements	Model claim, statements
Calcium and osteoporosis and calcium, vitamin D, and osteoporosis	For calcium and osteoporosis claim-high in calcium	The claim makes clear the importance of adequate calcium intake, or when appropriate, adequate calcium and vitamin D intake, throughout life, in healthful diet, are essential to reduce osteoporosis risk. The claim does not imply that adequate calcium intake, or when appropriate, adequate calcium and vitamin D intake, is the only recognized risk factor for the development of osteoporosis	Calcium and Osteoporosis: Adequate calcium throughout life, as part of a well-balanced diet, may reduce the risk of osteoporosis
	For calcium, vitamin D and osteoporosis claim-high in calcium and vitamin D	The claim does not attribute any degree of reduction in risk of osteoporosis to maintaining an adequate dietary calcium intake, or when appropriate, an adequate dietary calcium and vitamin D intake, throughout life	Calcium, vitamin D and osteoporosis: Adequate calcium and vitamin D, as part of a well-balanced diet, along with physical activity, may reduce the risk of osteoporosis
	– Assimilable (Bioavailable)		
	Supplements must disintegrate and dissolve, and		
	Phosphorus content cannot exceed calcium content		
Dietary fat and cancer	(Fish and game meats: “Extra lean”)	Required terms:	Development of cancer depends on many factors. A diet low in total fat may reduce the risk of some cancers
		“Total fat” or “Fat”	
		“Some types of cancers” or “Some cancers”	
		Does not specify types of fats or fatty acids that may be related to risk of cancer	
Sodium and hypertension	Low sodium	Required terms:	Diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors
		“Sodium,” “High blood pressure”	
		Includes physician statement (Individuals with high blood pressure should consult their physicians) if claim defines high or normal blood pressure	
Dietary saturated fat and cholesterol, and risk of coronary heart disease	Low saturated fat	Required terms:	While many factors affect heart disease, diets low in saturated fat and cholesterol may reduce the risk of this disease
	Low cholesterol	Saturated fat and cholesterol	
	Low fat	“Coronary heart disease” or “heart disease”	
		Includes physician statement (individuals with elevated blood total—or LDL—cholesterol should consult their physicians) if claim defines high or normal blood total—and LDL—cholesterol	

Fiber-containing grain products, fruits, and vegetables and cancer	A fruit, vegetable, or grain product that contains fiber	Required terms:	Diets low in saturated fat and cholesterol and rich in fruits, vegetables, and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors
	Low saturated fat	"Fiber," "Dietary fiber," "Some types of dietary fiber," "Some dietary fibers," or "Some fibers"	
	Low cholesterol	"Saturated fat" and "Cholesterol"	
	Low fat	"Heart disease" or "Coronary heart disease"	
	At least 0.6 g of soluble fiber per RACC (without fortification)	Includes physician statement ("Individuals with elevated blood total—or LDL—cholesterol should consult their physicians") if claim defines high or normal blood total—and LDL—cholesterol	
	Soluble fiber content provided on label		
Fruits and vegetables and cancer	A fruit or vegetable	Required terms:	Low fat diets rich in fruits and vegetables (foods that are low in fat and may contain dietary fiber, Vitamin A [as a precursor], or Vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors. Broccoli is high in vitamin A [as a precursor] and C, and it is a good source of dietary fiber
	Low fat	"Fiber," "Dietary fiber," or "Total dietary fiber"	
	Good source (without fortification) of at least one of the following	"Total fat" or "Fat"	
	• Vitamin A	"Some types of cancer" or "Some cancers"	
	• Vitamin C	Characterizes fruits and vegetables as "Foods that are low in fat and may contain Vitamin A, Vitamin C, and dietary fiber"	
	• Dietary fiber	Characterizes specific food as a "Good source" of one or more of the following: Dietary fiber, Vitamin A, or Vitamin C	
		Does not specify types of fats or fatty acids or types of dietary fiber that may be related to risk of cancer	
Folate and neural tube defects	"Good source" of folate (at least 40 mcg folate per serving)	Required terms:	Healthful diets with adequate folate may reduce a woman's risk of having a child with a brain or spinal cord defect
	Dietary supplements, or foods in conventional food form that are naturally good sources of folate (i.e., only non-fortified food in conventional food form)	Terms that specify the relationship (e.g., women who are capable of becoming pregnant and who consume adequate amounts of folate) "Folate," "folic acid," "folacin," "folate a B vitamin," "folic acid, a B vitamin," "folacin, a B vitamin," "neural tube defects," "birth defects, spinal bifida, or anencephaly," "birth defects of the brain or spinal cord—anencephaly or spinal bifida," "spinal bifida or anencephaly, birth defects of the brain or spinal cord"	
	The claim shall not be made on products that contain more than 100 % of the RDI for vitamin A as retinol or preformed vitamin A or vitamin D	Must also include information on the multifactorial nature of neural tube defects, and the safe upper limit of daily intake	
	Dietary supplements shall meet USP standards for disintegration and dissolution or otherwise bioavailable		
	Amount of folate required in Nutrition Label		

(continued)

Table 3.3 (continued)

Approved claim	Requirements for the food (including dietary supplements)	Claim requirements	Model claim, statements
Dietary noncariogenic carbohydrate sweeteners and dental caries	Sugar free	Required terms:	Full claim: Frequent between-meal consumption of foods high in sugars and starches promotes tooth decay. The sugar alcohols in [name of food] do not promote tooth decay.
	When a fermentable carbohydrate is present, the food must not lower plaque pH below 5.7.	"does not promote," "may reduce the risk of," "useful [or is useful] in not promoting" or "expressly [or is expressly] for not promoting" dental caries	Shortened claim (on small packages only): Does not promote tooth decay.
	Eligible substances	"dental caries" or "tooth decay"	
	1. The following sugar alcohols:xylitol, sorbitol, mannitol, maltitol, isomaltitol, lactitol, hydrogenated starch hydrolysates, hydrogenated glucose syrups, erythritol, or a combination of these	"sugar alcohol" or "sugar alcohols" or the name or names of the sugar alcohols	
	2. The following sugars:D-tagatose and isomaltulose	Note: D-tagatose may be identified as "tagatose"	
	3. The following non-nutritive sweetener: sucralose	When the substance that is the subject of the claim is a noncariogenic sugar (i.e., D-tagatose) the claim shall identify the substance as a sugar that, unlike other sugars, does not promote the development of dental caries	
		Includes statement that frequent between meal consumption of foods high in sugars and starches can promote tooth decay	
		Packages with less than 15 square inches of surface area available for labeling may use a shortened claim	
Soluble fiber from certain foods and risk of coronary heart disease	Low saturated fat	Required terms:	Soluble fiber from foods such as [name of soluble fiber source, and, if desired, name of food product], as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food product] supplies __ grams of the [necessary daily dietary intake for the benefit] soluble fiber from [name of soluble fiber source] necessary per day to have this effect
	Low cholesterol	"Heart disease" or "coronary heart disease"	
	Low fat	"Saturated fat" and "cholesterol"	

	The food product must include one or more of the following whole oat or barley foods: (1) oat bran, (2) rolled oats, (3) whole oat flour, (4) whole grain barley or dry milled barley, and the whole oat or barley foods must contain at least 0.75 g of soluble fiber per RACC of the food product	In specifying the substance the claim uses the term "soluble fiber" qualified by the name of the eligible source of the soluble fiber, which is either whole oat or barley or psyllium seed husk	
	Oatrim that contains at least 0.75 g of beta-glucan soluble per RACC of the food product	Claim specifies the daily dietary intake of the soluble fiber source necessary to reduce the risk of CHD	
	Psyllium husk that contains at least 1.7 g of soluble fiber per RACC of food product	Claim specifies the amount of soluble fiber in one serving of the product	
	Eligible sources of soluble fiber	Additional Required Label Statement	
	Beta-glucan soluble fiber from the following whole oat and barley sources:	Foods bearing a psyllium seed husk health claim must also bear a label statement concerning the need to consume them with adequate amounts of fluids; e.g., "NOTICE: This food should be eaten with at least a full glass of liquid. Eating this product without enough liquid may cause choking. Do not eat this product if your have difficulty in swallowing"	
	1. Oat bran		
	2. Rolled Oats		
	3. Whole Oat Flour		
	4. Oatrim		
	5. Whole Grain Barley and Dry Milled Barley		
	6. Barley Beta Fiber		
	7. Soluble fiber from psyllium husk with purity of no less than 95 %		
	The amount of soluble fiber per RACC must be declared in nutrition label.		
Soy protein and risk of coronary heart disease	At least 6.25 g soy protein per RACC	Required terms:	1. 25 g of soy protein a day, as part of a low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies __ grams of soy protein
	Low saturated fat	"Heart disease" or "coronary heart disease"	2. Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. One serving of [name of food] provides __ grams of soy protein

(continued)

Table 3.3 (continued)

Approved claim	Requirements for the food (including dietary supplements)	Claim requirements	Model claim, statements
	Low cholesterol	"Soy protein"	
	Low fat (except that foods made from whole soybeans that contain no fat in addition to that inherent in the whole soybean are exempt from the "low fat" requirement)	"Saturated fat" and "cholesterol"	
		Claim specifies daily dietary intake levels of soy protein associated with reduced risk	
		Claim specifies amount of soy protein in a serving of food	
Plant sterol/ stanol esters and risk of coronary heart disease	At least 0.65 g plant sterol esters per RACC of spreads and salad dressings	Required terms:	1. Foods containing at least 0.65 g per of vegetable oil sterol esters, eaten twice a day with meals for a daily total intake of least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies __ grams of vegetable oil sterol esters
	At least 1.7 g plant stanol esters per RACC of spreads, salad dressings, snack bars, and dietary supplements	"May" or "might" reduce the risk of CHD	2. Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 3.4 g of plant stanol esters in two meals may reduce the risk of heart disease. A serving of [name of food] supplies __ grams of plant stanol esters
	Low saturated fat	"Heart disease" or "coronary heart disease"	
	Low cholesterol	"Plant sterol esters" or "plant stanol esters"; except "vegetable oil" may replace the term "plant" if vegetable oil is the sole source of the sterol/stanol ester	
	Spreads and salad dressings that exceed 13 g fat per 50 g must bear the statement "see nutrition information for fat content"	Claim specifies plant stero/stanol esters are part of a diet low in saturated fat and cholesterol	
	Salad dressings are exempted from the minimum 10 % DV nutrient requirement	Claim does not attribute any degree of CHD risk reduction	
		Claim specifies the daily dietary intake of plant sterol or stanol esters necessary to reduce CHD risk, and the amount provided per serving	
		Claim specifies that plant sterol or stanol esters should be consumed with two different meals each a day	

Source: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064919.htm

Table 3.4 Good manufacturing practices for dietary supplements (72 Doc No 07-3039)

1.	Establishes minimum requirements for personnel, physical plant and grounds, and equipment and utensils
2.	Requires the establishment and use of written procedures for certain operations, including those related to equipment, physical plant sanitation, certain manufacturing operations, quality control, laboratory testing, packaging and labeling, and product complaints
3.	Requires the establishment of specifications in the production and process control system that will ensure dietary supplements meet the identity, purity, strength, and composition established in specifications and are properly packaged and labeled as specified in the master manufacturing record
4.	Requires testing of a subset of finished batches of dietary supplements based on a sound statistical sampling or, alternatively, testing all finished batches
5.	Requires implementation of quality control operations to ensure the quality of a dietary supplement
6.	Requires the preparation and use of a written master manufacturing record for each unique formulation of manufactured dietary supplement, and for each batch size, to ensure the manufacturing process is performed consistently and to ensure uniformity in the finished batch from batch to batch
7.	Requires the preparation of a batch production record every time a dietary supplement batch is made. The batch production record must accurately follow the appropriate master manufacturing record
8.	Requires the establishment and use of laboratory control processes related to establishing specifications and to the selection and use of testing and examination methods
9.	Requires identification and quarantine of returned dietary supplements until quality control personnel conduct a material review and make a disposition decision
10.	Requires a qualified person to investigate any "product complaint" that involves a possible failure of a dietary supplement to meet any cGMP requirement, with oversight by quality control personnel
11.	Requires records associated with the manufacture, packaging, labeling, or holding of a dietary supplement to be kept for 1 year beyond the shelf life dating (when such dating is used, such as expiration dating, shelf life dating, or "best if used by" dating), or if shelf life dating is not used, for 2 years beyond the date of distribution of the last batch of dietary supplements associated with those records

USP Dietary Supplements Verification Program (DSVP)

Since the launch of the DSVP in 2001, the unique logo has appeared on more than 400 million packages and products from 11 participating manufacturers. Volunteer manufacturers in this program submit products to USP for their evaluation against federally recognized USP–NF standards of quality, purity, potency, performance, and consistency and current FDA good manufacturing practices for dietary supplement (FDA 2010) [17].

The USP verification process includes four fundamental criteria (http://www.usp.org/usp-verification-services/usp-verified-dietary-supplements/manufacturers#processincludes).

- A thorough on-site manufacturing quality audit for compliance with USP's criteria for manufacturing practices for dietary supplements (USP <2750>) and compliance with FDA's GMP statutes (21CFR§111).
- An extensive review of manufacturing facility and quality control production documentation
- A comprehensive analytical assessment of product samples to verify compliance with USP–NF standards and manufacturing specifications
- A random sampling of products in the supply chain to confirm consistency with USP strict standards.

This program verifies active and inactive ingredients in the manufacturers' dietary supplements. Those ingredients include amino acids, botanical extract, nonbotanicals, excipients, vitamins, and minerals. Each of the analytical procedures applied to these products reflects an extensive methodological validation process. Importantly, these are the same analytical and quality standards applied to pharmaceutical agents that display the USP logo.

NSF Quality Program

NSF has a similar product certification program (http://www.nsf.org/services/by-industry/dietary-supplements). This certification program provides an assurance that dietary supplements do not contain unacceptable levels of contaminants. This certification and quality assessment is consistent with American National Standards Institute (NSF/ANSI 173-2008). The NSF quality certification is also consistent with FDA guidance (21CFR111), which includes compliance with the Bioterrorism Act of 2002, Adverse Event Reporting requirements, allergen testing, botanical ingredient (extract and non-extract) relative to composition and microbial limits, and heavy metals. Within the USA, NSF collaborates with many regulatory organizations, including, but not limited to the FDA, USDA, and CDC, and conducts courses on quality management and regulatory compliance germane to dietary supplements.

Adulteration

When it comes to adulteration, the fundamentals within US food regulations have not changed since passage of the Pure Food and Drug Act of 1906. With respect to dietary supplements, these products are considered adulterated if (a) the product presents a significant or unreasonable risk of illness or injury, (b) there is inadequate information to support a new dietary ingredient, (c) the Secretary declares the product or ingredient poses an imminent hazard to public health or safety, or (d) the product contains an ingredient that renders it adulterated (21 U.S. Code § 342). A critical area of concern with dietary supplements is the unacceptable prevalence of drugs in these products. A recent small study indicated nearly 10 % of 274 dietary supplements recalled between 2009 and 2012 contained one or more pharmaceutical adulterants [18]. An earlier study by authors commented dietary supplements adulterated and contaminated with undeclared medications pose a serious public health problem [19]. However, some contend these and earlier findings by these authors is not a reflection of existing statutes, but rather greater emphasis on regulatory enforcement by the FDA [20].

Contamination

Dietary supplements, like other foods, are subject to contamination. cGMPs for dietary supplements outline an array of guidances intended to eliminate contamination of these products. The presence of contaminants has resulted in numerous product recalls. The FDA posts all recalls of foods, drugs, dietary supplements. A survey of dietary supplement recalls from February 2012 to December 2014 indicates the majority of these actions involved the presence of undeclared pharmaceutical agents or unapproved drugs (Table 3.5), and microbial contaminants (Table 3.6).

Labeling

One critical aspect of product labeling of dietary supplements, like that of conventional foods, is that it must not be false or misleading. Specific labeling criteria of dietary supplements differ from that of conventional foods under the Nutrition Labeling and Education Act (NLEA) of 1990. Final regulations for dietary supplement were published in 1997 and amended in 2003 [21–24]. These regulations included label warning statement for those products containing ≥30 mg iron, but this stipulation was eliminated

Table 3.5 Undeclared pharmaceutical agents or Unapproved (UA) drugs detected in dietary supplements (alphabetical)

Pharmaceutical agent	Clinical application
Chlorpromazine	Antipsychotic
Chlorzoxazone	Muscle relaxant
Dapoxetine (UA)	Premature ejaculation
Desmethylcarbondenafil	Erectile dysfunction
Dexamethasone	Anti-inflammatory steroid
Diclofenac	NSAID
Dimethazine	Prodrug to Methasterone
Dimethyltestosterone	Androgenic steroid
DMAA (1,3-dimethylamylamine; methylhexanamine)	ADHD; Increased athletic performance (banned by WADA)
Doxepin	Anti-depressant; insomnia
Ephedrine alkaloids (UA)	Weight loss
Fluoxetine	SSRI
Geranium extract	Source of DMAA
Ibuprofen	NSAID
Indomethacin	NSAID
Methasterone (Schedule III; UA)	Anabolic steroid
Methocarbamol	Muscle pain management
Naproxen	NSAID
Nefopam	Non-opioid analgesic
Phenolphthalein (UA)	Laxation
Sibutramine (Schedule IV; UA)	Appetite suppressant
Sildenafil and its analogues	Erectile dysfunction
Tadalafil	Erectile dysfunction

Table 3.6 Microbial contaminants detected in dietary supplements

Microbe
Rhizopus oryzae
Salmonella

as a result of a court challenge by the Nutritional Health Alliance in January 2003, and ultimately withdrawn from the final rule [25]. Other regulations called for statement of identity, nutrition labeling, ingredient labeling, and nutrient content, including extracts and trans fats, and health claims.

DSHEA allows dietary ingredients for which recommendations have not been established, typically by the Institute of Medicine, to be listed as long as the label indicates this fact by an asterisk in the "% Daily Value" column that refers to the footnote "Daily Value not established." The nutrition information is provided in the "Supplement Facts" panel.

Like conventional foods, the nutrition information for dietary supplements must indicate "Serving Size" and declaration of all nutrients (vitamins and minerals) added to the product for explicit purposes of supplementation or when a claim is made relative to these nutrients. Unique to dietary supplements, the ingredients declaration may "proprietary blends" for which the total amount of the blend is indicated.

Important to nutrient content declaration, the FDA expects 100 % of the declared nutrient throughout the product's shelf life if it is part of the formulation. Naturally-occurring nutrients may be 80 % of declared value without triggering product misbranding by the FDA. In addition, the agency expects reasonable excesses within good manufacturing practices, but no more than 20 % excess with respect to calories, sugars, total fat, saturated fat, cholesterol, or sodium. These criteria are consistent with labeling of conventional foods.

New Dietary Ingredients (Post 1994)

Innovation often introduces new dietary components. With respect to dietary supplements, the FDA requires that manufacturers and distributors of these products must assure the reasonable safety of the ingredients under conditions of use, similar to that expected under GRAS requirements (21CFR170.30(a)–(c)) [26]. The FDA, under section 413(d) of the FD&C Act, notes that "new dietary ingredient" (NDI) refers to a dietary ingredient not marketed in the USA prior to October 15, 1994, which is 10 days prior to enactment of DSHEA. The 2011 draft guidance on NDI stipulated a 75-day premarket approval regarding safety and unaltered chemistry of the ingredient of interest. Yet under current regulations, it is the FDA's responsibility to demonstrate that a particular product or specific ingredient is not safe [27]. Noteworthy is that dietary ingredients marketed prior to this date are considered old dietary ingredients for which no "reasonable safety" data are required since they have a history of use and thus are "grandparented" into the dietary supplement supply chain. Interestingly, during the past 20 years, the agency has not enforced the NDI requirement, basically exercising enforcement discretion, and has only issued few warning letters, such as due to insufficient documentation, therapeutic claims or failure to declare ingredients, even though more than 800 NDIs have been filed since 1995 [28–30].

It is important to note that under current US regulations (DSHEA 1994), it is the responsibility of the manufacturer to demonstrate the safety and labeling of dietary supplements; however, it is obligation of the FDA to demonstrate that such products are not safe. At this time, FDA's Center for Food Safety and Applied Nutrition does not approve dietary supplements or their ingredients per se except through the NDI notification process.

Changing Regulatory Environment

The regulatory environment of dietary supplements has not changed in the USA since 1994. As indicated by the FDA, there is a concern that more than 85,000 dietary supplements now on the US market are not registered and many products make false and misleading health claims. [31] According to NHANES data (2003–2006), these products impact the majority of those living in the USA who takes at least one dietary supplement daily [32].

The utility and safety of dietary supplements is under considerable scrutiny by the medical community and legislatures. Some argue that dietary supplement usage is not warranted and should be avoided [33]. Several recent studies indicate that numerous forms of dietary supplements do not reduce the risk of cardiovascular events, prevent cancer or improve cognitive function among high-risk patients [34–36]. Upon examination of these studies, the study population groups presented compromised health conditions prior to initiation of the respective interventions. It is imperative that, with the exception of nutrient insufficiencies, dietary supplements, by statutory definition, are not intended to treat or prevent classic noncommunicable diseases. The preponderance of clinical evidence indicates dietary supplements can be beneficial when consumed by populations at risk for conditions such as iron, folic acid, calcium, and vitamin D inadequacies as noted by the 2010 Dietary Guidelines Advisory Committee [37].

The safety and composition of herbal dietary supplements was recently challenged in New York. The state attorney general's office issued four cease-and-desist letters based on that premise that the products were apparently contaminated, contained substituted ingredients, falsely labeled [38]. The specific report on genetic fingerprinting of the 44 herbal products represented 12 companies and 30 different species common in the Canadian and USA markets [39]. The targeted dietary supplements suppliers either affirmed quality control measures in the production of their respective

products. Meanwhile, the action by the New York AG was criticized by at least one dietary supplement trade association, while New York legislators and CSPI called for adequate standards, better enforcement, improved labeling and regulatory reform. [40, 41] While the investigators cite similar studies, the methods used in this study were not validated, and the results have not been corroborated. Importantly, the authors noted that standards for authentication of herbal products and their ingredients do not exist.

Conclusions

A recent publication challenges the future regulations of dietary supplements [42]. In the opinion of this author, four significant changes to dietary supplements regulations were proposed. Those changes included premarket approval, modified label claims, cGMP compliance, and adverse event reporting. Premarket approval would transfer the no-harm burden from the FDA and placed it firmly on the shoulders of the manufacturers. This would strengthen the agency's effectiveness in removing inappropriate products from the market. The FDA should provide specific guidelines surrounding the quality and quantity of scientific evidence for structure/function claims for dietary supplements, similar to the manner that is used for conventional foods. These guidelines and evidence review could be the responsibility of the agency, the Food and Nutrition Board, the Institute of Medicine or a third party of experts, such as those currently specified for GRAS self-affirmation.

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Chapter 4
The Trans Pacific Partnership: Global Nutrition at Risk

Henry Greenberg and Stephanie Shiau

Key Points

- Global trade is controlled by regional trade agreements, arrangements no longer under the strict control of the World Trade Organization.
- The details of these agreements more and more focus on the role of transnational corporations and their insistence on supportive regulations.
- The agreements appear to infringe upon roles that usually fall under the banner of national sovereignty.
- A nation's control of the scope of corporate activity appears threatened.
- The content of the new agreements have less and less to do with trade and tariff issues and more to do with corporate function once goods and services are in country.
- The details of the agreements could be interpreted to show that the primary purpose is no longer trade and tariff regulation but infiltration of transnational corporate policy into national policy.

Keywords Global trade • Trade agreements • Tariffs • Trans Pacific Partnership

Abbreviations

FDI	Foreign direct investment
GATT	General Agreement on Trade and Tariffs
MNC	Multinational corporation
NCD	Noncommunicable diseases
RTA	Regional trade agreements
TPPA	Trans Pacific Partnership Agreement
WTO	World Trade Organization

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_4

Introduction

In 2014 over half of the world's population lived in urban areas, and the shift is anticipated to continue such that by 2030 the world will be 60 % urban [1]. This urban migration has produced a dramatic transformation in lifestyle. The new environment has led to changes in physical activity, employment, the role of women both at home and in the work place, the role of education, the very concept of home life, and not least important, diet and nutrition. This is rightly called a global nutritional transition. There is less time at home for food preparation, less structure to family life and meal time, and a marketplace replete with options, temptations, and convenience. Urban environments provide access to foods prepared outside the home; this includes restaurants, street vendors, and "fast-food" outlets. "Wet markets," a source of fresh fruit and vegetables are often in short supply. Migrating from an environment in which caloric and/or nutrient deprivation was either real or not far away, most urban dwellers are now exposed to a wide array of cheap, high caloric, nutritionally empty food. This nutritional transition is bolstered by several important externalities related to the ubiquitous globalization in trade, communication, industry, and agriculture. The multinational food companies have prepared well for this population transition within modern globalization. New technologies in food preservation have extended shelf life for many foods. The multinational food industry has developed an efficient and sophisticated distribution system, used its technological strength to develop an effective "cold train," with obvious spin-offs for the health sector, and has greatly improved food safety for the products it sells in developing countries.

To be sure, globalization paints with a broad brush, with many anticipated but many unforeseen outcomes. Regarding trade liberalization, each country, and each subpopulation within the country, will need to accommodate to myriad changes in secondary effects of product availability, new investments that come with new agreements, and technological enhancements that branch out in new areas.

The impact of tariff reductions on food stuffs, agricultural products, and processed food has been, is, and will be profound. The scope of product availability, the expansion of food choice, the potential for local production to gain new markets, and the reduction in consumer cost for food are benefits that can accrue to all. Global agricultural trade has increased by more than a factor of 10 in the past six decades [2] and shows no evidence of slowing down. As efficiency and new technology combine to make the enterprise an ongoing growth industry, the potential impact of food and agricultural trade to create change can only increase. But there are downsides to this enhanced global trade, and the current environment suggests more downside than upside to come.

Background

The World Trade Organization (WTO) emerged from the post-World War II General Agreement on Trade and Tariffs (GATT) which was designed to help the global economy grow and thrive following the worldwide devastation of the war. Its well-intentioned origins led to a succession of tariff reduction agreements over the intervening half century that has had a dramatic net positive impact on global economic development. Trade agreements are complex with multiple stake holders each with a detailed set of requirements, demands, and political realities to contend with. Notwithstanding the persistence of subsidies, the requirements of increasing powerful global corporations, the Cold War and now post-Cold War alignments, tariffs have fallen in each of the four major negotiating periods of the GATT-WTO by more than a third [3]. One estimate is that GATT/WTO has increased world imports by 120 % of world trade [3].

A corollary of this is that after nearly 70 years of nearly continual negotiations, the details and capacities of trade and tariff agreements are well known, even to the developing nations that more and more are participating in the process. Most of the issues must now be seen as "routine."

As the importance of trade increased, the number of countries wishing to join the global community has increased. The GATT and then WTO tend to work by consensus. As the number of countries went from 23 to 160, this negotiating style became less and less workable. In hand with this, the realignments that followed upon the end of the Cold War eased a transition away from WTO to regional trade agreements (RTA). The monopolization of world trade by the WTO diminished and now most new agreements are RTAs, or simply bilateral arrangements. These RTAs are not bound by the rules established by the founding concepts of GATT carried forward in the WTO and have lost that foundational beneficence that was built into the global trade arrangements at the beginning. The RTAs reflect a new era.

The Non-Trade, Non-Tariff Role of Regional Trade Agreements

The RTAs have become more and more focused on regulations needed by global corporations as they increasingly become involved in local economies. For the food industry this includes production, growing crops, packaging, marketing, and financial arrangements to support each and all of these activities. Also, the thrust of new RTAs is on intellectual property protection, patents and guaranteed protection of investments. There is more concern with what happens to a product once it arrives, or is prepared, in a foreign country than how it got there. This was not a locus of great concern when GATT was founded.

There is now a sense that the USA is escalating its concerns with these non-tariff issues[4]. In turn, this plays to the concerns of the multinational corporation (MNC) that are now expending enormous sums of foreign direct investment (FDI) so as to become local players globally. While these developments can be seen, perhaps, as a logical extension of globalization and investment, this new corporate reach has begun to raise serious questions about the restrictions binding local governments from exercising what are usually seen as sovereign prerogatives [5].

Thow and Hawkes have presented a case study in Central America demonstrating the impact of trade policy changes in food imports [6]. Well before the implementation of Central American Free Trade Agreement (CAFTA), but accelerated after its inauguration in 2005, trade liberalization led to a doubling of food imports between 1990 and 2005 related to tariff reductions from 45 % in 1985 to 6 % in 2000. The food imports included meat (primarily chicken and pork), dairy (cheese), French fries, and snacks. During this same time frame the nutrition transition in Latin America documented a rise in obesity and cardiovascular diseases [7]. These trade changes occurred under the oversight of the WTO, not an RTA. However, it is important to note that the overall tariff was reduced to 6 % before the inauguration of the RTA, in this case, CAFTA. While there were outliers of high tariffs remaining in 2004, CAFTA could only have a marginal role in tariff reduction.

There are three categories of products in RTAs that have attracted increasing attention from concerned observers outside of the negotiating process; these are tobacco, generic drugs, and food. While our focus is on food and nutrition, the most cited example, and one that gives a flavor of the arguments, is tobacco. Philip Morris Asia is suing Australia (and others in separate cases) because sales and profits will be impacted by Australia's package labeling [5]. If corporate profits will suffer because of country regulations, corporations, no longer only other sovereign nations, can challenge the regulations in an extraterritorial tribunal that is designed to favor the challenger. The offence is considered "expropriation." And the time and expense involved will exceed the capacity of many small, emerging economies to even defend their policies.

Friel et al. [8] argue that the new RTAs have gone substantially beyond easing the flow of raw materials and finished products between nations. The thrust now involves, "integrated flows of goods, services, people, ideas, and investments in physical, human, and knowledge capital." In turn, this broad new scope can influence national control of policy, particularly as it concerns public health and

societal well-being. Under WTO, an "enabling clause" permitted developing countries to carve out for protection from trade-related competition selected sectors of their economies such as new agricultural initiatives. This "enabling clause" holds no sway with the new RTAs.

Nutrition, they argue, can be impacted through multiple pathways emanating from these new RTAs. Regulations that commit countries to transparency and coherence permit input to policy generation by any interested stakeholders, including MNCs, thus compromising government efforts to propagate nutrition policy. Investor protection provisions can inhibit, or even prohibit, active interventions to decrease consumption of unhealthy foods. Regulations may constrain governments from efforts to reduce affordability of unhealthy foods. Rules or regulations may proscribe government regulations to limit consumption by package labeling. Consumer education can be limited by binding commitments to facilitate trade.

We have argued that the Trans Pacific Partnership Agreement (TPPA), an RTA currently under negotiation, poses significant threats to global public health, perhaps most structurally and long term in the area of food and nutrition, and that the American public health community, for a variety of reasons, has failed to appreciate these [9]. The Australians have, however, continued to explore the implications of this agreement and other RTAs. Of note, they argue that the USA has escalated its insistence that RTAs include more and more restrictions of local agencies—corporate or government. Overall, they argue that the TPPA is more concerned with investment than trade and that it is designed to shift policy making power from national governments to global corporations.

The concern has stimulated the formation of a new entity, INFORMAS (International Network for Food and Obesity/non-communicable disease Research, Monitoring and Action Support) which "aims to monitor, benchmark, and support public and private sector actions to create healthy food environments and reduce obesity, NCDs, and their related inequalities." The group first convened in 2012 and has published a manifesto in 2013 as supplement to Obesity Reviews [10]. There is now a template for monitoring and assessing RTA activities and actions, hopefully leading to a capacity to highlight concerns early in the RTA negotiations rather than reacting to agreements inked in binding agreements.

The American public health community is not the only relevant entity that has failed to appreciate the tight relationship between trade and nutrition. A recent report by the International Food Policy Research Institute, *Global Nutrition Report 2014: Actions and Accountability to Accelerate the World's Progress on Nutrition*, compiled by an international array of renown public health activists, mentions trade or trade agreements as potentially playing a role in nutrition in only the weakest possible way [11]. While much of the report focuses on undernutrition¸ itself not immune to influences of trade agreements, there are substantial discussions of obesity and diet, but no recommendations that trade agreements be examined as a potential arena for intervention. On the other hand, the recent Council on Foreign Relations release on noncommunicable diseases (NCD), *The Emerging Global Health Crisis: Non-communicable Diseases in Lower and Middle Income Countries,* does discuss the negative impact of trade patterns on the emergence of NCDs [12].

RTAs favor the MNC importing processed food, much of it being calorie rich and nutrient poor, making them cheaper, more plentiful, and likely safer than traditional foods. As the MNCs control markets, they can dictate via price and advertising the dietary trends, initially in urban settings but eventually throughout the country. Because tariffs are reduced, national tax income will fall thus further jeopardizing a government's ability to fund health initiatives and other social programs. FDI enables greater penetration of the MNC to extend their supply chain thus controlling product, processing, distribution, and marketing. This trend, that of increasing local consumption of animal products and highly processed food has been seen with other RTAs, notably the North American Free Trade Agreement and CAFTA [13].

Of the 29 sections, called chapters, of the TPPA, several deserve specific mentions. As the TPPA has not been ratified and as the details are only known through leaks, these concerns are potential threats, still capable of being modified or eliminated. The Cross Border Service Chapter may limit

controls a country can exert over the growth of a particular market or the magnitude of a service operation thus preventing any restrictions of market access for products deemed unhealthy. The Government Procurement Chapter could demand that government tenders for food services in schools or hospitals be open to any company in any TPPA country, thus compromising the ability to foster healthy food consumption. The Regulatory Coherence Chapter may mandate a centralized policy formulation entity that would support pro-market initiatives that governments should adhere to and which could give MNCs access to regulatory policy making. The Intellectual Property and Technical Barriers to Trade Chapters could cause governments to lose authority to regulate food labeling and advertising. The Investment Chapter permits corporation to sue governments if any domestic laws or regulations are perceived to diminish the value of investments, i.e., profits. Lastly, the Investor-State Dispute Settlement mechanism, championed by the USA, allows individual corporations to sue governments for any regulation that diminished profits. The venue is an extraterritorial hearing that lacks the usual safeguards of due process and is designed to favor industry. Even the Economist balks at this perversion of a mechanism originally designed to protect foreign investors from unfair or arbitrary expropriations [14].

The risk to NCD prevention is significant, and the risk will fall heavily on nutritional risks such as excess calories, salt, animal fat, and diminished fresh fruit and vegetables. There has been pushback against many of these provisions in the TPPA and other RTAs; they just have not come from American public health. The Australian public health groups have been the leaders. But lawyer groups, medical groups such as Medecins sans Frontiere and even the American Medical Association, social activist groups, and New York City's former mayor have all sounded alarms of varying intensity and effectiveness [9]. This open society response may have had an impact. For whatever reasons, and the unrelenting political antagonism in Washington being most dominant, the TPPA is unlikely to get the "Fast Track" authorization from Congress that it would need were it to have any chance of passing. However, there are nearly 400 RTAs in force today and over 600 in varying stages of negotiation according to the WTO web site [15], with much heightened activity beginning in the early 1990s. The issues raised here retain an ongoing relevance.

Changing the trajectory of the trade-nutrition linkage toward an enlightened pathway to improved nutrition poses many challenges. In a detailed and idealized analysis that assumed economic, political, and corporate considerations would bend to the goal, Lock et al. studied the requirements to establish a healthy diet in the UK and Brazil [16]. The changes in eating habits, agricultural activity in multiple realms, corporate realignments, and national productivity, i.e., wealth, are profound. This idealized scenario will not be rapidly, readily, or easily engaged. Even a modest tilt toward improved nutrition through trade will require much effort on many fronts and time.

A Dark Overview

As we have seen, the GATT, and then WTO, oversaw 50 years of gradual but progressive reduction in tariffs as a major part of global trade liberalization. More and more countries joined the process. Surely, over these decades the problems, barriers, pitfalls, risks, and dangers have been clearly perceived and dealt with. Signing up new countries to the global network or accommodating the new operational aspirations of the MNCs cannot be such overwhelming problems that require 6+ years for 11 countries to find common ground. Is something else afoot?

Perhaps tariffs and even trade are not the issue but the diversion. The real issue is the penetration of the global corporation into the regulatory mechanisms of otherwise sovereign nations. While called trade agreements, these new RTAs focus primarily on manipulation of national policies that relate to the needs for efficiency and integration on the part of major global corporate entities. While US congressional committees can get no substantive information from the TPPA negotiators, there are

roughly 600 trade advisors, representing corporations, lobby groups, state chambers of commerce, and law firms that are privy to some or all of the negotiations [17].

A Public Health Response

While the threats to global public health from the TPPA in general and nutrition in particular are genuine, they are unlikely to be implemented soon for topical political reasons and the pushback by civil society forces. However, the trajectory of progressive RTA encroachment on sovereign prerogatives is real and growing [5].

There is no obvious quick fix that can reverse the influence and impact of this new mercantilist world view. Public health needs to reconfigure its approach to public policy, as opposed to an overweighted focus on health policy. The current approach is reactive not proactive. The proactive initiatives directly related to public health are too often inaugurated outside of public health by civil society groups such as Mothers against Drunk Driving and the heart associations, or politicians such as Michael Bloomberg.

However, there is a lesson to be learned from the response of the Australian public health community to RTAs. The leaders have been, if not ahead of the curve, not far behind it. They have given a responsible voice to the somewhat unsophisticated blogosphere and may or may not have had an impact on the progression of acceptance of the TPPA in Australia and other TPPA countries. It is unclear if this is a one trick pony show or if the community is vigilant in all spheres of public policy. The message, however, is that broad vigilance is necessary. To establish this as an ongoing, routine component of public health, it needs to be installed in the curriculum of schools of public health. The current and future generations of public health professionals need to know that part of their mandate is to scan the political arena looking for opportunities to participate in policy generation that fosters the public health. Without criticizing the work of the Australian group, it can be argued that global trade triggered minimal, if any, public health input for a long time; the TPPA is a welcome wakeup call but clearly not the first trade agreement with important public health implications.

Conclusion

The public health establishment via its curriculum and training initiatives needs to enhance its focus on engaging policy formulation in those spheres that impinge upon health. This "consequentialist" approach has been introduced, and forcefully so, by Galea [18]. Our argument also falls under the umbrella of a "health in all policies" approach to public health [19]. The TPPA has become a bell weather agreement. Whatever happens here will happen again [8]. This agreement should it not be stopped will become the bedrock upon which future RTAs will be built. Our argument here highlights a specific example of an issue that in a modern public health environment should have been embraced earlier and by all public health communities. Going forward the embrace needs to be firm.

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Chapter 5
Nutrient Density and Health: How to Develop Global Nutrient Density Metrics

Adam Drewnowski

Key Points

- Nutrient profiling is the science of ranking or rating foods according to their nutritional value.
- Nutrient profiling separates foods that are energy dense from those that are nutrient rich.
- The Nutrient-Rich Foods (NRF) Index and the French SAIN,LIM score are examples of nutrient profiling systems in the public domain.
- Nutrient profiles have been used by government agencies to regulate marketing to children and by the food industry to review the quality of product portfolios.
- Profiling techniques can be applied to data from low- and middle-income countries.
- These new metrics allow consumers to identify foods that are nutrient rich, affordable, sustainable, and appealing.

Keywords Nutrient density • Nutrient profiling • Cost • Sustainability • Diet quality • Front-of-pack Dietary guidelines • Global health

Abbreviations

%DV	Percentage daily values
FNDDS	Food and Nutrient Database for Dietary Studies
FOP	Front-of-pack
GHGE	Greenhouse gas emissions
HEI	Healthy Eating Index
MRV	Maximum recommended values
NHANES	National Health and Nutrition Examination Survey
NRF	Nutrient-rich foods
RACC	Reference amounts customarily consumed
USDA	US Department of Agriculture

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_5

Introduction

The method of rating or classifying individual foods based on their nutritional value has become known as nutrient profiling [1]. Nutrient profile models provide composite ratings of nutrient density of foods per reference amount, variously defined as 100 g, 100 kcal, or serving [2]. Nutrient density calculations have been based on nutrients of public health concern such as fats, sugar, and sodium; on shortfall nutrients such as fiber, vitamins, and minerals; or on some combination of both [2, 3]. In several models, nutrient density score was calculated as the difference between nutrients to encourage and the nutrients to limit [4–6]. Given that most foods contain multiple nutrients, developing new metrics of nutrient density of foods represents a conceptual and methodological challenge.

First, the concept of nutrient profiling itself runs counter to the long standing dogma that there are no good or bad foods, only bad diets [3]. Here, the impetus for ranking and rating nutrient quality of individual foods came from regulatory agencies in the European Union [1]. Nutrient profiling was to be the scientific basis for allowing nutrition and health claims and for regulating food marketing and advertising to children [7]. The subsequent development of nutrient profiling models in the USA was largely driven by the retail sector [2, 8]. Nutrient profiling of packaged foods was expected to aid consumers to make better food choices and create healthier diets [7, 9]. As described by the Institute of Medicine (IOM), the intent of new front-of-pack (FOP) labels was to help shoppers identify nutrient-rich foods [8].

Nutrient profiling models have also been used by the global food industry to screen product portfolios for nutritional value [10, 11]. Nutrient profile models, developed by different companies, have become the scientific basis for addressing nutrients of public health concern in a wide range of food products [10]. In many cases, nutrient profiling was followed by product reformulation.

Second, nutrient profiling techniques may need to be adapted to evaluating the quality of composite meals, or the total diet [7]. New metrics had to be developed to score individual meals, such as MyPlate options, for energy and nutrient density, and for nutrient balance. Extending the nutrient density concept to the economics of food choice behavior is another innovation [12]. This econometric nutrient profiling of individual foods and total diets [13, 14] has now addressed the interrelations between nutrient density, energy density, and monetary cost. Combining nutrient density calculations with food prices data has allowed researchers to create novel metrics of food affordability, identifying those foods that provide most nutrients per penny [13, 14]. Comparable studies of diets have explored the relations between diet quality and daily diet cost. The most recent studies on dietary nutrient density have explored the environmental impact of different-quality diets, as defined by greenhouse gas emissions (GHGEs) [15, 16]. Implementing national food and nutition policies can be aided by identifying those foods and food patterns that are sustainable, affordable, socially acceptable and nutrient rich [7, 9].

Third, there is a clear need to address the dietary concerns of developed countries. Most existing models have been developed in France [17], the UK [6], and the USA [4]. Applying nutrient profiling techniques to screen the food supply of low- and middle-income countries poses further challenges, given different patterns of consumption of shortfall nutrients and nutrients to limit [18]. Here, the main challenge is to balance the risk of malnutrition and nutrient deficiencies against excessive energy intakes and the growing presence of nutrients to limit in the food supply. To add to the challenge, accurate data on nutrient composition of foods and population-level dietary intake and health data may not be available. Nonetheless, identifying foods and food patterns that are both affordable and nutrient rich is a priority issue that has multiple implications for improving global public health [18].

Principles of Nutrient Profiling

Composite nutrient density scores are intended to capture multiple nutritional attributes of a given food [2, 3]. The goal is to create an objective metric that would award higher scores to wholesome nutrient-rich foods and award lower scores to energy-dense foods of minimal nutritional value [3]. To accomplish this, some nutrient profiling models have focused exclusively on nutrients of public health concern, otherwise known as nutrients to limit. However, the inclusion of beneficial nutrients in nutrient profiling models is one way to convey positive information about nutritional value of foods to the consumer [7, 9]. For that reason, both the nutrient-rich food (NRF) family of indices and the French SAIN,LIM system have included both nutrients to encourage and nutrients to limit [5, 17]. In those balanced models, the presence of nutrients of concern was offset by the presence of nutrients that were essential to health. However, whereas the NRF model assigned each food a single numerical score, the SAIN,LIM system assigned foods into one of four groups [17].

For nutrient profiling to remain a science, it must follow scientific rules [4]. To date, the procedures for developing, testing, and validating nutrient profile models have not been standardized. The European Food Safety Authority (EFSA) has provided a useful blueprint for nutrient profiling, outlining some important decisions that need to be made [1]. The first question is whether nutrient profiling models should be across-the-board or category specific. Across-the-board models apply the same nutrient standards to all foods; category-specific models may relax some standards or look for specific nutrients in some food categories, e.g., calcium in dairy. Further decisions to be made include the selection of relevant index nutrients, both positive and negative, the choice of reference daily values, and the basis of calculation: 100 g, 100 kcal, or serving size [19, 20]. Both continuous formulas and threshold-based algorithms have been used to calculate the food's overall nutritional value [5]. The resulting nutrient density scores then need to be tested against other food attributes such as energy density and cost [20]. Scores created using alternative profiling algorithms need to be validated further with respect to independent measures of a healthy diet [5, 17]. Ideally, the impact of nutrient profiling on consumer behavior ought to be tested as well.

The basic principles of nutrient profiling have been described before [2, 21]. There is clearly a need for objectivity, transparency, simplicity, and validation. The development of nutrient profile models needs to be based on objective nutrition science and should be based on open-source nutrient composition and dietary data. The number of index nutrients should be limited and needs to reflect the needs of each population, especially if the profiling model is to find applications worldwide. Simple algorithms are preferable to more complex ones, with formulas based on sums and means performing better than ratio-based metrics. Alternative scoring algorithms need to be tested against other food attributes, such as food energy density (kcal/g) and per calorie food or diet cost [19, 20]. Importantly, alternative profiling models need to be validated against independent measures of a healthy diet and, wherever possible, related to specific long-term health outcomes [22, 23].

For those reasons, the development and validation of a nutrient profiling system rely on a variety of databases. Having access to a nutrient composition database for individual foods and beverages is a prerequisite. These data need to be paired with nutrition standards (recommended values) to calculate nutrient density per reference amount. The selection of index nutrients, positive and negative, should be guided by basic biological needs and by population-based data on diets and health [24]. It is important to note that the development of nutrient profiling models with potential global applications can be constrained by the availability of the necessary datasets.

Nutrient-Rich Foods Index

The development of the NRF family of indices followed the principles of transparency and openness [2]. The first decision was to make the NRF indices across-the-board, with the same nutrient standards applied to foods in every food group. The second decision was to include beneficial nutrients to encourage as well as nutrients to limit. The selection of nutrients to encourage closely followed the federal regulatory standards in the USA. The US Food and Drug Administration (FDA) allows certain foods to carry nutrition and health claims, based on their content of protein, fiber, calcium, iron, and vitamins A and C. The 2005 Dietary Guidelines [25] further identified potassium, magnesium, and vitamin E as shortfall nutrients in the American diet. The positive component of the NRF9.3 score was accordingly based on nine nutrients: protein, fiber, vitamins A, C, and E, calcium, iron, potassium, and magnesium.

Foods are disqualified by the FDA from carrying nutrition and health claims if they contain higher-than specified amounts of fat, saturated fat, trans fat, cholesterol, or sodium. The European Commission mandate for nutrient profiling specifically identified added sugar as nutrient of concern. Both the NRF and the French SAIN,LIM system based the negative component of the score on the same three nutrients: saturated fat, added sugar, and sodium. Since that time, the Food and Agriculture Organization (FAO) of the United Nations has developed a definition of "free" sugars that would include 100 % fruit juices, honey, and molasses among the sources of nutrients of concern.

The NRF was designed to be consistent with the FDA-regulated Nutrition Facts panel, the principal source of nutrition information for the US consumer. Accordingly, reference daily values for each nutrient, based on a 2000 kcal diet, were obtained from FDA sources. The values were for protein (50 g), fiber (25 g), vitamin A (5000 IU), vitamin C (60 mg), vitamin E (30 IU), calcium (1000 mg), iron (18 mg), potassium (3500 mg), and magnesium (400 mg). These values were not adjusted by age and gender as is the case with reference daily values from the Institute of Medicine (IOM). It should also be noted that the corresponding French values for the same nutrients can be different, and so are the default values from the FAO.

Nutrient composition data or individual foods and beverages were obtained from the Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS), which is used to code, process, and analyze the What We Eat in America dietary surveys [26]. For each nutrient, nutrient content per 100 g of food was converted to percentage daily values (%DV) per reference amount and capped at 100 % DV so that foods containing high amounts of a single nutrient would not obtain a disproportionately high NRF score [3]. For nutrients to limit, maximum recommended values (MRVs) were 20 g for saturated fat, 125 g for total sugar, 50 g for added sugar, and 2400 mg for sodium. All scores were initially calculated for three different reference amounts: 100 g, 100 kcal, and serving size of food [19, 20].

The FDA-mandated serving sizes, listed on the Nutrition Facts panel, are also known as reference amounts customarily consumed (RACC). The FDA uses 139 different RACC values that are set lower for energy-dense sugar (4 g), fats and oils (15 g), and cheeses (30 g) than for meats (85 g), vegetables and fruit (120 g), yogurts (220 g), or milk, juices, and other beverages (240 g). Given that a serving of food can vary from 4 to 240 g or more, and energy density can vary as well, nutrient profiling algorithms can produce very different results when based on 100 g, 100 kcal, or food serving. RACC values were developed for 5096 foods in the FNDDS database.

The publicly available FNDDS database included detailed food descriptions, common portion sizes (g), and nutrient content per 100 g for a wide variety of nutrients. The FMDDS data base needed to be supplemented with data on added sugars from other USDA sources and did not at the time include data on vitamin D. The FNDDS nutrient composition databases can also be linked to the USDA Standard Release (SR) databases that have been used to provide information about the nutritional content of foods worldwide.

In developing the family of NRF indices, we first created the nutrient-rich (NR) subscore based on a variable number *n* of beneficial nutrients (NR*n*). The NR*n* components were expressed as unweighted sums of percent daily values (SUM) or as means of percent daily values (MEAN) of multiple nutrients per reference amount. The negative LIM component was based on three nutrients only: saturated fat, added sugar, and sodium, also expressed as percent daily values per reference amount NRF indices were calculated as the arithmetic differences between the positive (NR*n*) and the negative (LIM) components. A ratio-based algorithm was also tested. Food scores obtained using alternative NR*n*, LIM, and NRF indices were then compared with the energy density (kcal/100 g), energy cost ($/100 kcal), and the presence of other nutrients in the food. Varying algorithms and calculation methods developed in past research are shown in Table 5.1. The final NRF algorithm was the sum of 9 %DVs for positive nutrients minus the sum of 3 %DVs for the three nutrients of concern. By contrast, the French SAIN,LIM algorithm used the means (rather than the sums) for both positive nutrients and nutrients of concern. Furthermore, the French system calculated the positive component SAIN based on 100 kcal and the negative component LIM based on 100 g. The initial NRF subscores were both based on 100 kcal as the reference amount, though serving-based scores were tested as well.

Calculations showed that the NRF scores based on 100 kcal and 100 g or serving size produced very different results. Foods that benefitted the most from the 100 g-based calculations were NRFs that had low RACC values and were normally consumed in small amounts such as protein powder, fortified cereals, and nuts and seeds. By contrast, as noted later, foods containing saturated fat and sodium, such as cheese (30 g RACC), were disproportionately penalized when the nutrients were expressed per 100 g. It is worth noting that the British FSA-Ofcom nutrient profiling system, based on 100 g reference amounts, needed to have multiple adjustments to allow for the inclusion of both oils and beverages.

Basing NRF calculations on the 100 kcal reference amount was not without problems. Foods that benefitted the most were vegetables and salad greens such as spinach, lettuce, endive, watercress, and cabbage. Here, the calculation of the nutrient-to-calorie ratio was sometimes driven not so much by nutrient content as by the foods' very low energy density. When high nutrient content and low energy density came together, some vegetables delivered several hundred percent of vitamin C per 100 kcal.

Basing NRF calculations on serving sizes, as guided by FDA RACC values, benefitted foods that were consumed in amounts >100 g, including fruit and fruit juices, cooked vegetables and juices, milk

Table 5.1 Algorithms for NR*n* and LIM subscores, and for the composite NRF nutrient profile models

Model	Algorithm	Reference amount	Comment
Subscores NR*n*			
NR*n*_100 g	$\sum_{1-n}$ (Nutrient*i*/DV*i*) * 100	100 g	Nutrient*i* = content of nutrient *i* in 100 g
			DV = Daily value
NR*n*_100 kcal	(NRn_100 g/ED) * 100	100 kcal	ED = energy density (kcal/100 g)
NR*n*_RACC	(NRn_100 g/100) * RACC	serving	RACC = FDA serving size
Subscores LIM			
LIM_100 g	$\sum_{1-3}$ (L*i*/MRV*i*) * 100	100 g	*Li* = content of limiting nutrient *i* in 100 g
			MRV = Maximum recommended value
LIM_100 kcal	(LIM_100 g/ED) * 100	100 kcal	ED = energy density (kcal/100 g)
LIM_RACC	(LIM_100 g/100) * RACC	serving	RACC = FDA serving size
Composite NRF*n*.3			
NRF*n*.3_sum	NR*n*_100 kcal − LIM_100 kcal	100 kcal	Difference between sums
NRF*n*.3_mean	NR*n*/*n* − LIM/3	100 kcal	Difference between means
NRF*n*.3_ratio	NR*n*/LIM[a]	None	Ratio

NRF nutrient-rich food, *LIM* limited nutrient score, *RACC* reference amounts customarily consumed

[a]NR*n*_100 g/ LIM_100 g was equivalent to NR*n*_100 kcal/LIM_100 kcal and to NR*n*_RACC/LIM_RAC

and yogurts, and other beverages and mixed foods. In contrast, foods that were consumed in amounts <100 g, such as nuts and seeds, and fortified cereals received lower scores under a food serving based system. It needs to be noted that RACC values are specific to the USA and that government mandated serving sizes do not exist in the European Union.

The NRF LIM subscore performed differently when calculated per 100 g or per serving. Calculations based on 100 g strongly penalized foods that contained saturated fat and sodium but were regularly consumed in serving sizes well below 100 g. RACC-based LIM scores penalized beverages that contained added sugar and were consumed in 246 g portion sizes, as opposed to 100 g. A system based on 100 g was more lenient toward sugar-sweetened beverages than a system based on serving size (240 g in the USA). The largest differences in LIM scores, calculated using different reference amounts, were obtained for fats, mixed foods, and beverages.

Validation of Nutrient Profile Models

Selecting the best-fitting nutrient profile model from among several potential alternatives presents another scientific challenge [4]. Some studies compared food rankings generated by different nutrient profile models with mean scores for the same foods as rated by health professionals or by expert panels. However, testing objective nutrient profiling models against prevailing is a poor way to decide whether a model accurately captures the foods' nutritional value or not.

Only three published and fully transparent models have been validated with respect to objective measures of diet quality: the French SAIN/LIM [17], the British FSA-Ofcom model [22], and the NRF family of indexes [5].

The family of NRF indices was tested with respect to a diet quality measure, the Healthy Eating Index (HEI 2005) that measures compliance with US dietary guidelines [5]. The HEI 2005 is based on a 100-point scale that is largely food- as opposed to nutrient based [5]. In the validation study, each food reported by subjects in the NHANES 1999–2002 database was scored using NR*n*, LIM, and NRF*n*.3 algorithms. The NR*n* and NRF*n*.3 indices were based on a variable number *n* of beneficial nutrients (where $n = 6–15$). An average NRF-based nutrient density score was calculated for each person using either 100 kcal or RACC as the reference amounts. HEI 2005 scores for the same NHANES respondents were also calculated. Average NRF nutrient density scores were then regressed against HEI, adjusting for gender, age, and ethnicity. The percentage of variation in HEI (R^2) that was explained by each NRF index was a measure of index performance [5]. The index that explained the most variation in HEI (44.5 % of the variance) was NRF9.3, based on nine nutrients to encourage (protein, fiber, calcium, iron, potassium, magnesium, and vitamin A, C, and E) and three nutrients to limit (saturated fat, added sugars, and sodium).

In other analyses, we found that the NRF models that balanced beneficial nutrients against nutrients to limit performed better than did indices based on nutrients to limit only. The LIM score alone predicted about 32 % of the variance in HEI. Furthermore, adding more nutrients to the model did not predict a better fit with diet quality measures. Only six to nine nutrients to encourage were sufficient to predict maximum variance in HEI 2005 scores; the proportion of variance explained actually declined with the inclusion of additional index nutrients, vitamins and minerals. The data confirmed previous reports [14, 15] showing that adding nutrients above 9–10 in a nutrient profile model provided no additional benefit in predicting overall diet quality. In other words, there is no particular advantage in basing a nutrient profile model of 30 plus nutrients as opposed to only six. That last finding has major implications for the development of global nutrient profiles for countries where nutrient composition data can be severely limited.

In other analyses, NRF indices based on 100 kcal (418 kJ) performed similarly to indices based on RACC. Algorithms based on sums or means of nutrient-based subscores performed better than

algorithms based on dividing one subscore by another. Ratio-based scores are inherently problematic and may need to be radically transformed before they will be useful to consumers.

Whether nutrient profiles ought to be weighted by the relative importance of different nutrients is another good question. The NRF family of indices provides an example of unweighted scores, where every nutrient (expressed as %DV) is treated equally. However, instances of weighted nutrient density scores do exist. Weighting mechanisms have been justified in a variety of ways: biological quality of nutrients, ubiquity in the food supply, bioavailability, and relative health effects. In previous studies, weighting has been based primarily on expert opinion. However, new analyses point to novel approaches to weighting nutrients for inclusion in nutrient profiling schemes, based on their estimated importance in the population diet [24].

Identifying Nutrient-Dense Foods

What nutrient profiling methods do is to assess nutrient density of foods in relation to calories. One approach is nutrient-by-nutrient profiling. For example, a 6 oz serving of plain skimmed milk yogurt provides less than 5 % DV of daily calories but >30 % DV of calcium, >25 % DV of phosphorous, >10 % DV of potassium and zinc, and >5 % DV of magnesium. Similarly, fruited low-fat yogurt provides <10 % of dietary energy but >25 % DV for calcium, >20 % DV for phosphorous, approximately 15 % DV for protein, and >10 % DV for potassium. Using this method, yogurt can be defined as a NRFs, providing relatively more individual nutrients than calories.

Nutrient profiling models such as the NRF calculate a composite nutrient density score that is based on multiple nutrients. The overall nutrient density of foods, as rated by the NRF system, falls along a continuum that ranges from sugars to spinach, as indicated in Fig. 5.1. The data, presented as medians for major USDA food groups in the FNDDS database, show that sugars and sweets and fats and oils got the lowest NRF scores overall. By contrast, the highest NRF scores went to vegetables and fruit, followed by dried beans, legumes, nuts and seeds, and eggs.

There were large individual variations within each food group. Fresh fruit had higher NRF scores than either dried fruit or canned fruit in syrup and 100 % fruit juices scored higher than did soft drinks with added sugar. Low-fat dairy products, including fluid milk and plain yogurt scored higher than did ice cream or hard cheese, given the content of sugar or saturated fat. For example, skimmed milk scored 123 on the NRF9.3 score, chocolate skimmed milk scored 56, milk with 2 % fat (semi-skimmed) scored 43, and whole milk had an NRF score of 38. Plain nonfat yogurt scored 94, whereas vanilla flavored nonfat yogurt scored 38. Lower NRF scores were obtained for ice cream and for some dairy desserts. Salad greens, carrots, and green peppers scored higher than did corn.

Figure 5.2 shows that foods of higher energy density and lower nutritional value provided cheaper calories. The y-axis shows the differential per calorie cost for foods in each food group. The data are expressed as median cost per 100 kcal, based on the national food prices database released by the USDA Center for Nutrition Policy and Promotion (CNPP). First, it can be seen that grains, fats and oils, and sugars and sweets were relatively inexpensive on a per calorie basis. By contrast, meat, poultry and fish, and fruit and vegetables were associated with higher per calorie food costs. In general, there was a positive association between nutrient density of foods and their per calorie diet cost. The present findings echo previous reports from both the USA and France that energy-dense foods of low nutritional value tend to be cheap, whereas the recommended nutrient dense foods tend to be more expensive [23, 28]. The most recent value metrics have explored the concepts of nutrients per unit cost: although vegetables and fresh produce are expensive sources of calories, they supply key nutrients at an affordable cost [12].

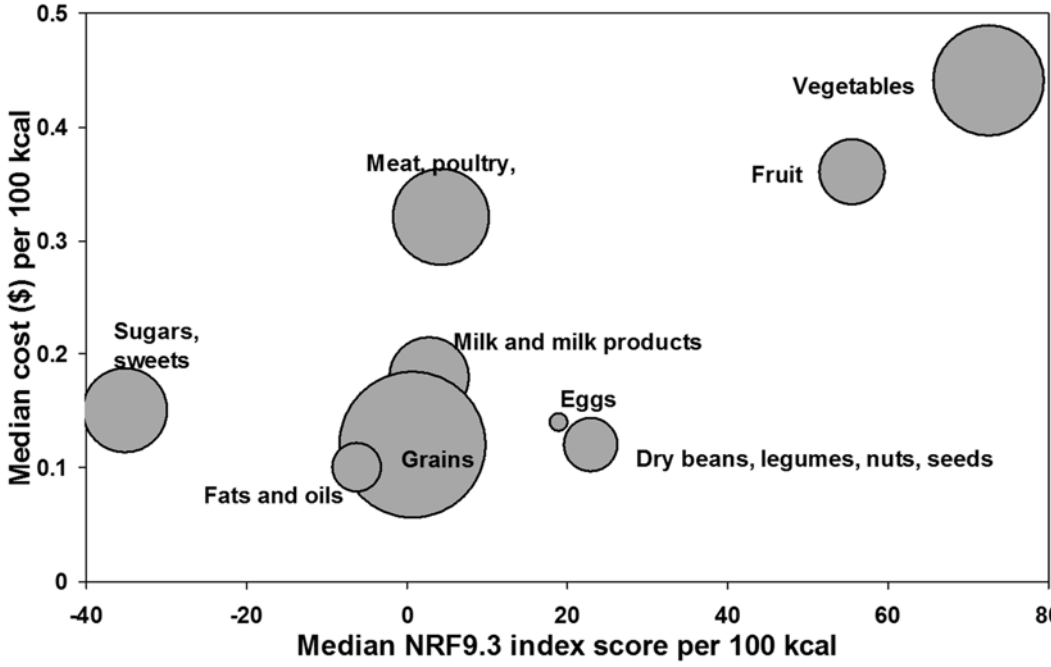

Fig. 5.1 A plot of median NRF9.3 index scores for major US Department of Agriculture food groups plotted against the foods' median energy cost ($/100 kcal). Size of bubble denotes the number of foods per food group [27]

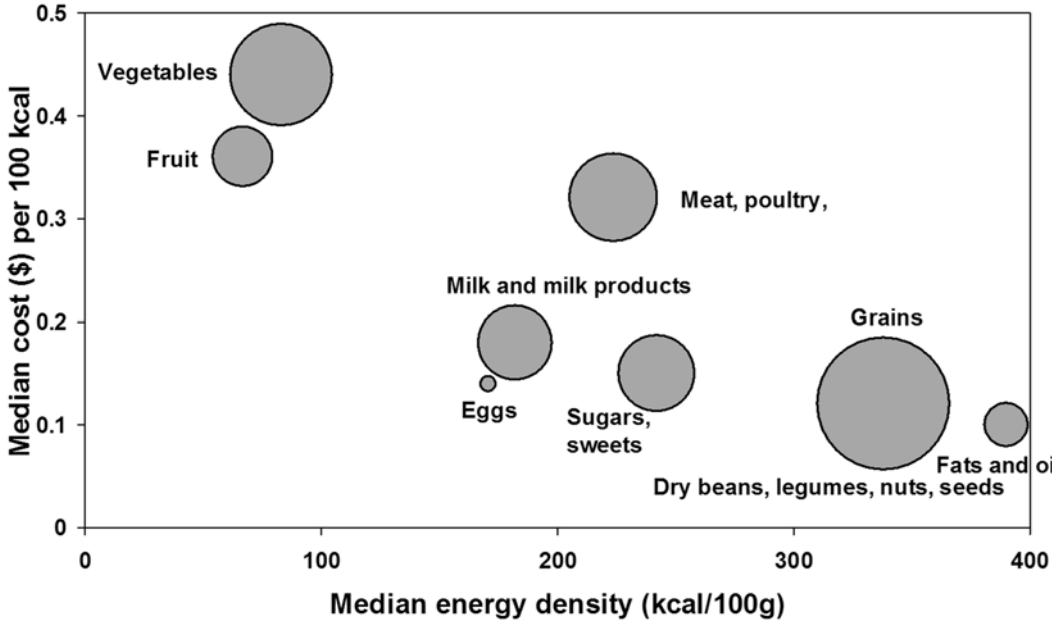

Fig. 5.2 A plot of median energy density (kcal/100 g) for major US Department of Agriculture food groups plotted against the foods' median energy cost ($/100 kcal). Size of bubble denotes the number of foods per food group [27]

Building Healthier Diets

Nutrient density metrics can distinguish between foods that are energy dense and those that are nutrient rich [17]. As shown in Fig 5.3, grains, sweets and fats had higher energy density and lower nutrient density, whereas vegetables and frut had lower energy density and were nutrient rich. The same pronciples have been applied to evaluating nutrient density of the total diet. Participants in the 1999–2002 NHANES were assigned to quintiles based on their dietary NRF9.3 scores. Individuals in the top quintile of NRF9.3 scores consumed more beneficial nutrients, including some nutrients that were not part of the model (vitamin B_{12} and zinc). Higher consumption of whole grains, low-fat dairy, vegetables, and fruit also characterized diets of individuals in the highest quintile.

There was a further connection to some existing diet quality metrics. Quintiles of NRF9.3 scores easily translated into a consumer friendly 5-point scale. Preliminary data suggest that each point on a 5-point scale was roughly equivalent to 10 % DV, a criterion favored by the FDA in regulating nutrition and health claims. Interestingly, lower LIM subscores alone did not predict more nutrient-rich diets. Diets in the bottom quintile of LIM scores were not much higher in nutrients or in food groups to encourage. In other words, diets that were lower in saturated fat, added sugar, and sodium were not necessarily higher in vitamins and minerals, whole grains, vegetables, or low-fat dairy. Focusing only on nutrients to limit may not necessarily guide consumers toward healthier dietary options, especially if those options are associated with higher per calorie diet cost.

More studies are needed to confirm that nutrient density labeling can lead to positive changes in consumer food purchase behavior. Nutrition experts agree that the diets in the USA are increasingly energy dense but nutrient poor [25]. Paradoxically, dietary advice tends the stress the importance of avoiding certain nutrients. The idea of what constitutes a "healthful" food appears to be based on the *absence* of saturated fat, added sugars, and sodium rather than on the *presence* of beneficial nutrients

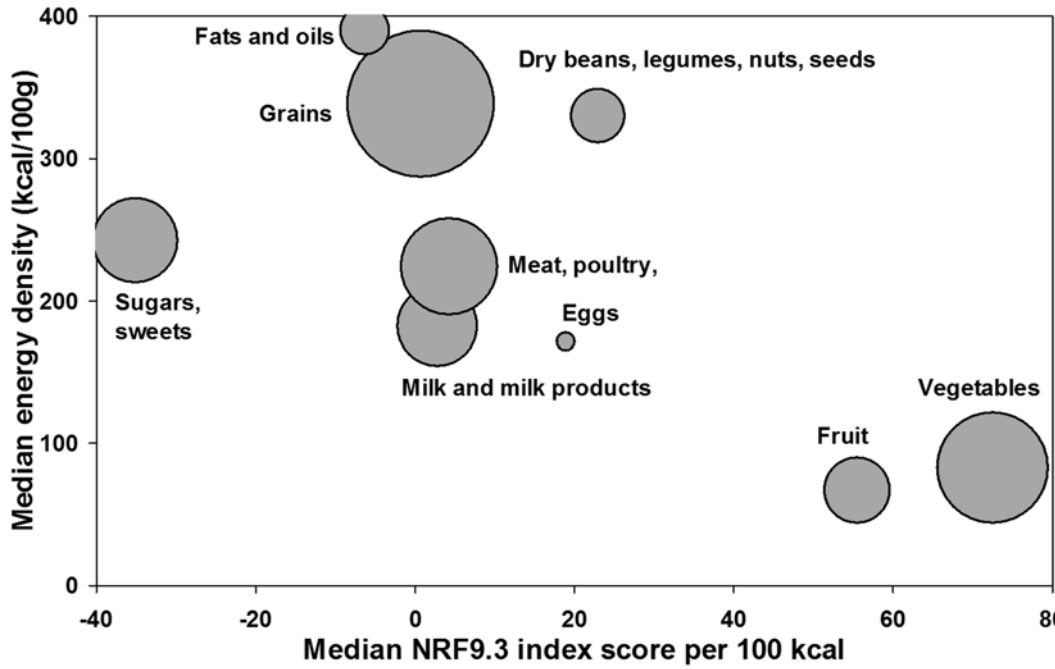

Fig. 5.3 A plot of median NRF9.3 index scores for major US Department of Agriculture food groups plotted against the foods' median energy density (kcal/100 g). Size of bubble denotes the number of foods per food group [27]

[3]. As observed by dramatic increases in the rates of obesity and diabetes, such negative dietary advice has been largely ineffective. A more positive approach would be to focus on the nutrient density of foods and their nutritional value [7, 9]. The goal of nutrient profiling models was to promote the consumption of more beneficial nutrients and fewer calories.

Conclusions

Identifying foods that are affordable, sustainable, and nutrient rich is the goal of nutrient profiling [7]. Incorporating the concept of nutrient density into everyday diets requires the combination of nutrient profiling methods with other strategies toward improving food habits and food choice. Studies need to relate individual food ratings to total diet quality and to explore nutrient density in relation to monetary cost [14] and GHGE [15, 16]. Linking nutrient profile models to the prevailing food prices opens the door to global studies on the relation between diet quality and diet cost.

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Chapter 6
The Influence of Polypharmacy on Nutrition

Korinne M. Piccolo and Joseph I. Boullata

Key Points

- About 82–91 % of adults use at least one medication on a regular basis, many taking five or more.
- Medication use is a significant, although seldom recognized, factor for altering nutrition status that is not routinely assessed prior to marketing.
- Drug-induced poor nutrition status can be manifest by changes in body mass or composition, in metabolic function, or in nutrient biomarkers.
- Mechanistically, drugs can impact food preparation/intake, gastrointestinal structure/function, nutrient absorption, distribution, metabolism, or elimination.
- A thorough nutrition assessment should take place prior to initiating a new medication and periodically during chronic therapy.

Keywords Adverse effect • Drug • Interaction • Malnutrition • Medication • Nutrient • Nutrition • Outcome • Polypharmacy

Introduction

There is deservedly much attention being paid to malnutrition and nutrition assessment in clinical practices. Nutrition status may be altered by many things especially in persons with recognized risk factors. High on the list of contributing factors are medications. This chapter will provide a perspective for clinicians on the potential changes to nutrition status resulting from medication use. Many examples from drugs approved in the last 5 years will be provided.

Use of medication is a part of daily life for many people. It is estimated that about 82 % of American adults use medication regularly [1]. It should not come as a surprise that so many people use pharmaceutical products including prescription drugs, over-the-counter medicines, or dietary supplement products. But aside from the promise of benefit, these products each have their own set of potential adverse

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_6

effects—whether dose related or not. For example, falls associated with medication use occur in young and middle-aged adults as well as in the older adult [2]. Many of the adverse effects attributed to drugs can influence nutrition status. In fact, specific drug-induced changes to nutrition status may be considered as a subclass of adverse drug effects.

The economic and health quality-of-life impact of adverse effects of new medications are taken into account [3]. Given the enormous economic impact of drug use, and their untoward consequences, it is unfortunate that more attention is not regularly paid to their influence on nutrition status. Of the many issues that affect patient safety within the healthcare system that were cataloged in a national report, these potential effects were not included among them [4]. Even the Patient Safety Act of 2005 and subsequent regulations fail to address medication and nutrition status specifically [5, 6].

Although the term polypharmacy once referred to the mixing of several medicines into one prescription, it has come to mean too many medications for a single patient. Rather than defining polypharmacy as a value beyond some threshold number of prescribed medications or number of dosage units daily, there may be a progressive impact on outcome with increasing medication use. What has become clear is that increases in numbers of medication increases the risk for interactions and adverse effects. These consequences especially in older adults include declines in cognitive capacity, functional ability, and nutrition status [7]. Polypharmacy does not develop overnight in a patient, but rather develops over time as predicted by age and comorbidities [8]. Cardiovascular disease is among the comorbidities with high medication use in those afflicted. For example, in the acute post-myocardial infarction period the pill burden may near 20 daily even if adherence to complicated regimens is poor [9]. The appropriateness of a large number of medicines will depend on the patient.

Although 82 % of adults in the USA take at least one medication on a regular basis, 29 % take five or more medications [1]. Looking specifically at a cohort of adults aged 57 years and older, 91 % regularly use at least one medication, with over half using five or more medications [10]. Adverse effects of single medications may include depression, change in mental status, loss of appetite, or gastrointestinal disturbances. These untoward effects each influence nutrition status and may be multiplied when more than one medication or an interaction increases the risk. In fact, the number of medications has been correlated with deficits or excesses of specific nutrients [11].

In addition to the number of medications, drug–nutrition interactions are an important associated clinical concern. These interactions result from the physical, chemical, physiological, or pathophysiological relationships between a drug and a nutrient, multiple nutrients, food in general, specific food components, or nutrition status [12]. In this broad view, a drug may be the object of an interaction when a food or food component alters drug bioavailability, a specific nutrient effects drug clearance, or nutrition status changes drug disposition (Table 6.1). This chapter will focus on medication as the precipitating factor influencing nutrition and metabolic status in general or the status of specific nutrients. We provide descriptions using examples of medication that have come to the US market place since the publication of the previous edition of this reference work [13].

Table 6.1 Categorizing drug–nutrition interactions [12]

Precipitating factor	Object of clinical interaction	Example
Food or food component	Drug	Grapefruit juice reduces bioavailability of levothyroxine
Specific nutrient	Drug	Vitamin D reduces concentration of atorvastatin
Nutrition status	Drug	Obesity lowers concentrations of ertapenem
Drug	Nutrition status	Quetiapine induces weight gain
Drug	Metabolic status	Capecitabine may cause hypertriglyceridemia
Drug	Specific nutrient	Carbamazepine lowers status of biotin

The data on adverse effects of medication come largely from clinical studies in which subjects or patients self-report their complaints, together with objective measures that are also collected and reported by investigators. The frequency of an adverse effect can be compared with a placebo as well as against the expected frequency as part of the disease process under study. This provides the likelihood that, and the degree to which, the effects are attributable to the drug under study. Studies in which a combination of treatments is used are difficult for characterizing the adverse effects attributed to each medication. Although general nutritional and metabolic parameters are often available from these investigations, the many nutrient biomarkers of interest are rarely included. Post-marketing case series and studies add to the available information with time. This is where nutrient-specific effects of new medication may be identified and reported.

Methods of Altered Nutrition Status

Clinical Consequences

There are a number of ways in which a drug can influence nutrition status. It is not always the most common or obvious adverse effects that may influence nutrition status. The recognized clinical consequences will include common global parameters (e.g., changes in body weight, body mass index [BMI], volume status), related metabolic parameters (e.g., dyslipidemia, hyperglycemia), or nutrient-specific biomarkers (e.g., biotin insufficiency, zinc deficiency, hypokalemia).

An individual drug may set the stage for worsening nutrition status by affecting numerous factors. For example, the HER2 receptor antagonist pertuzumab is associated with significant fatigue (reported in 34–45 % of treated patients), nausea (34–38 %), vomiting (20–34 %), abdominal pain (38 %), peripheral edema (20–22 %), decreased body weight (15 %), and hypokalemia (12 %) [14, 15].

Mechanisms at Play

Drugs can directly influence food preparation and intake through effects in the central nervous system or at the gastrointestinal (GI) tract. These may include commonplace adverse effects (e.g., anorexia, nausea, vomiting, diarrhea) or more subtle influences on digestion and absorption. For example, by inducing a general malabsorption, colchicine increases loss of fat, nitrogen, sodium, and potassium in the feces with decreases in absorption of xylose, vitamin B_{12}, and possibly some carotenoids [16]. Both oral colchicine and neomycin are associated with fat and carbohydrate malabsorption. Neomycin produces the classic example of dose-related drug-induced malabsorption [17]. By causing brush-border damage, an enteropathy arises in part the result of disaccharide intolerance and bile acid precipitation. Malabsorption of carbohydrate and fat is also an expected therapeutic effect from the use of acarbose and orlistat, respectively.

Medications may indirectly alter food preparation and intake through drug-induced cognitive, visual, or movement disturbances especially when severe. Many psychoactive (e.g., benzodiazepines, opioids) and anticholinergic (e.g., diphenhydramine) drugs are classically associated with acute and chronic confusional states [18]. There are other more subtle indirect influences of serious adverse effects (e.g., peripheral neuropathy, myalgia) on nutrition status.

Influences of medication on metabolic function in particular body weight and composition, as well as lipid and glycemic control are important to recognize. The second-generation antipsychotics are a typical example. These drugs may cause weight gain of up to 4 kg more than placebo as well as disturbances of glucose, lipid, and prolactin homeostasis [19, 20]. Metabolic changes are in many cases

transient but can also be life-threatening. Lipodystrophy, including fat redistribution syndrome, associated with the highly active antiretroviral therapy regimens used in patients with HIV infection is likely multifactorial [21]. Medications with a primary therapeutic effect of reducing blood glucose, triglycerides, or cholesterol are well known.

Then the status of specific nutrients may be influenced by drugs. The absorption, distribution, metabolism, and excretion of a nutrient can be altered but may only be recognized with the availability of appropriate biomarkers. Although classic deficiency syndromes are rare, the clinical manifestations in practice may be patient specific. The influence of drugs on nutrient disposition has been well described [22]. A classic example includes the influence of isoniazid on vitamin B_6 metabolism [23]. Hundreds of genes have been identified in the human genome that express transporter proteins and sensing receptors for specific nutrients or groups of nutrients some of which are also targets of drugs [24–26]. This provides the potential for many drug–nutrition interactions not yet investigated.

Mechanistically the drug-induced changes to nutrition status can be diverse. Any drug that causes altered taste, dry mouth, oral pain, anorexia, nausea, vomiting, altered gastric emptying, diarrhea, or constipation can lead to impaired oral intake and nutrient absorption, increasing the risk for malnutrition. There may be physicochemical reactions that take place within the GI tract; actions at membrane transporters or metabolizing enzymes; or antagonistic, additive, or synergistic actions on wide-ranging physiologic functions [27]. Given the wide range of innervating neural pathways affecting the function of the GI tract, it is easy to appreciate that any medication with pharmacologic effects on cholinergic, histaminic, dopaminergic, opiate, serotonergic, or benzodiazepine receptors can influence GI function.

Complexity and Clinical Interpretation

As described above, several mechanisms exist to explain drug-induced changes to nutrition status. More than one mechanism can be causative for a given drug and nutrient. For example, the antiepileptic drug carbamazepine decreases biotin status both by inhibiting intestinal absorption and accelerating metabolism to inactive catabolites [28, 29]. Then, a single nutrient may be influenced by more than one drug through a combination of mechanisms. For example, folate status may be affected by drugs that impair its absorption, alter protein binding in the circulation, block release from tissue sites, or enhance metabolism [30]. A single drug can have an influence on more than one nutrient through either a single or multiple mechanisms. Corticosteroids have been noted to reduce concentrations of several nutrients (e.g., folate, vitamin B_{12}, selenium, and calcium) [31, 32]. The exact mechanism of altered nutrition status is not always fully understood. For example, the use of kava (*Piper methysticum*) may cause a yellowing pellagrous dermopathy unrelated to the hepatotoxic potential of this herbal medicine. It has been suggested that this may occur due to a niacin deficiency, although the exact mechanism remains unclear [33]. Of course, the influence of some drugs on nutrition status is the primary therapeutic action sought clinically (e.g., orlistat decreases fat absorption, pamidronate lowers serum calcium, warfarin reduces availability of reduced vitamin K).

The impact of drugs on nutrition status is not always predicted from animal studies or in vitro data. It is not routinely assessed during the drug approval process. Given the poor likelihood that each new drug will be evaluated for nutritional effects prior to marketing, clinicians should operate on the assumption that any variability in nutrition status is the result of a drug-induced change unless proven otherwise. The clinician should be prepared to ask: if my patient presents with an unexpected change in nutrition status, could this be related to one of their medications?

Clinical manifestations are typically acute for a drug that interferes with a nutrient's metabolic activities, compared with that involving food intake, absorption, or clearance which are expected to take longer to manifest. Of course the patient's gender, age, genetic factors, and underlying nutrition

status all need to be taken into account. There is increased risk if the effects on nutrition status are additive, if medication use is chronic, if the patient is an older adult, or if they have marginal nutrition status to begin with. With advances in recognizing adverse drug effects that influence nutrition status, understanding their mechanisms, and learning how best to manage individual situations, patient care can be further improved.

Using This Chapter

The remainder of the chapter and the accompanying tables will focus on the medications that have been approved in the USA for systemic use since 2010. A more comprehensive review of older medication is found in the previous edition of this reference book to which readers are also referred for a more inclusive and historic accounting [13].

Although these newer medications may have a number of significant adverse reactions, only those relevant to nutrition status are discussed. For example, macitentan does not appear in any of our tables despite 13 % of patients having a drop in hemoglobin apparently unrelated to nutrition. And apixaban and vorapaxar do not appear in any of the tables in this chapter because their main adverse event is bleeding. The data in the tables come from clinical trials and early post-marketing experiences as reported by the manufacturer to the FDA and included in FDA-approved product labeling [34]. For some the percent frequency for a described adverse effect was not included in the product information. These values may not necessarily reflect what will be observed in widespread clinical practice which may be higher or lower. The severity of adverse effects can be described using the Common Terminology Criteria for Adverse Events [35]. Although most often applied to cancer treatments, these criteria are more universally applicable. Criteria that meet grade 3 or higher are the most clinically severe.

Drug Influence on Food Preparation or Intake

A wide number of medications have the potential to interfere with obtaining and preparing food or altering food intake each of which contributes to the risk for poor nutrition status. Table 6.2 provides a list of select drugs that influence food preparation or intake. Besides drugs with an obvious impact on appetite control, are those that influence mood and cognitive function or oropharyngeal structure and function. In the case of dalfampridine the change in mental status can include insomnia and seizure activity, while for levomilnacipran it can include serotonin syndrome [36].

Appetite

A number of new medications have been reported to influence appetite (Table 6.2). The rates of decreased appetite approach 50 % of patients for cabozantinib or regorafenib, but are <10 % for several others (e.g., roflumilast, tapentadol). The central processing of other afferent inputs can also influence feeding behavior. The brain integrates multiple inputs including those from the GI tract to determine feeding behavior [37]. Although decreased appetite was not described in the product labeling it has now been reported for enzalutamide and ipilimumab—seen in 18 % and 25–31 % of patients, respectively [38–40].

Table 6.2 Select drugs that may interfere with food preparation or intake

Decreased appetite	
Afatinib	29 %
Axitinib	34 %
Boceprevir	25–26 %
Bosutinib	13 %
Brentuximab	11–16 %
Cabozantinib	48 %
Carglumic acid	9 %
Ceritinib	34 %
Crizotinib	27 %
Eribulin	20 %
Glycerol phenylbutyrate	4 %
Ibrutinib	17–21 %
Levomilnacipran	3 %
Lorcaserin	2 %
Miltefosine	11–23 %
Omacetaxine	10–13 %
Pasireotide	10 %
Pertuzumab	29 %
Pomalidomide	22 %
Ponatinib	8–31 %
Regorafenib	31–47 %
Rilpivirine	pnr
Roflumilast	2 %
Siltuximab	4 %
Sofosbuvir	6 %
Tapentadol	2 %
Tranetinib	22 %
Vandetanib	21 %
Vemurafenib	18 %
Vismodegib	25 %
Increased appetite	
Deferiprone	4 %
Lurasidone	3 %
Teduglutide	7 %
Altered mental status	
Clobazam	26 %
Dalfampridine	9 %
Droxidopa	pnr
Eslicarbazepine	26–38 %
Ezogabine	4–16 %
Glycerol phenylbutyrate	pnr
Levomilnacipran	pnr
Lorcaserin	4 %
Lurasidone	8–26 %
Pomalidomide	12 %
Roflumilast	6 %
Tapentadol	15 %
Vilazodone	3–9 %

(continued)

Table 6.2 (continued)

Vortioxetine	6–9 %
Depression	
Belimumab	5 %
Eslicarbazepine	1–3 %
Fingolimod	8 %
Rilpivirine	9 %
Tesamorelin	2 %
Vandetanib	10 %
Fatigue	
Abiraterone	39 %
Axitinib	39 %
Boceprevir	55–58 %
Bosutinib	24 %
Brentuximab	41–49 %
Cabozantinib	41 %
Carfilzomib	56 %
Ceritinib	52 %
Crizotinib	27 %
Elosulfase-α	10 %
Enzalutamide	51 %
Eribulin	54 %
Eslicarbazepine	4–7 %
Everolimus	9 %
Ezogabine	13–16 %
Ibrutinib	31–41 %
Ipilimumab	41 %
Lomitapide	17 %
Lorcaserin	7 %
Metreleptin	8 %
Mipomersen	15 %
Omacetaxine	29 %
Pasireotide	19 %
Perampanel	10 %
Pertuzumab	38 %
Pomalidomide	55 %
Ponatinib	31–39 %
Regorafenib	52–64 %
Sofosbuvir	30–38 %
Tapentadol	3 %
Telaprevir	56 %
Ticagrelor	3 %
Vedolizumab	6 %
Velaglucerase-α	13 %
Vemurafenib	38 %
Vismodegib	40 %
Dysgeusia	
Boceprevir	35–44 %
Cabozantinib	34 %
Carglumic acid	9 %

(continued)

Table 6.2 (continued)

Crizotinib	26 %
Pertuzumab	18 %
Telaprevir	10 %
Vandetanib	8 %
Vemurafenib	14 %
Vismodegib	55 %
Stomatitis/oral mucositis	
Afatinib	71 %
Cabozantinib	51 %
Ibrutinib	17–21 %
Omacetaxine	pnr
Pertuzumab	19 %
Ponatinib	9–23 %
Regorafenib	33–40 %
Tocilizumab	pnr
Trametinib	15 %
Dysphagia/oropharyngeal pain	
Bazedoxifene	7 %
Cabozantinib	36 %
Ibrutinib	15 %
Ivacaftor	22 %
Omacetaxine	pnr
Rivaroxaban	1 %
Siltuximab	8 %
Trametinib	13 %
Vedolizumab	3 %

Pnr percent not reported

Fatigue

Although a number of medications are associated with fatigue (Table 6.2), the mechanism for drug-related fatigue is not always clear. Well beyond the complaints of fatigue in the general population, this effect reaches above 10 % and as high as 92 % following cytotoxic medication [41]. Self-report does not provide causal links to inhibition of CNS excitatory activation or stimulation of inhibitory pathways, neurotransmitter related or signal conduction abnormalities, or a more peripheral source (e.g., musculoskeletal, metabolic disturbances, hematologic toxicity). In any case, fatigue easily influences quality of life including activities of daily living. Thereby interfering with the ability to gather, prepare, or consume a regular diet.

The report of fatigue seen with abiraterone (~44 %) is not significantly different than seen with placebo in patients treated for metastatic prostate cancer [42]. A high frequency of fatigue is also reported for telaprevir (>50 %) but this is not significantly different from placebo in patients treated for hepatitis C virus infection [43]. Conversely a number of medications are reported to induce fatigue beyond the disorder being managed, and is often distinguished from affective disorders. It can be as high as 24 % with omacetaxine and can occur in >50 % of patients receiving eribulin, pomalidomide, and regorafenib [44, 45]. The fatigue attributable to pomalidomide (73 %, with 18 % grade 3 or above) may be in part related to drug-induced anemia [46]. Regorafenib is associated with fatigue 47–53 % (8–17 % grade 3 and higher) which is significantly greater than seen with placebo [47–49]. Fatigue may present in up to 70 % of refractory patients treated for multiple myeloma with

carfilzomib related to treatment and beyond what might be expected from the disorder [50, 51]. Vemurafenib includes grade 3 and higher fatigue (34–42 %) [52, 53]. Fatigue is also seen in 32–41 % of older patients receiving ibrutinib [54, 55] and 36–41 % with vismodegib [56]. Axitinib is associated with fatigue in 59 % of patients (22 % grade 3 or worse) [57]. Fatigue with this drug may be associated with hypothyroidism, and has been seen more commonly in Japanese patients (44 % vs. 19 %) [58, 59]. The fatigue may not appear for at least 4 weeks into treatment given long half-lives for some drugs (e.g., enzalutamide) [60].

Antiepileptic drug-related fatigue is considered an extension of decreased neuronal excitability induced by the medication, but could also be due to multiple central and peripheral mechanisms [41]. The frequency varies by the drug with eslicarbazepine low (5 %) compared with ezogabine (16 %). In fact ezogabine (aka retigabine internationally) is also associated with dizziness (41 %), somnolence (31 %), confusion (14 %), ataxia (12 %), blurred vision (12 %), disorientation (5 %), depression, and fatigue [61]. All of these could contribute to impaired obtaining, preparation, and consumption of food. This adverse effect can be dose- and concentration-related for perampanel with an overall incidence of 14–21 % and may further be associated with gait disturbances in 9 % [62, 63]. As mentioned earlier, drugs that alter gait or vision may also limit the ability to gather food and prepare meals. Blurred vision is also reported in 10 % of patients treated for chronic lymphoid leukemia with ibrutinib [64].

Adverse Oral Effects

Although drug-induced nausea, vomiting, abdominal pain, constipation, and diarrhea are likely to alter eating behavior, these will be discussed in a subsequent section. Some medications can cause more proximal effects including dysgeusia, stomatitis, oropharyngeal pain, and dysphagia.

Change in taste perception, as seen with several newer drugs (Table 6.2), can have a significant influence on subsequent dietary intake. This dysgeusia can lead to decreased appetite and possible weight loss, especially if the patient is unable to maintain hydration and saliva production. Medications can cause loss of taste (ageusia), distortion of taste (dysgeusia), decreased sense of taste (hypogeusia), and even gustatory hallucination (phantogeusia) [65]. Xerostomia as a result of inhibited saliva production, most commonly a result of drugs with anticholinergic properties, can also alter taste sensation. If the causative agent cannot be discontinued or reduced in dose, use of breath mints, lozenges, and sugarless gum may offer relief. Oral care regimens should be encouraged for those using metered-dose inhalers, and appropriate treatment implemented for candidiasis.

Patients who receive hedgehog inhibitors are known to exhibit dysgeusia/ageusia (51–71 %), muscle spasms (68–72 %), decrease in appetite (17–25 %), and weight loss (45–46 %) during treatment for basal cell carcinoma or ovarian cancer [66–68]. Frequent dysgeusia (47–85 %) has been reported with vismodegib including many patients with ageusia [56]. This adverse effect may reflect the presence of residual hedgehog signaling in adults [56]. These pathways are involved in regulating progenitor cell turnover in the adult tongue epithelium beyond the known role of tongue development in embryogenesis.

A number of the newer agents have been reported to cause stomatitis (i.e., oral mucositis) (Table 6.2). Afatinib is a significant offender as reported in over two-thirds of patients with 9 % at grade 3. In fact stomatitis is reported in as many as 86 % of afatinib-treated patients; enough to decrease appetite and food intake [69]. Unlike the parallel frequencies of stomatitis with reduced appetite and weight loss seen with regorafenib, the afatinib-induced stomatitis does not seem to be associated with weight loss. As with many older cytotoxic drugs these agents can create inflammation of the oropharyngeal mucosal surface. This stomatitis severely curtails food intake because of the pain caused with eating. Oral mucositis (43 %), anorexia (29 %), and nausea (27 %) in patients treated with the multikinase inhibitor regorafenib occur between 5 and 14 days into therapy [47].

Drug-Induced Effects on the Gastrointestinal Tract

A large number of medications have an adverse impact on the GI tract. The most recently approved systemic drugs are no exception (Table 6.3). Some have multiple adverse effects on the GI tract. For example, although miltefosine is a valuable treatment for leishmaniasis, at least 40 % of patients exhibit vomiting, many with anorexia and nausea, and as many as 23 % experience abdominal pain, and about 10 % report diarrhea [70, 71]. For other drugs, the complaints may be confined to nausea as is the case in 23–48 % using the opioid analgesic tapentadol while only 7–10 % of patients complain of constipation [72, 73]. A medication need not be administered orally for those drug-related events. For example, following subcutaneous injection, perampanel is associated with nausea (17–30 %), vomiting (15 %), abdominal pain (19–20 %), and diarrhea (29–42 %) [63, 74, 75]. Approximately 12 % of patients receiving ferric carboxymaltose report GI disorders despite being administered as a single intravenous dose [76]. However this is less common than when receiving the equivalent therapeutic dose of oral ferrous sulfate [77].

Table 6.3 Drug-induced effects on the gastrointestinal tract

Nausea/vomiting	
Abiraterone	pnr
Apremilast	44 %/16 %
Axitinib	32 %/24 %
Bedaquiline	38 %/–
Belatacept	24 %/22 %
Belimumab	15 %/–
Boceprevir	43–46 %/15–20 %
Bosutinib	46 %/39 %
Brentuximab	38–42 %/17–22 %
Cabozantinib	43 %/24 %
Carfilzomib	45 %/22 %
Carglumic acid	–/26 %
Ceftaroline	4 %/2 %
Ceritinib	80 %/60 %
Clobazam	–/7 %
Crizotinib	55 %/47 %
Dalbavancin	6 %/3 %
Dalfampridine	7 %/–
Deferiprone	13 %/10 %
Dimethyl fumarate	12 %/9 %
Elosulfase-α	24 %/31 %
Eribulin	35 %/18 %
Eslicarbazepine	10–16 %/6–10 %
Everolimus	29 %/15 %
Ferric carboxymaltose	7 %/2 %
Fidaxomicin	11 %/7 %
Glucarpidase	2 %/2 %
Glycerol phenylbutyrate	7 %/4 %
Ibrutinib	31 %/23 %
Ivacaftor	12 %/–
Levomilnacipran	17 %/5 %

(continued)

Table 6.3 (continued)

Liraglutide	28 %/11 %
Lomitapide	65 %/34 %
Lorcaserin	8 %/4 %
Lurasidone	7–17 %/6–9 %
Miltefosine	36 %/38 %
Omacetaxine	29–35 %/12–16 %
Pasireotide	52 %/7 %
Peginesatide	17 %/15 %
Pegloticase	12 %/5 %
Perampanel	8 %/4 %
Pertuzumab	42 %/24 %
Pomalidomide	36 %/14 %
Ponatinib	22–32 %/13–24 %
Regorafenib	20 %/17 %
Riociguat	14 %/10 %
Roflumilast	5 %/pnr
Sapropterin	–/8 %
Simeprevir	22 %/–
Sofosbuvir	13–22 %/–
Sucroferric hydroxide	10 %/–
Tapentadol	30 %/18 %
Teduglutide	25 %/12 %
Telaprevir	39 %/13 %
Teriflunomide	9–14 %/–
Ticagrelor	4 %/–
Trametinib	44 %/40 %
Vandetanib	33 %/15 %
Vemurafenib	35 %/18 %
Vilazodone	23 %/5 %
Vismodegib	30 %/14 %
Vortioxetine	21–32 %/3–6 %
Abdominal pain	
Axitinib	14 %
Bazedoxifene	7 %
Belatacept	19 %
Bosutinib	37 %
Brentuximab	9–25 %
Cabozantinib	27 %
Carglumic acid	17 %
Ceritinib	54 %
Dabigatran	35 %
Deferiprone	10 %
Dimethyl fumarate	18 %
Elosulfase-α	21 %
Everolimus	13 %
Fidaxomicin	6 %
Glycerol phenylbutyrate	7 %
Ibrutinib	15–24 %
Ivacaftor	16 %

(continued)

Table 6.3 (continued)

Linaclotide	7 %
Lomitapide	34 %
Metreleptin	10 %
Miltefosine	8–11 %
Mirabegron	2 %
Omacetaxine	23 %
Pasireotide	24 %
Ponatinib	34–49 %
Regorafenib	pnr
Rivaroxaban	2 %
Siltuximab	12 %
Teduglutide	38 %
Trametinib	13 %
Vandetanib	21 %
Velaglucerase-α	19 %
Acute pancreatitis	
Alogliptin	1 %
Dabrafenib	<10 %
Denosumab	1 %
Linagliptin	pnr
Liraglutide	pnr
Ponatinib	6 %
Increased liver enzymes	
Abiraterone	31–42 % (ALT > AST > TB)
Axitinib	30 % (AP > ALT, AST)
Cabozantinib	86 % (AST, ALT > AP > TB)
Carfilzomib	13 % (AST)
Ceritinib	80 % (ALT > AST > TB)
Crizotinib	76 % (ALT > AST)
Deferiprone	8 % (ALT)
Fingolimod	14 %
Lomitapide	17 % (ALT)
Miltefosine	5 %
Mipomersen	5–30 %
Obinutuzumab	28 % (AST > ALT > AP)
Pasireotide	10 %
Ponatinib	53 % (ALT > AST, AP > TB)
Regorafenib	58–65 % (AST > ALT, TB)
Rilpivirine	pnr
Simeprevir	50 % (TB > ALT, AST, AP)
Tasimelteon	10 % (ALT)
Teriflunomide	12–14 % (ALT)
Tocilizumab	36 % (ALT > AST)
Trametinib	60 % (AST > ALT > AP)
Constipation	
Brentuximab	16–19 %
Cabozantinib	27 %
Carfilzomib	21 %
Ceritinib	29 %

(continued)

Table 6.3 (continued)

Clobazam	5 %
Crizotinib	42 %
Dabrafenib	11 %
Eribulin	25 %
Everolimus	38 %
Ibrutinib	23 %
Levomilnacipran	9 %
Lomitapide	21 %
Pomalidomide	36 %
Ponatinib	24–47 %
Tapentadol	8 %
Vemurafenib	12 %
Vismodegib	21 %
Diarrhea	
Abiraterone	18–22 %
Afatinib	96 %
Apremilast	38 %
Axitinib	55 %
Azilsartan	2 %
Bazedoxifene	8 %
Belatacept	39 %
Belimumab	12 %
Boceprevir	24–25 %
Bosutinib	82 %
Cabozantinib	63 %
Carfilzomib	33 %
Carglumic acid	13 %
Ceftaroline	5 %
Ceritinib	86 %
Crizotinib	60 %
Dalbavancin	4 %
Dimethyl fumarate	14 %
Enzalutamide	22 %
Everolimus	19 %
Fingolimod	12 %
Glycerol phenylbutyrate	7 %
Ibrutinib	51–63 %
Ipilimumab	32 %
Ivacaftor	13 %
Linaclotide	16–20 %
Liraglutide	17 %
Lomitapide	79 %
Miltefosine	15–20 %
Omacetaxine	35–41 %
Pasireotide	58 %
Peginesatide	18 %
Pertuzumab	67 %
Pomalidomide	34 %
Ponatinib	13–26 %

(continued)

Table 6.3 (continued)

Ramucirumab	14 %
Regorafenib	43–47 %
Riociguat	12 %
Roflumilast	10 %
Sapropterin	8 %
Sofosbuvir	9–12 %
Sucroferric-oxyhydroxide	24 %
Telaprevir	26 %
Teriflunomide	15–18 %
Ticagrelor	4 %
Trametinib	36–43 %
Vandetanib	57 %
Vemurafenib	28 %
Vilazodone	28 %
Vismodegib	29 %
Gastrointestinal perforation	
Axitinib	1 %
Cabozantinib	3 %
Ipilimumab	1 %
Ramucirumab	1 %
Regorafenib	1 %
Siltuximab	pnr
Tocilizumab	pnr

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *AP* alkaline phosphatase, *TB* total bilirubin, *pnr* percent not reported

Nausea and Vomiting

Nausea is a common finding with many oral medications but severity varies with the individual drug accompanied by vomiting in many cases. The frequency of nausea is generally greater than that for vomiting, but for many medications this is not much greater than placebo and often with an incidence of 5–10 % (Table 6.3). For example, ticagrelor causes nausea in 7 % which may be related to adenosine receptor antagonism [78]. More typically are drugs like omacetaxine which is associated with nausea in 30 % (vomiting in 15 %) [44, 45]. Others are reported to have even higher frequencies. Significant nausea may be centrally mediated (e.g., cytotoxic agents, opioid analgesics), or the result of local GI irritation. Nausea especially when accompanied by vomiting will interfere with food intake. Interference with nutrition status is observed with severe and prolonged emesis as seen with cytotoxic chemotherapy. Even transient nausea/vomiting as seen with liraglutide is associated with weight loss [79]. Poorly controlled nausea and vomiting significantly impact not only nutrition status but also quality of life. Vomiting may be a reported side effect of many medications; however continued intolerance to the medication only occurs with a select number of these drugs in most patients.

Patients may be less willing to tolerate nausea and vomiting when managing depression than a malignancy. For example, 20 % of patients receiving the antidepressant vilazodone will discontinue therapy because of adverse effects (nausea 32 %, diarrhea 36 %) [80, 81]. The nausea associated with another antidepressant levomilnacipran (17–22 %) is considered severe enough for many to discontinue therapy [82, 83]. Although levomilnacipran is considered weight neutral overall, it may lead to reduced intake in some patients at a time when depression should be improving.

Abdominal Pain

Drugs that alter GI motility are most likely to cause abdominal pain and constipation or diarrhea that, if severe or prolonged, will impair nutrition status by reducing intake, limiting absorption, and increasing nutrient losses. Despite a tablet coating, the GI complaints (nausea, abdominal pain, diarrhea) with dimethyl fumarate appears dose related in up to 41 % of patients with multiple sclerosis and can result in drug discontinuation [84]. These complaints may decrease in frequency with ongoing treatment after the first month [85]. Abdominal pain is reported to be 27 % (7 % grade 3 or above) with ponatinib [86]. Dabigatran has been reported to cause severe abdominal pain only sometimes associated with bleeding [87–89].

The impact of most medications on digestive processes is not well documented. Drugs that are associated with pancreatitis or that cause cholestatic or hepatocellular injury/dysfunction can play a role in altering digestion and absorption. Many drugs have been reported to cause pancreatitis but the association is strongest for only a few [90]. Pancreatitis has been associated with the use of several of the newer agents (Table 6.3). Linagliptin is associated with pancreatitis but the risk may not be different from comparator drugs (OR 1.62 [0.37, 7.02], NS) [91]. Cases of pancreatitis have also been reported with another agent used in diabetes—the GLP-1 receptor agonist liraglutide [92, 93]. This despite a meta-analysis suggesting no increased risk of pancreatitis (OR 1.01 [0.37, 2.76]) [94]. In addition to these 2 drugs, used in diabetes, cases of pancreatitis associated with denosumab have reported fatal outcomes.

Constipation and Diarrhea

Constipation is commonly caused by agents such as opioid analgesics, anticholinergics, calcium channel blockers, and iron preparations [95]. The newer opioid analgesic tapentadol is associated with a low incidence of constipation. A drug used to treat chronic constipation (i.e., linaclotide) by acting locally would be expected to cause abdominal pain (5 %) and diarrhea (8–20 %), usually occurring in the first few weeks of therapy [96–99].

While many drugs are reported to cause diarrhea by one or more mechanisms, a recalcitrant few cause the most severe presentation. It can be expected that rapid intestinal transit induced by drugs would also increase the risk for maldigestion and subsequent malabsorption. Diarrhea may be secretory or osmotic in nature, but in either case, if severe, will decrease dietary intake and create fluid, electrolyte, and other mineral losses. Drug-induced diarrhea can lead to the unrecoverable loss of macronutrients and micronutrients possibly with significant consequences. Drugs commonly associated with diarrhea have included magnesium-containing antacids, antibiotics, antineoplastics, colchicine, mycophenolate mofetil, and laxatives [95].

Medications may be more likely to impact lower GI function (e.g., ticagrelor causes diarrhea in 7 %) compared with placebo [78]. The GI complications are more significant for many other drugs. Diarrhea may be as high as 44 % as with omacetaxine, including >5 % grade 3 or above in addition to other GI complaints [44, 45]. Ipilimumab is associated with significant GI distress including diarrhea (51 %, 17 % grade 3 or higher), nausea/vomiting (32/28 %), abdominal pain (9 %), and constipation (18 %) [40]. The tyrosine kinase inhibitor ibrutinib is also associated with significant GI complaints: diarrhea (68 %, 13 % grade 3 or above), nausea (48 %), vomiting (23 %), dyspepsia (26 %), gastro-esophageal reflux (19 %), stomatitis (16 %), and constipation (23 %) [55]. The diarrhea often resolves without needing to stop the drug. The frequency of nausea (11–18 %), vomiting (3–5 %), and diarrhea (7–14 %) with apremilast is considered transient in the first few weeks of treatment [100].

When used as monotherapy for prostate cancer pertuzumab is reported to cause diarrhea in 61 % [15], and in combination regimens rates of 67–69 % have been reported with 8–11 % grade 3 [14, 101].

Combining HER targeted therapy is only slightly more likely to cause diarrhea compared with monotherapy in breast cancer [102]. The severity of adverse GI events can be dose related as is the case with crizotinib with doses ≥250 mg twice daily associated with nausea (57 %), vomiting (46 %), and diarrhea (49 %) [103]. The incidence of diarrhea is much more common with this agent than with alternate chemotherapy (61 % vs. 13 %) [104]. Ceritinib doses up to 750 mg daily have been tolerated in patients with incidence of nausea (82 %), vomiting (65 %), and diarrhea (75 %) accompanied by dehydration [105]. Dose-related adverse GI effects occur with bosutinib too but improved tolerance is seen at higher doses when administered with food [106]. The diarrhea occurring in bosutinib-treated patients resolved in 83 % following dose reduction, interruption, and/or concurrent antidiarrheal medications [107, 108]. The diarrhea reported with cabozantinib (63 %) can be severe with 16 % at grade 3 or higher [109]. Vandetanib is reported to cause diarrhea in 46–60 % (5–11 % grade 3 or above) [110, 111].

Despite its frequency, diarrhea (55 %) is not always predictable with axitinib, usually taking 3–8 weeks to develop [112]. It may be due to direct damage to the intestinal mucosa or altered microbiota, requiring oral rehydration and loperamide as needed. The diarrhea accompanying boceprevir is associated in practice with anorectal discomfort (i.e., burning sensation) in about one-third of patients requiring changes in diet (increasing fat intake and fiber) [113].

Diarrhea is only experienced by 5–7 % of patient receiving ceftaroline in phase III trials, but comes with the risk for *Clostridium difficile* infection [114, 115].

Perforation

Fistula formation and GI perforation are associated with inhibition of VEGF pathways (e.g., axitinib, cabozantinib) leading to drug discontinuation if not fatal [57, 109]. This risk may be associated with metastases to the abdominal cavity. The anti-CTLA4 monoclonal antibody ipilimumab has also been reported to cause colitis and GI perforation requiring surgical intervention again in the presence of abdominal metastases [116, 117]. Pathologic examination revealed multifocal deep ulcerations and fissures that had penetrated into the muscular layer; epithelial damage and dilated crypts were present without granuloma [116, 117].

Drugs Influencing Volume Status and Body Weight

Volume Status

A number of medications are associated with edema or hypovolemia. The latter may be expected for those drugs which can cause significant vomiting or diarrhea if not managed. However from the recent drug approvals the two SGLT2 inhibitors (canagliflozin, dapagliflozin) available to treat type 2 diabetes are associated with hypovolemia. The volume losses are due to the dose-independent drug-induced osmotic diuresis that may also account for some weight loss [118]. This is most likely to occur in patients with renal impairment or with concurrent use of loop diuretics.

Several of the newer medications are associated with edema (Table 6.4). This may occur in up to one-third of patients receiving belatacept, crizotinib, ibrutinib, or trametinib. The specific mechanism for each is not clear. Tesamorelin-associated edema may be related to the drug's induction of growth hormone secretion. Of note, the edema attributed to ponatinib has included fatal brain edema. Noncardiac-related peripheral edema has also been reported in a considerable number of patients treated with everolimus [119]. Edema (31–33 %) and hypokalemia (17–18 %) reported with abiraterone follow from the drug's blockade of CYP17 thereby elevating mineralocorticoid concentrations as it reduces testosterone [42, 120].

Table 6.4 Drugs that influence volume status

Edema	
Abiraterone	25–27 %
Belatacept	34 %
Bosutinib	14 %
Carfilzomib	24 %
Crizotinib	31 %
Denosumab	5 %
Enzalutamide	15 %
Everolimus	45 %
Ibrutinib	23–35 %
Lorcaserin	5 %
Mipomersen	5 %
Omacetaxine	16 %
Pasireotide	10 %
Pertuzumab	23 %
Pomalidomide	23 %
Ponatinib	13–22 %
Siltuximab	16–26 %
Teduglutide	12 %
Tesamorelin	2–6 %
Tocilizumab	pnr
Trametinib	31 %
Vemurafenib	17 %
Hypovolemia	
Canagliflozin	2–8 %
Dapagliflozin	1–10 %

Pnr percent not reported

Weight Loss

All the aforementioned sections describing the effect of medication on food intake, GI function, and volume status may ultimately influence body weight. However there are a number of medications specifically noted to affect body weight (Table 6.5). Weight loss has been associated with a number of medications beyond those causing GI dysfunction. Other causes of weight loss still need to be ruled out in patients using any of these medications. It is interesting to note that metreleptin (an analog of leptin administered subcutaneously), used to improve insulin sensitivity, hyperglycemia, and hypertriglyceridemia, does not necessarily cause weight loss beyond that from a hypocaloric diet [121–123]. Lorcaserin, a selective serotonin receptor (subtype 2C) agonist, decreases appetite and causes weight loss in obese patients. Although this is the drug's indication it may be used for other indications for which weight loss may not be welcome [124].

Vismodegib is reported to cause weight loss of 5 % or more in 27–46 % of patients [56]. The phosphodiesterase-4 inhibitor roflumilast is reported to cause weight loss significantly greater than seen with placebo in patients with chronic obstructive pulmonary disease (mean weight loss 1.63 kg [2.08, 1.18]) in patients with a mean BMI of 21 kg/m^2 [125]. Obese patients are more likely to experience more significant weight loss (mean 3.6 kg). At 1 year of treatment mean weight loss 2.09 kg (2.7 % of baseline weight) was seen in 62 % of patients, with 20 % having moderate losses (5–10 % weight), and 7 % losing more than 10 % of baseline weight [126]. Most weight loss seems to occur in the first couple of months, but any weight loss is typically not welcome in patients with chronic obstructive pulmonary disease.

Table 6.5 Drug-induced changes to body weight

Increased body weight	
Deferiprone	2 %
Ezogabine	2–3 %
Lurasidone	3 %
Perampanel	4 %
Ruxolitinib	7 %
Siltuximab	19 %
Tocilizumab	pnr
Decreased body weight	
Apremilast	10 %
Afatinib	17 %
Axitinib	25 %
Brentuximab	6–12 %
Cabozantinib	48 %
Carglumic acid	9 %
Crizotinib	10 %
Eribulin	21 %
Fingolimod	5 %
Lomitapide	24 %
Metreleptin	13 %
Pomalidomide	pnr
Ponatinib	5–13 %
Regorafenib	14–32 %
Roflumilast	8–20 %
Vismodegib	45 %

Pnr percent not reported

Weight Gain

A number of drugs are associated with weight gain (Table 6.5). This is most commonly reported with psychotropic medications, with most attention given to the second-generation antipsychotic agents as mentioned earlier in the chapter. Drug-induced weight gain can be accompanied by the same increased risk of morbidity associated with increased body weight from other causes. This weight gain may be difficult to reverse given that much of it is adipose tissue gain. The newest agent used to treat bipolar disorder, lurasidone, is the least likely to cause weight gain, defined as more than a 7 % increase from baseline. However, at a frequency of 4 % (with a gain of 0.5 kg in short-term studies) it is still greater than seen with placebo and is accompanied by hypertriglyceridemia in 12 % of treated patients [127, 128]. The antiepileptic drug perampanel causes weight gain in 38 %, with a mean increase of 2.2 kg by year 2 in all adults with exposure data available [62, 129]. In early clinical trials weight gain was not noted with perampanel, in fact weight loss was reported to occur in 20 % of patients using perampanel [75].

Drugs Influencing Metabolic Control

A number of medications may influence glycemic and lipemic status (Table 6.6). The exact threshold values of blood glucose and lipid profile used to define rates of these events vary modestly between drugs and studies.

Table 6.6 Drugs that influence metabolic control

Hypoglycemia	
Alogliptin	5 %
Canagliflozin	4 %
Dalbavancin	<2 %
Dapagliflozin	1–43 %
Linagliptin	7 %
Liraglutide	11 %
Lorcaserin	29 %
Metreleptin	13 %
Pasireotide	9 %
Ponatinib	24 %
Vandetanib	24 %
Hyperglycemia	
Abiraterone	57 %
Axitinib	28 %
Carfilzomib	12 %
Ceritinib	49 %
Dabrafenib	50 %
Everolimus	12 %
Lurasidone	pnr
Omacetaxine	11 %
Pasireotide	40 %
Pomalidomide	12 %
Ponatinib	58 %
Tesamorelin	pnr
Trametinib	58 %
Hypertriglyceridemia	
Abiraterone	63 %
Everolimus	21 %
Siltuximab	8 %
Tocilizumab	pnr
Hypercholesterolemia	
Abiraterone	62 %
Canagliflozin	pnr
Dapagliflozin	pnr
Denosumab	7 %
Everolimus	17 %
Pasireotide	10 %
Siltuximab	4 %
Tocilizumab	19 %
Fat accumulation/redistribution	
Rilpivirine	pnr
Proteinuria	
Axitinib	11 %
Cabozantinib	2 %
Dimethyl fumarate	6 %
Everolimus	pnr
Regorafenib	33–60 %
Trametinib	42 %
Vandetanib	10 %

Pnr percent not reported

Glycemic Control

Newer classes of medication to manage hyperglycemia (e.g., canagliflozin, dapagliflozin) are no more likely than placebo to be associated with hypoglycemia [118, 130]. Weight loss that may accompany better glycemic control reflects reduction in fat mass [131]. As may be expected the DPP-4 inhibitor linagliptin has been reported to cause hypoglycemia, but again this is not significantly different compared with placebo when used as monotherapy (OR 0.93 [0.23, 3.77]), although risk is greater when used in combination therapy (OR 1.3 [1.00, 1.68]) [132].

Severe hyperglycemia has been reported with the use of omacetaxine including 11 % grade 3 and 4 hyperglycemia in the treatment of non-lymphocytic leukemia [44, 45]. Hyperglycemia (16–22 %) has been noted to be dose related for perampanel in otherwise healthy subjects [63, 74, 75]. Hyperglycemia was also reported with ipilimumab in 11 % of patients treated for melanoma [39].

Lipemic Control

Some drugs are associated with both hypertriglyceridemia and hypercholesterolemia requiring statin management. For example, everolimus was associated with dyslipidemia in 36 % of hepatic transplant patients, and with hypercholesterolemia in as many as 75 % of renal transplant patients [133, 134]. The risk for a 30 % increase in LDL:HDL with tocilizumab (RR 1.7 [1.2, 2.2]) exists for the monoclonal antibody against the IL-6 receptor [135]. After 6 weeks the LDL-cholesterol rises by 22 % with a 48 % rise in fasting triglycerides and a significant rise in postprandial triglycerides as well. This is accompanied by a decrease in hepatic LDL-receptor expression (i.e., a direct hepatic effect) [136]. Some weight gain is also seen, 24 % of patients had a BMI increase of at least 2 units over 16 weeks [137]. Another anti-IL-6 monoclonal antibody (siltuximab) is associated with increased triglycerides (19 %) and total cholesterol (15 %) but no reported weight gain [138].

Azilsartan is associated with dyslipidemia in up to 6 % of patients [139]. Mipomersen is a valuable addition to the management of dyslipidemia, resulting in significant lowering of serum LDL-cholesterol and triglycerides but at the cost of significant hepatic transaminitis (>30 %) [140]. Tesamorelin is an analog of human GHRH specifically indicated for reducing visceral adipose tissue in patients with HIV-associated lipodystrophy [141]. The drug enhances lipolysis and reduces triglyceride accumulation, while improving adiponectin, total cholesterol, and triglycerides.

Others

Interfering with mTOR (e.g., everolimus) is associated with surgical wound complications as well as dyslipidemia and proteinuria. The latter occurs because of the antiproliferative action on endothelial cells and fibroblasts but additional factors may be involved. Although the frequency of proteinuria (>300 mg protein per g creatinine) is not provided in FDA-approved labeling for everolimus, it has been reported and considered to be dose dependent [142]. After 3 months of everolimus exposure, this can be severe (>1 g protein per g creatinine), and the glomerular damage appears to be non-immunologic with diffuse endocapillary proliferation with endothelial cell swelling [143]. Others have reported patients with nephrotic range proteinuria (>3 g per g creatinine) at 12–24 months of exposure which appears to be associated with elevated trough concentrations of the drug [144]. Eleven percent of patients receiving dimethyl fumarate develop proteinuria although reportedly mild and reversible [85].

Another metabolic effect has been described for the proteasome inhibitor carfilzomib. This drug may improve osteoblast differentiation and contribute an anabolic role in myeloma associated bone disease [145].

The Status of Specific Nutrients

Historically a number of medications have been associated with alterations in the status of specific nutrients; these usually become recognized after years of use [13]. This is rarely an end point of any drug trial so it is difficult to quantify and even so would likely underestimate an effect. Medications may influence the disposition of individual or related groups of vitamins, minerals, amino acids, and fatty acids. What is known thus far with the recently approved drugs is found in Table 6.7.

The decrease in vitamin E absorption reported with lomitapide occurs despite concurrent daily vitamin supplementation. The mechanism is not yet clear.

As an orally active iron (Fe^{3+}) chelator deferiprone is expected to lower circulating iron and ferritin concentrations [146]. However, it also binds zinc enough to cause deficiency, although the frequency is unknown. This can occur without an increase in zinc-binding capacity in patients with thalassemia. Given deferiprone's physicochemical properties it is distributed intracellularly making stores of zinc as well as iron potential targets. Serum zinc is significantly lower in patients using deferiprone than control (mean 66.5 μg/dL vs. 149 μg/dL, $p < 0.05$), with urinary zinc losses exceeding 1 mg per day in the drug-treated group [147].

Electrolyte (macromineral) abnormalities are reported more commonly. Hyponatremia is seen in patients treated with regorafenib [47]. The rates are higher (>50 %) with trametinib especially when used in a combination regimen. Hypomagnesemia was documented in 12 % of patients treated with vismodegib [67]. Although common (86 %) hypophosphatemia seen with ferric carboxymaltose can be transient at 1.1–1.3 mg/dL change from baseline in the first few weeks of therapy [76, 77]. Hypophosphatemia has also been reported in 10 % of brentuximab-retreated patients [148]. Hypokalemia has been reported with a wide number of newer drugs. Although the frequency is low with ceftaroline, it included severe/life-threatening hypokalemia in a phase 3 clinical trial [114]. Significant treatment-emergent electrolyte abnormalities beyond the initial data submitted to the FDA for bosutinib includes hypophosphatemia (50 %), hypocalcemia (48 %), and hypokalemia (18 %) [149].

Several medications used for malignancies may rarely cause tumor lysis syndrome which manifests as hyperkalemia, hyperphosphatemia, and hypocalcemia. This has been reported to occur with carfilzomib and obinutuzumab [150]. The bone-modifying agent denosumab used in patients with bone metastases helps manage hypercalcemia. However an extension of this is hypocalcemia seen in up to 10 % and hypophosphatemia in 15 % of patients treated with denosumab [151, 152]. Hypocalcemia may manifest as oral and extremity paresthesias, as well as myalgia and spasms that may themselves interfere with food preparation and intake. Fatal hypocalcemia has been reported. Despite concurrent daily calcium (1250 mg) and vitamin D (400 IU), hypocalcemia (<2 mmol/L) occurred in 50 % of patients including 9.6 % grade 3 or above, and may present up to 28 days after the last dose of medication [152]. This risk may be even greater for patients with renal impairment [153].

Other Medication with the Potential to Influence Nutrition Status

There are additional and diverse adverse drug effects which may indirectly influence a patient's ability to maintain their nutrition status (Table 6.8).

Arthralgias can vary in severity by drug. For example, dabrafenib can affect joints of fingers, hands, elbows, knees, and ankles, which have the potential to be very debilitating especially if long-lasting

Table 6.7 Drug-induced effects on specific nutrients

Decreased Vitamin E absorption	
Lomitapide	pnr
Vitamin B12 deficiency	
Everolimus	pnr
Hypozincemia	
Deferiprone	pnr
Iron deficiency	
Everolimus	pnr
Hyponatremia	
Eslicarbazepine	5 %
Obinutuzumab	29 %
Pomalidomide	10 %
Ponatinib	29 %
Ramucirumab	6 %
Regorafenib	30 %
Trametinib	55 %
Vortioxetine	pnr
Hypokalemia	
Abiraterone	20–28 %
Afatinib	11 %
Belatacept	21 %
Cabozantinib	18 %
Carfilzomib	14 %
Ceftaroline	2 %
Crizotinib	18 %
Eribulin	5–10 %
Everolimus	12 %
Obinutuzumab	13 %
Pasireotide	6 %
Pomalidomide	10 %
Ponatinib	16 %
Regorafenib	21–26 %
Hypocalcemia	
Axitinib	39 %
Belatacept	13 %
Cabozantinib	52 %
Denosumab	2 %
Obinutuzumab	32 %
Pomalidomide	6 %
Ponatinib	52 %
Regorafenib	17–59 %
Vandetanib	11–57 %
Hypomagnesemia	
Belatacept	7 %
Cabozantinib	19 %
Carfilzomib	14 %
Eribulin	pnr

(continued)

Table 6.7 (continued)

Everolimus	14 %
Trametinib	18 %
Vandetanib	7 %
Hypophosphatemia	
Abiraterone	24 %
Axitinib	13 %
Belatacept	19 %
Cabozantinib	28 %
Carfilzomib	11 %
Ceritinib	36 %
Crizotinib	28 %
Dabrafenib	37 %
Everolimus	13 %
Ferric carboxymaltose	27 %
Ponatinib	57 %
Regorafenib	55–57 %
Teriflunomide	18 %
Hyperkalemia	
Axitinib	15 %
Belatacept	20 %
Canagliflozin	12–27 %
Everolimus	18 %
Obinutuzumab	31 %
Peginesatide	11 %
Pomalidomide	pnr
Ponatinib	15 %
Teriflunomide	1 %
Hypercalcemia	
Carfilzomib	11 %
Pomalidomide	21 %
Ponatinib	5 %
Trametinib	15 %
Hyperphosphatemia	
Canagliflozin	pnr
Dapagliflozin	2 %
Obinutuzumab	pnr

Pnr percent not reported

[154]. Patients with prostate cancer treated with enzalutamide have been reported to have arthralgia (21 %), musculoskeletal pain (15 %), muscle weakness (10 %), and paresthesias (7 %) [155]. All of which could ultimately contribute to poor nutrition status. Ongoing muscle spasms/cramps can also be seen with vismodegib (47–85 %) [56]. Excessive hypotension and dizziness caused by medication may also restrict food preparation and consumption. Consideration should even be given to drug-induced tremor as a cause for reducing food intake.

Most of the medications associated with peripheral neuropathy in this table are used to treat cancer. Peripheral neuropathy is common in patients with refractory multiple myeloma, so although carfilzomib is associated with an incidence of 85 % most of this is premorbid [51]. Eribulin and brentuximab had highest frequency of this serious and painful adverse effect. Although the mechanisms are unclear,

Table 6.8 Other drug-induced effects that may impact nutrition

Arthralgia	
Bedaquiline	33 %
Dabrafenib	27 %
Deferiprone	10 %
Enzalutamide	21 %
Icosapent ethyl	3 %
Ibrutinib	11 %
Metreleptin	8 %
Omacetaxine	19 %
Pasireotide	8 %
Peginesatide	11 %
Pertuzumab	16 %
Pomalidomide	16 %
Ponatinib	13–31 %
Taliglucerase-α	11–13 %
Tesamorelin	13 %
Vedolizumab	12 %
Vemurafenib	53 %
Vismodegib	16 %
Gait disturbance	
Perampanel	12–16 %
Myalgia	
Denosumab	3 %
Eribulin	22 %
Ibrutinib	37 %
Mipomersen	pnr
Omacetaxine	11 %
Pasireotide	9 %
Pertuzumab	23 %
Ponatinib	6–22 %
Simeprevir	16 %
Sofosbuvir	6–9 %
Teriflunomide	3–4 %
Tesamorelin	6 %
Vemurafenib	13 %
Pain in extremities	
Belimumab	6 %
Brentuximab	10 %
Deferiprone	2 %
Denosumab	12 %
Everolimus	12 %
Mipomersen	7 %
Omacetaxine	11–13 %
Peginesatide	11 %
Taliglucerase-α	11 %
Tesamorelin	3–6 %
Vedolizumab	3 %

(continued)

Table 6.8 (continued)

Peripheral sensory neuropathy	
Brentuximab	54 %
Cabozantinib	7 %
Carfilzomib	12 %
Crizotinib	19 %
Dalfampridine	7 %
Enzalutamide	7 %
Eribulin	35 %
Glucarpidase	2 %
Ibrutinib	10 %
Pertuzumab	26–32 %
Pomalidomide	9 %
Ponatinib	13 %
Teriflunomide	3 %
Wound healing complications	
Cabozantinib	pnr
Everolimus	35 %
Ramucirumab	pnr
Regorafenib	pnr

Pnr percent not reported

the neuropathy may be associated (eribulin) with inhibition of anterograde fast axonal transport [156]. This may take about 6 months to manifest but lasting months after completion of eribulin therapy [157]. Time to improvement or resolution following discontinuation of brentuximab can be several months as well [158]. Some patients may not fully recover.

Given the severity of drug-induced peripheral neuropathy and the association of a number of micronutrient deficits with neuropathy, it should be noted that nutrient interventions have not offered a significant improvement when evaluated [159].

Conclusions and Recommendations

A variety of the adverse effects attributed to medication recently entering the marketplace may influence nutrition status. As the precipitating factor to interactions these drugs may alter general nutrition or metabolic status as well as the status of specific nutrients. The data on new drugs may be limited which leaves the clinician having to remain vigilant in recognizing additional, perhaps subtle, changes to a patient's nutrition status especially after introducing newer medication. Patients requiring medication should have a thorough nutritional assessment performed at baseline and periodically during chronic treatment.

A nutritionally focused patient history, physical examination, and appropriate laboratory marker evaluations are important to correctly identify altered nutrition status in patients using medication. No one clinician specifically looks for nutritional aspects of drug use routinely. Any change in nutrition status identified by a clinician should be worked up for a drug-induced etiology. The clinical relevance of any drug-induced changes to nutrition status is interpreted by the clinician with the patient. This is then followed up with an appropriate plan. When a drug-induced change poses a significant concern for a patient, a therapeutically equivalent or alternative agent may be selected.

More clinical drug studies that account for nutrition outcomes are needed. Ideally all new drugs should be evaluated for effects on nutrition status. Applying available instruments during clinical trials that could consider the impact of drugs on the ability to prepare and consume meals, to alter body weight or composition, to impact metabolic homeostasis, or the biomarkers of specific nutrients would be welcome. In the meantime, clinicians should be sensitized to identify and report these otherwise unrecognized effects of drugs.

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Chapter 7
Diet–Gene Interactions: Haptoglobin Genotype and Nutrient Status

Leah E. Cahill and Eric B. Rimm

Key Points

- A gene is a segment of DNA that contains the instructions for making proteins (enzymes, transporters, receptors, hormones, etc.)
- Variations within genetic code can explain why certain processes, including the metabolism of nutrients, function differently among people.
- Studying interactions between nutrition and genetics provides insights into the variability in biological response to foods and nutrients, and may explain inconsistent study results in the field of nutrition research.
- The study of diet–gene interactions can elucidate the function of both genes and nutrients and their roles in the pathophysiology of disease.
- There is a common polymorphism in the haptoglobin gene that can influence an individual's status of vitamin C, vitamin E, and iron.

Keywords Allele • Diet–gene interaction • Gene • Genetic variation • Genotype • Haptoglobin • Nutrigenomics • Nutritional genomics • Phenotype • Polymorphism

Abbreviations

A	Adenine
C	Cytosine
CHD	Coronary heart disease
CNV	Copy number variant

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_7

DNA	Deoxyribonucleic acid
G	Guanine
GWAS	Genome-wide association study
Hb	Hemoglobin
HDL	High-density lipoprotein
Hp	Haptoglobin
LDL	Low-density lipoprotein
MI	Myocardial infarction
PKU	Phenylketonuria
RDA	Recommended dietary allowance
SNP	Single nucleotide polymorphism
T	Thymine

Introduction

Many disease states are independently influenced by both diet and genes. Dietary and genetic factors can also act synergistically to have a joint effect on human health through diet–gene interaction. For example, the protein products of genes (enzymes, transporters, receptors, hormones, etc.) may interact with nutritional constituents of foods to influence metabolic processes and health status. Commonly occurring polymorphisms in the genes that code for these protein products can lead to differences both in the amount of protein produced and in how efficiently that protein functions, thus leading to individual differences in processes such as digestion and metabolism. Among researchers and healthcare practitioners, there is a growing interest in utilizing genetic information to predict and manage the large interindividual differences in response to intake of food and nutrients. Recent advances in human genomics have enabled cost-effective and rapid detection of variations in genes affecting nutrient metabolism, but their full impact on nutrient requirements remains to be elucidated. The common haptoglobin polymorphism is an example of a polymorphism which may have an important influence on chronic disease via its impact on nutrient status. The objective of this chapter is to introduce the concept of diet–gene interactions and to discuss the various effects that genes can have on metabolic responses to food, focusing on the common haptoglobin polymorphism as an example.

Diet–Gene Interaction Nomenclature and Terminology

The study of diet–gene interactions has been given several names, including ***nutrigenomics*** and ***nutritionalgenomics*** [1–3]. These names are usually used as umbrella terms for two complimentary approaches: how nutrition affects gene function, and how genetic variation affects nutritional response or status. The latter also includes the study of how genetic variations affect food intake and eating behaviors [4, 5].

Training in genetics is not required to understand the field of diet–gene interaction, but knowledge of a few simple genetics terms and concepts is important. A ***gene*** is a segment of deoxyribonucleic acid (DNA) that contains the instructions for making a specific protein in the human body such as an enzyme, hormone, transporter, or receptor. Many genes exist in different forms depending on the sequence of ***nucleotides*** (A: adenine; C: cytosine; G: guanine; and T: thymine) that encode them. A single gene may be thousands or hundreds of thousands of nucleotides long. Over many generations

of cells replicating and being exposed to environmental pressures, changes in sequences of nucleotides occur, and they are called ***polymorphisms*** ("*poly*" means many while "*morph*" means form). A ***single nucleotide polymorphism*** (SNP, pronounced "snip") represents a single nucleotide difference (for example a C nucleotide replacing an A nucleotide) in a gene. Some genes have large segments of nucleotides that are missing (referred to as "deleted") or have additional nucleotide sequences that are inserted or repeated. Repeated polymorphisms are often referred to as ***copy number variants*** (CNVs). Most polymorphisms have been given a unique number called an "rs number" to serve as an identifier in research papers and in commonly used databases that catalogue all known human genetic variation, such as the Single Nucleotide Polymorphism Database (known as "dbSNP") [6]. Research studies can use a ***candidate gene approach*** to test a hypothesis about a specific polymorphism or set of polymorphisms in one gene, or can assess millions of SNPs at once in a ***genome-wide association study*** (GWAS).

The different forms of a polymorphism at a particular location on a gene are called ***alleles***. For each polymorphism there are usually two possible alleles (for example: A or C, present or deleted, repeated or not repeated). We inherit two alleles, one from each parent. Therefore, there are usually three possible versions of a gene, which are called ***genotypes*** (for example: AA, AC, and CC; or present/present, present/deleted, and deleted/deleted). In most cases, a change in a nucleotide or in small sections of nucleotide do not change the actual function of the gene and thus while they have an rs number and are catalogued, they have no known change in human physiology. However, some SNPs, especially those located in coding regions of genes, do impact function. Genes function to produce proteins, and the different genotypes for a given polymorphism may produce proteins that function differently from one another. These functional differences in proteins can alter the metabolism of nutrients, thus producing different ***phenotypes***. The phenotype is the resulting observable physical or biochemical characteristic, and it can be determined by both genetic makeup and environmental influences. A health outcome is considered to be ***monogenic*** if it is related to one single gene, and is called ***polygenic*** if it is influenced by multiple genes.

Genetic Variations Influence Response to Diet

The daily ingestion, absorption, digestion, transport, biotransformation, and excretion of nutrients and food bioactives by humans involve many proteins such as enzymes, receptors, transporters, ion channels, and hormones. Polymorphisms in the genes encoding these proteins can alter both the amount of the protein produced and how efficiently that protein functions. Therefore, there are many potential ways through which nutritional status may be affected by genetics (Fig. 7.1).

Genetics can influence our food preferences through taste (Table 7.1). For example, the gene *TAS2R38* codes for a bitter taste receptor located in the tongue's taste buds and has a common polymorphism that leads people to experience bitter foods such as Brussels sprouts differently. For some people, Brussels sprouts are incredible bitter, while others cannot taste the bitterness. This discrepancy is mostly due to an individual's ability to taste the bitter compounds phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), an ability that is strongly influenced by *TAS2R38* genotype and by age (bitter sensitivity decreases with age) [7, 8]. Bitter taste sensitivity and subsequent rejection of bitter foods likely evolved to prevent humans from ingesting the many poisonous bitter compounds found in plants. In addition to humans' large and diverse family of bitter taste receptors, common polymorphisms have also been identified in genes involved in perception of other tastes, such as sweetness, and so genetics can lead to differences in food preferences [5]. Genetic variability in taste could therefore affect food choices and dietary habits, which may subsequently influence nutritional status, health standing, and the risk of chronic disease.

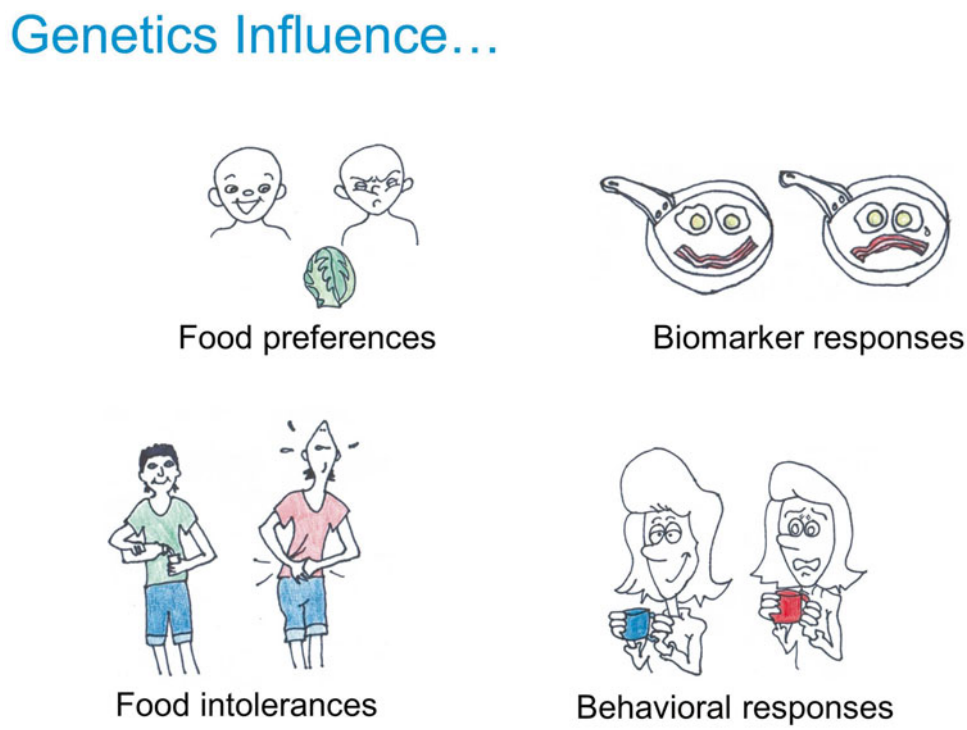

Fig. 7.1 How genetics can influence our response to food and beverages

Table 7.1 Examples of genes that cause interindividual response to diet

Example	Gene	Cause and effect
Food preference	*TAS2R38* is the gene for a bitter taste receptor in the mouth that can taste the bitter compounds phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP)	Genetic variation causes some people to taste the bitter compounds in foods such as Brussels sprouts that other people cannot taste
Food intolerance	*MCM6* is a gene that regulates *LCT*, the gene for lactase (the digestive enzyme that breaks down the milk sugar lactose)	Variation in *MCM6* causes some people to be able to produce lactase and therefore digest milk, while others do not produce enough lactase and subsequently feel considerable discomfort after consuming milk
Behavioral response	*ADORA2A* is the gene for the adenosine A_{2A} receptor, which is a primary target of caffeine in the central nervous system	Genetic variation in the adenosine receptor is associated with caffeine-induced anxiety
Metabolic response	*CYP1A2* is the gene for the CYP1A2 protein, which is the primary enzyme that metabolizes caffeine in the body	Different *CYP1A2* genotypes have different amounts of CYP1A2 enzyme activity and can thus be used to classify people as fast or slow caffeine metabolizers
Biomarker response	*LDLR* is the gene that encodes the low-density lipoprotein (LDL) receptor, which normally removes LDL from the body's circulation	Variation in the *LDLR* gene can cause familial hypercholesterolemia, which is characterized by high concentrations of LDL cholesterol at a young age, and so a diet low in cholesterol is prescribed

Genetic variation can explain food intolerances, and a well-known example is lactose intolerance. Lactose intolerance is caused by limited production of lactase, the enzyme that breaks down lactose (the primary sugar found in milk) due to genetic variation in the gene that codes for lactase [9]. Lactose intolerance is considerably more common globally than lactase persistence, which is the ability of adult humans to digest lactose. Lactase persistence is thought to be a dominant Mendelian trait developed through a natural selection process that favored lactase-persistent individuals in populations in which dairy products were a food source. Lactose persistence has been traced to a number of polymorphisms that occurred independently in different parts of the world, and it is likely that more lactase persistence-causing polymorphisms remain to be discovered [10]. The polymorphisms linked to lactase persistence that have been identified have not been in the lactase gene (*LCT*), but rather are in the gene *MCM6* which contains two of the regulatory regions for *LCT* [10]. Individuals with lactose intolerance can feel substantial gastrointestinal distress when then consume dairy products, while individuals with lactose persistence do not experience the same discomfort [11].

We have all experienced examples of how peoples' behavior can react differently to caffeine, the most widely consumed stimulant in the world. After drinking a cup of coffee, some people experience an elevated mood and feel calm, while other people become jittery and anxious. A review of twin studies reveals that genetics play a role in individual variability in caffeine consumption and also in determining the direct effects of caffeine [12]. Genome-wide association studies (GWAS) have linked variations in adenosine and dopamine receptor genes to caffeine-induced anxiety and sleep disturbances [12]. In particular, a common polymorphism (rs5751876) in the adenosine A_{2A} receptor gene (*ADORA2A*) has been linked to caffeine-induced anxiety [13], which is likely the cause for the repeated observation that this polymorphism is associated with the amount of caffeine that individuals choose to consume [14, 15]. These findings fit with biology because many of the physiologic effects of caffeine are mediated through the antagonism of the adenosine A_{2A} receptor, which is a primary target of caffeine in the central nervous system.

In addition to the behavioral differences that people experience after consuming caffeine-containing foods and beverages, there are genetic differences in the speed of caffeine metabolism. The clearance of caffeine can vary up to 40-fold within and between individuals, due to factors that include medications, smoking, and genetics [12]. Caffeine is mainly metabolized by the cytochrome P450 1A2 (CYP1A2) enzyme, and a common polymorphism in the *CYP1A2* gene influences the rate of caffeine metabolism. Individuals can be classified as either "rapid" caffeine metabolizers (those who are homozygous for the rs762551 A allele and therefore have the AA genotype) or "slow" caffeine metabolizers (carriers of the rs762551 C allele and have either the AC or the CC genotype). The difference in rate of caffeine metabolism is of interest because a Costa Rican study reported that high coffee consumption is associated with an increased risk of myocardial infarction (MI) only among individuals with slow caffeine metabolism [16]. No attempts to replicate this finding in other populations have been published since, but individuals with the CYP1A2 slow caffeine metabolism genotype who consume a lot of coffee have been reported to have high risk of risk factors for MI, such as hypertension [17] and impaired fasting glucose [18]. Before the incorporation of genetic data into the study of coffee and risk of MI, the association between coffee intake and MI had been inconsistent and controversial, and distinguishing between the effects of caffeine and other compounds found in coffee had been difficult given the strong association between caffeine and coffee intake in many populations. However, the discovery that intake of coffee was associated with an increased risk of MI only among individuals with the slow caffeine metabolism *CYP1A2* genotype [16] suggests that caffeine is the compound that increases risk of MI since it is the only major compound in coffee that is known to be detoxified by the CYP1A2 enzyme.

As a final example, genetics can influence blood biomarkers in response to food. For instance, blood concentrations of cholesterol are commonly measured and tracked as a risk factor for cardiometabolic disease, and people differ in the speed and extent to which their cholesterol concentrations rise or fall in response to different dietary fats. Some monogenic conditions such as familial hypercholesterolemia already have customized dietary recommendations to decrease intake of saturated fat and cholesterol

under the supervision of a dietitian [19]. Familial hypercholesterolemia is characterized by high cholesterol levels, specifically very high levels of low-density lipoprotein (LDL) cholesterol. Multiple polymorphisms can cause familial hypercholesterolemia, most notably variation in the *LDLR* gene that encodes the LDL receptorprotein (which normally removes LDL from circulation) or variation in the *APOB* gene that encodes apolipoprotein B. These forms of familial hypercholesterolemia are extreme cases of a monogenic condition that affects only a minority of people. However, there is also considerable evidence of wide interindividual variation in the lipid and lipoprotein responses to dietary intervention, suggesting that common genetic variations in multiple genes influence the blood lipid response to food intake [20]. For example, research has identified several interesting polymorphisms that may modify blood cholesterol concentrations in response to dietary fat, within cholesterol-related genes such as the cholesterol ester transfer protein gene (*CETP*) [21], the hepatic lipase gene (*LIPC*) [22, 23], and lipoprotein lipase (LPL) gene [24]. However, results of research in this complex area of lipid metabolism have been inconsistent, as is usual when multigenic metabolic pathways intertwine. It remains undetermined as to whether recommendations for dietary intake will ever be genotype specific in order to manage polygenic outcomes such as hypercholesterolemia that is not familial.

Thinking about the interindividual differences in response to food intake leads one to wonder about how nutrition study results are influenced by the genetics of participants.

The Application of Genetics to Nutrition Research

Studies in the field of nutrition have taught us much about the etiology of disease, but often have yielded inconsistent results. Nutrition studies are notoriously difficult to conduct, due in part to issues surrounding experimental study design and measurement error in dietary assessment. The mechanisms responsible for the between-person differences in dietary response are very complex and often poorly understood, likely in part due to genetic heterogeneity in study participants. Intake of a specific amount of a food or nutrient does not necessarily result in the same response (in blood concentrations of a biomarker for example) because substantial individual variability in the absorption, distribution, metabolism, and elimination of food and nutrients can exist. For example, in addition to sun exposure (a primary source of endogenous production of vitamin D) and dietary vitamin D, polymorphisms in genes that code for the vitamin D binding protein and vitamin D receptors have been associated with circulating levels of vitamin D [25–28]. Therefore, applying genetics to the field of nutrition and thus studying diet–gene interactions is a way to reduce the bias associated with variability in response to foods and nutrients consumed, and can explain inconsistent study results in the field of nutrition research. Genetic association studies that link polymorphisms to health outcomes have also yielded inconsistent results [29], even though genetic variants can be measured much more reliably and accurately than dietary exposures. Thus, not only can inconsistencies among nutrition studies be reconciled by the incorporation of genetic data, but contradictory gene-association studies could also be explained by incorporating measures of environmental exposures such as diet [30]. Additionally, as discussed in previous sections of this chapter, applying genetics to nutrition research can identify the specific bioactive or nutrient in a food that is behind the biological effect observed, and can elucidate the biological mechanisms at play at the molecular level. In addition to these strengths of applying genetics to nutrition studies, there are also limitations. In general, studying diet–gene interactions often requires large study populations to disentangle the associations between diet and the health outcome for each of the different genotypes. Additionally, because genetic information can be used to identify individuals, additional ethical approval is necessary to include genetic analysis in medical studies (Fig. 7.2).

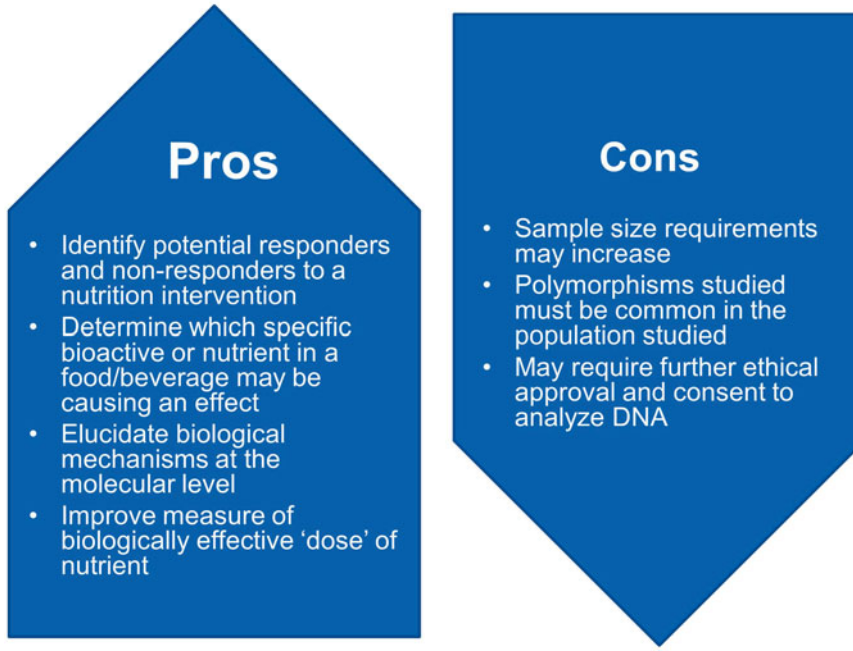

Fig. 7.2 Pros and cons of incorporating genetic variation into nutrition studies

The Application of Genetics to Dietary Recommendations

There already exist some evidence-based dietary recommendations that are constructed based on genetics. Phenylketonuria (PKU) is a classic example. PKU can result from a polymorphism in the gene for phenylalanine hydroxylase (the enzyme needed to convert phenylalanine to tyrosine) that results in a decrease in phenylalanine hydroxylase activity [31]. Individuals with PKU can develop neurological damage and experience seizures from excess phenylalanine [32] unless they follow the recommended low-phenylalanine diet [33]. As previously mentioned in this chapter, some rare forms of monogenic dyslipidemia are associated with specific dietary prescriptions, such as a diet low in saturated fat and cholesterol among individuals with familial hypercholesterolemia [19], and individuals with lactose intolerance are recommended to avoid lactose-containing foods and beverages. Unlike these examples, however, many nutrition-related chronic diseases are polygenic, having complex etiologies that often take years or decades to develop and may involve hundreds of gene products which could theoretically be influenced by thousands of polymorphisms. Therefore, it may take very sophisticated bioinformatics techniques to determine the joint effects of diet and genes in the development of complex chronic diseases such as diabetes, osteoporosis, cancer, and cardiovascular disease. The extent to which genetics will continue to be incorporated in nutrition therapy in the future remains unknown, but examples already exist where revealing genetic information to study participants in a clinical trial of personalized nutrition resulted in greater changes in intake for some dietary components compared to general population-based dietary advice [34].

The Common Haptoglobin Polymorphism

Haptoglobin is a circulating acute phase protein that rises in response to inflammation. However, the primary function of haptoglobin is to bind free hemoglobin [35] and thereby prevent heme-driven oxidative damage [36]. There is a common CNV polymorphism (rs72294371) in the haptoglobin gene (*HP*) with genotype frequencies that differ worldwide [37]. This polymorphism has been associated with risk of infections, autoimmune diseases, and chronic diseases including incident cardiovascular disease, suggesting broad clinical significance [38]. The CNV polymorphism in the haptoglobin gene consists of two structurally different alleles, Hp1 and Hp2. A large DNA segment of ~1700 nucleotides is duplicated in the Hp2 allele, but not in the Hp1 allele, and this difference in allele length results in structurally and functionally different proteins being formed by each of the three common genotypes: Hp1-1, Hp2-1, and Hp2-2 [39].

The small linear haptoglobin protein made by the Hp1-1 genotype is biologically the most active. The heterozygote genotype, Hp2-1, produces a medium-sized protein that is moderately active, and Hp2-2 homozygote individuals produce a protein that is large in size, cyclic in structure, and biologically the least active. These varying levels of activity are due to the ability of the small, linear Hp1-1 protein to enter intracellular and extravascular spaces more easily than the Hp2-2 protein, which is restricted by its higher molecular mass and cyclic shape. The phenotypes and genotypes for this polymorphism show full concordance [40], and are presented in Table 7.2.

Haptoglobin has several functions in the human body (Fig. 7.3). While both Hp1-1 and Hp2-2 proteins are able to bind free hemoglobin, the Hp1-1 protein has been shown to be superior to the

Table 7.2 The genotypes of the common haptoglobin polymorphism

	Haptoglobin (Hp) genotypes		
	Hp1-1	Hp2-1	Hp2-2
Shape	Dimers	Linear polymers	Cyclic polymer
Molecular weight	Lightest	Medium	Heaviest
Antioxidant capacity	Highest	Medium	Lowest

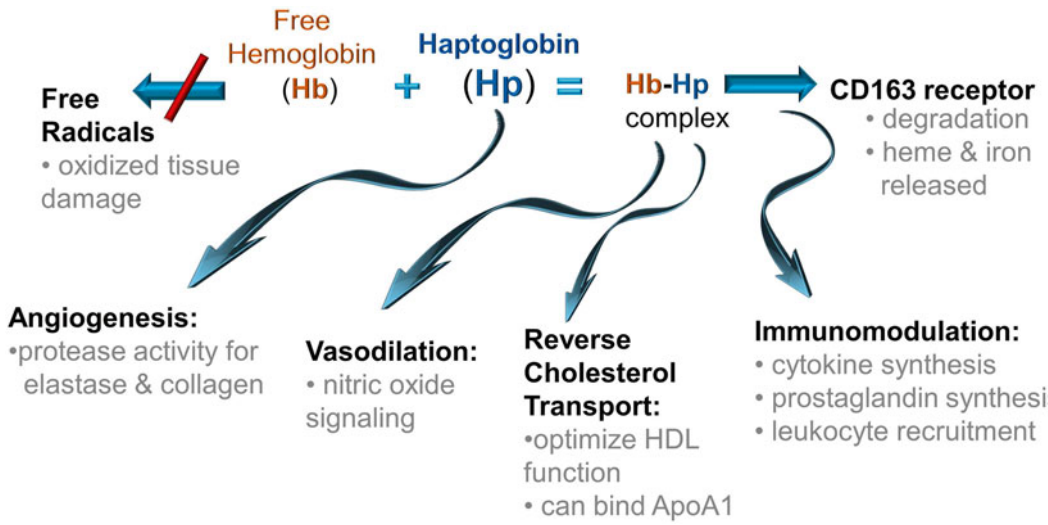

Fig. 7.3 Functions of the haptoglobin (Hp) protein

Hp2-2 protein in mediating the clearance of hemoglobin via the CD163 pathway, which is the only means of clearing hemoglobin in the extravascular compartment [41]. As the Hp-Hb complex is cleared by CD163, beneficial immunomodulatory effects can occur, although the exact roles of haptoglobin in immunomodulation are still unknown. The haptoglobin–hemoglobin complex is also involved in reverse cholesterol transport, promoting the movement of cholesterol from tissues to the liver for excretion [42]. One final set of functions of haptoglobin, which is also not well understood yet, is that it promotes angiogenesis [43] and vasodilatation [44].

Interestingly, the different haptoglobin genotypes offer different forms of protection. The haptoglobin polymorphism is posited to have arisen early in human evolution from a selective advantage of Hp2 against malaria and various forms of infectious disease, but in modern times may confer increased risk of several noninfectious, inflammatory, and chronic disease complications [45]. The weaker antioxidant capacity of the Hp2-2 haptoglobin protein creates a more pro-oxidant environment in which many microorganisms that cause illness are vulnerable and less likely to thrive. However, in the case of noninfectious diseases, the lower antioxidant activity of the Hp2-2 genotype can create metabolic disturbances and tissue damage, which has led to the hypothesis that the Hp2-2 genotype may be a risk factor for chronic disease characterized by inflammation and oxidative stress. Not only is the Hp2-2 protein inferior to the Hp1-1 protein in preventing the oxidation of a variety of lipid and protein substrates by free hemoglobin [33, 46], but also the dysfunctional haptoglobin protein produced by Hp2-2 individuals leads to reduced ability of HDL to promote reverse cholesterol efflux, and to plaque instability in several in vitro and in vivo systems [33, 35, 41, 47, 48]. These dysfunctions evident in the Hp2-2 protein are accentuated when hemoglobin is glycosylated [35, 41], which commonly occurs in individuals with diabetes mellitus or pre-diabetes. Further, the complex formed by glycosylated hemoglobin and 2-2 haptoglobin can become a pro-atherogenic, pro-inflammatory compound [49], oxidatively modifying the HDL of Hp2-2 individuals with elevated blood sugar, and resulting in increased oxidative damage that increases susceptibility to atherosclerosis and the deterioration of cardiac function [41].

The Hp2-2 genotype has been observed to be a significant predictor of CHD among individuals with elevated HbA_{1c} (HbA_{1c} is glycosylated hemoglobin, and a blood concentration $\geq$6.5 % is a marker of high blood glucose over time) in multiple populations [50]. The Hp2-2 genotype has also been associated with increased risk of (or severity of) other health outcomes, including chronic kidney disease [51], lupus [52], celiac disease [53], anemia [54], and inflammatory bowel disease [55]. Meanwhile, the Hp1-1 genotype has been reported to be an independent predictor for malaria [56] and some clinically significant bacterial infections [57]. However, research into the haptoglobin polymorphism is sometimes inconsistent and complicated, potentially due to bias of survivorship, as suggested by studies inferring increased longevity among individuals with the Hp1-1 genotype [58, 59]. Further, the haptoglobin polymorphism is a CNV that is not included in currently available versions of GWAS technologies, and nor is it currently "taggable" by SNPs [60], so it is not incorporated in current GWAS analyses of health outcomes. Substantial further research is required to fully understand the complexities of the associations between this polymorphism and various health outcomes. Future research will include assessing interactions with diet in order to maximize nutritional status and reduce disease risk.

Haptoglobin Genotype Can Influence Nutritional Status

Nutritional status is influenced by several factors, including body size, age, sex, smoking status, diet, and genetics. The common haptoglobin genotype has been repeatedly and consistently associated with three nutrients: vitamin C, vitamin E, and iron. Because the three haptoglobin genotypes produce varying levels of functioning haptoglobin protein, the antioxidant cycles and pathways (including the oxidation and reduction of vitamin C) are differently affected. For example, when the antioxidant

function of haptoglobin is insufficient, vitamin C may act in its place, subsequently diminishing blood concentrations of vitamin C [61]. By studying the interactions between the haptoglobin genotypes and dietary variables, it is plausible that we can elucidate how to use diet as a tool by which we can decrease the burden of disease and optimize health for each genotype.

Vitamin C

Vitamin C (ascorbic acid) is an essential nutrient and a strong reducing agent that inhibits oxidative damage [62]. Recent studies show that deficient blood (or serum) concentrations of ascorbic acid are common in many parts of the world, including North America [63, 64] and the UK [65, 66]. These high deficiency rates are a concern because an inverse relationship has been observed between serum ascorbic acid concentrations and several markers of chronic disease including glucose homeostasis [67], blood pressure [68], oxidative stress [69, 70], high sensitivity C-reactive protein [71], and indicators of obesity such as body mass index (BMI) and waist-to-hip ratio [69]. Serum ascorbic acid is also inversely associated with risk of cardiovascular disease [72, 73], diabetes [67] and all-cause mortality [74]. However, the findings of studies on dietary vitamin C and the prevention of chronic diseases remain inconsistent and controversial [75], potentially because of individual variability in serum ascorbic acid response to dietary vitamin C.

Haptoglobin is a glycoprotein that functions to bind to the free hemoglobin molecules formed after hemolysis, which would otherwise cause iron-mediated generation of free radicals [76]. However, if haptoglobin is not available or functional, then vitamin C will serve the same antioxidative function and become dehydroascorbic acid (Fig. 7.4), thereby lowering serum ascorbic acid concentrations [61]. Both in vivo and in vitro experiments have shown a significantly lower concentration and decreased stability of ascorbic acid in blood taken from subjects with the Hp2-2 genotype compared to those who carry the Hp1 allele [76–78]. The capacity of Hp2 to inhibit oxidation and vitamin C depletion is less than that of Hp1, and thus the Hp2-2 genotype has the lowest capacity to inhibit oxidation and vitamin C depletion [79].

The observation that the haptoglobin polymorphism is an important non-nutritional modifying factor in the pathogenesis of vitamin C deficiency has contributed to a hypothesis that the success of long-range human migration has been partially determined by the haptoglobin polymorphism, through the natural selection of populations characterized by high Hp1 allele frequencies who would have been less prone to scurvy [79]. The advantage of the Hp1-1 genotype as a genetic factor favoring survival in long-distance sea voyages is illustrated by the haptoglobin genotype distribution among the indigenous populations of remote islands. For example, Easter Island is one of the remotest places

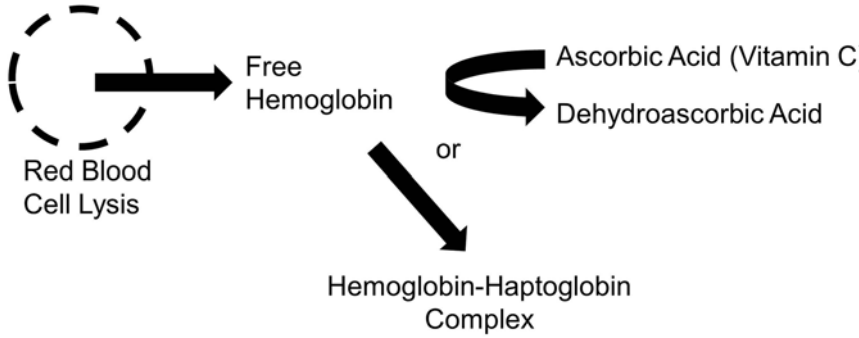

Fig. 7.4 Haptoglobin prevents the hemoglobin-related oxidation of vitamin C

on earth, and its indigenous Rapa Nui population is characterized by the highest Hp1 allele frequency known (0.86) [61]. Unquestionably, early inhabitants of Easter Island would have been subject to challenging vitamin C depletion (scurvy) during their long voyage there. Among Western European populations, Hp1 and Hp2 allele frequencies are ~0.40 and 0.60, respectively [45]. In human evolution, the Hp2 allele originated in Asia, which explains why the highest Hp2 and the lowest Hp1 allele (~0.25) frequencies are found in South and East Asian populations. Historical documents note that Chinese and Japanese sailors were particularly prone to scurvy [80, 81], and it has been hypothesized that this susceptibility accounts for why Europeans settled the Americas before Asian countries could, despite the advanced sailing technologies of Asian countries [61].

Because of the differences between the haptoglobin genotypes in susceptibility to scurvy, the idea of genotype-specific recommended dietary allowances (RDA) for daily vitamin C intake has been suggested [79]. However, only one study to date has investigated this hypothesis, and it reported that individuals with the Hp2-2 genotype had increased odds of serum ascorbic acid deficiency if they did not meet the RDA for vitamin C (while carriers of the Hp1 allele did not) [82], but that as long as the RDA for vitamin C (75 mg/day for women, 90 mg/day for men, with an additional 35 mg/day recommended for smokers) was met, vitamin C deficiency was prevented in all three genotypes [82]. Clinical trials using doses of vitamin C well above the RDA have reported no additional genotype-specific benefit [83, 84]. Taken together, these findings suggest that the differences in vitamin C status between haptoglobin genotypes are only present when dietary vitamin C is inadequate.

Vitamin E

Similar to vitamin C, vitamin E is an antioxidant that inhibits oxidative damage in the human body. Although no studies published to date have directly assessed blood concentrations of vitamin E in relation to the common haptoglobin polymorphism, it has been hypothesized that levels of vitamin E could be depleted in the Hp2-2 genotype if vitamin E performs the antioxidant functions in place of haptoglobin [85]. Studies of vitamin E in relation to the haptoglobin polymorphism have focused on the use of supplemental vitamin E in the prevention of diabetic cardiovascular events [86], as the Hp2-2 genotype carries a high risk of cardiovascular events in diabetic patients. It has been reported that vitamin E significantly improves the quality of HDL in Hp2-2 diabetic individuals [49]. A meta-analysis of the medical literature (5 studies) suggests that a pharmacogenomic approach towards treatment of Hp2-2 diabetic patients with vitamin E may be warranted [87].

Iron

Iron status is affected by the haptoglobin polymorphism, because the Hp2-2 genotype is least efficient in the clearance of hemoglobin from circulation, and consequently Hp2-2 individuals show iron retention in macrophages and present higher serum iron and ferritin concentrations and increased transferrin saturation compared with the other haptoglobin phenotypes [88]. This effect is strongest in males, as iron is generally more abundant in men than in women. The association between the haptoglobin genotype and iron status partially explains why the haptoglobin polymorphism is related to the prevalence and outcome of various pathological conditions with altered iron metabolism, such as hemochromatosis, infections, anemia, and atherosclerotic vascular disease [54, 89]. However, to the best of our current knowledge, no studies to date have investigated whether there is an interaction between dietary iron and haptoglobin genotype on the risk of health outcomes. So it is unknown whether optimal dietary recommendations for iron intake could be genotype specific.

Conclusion

Genetics can interact with diet to modulate nutritional status. An understanding of these diet–gene interactions has the potential to support disease prevention through the optimization of dietary recommendations. However, the extent to which genetics will be incorporated into nutrition therapy and health promotion currently remains unknown. The study of diet–gene interactions has emerged as a rapidly developing research area, and the replication of diet–gene interaction findings in multiple populations and study designs is necessary. Regardless of whether genotype-specific dietary recommendations will be developed outside of monogenic conditions like lactose intolerance and PKU, the incorporation of markers of genetic variation, such as the common haptoglobin polymorphism, into studies of nutrition and health will assist with some of the main challenges and goals of nutrition research, including coping with the inter-variability in response to diet, and providing sound biological and mechanistic evidence linking diet to health.

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Part II
Cancer Prevention

Chapter 8
The Role of Diet and Nutrition in Lung Cancer

Anthony J. Alberg and John M. Wrangle

Key Points

- Cigarette smoking is the single major determinant of lung cancer risk.
- Cigarette smokers are more likely to have unhealthful diets than nonsmokers, which makes it complex to study nutrition and diet in relation to lung cancer.
- Higher dietary intakes of fruits, and to a lesser extent vegetables, are inversely associated with the risk of lung cancer.
- Factors with weak evidence of association with increased risk of lung cancer include consumption of red and processed meat, total fat, and lower body mass index.

Keywords Lung cancer • Epidemiology • Cigarette smoking • Tobacco • Diet • Nutrition • Fruits • Vegetables • Micronutrients • Chemoprevention • Body mass index • Beverages

Introduction

Lung cancer is currently the leading global cause of cancer death, accounting for 18 % of all cancer deaths [1]. The global lung cancer epidemic is largely due to a single predominant cause: cigarette smoking. In addition to cigarette smoking and other forms of combustible tobacco, many other environmental risk factors and clinical risk indicators for lung cancer have been identified. The potential influence of dietary factors on lung cancer risk has been a topic of considerable interest in recent decades, and a substantial body of evidence has now been generated on this topic.

Descriptive Epidemiology of Lung Cancer

Lung cancer is a significant public health problem. In the USA, lung cancer is the third most common form of cancer and is by far the leading cause of cancer mortality. Approximately 224,000 new cases of lung cancer were diagnosed in the USA in 2014 [2]. By itself, lung cancer accounts

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_8

for as many deaths as the next four leading causes of cancer death (breast, prostate, colorectal, and pancreatic cancer) combined [2]. Lung cancer is the leading cause of cancer death among both men and women [3]. Lung cancer is a leading global health problem as the leading cause of cancer death in world [1].

The high mortality rate for lung cancer is a function of a high incidence rate combined with a poor survival rate. The 5-year relative survival rate for lung cancer has improved only slightly during the past decades, increasing from 14 % in 1985–1989 to 18 % in 2004–2010 [3]. Five-year relative survival is considerably better for local disease (54 %) compared to a lung cancer diagnosis with regional spread (27 %) or distant disease (4 %) [3].

Lung cancer rarely occurs in individuals younger than 45 years of age, but the incidence rates start to increase among those 45–64 years old and peak in the elderly, with rates of 272 per 100,000 or higher for all age groups 65 and older [3]. Men have greater risk of developing and dying from lung cancer than women. This is not due to an inherent difference in susceptibility to lung cancer risk factors, but rather due to gender differences in historical patterns of smoking prevalence and exposure to other lung cancer risk factors [4]. African Americans have the highest lung cancer incidence rates of any racial/ethnic group, which is due specifically to extremely high rates in African American men [5]. With respect to trends over time in the USA, there has been a steady decrease in the age-adjusted incidence rates (per 100,000) from 69.5 in 1992 to 56.6 in 2011 [3]. This decrease can largely be attributed to the decreased smoking prevalence that began in the mid-1960s [6].

Socioeconomic Status

Indicators of lower socioeconomic status (SES) such as lower income and education have been consistently associated with increased lung cancer risk. The association between lower SES and increased lung cancer risk was observed decades ago in the USA [7], but is also a consistent finding across countries and cultures [8–11]. Lower SES is associated with a high-risk profile for many factors associated with lung cancer risk including smoking and exposures to inhaled carcinogens in the workplace and general environment. Lower SES is also associated with less healthful diets [12]. Nutrition and dietary factors may therefore be a contributing factor to the disproportionate burden of lung cancer observed in the lower socioeconomic status groups.

Histopathology

As classified by light microscopy, the four major histologic types of lung cancer are adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and small cell carcinoma. All four histologic types are caused by cigarette smoking, but the magnitude of risks across types varies [13, 14]. Clinically, diagnosis is dichotomized into classifications of small cell (about 15 % of lung cancers) or non-small cell lung cancer (about 85 %) because small cell lung cancer has high metastatic potential to the central nervous system and, as opposed to non-small cell lung cancer, surgery is not the backbone of curative intent therapy.

The histologic characteristics of lung cancer have changed in recent decades, with an increase in adenocarcinoma and decrease in squamous cell carcinoma. The shift to adenocarcinoma is thought to be due to alterations in cigarette manufacturing over time leading to changes in (1) smoking-delivered carcinogens and (2) how cigarettes are smoked, such as depth of inhalation [14].

Beyond the histopathologic definitions of cancer, molecular characterization plays an increasingly important role in the definition of cancers of the lung and guiding its treatment. Adenocarcinoma of the lung is now routinely tested for mutations and genetic events in genes such as the epidermal growth factor receptor (*EGFR*) and translocations involving anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) which predict clinical sensitivity to oral tyrosine kinase inhibitors.

Risk Factors for Lung Cancer

Epidemiologic research has succeeded in identifying several environmental risk factors that are causally associated with the risk of developing lung cancer. A brief consideration of well-established risk factors for lung cancer is essential to provide context for interpreting the evidence on the potential role of diet and nutrition in human lung carcinogenesis.

Tobacco Products

Cigarette Smoking

Cigarette smoking is the predominant cause of lung cancer [15]. The link between cigarette smoking and lung cancer is so strong that after accounting for a latency period of approximately 20 years, smoking prevalence and rates of lung cancer track closely at the population level [16]. Cigarette smoking is primarily responsible for the worldwide lung cancer epidemic as it is associated with such enormous increases in risk of developing lung cancer and cigarette smoking is so common [17].

The risk of lung cancer associated with cigarette smoking follows clear dose-response relationships [14]. This remains true with respect to the number of cigarettes smoked per day and the duration of smoking. Lung cancer risk decreases in those who quit smoking compared to persistent smokers, but not to the level of those who never smoked [18].

Menthol is a flavoring agent added to cigarettes, and menthol cigarettes have been hypothesized to be associated with higher risk of lung cancer than non-menthol cigarettes. The evidence, however, does not support this hypothesis. Numerous case–control [19–23] and cohort [24–26] studies indicate that menthol cigarettes are not associated with a greater risk of lung cancer than non-menthol cigarettes.

The trends in the composition of cigarettes over time, such as the addition of filters and advertised lower tar and nicotine concentrations, would suggest the hypothesis that risks of lung cancer associated with cigarette smoking would have decreased over time. However, contrary to expectation, the smoking-associated risks of lung cancer have actually *increased* over time [27]. Three US cohorts were followed from 1959 to 1965, 1982 to 1988, and 2000 to 2010. Among women, the relative risks of lung cancer in current versus never smokers increased across these time periods from 2.7 to 12.6 to 26.2, respectively; among men, the relative risks increased from 12.5 to 25.3 to 27.3, respectively. The reasons underlying the increase in risk over time are uncertain, but are hypothesized to be related to changes in tobacco processing and additives.

Other Combustible Tobacco Products

Combustible tobacco products other than cigarettes are also associated with increased risk of lung cancer. Pipes and cigars are causally associated with lung cancer, even though the risks are less than observed for cigarette smoking due to differences in smoking frequency and smoking topography in

pipe/cigar smokers compared to cigarette smokers [15]. Pipe and cigar smoke contain a similar profile of harmful toxins as cigarette smoke, but a key determinant of the observed epidemiologic differences in health risks is that compared to cigarette smokers, the tendency is for pipe and cigar smokers to smoke less frequently and to inhale the smoke less deeply. As a result of the difference in the way pipes and cigars are smoked compared with cigarettes, the end result is that the dose of toxins delivered to the smoker is often less for pipes and cigars than for cigarettes [15].

Smoking tobacco through a waterpipe is referred to by a number of terms including "hookah." In a meta-analysis of data from four studies waterpipe tobacco smoking was significantly associated with increased lung cancer risk (OR 2.1; 95 % CI 1.3–4.2) [28]. The relatively sparse evidence thus far indicates that waterpipe tobacco smoking is associated with a significantly increased risk of lung cancer, but further research on this topic is needed.

Vaporized Nicotine Products

The marketplace for tobacco products and devices that deliver nicotine has been expanding during the past decade [29–32]. Vaporized nicotine products such as electronic cigarettes (or "e-cigarettes") are nicotine delivery devices that have experienced a rapid upsurge in use and are now marketed by the major US tobacco companies [29, 30]. There is currently a lack of data on whether these products are associated with lung cancer risk. A product such as the e-cigarette that would decrease delivery of tobacco toxins would ostensibly also reduce the risk of developing lung cancer if current cigarette smokers were to switch from cigarettes to exclusive use of the e-cigarette. By contrast, however, the risk of lung cancer could be increased if the e-cigarette maintained nicotine addiction and its users also continued to smoke cigarettes as well as use multiple products that deliver nicotine or if e-cigarette use served as a gateway to the uptake of combustible tobacco products such as cigarettes. Additionally, the risks of nicotine exposure are not eliminated by electronic nicotine delivery systems as nicotine itself has been reported to promote tumor growth and the nonuniform standard of manufacture of device refills and vaporizer delivery technology has led to concerns about the generation of delivery of carcinogenic materials and combustible toxins [33, 34].

Secondhand Tobacco Smoke Exposure

There is currently no "safe level" of exposure to cigarette smoke, as even the secondhand tobacco smoke inhaled by nonsmokers involuntarily in ambient air is causally associated with lung cancer [35]. As expected given the fact that the doses of exposure to cigarette smoke are much lower for secondhand smoke exposure than for active cigarette smoking, the risk of lung cancer is also much less than for active cigarette smoking. This association has had major policy implications such as by providing justification for smoke-free workplace legislation.

Factors Other than Tobacco

Smoking cigarettes and other combustible tobacco products are the major determinants of the population burden of lung cancer. However, many other risk factors for lung cancer have been identified. The most important of these are briefly summarized below.

Occupational Exposures

Numerous occupational lung carcinogens have been identified; the substances involved include radon, arsenic, asbestos, chromium, chloromethyl ethers, nickel, and polycyclic aromatic hydrocarbons [36, 37]. Synergism with smoking has been shown for several of these agents, such as asbestos and radon [38]. Many other agents are suspected occupational carcinogens.

Indoor and Outdoor Air Pollution

By exposing the lung to air contaminants from combustion sources that generate polycyclic aromatic hydrocarbons and radionuclides, outdoor air pollution is associated with increased risk of lung cancer [39]. Indoor air pollution is also associated with lung cancer risk. The sources and composition of lung carcinogen exposure in indoor air vary with the setting but may include radon, tobacco smoke, smoke from wood or coal burning, and cooking fumes [40–43].

Family History and Inherited Predisposition

A positive family history of lung cancer is a clinically useful risk indicator. In analyses of pooled data from 24 case–control studies, those with a positive history of lung cancer in a first-degree relative had a 1.5-fold increased risk of lung cancer (95 % CI 1.4–1.6). When analyses were limited to never smokers the association was weaker but still statistically significant (OR 1.3; 95 % CI 1.03–1.5) [44].

The observed familial aggregation of lung cancer suggests that genetic factors may influence susceptibility. There have been no clinically relevant high-risk mutations with high penetrance for lung cancer identified as with disease-conferring *BRCA1* and *BRCA2* mutations in breast cancer. However, data from genome-wide association studies (GWAS) have provided promising leads with respect to low-penetrance germline variants that are associated with small increases in lung cancer risk [45]. For example, the results of GWAS have been remarkably consistent in identifying genetic variants within a region on the long arm of chromosome 15 that are associated with lung cancer risk; those with at least one variant allele of a specific SNP in this region (rs8034191) had a 1.3-fold greater risk of lung cancer than those homozygous for the wild-type allele.

Physical Activity

In a consideration of the role of diet in relation to lung cancer it is important to consider the evidence on physical activity because it is also a lifestyle factor and because of the interrelationship between diet and physical activity. A meta-analysis of leisure-time activity observed that both moderate and high levels of physical activity were associated with a 13–30 % decrease in lung cancer risk [46]. In a meta-analysis of physical activity and lung cancer specific to smokers, the overall association was consistent with an 18 % reduction in risk; there was some heterogeneity in the magnitude of the associations across categories of physical activity, smoking intensity, and gender but the associations remained statistically significant in all of these subgroups [47]. In a thorough narrative review of the epidemiologic evidence, physical activity was consistently observed to be associated with a >20 % reduction in lung cancer risk across studies [48]. The mechanistic basis for how physical activity could protect against lung cancer remains to be established, but current hypotheses include (1) improved lung function accelerating clearance of

Table 8.1 Key risk factors and clinical risk indicators for lung cancer

A.	Single most important causal determinant of risk, strongest indicator of clinical risk[a]
	Active smoking of cigarettes and other tobacco products
B.	Other risk factors causally associated with lung cancer[a]
	Secondhand smoke exposure
	Ionizing radiation, including radon
	Occupational exposures, such as arsenic, chromium, nickel, asbestos, tar, and soot
	Indoor and outdoor air pollution
C.	Additional clinical risk indicators[b]
	The risk factors listed above, plus:
	Older age
	Male sex, particularly among those of African American ancestry
	Family history of lung cancer
	Acquired lung disease: COPD, tuberculosis, pneumoconioses, idiopathic pulmonary fibrosis, systemic sclerosis
	Occupational exposures, such as to silica dust
	Human immunodeficiency virus (HIV) infection

[a]The evidence for factors listed in these categories meet epidemiologic criteria for causality

[b]The factors included in clinical risk indicators are strongly associated with increased risk of lung cancer, but are listed in this category either because they are intrinsic patient characteristics (age, sex, ethnic ancestry, family history) or are consistently associated with increased risk but with evidence that falls short of being rated as causal at the present time

carcinogens; (2) reduced inflammation and enhanced immune status; and (3) changes in concentrations of growth factors [48].

An inverse and relatively strong statistical relationship between physical activity and lung cancer risk has clearly been documented. The inferences from this evidence will be strengthened if the specific biologic mechanism whereby physical activity reduces lung cancer risk can be pinpointed and the potential for residual confounding by cigarette smoking is more strongly addressed.

Clinical Risk Indicators for Lung Cancer

Several clinical risk indicators have been documented to be more prevalent in lung cancer patients than the general population. Even though the exact etiologic significance of these associations has yet to be clearly elucidated, these are useful clinically. In addition to a family history of lung cancer, increased risk of lung cancer has been noted for several acquired lung diseases. This includes obstructive lung disease, such as chronic obstructive pulmonary disease, as well as fibrotic disease, such as idiopathic pulmonary fibrosis and systemic sclerosis [36]. Further, the presence of infections, such as with tuberculosis or HIV, is associated with increased risk of lung cancer [36]. Known risk factors and clinical risk indicators for lung cancer are summarized in Table 8.1.

Diet and Nutrition

The background provided above has emphasized the known risk factors for lung cancer outside the domain of diet and nutrition. This background provides essential contextual information for considering the potential role of dietary factors in the etiology of lung cancer, highlighting the fact that many well-established risk factors for lung cancer have been identified (Fig. 8.1). The key message from

this background information is that cigarette smoking is far and away the leading cause of lung cancer. This is of central importance to a consideration of the role of diet and lung cancer for several reasons. Not only is cigarette smoking the overwhelming cause of lung cancer, but smokers also tend to eat less healthful diets than nonsmokers [49]. Cigarette smoking thus poses significant inferential challenges to establishing a clear role for dietary factors in the etiology of lung cancer. Specifically, this set of circumstances means that cigarette smoking is a major potential confounder that needs to be carefully considered when evaluating a potential role for dietary factors in relation to lung cancer risk, making it difficult to disentangle the potential impact of cigarette smoking on any observed association between a dietary factor and lung cancer (Fig. 8.2). Complicating matters further is that cigarette smoke can directly affect nutritional biomarkers; for example, smokers tend to have lower levels of circulating antioxidant micronutrients even after accounting for differences in dietary intake, implying that the oxidative stress from cigarette smoke leads to depletion of antioxidant micronutrients [49, 50]. Similar associations have even been noted for secondhand smoke exposure [50, 51].

Additionally, associations between dietary factors and lung cancer risk are likely to be far weaker than the association with active smoking. As discussed below, even for dietary factors that have a robust inverse association with lung cancer the associations are very weak compared to the strong increased risk caused by cigarette smoking. Thus, in interpreting evidence on the associations between dietary factors and lung cancer, residual confounding by cigarette smoking is not easily dismissed as a potential explanation. The facts that cigarette smoking is so closely intertwined with risk of lung cancer and with diet and biomarkers of diet underscore not only the complexities involved in studies of diet in relation to lung cancer, but also the imperative to control cigarette smoking as carefully as possible.

The potential role of dietary factors on risk of lung cancer has been extensively investigated. To provide a synopsis of the research in this area, evidence concerning relationships between lung cancer and fruits, vegetables, micronutrients, phytochemicals, fat, body mass index, beverages, and meat intake is described below. To provide a guide for assessing the evidence for each dietary factor, evidence ratings from an objective assessment of the world's evidence on these topics, summarized in a seminal 2007 report of the World Cancer Research Fund (WCRF), are used for factors that were assigned evidence ratings. The rating scale used included evidence ratings of "convincing," "probable," and "limited—suggestive" for whether a dietary factor was associated with increased or decreased risk of lung cancer. Key research published since the 2007 WCRF report are used to augment the review.

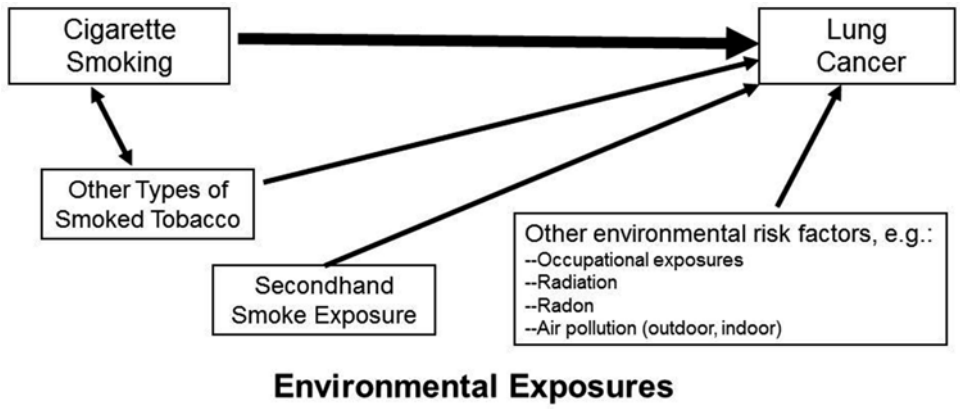

Fig. 8.1 Major established environmental causes of lung cancer, with active cigarette smoking the primary determinant of individual and population risk

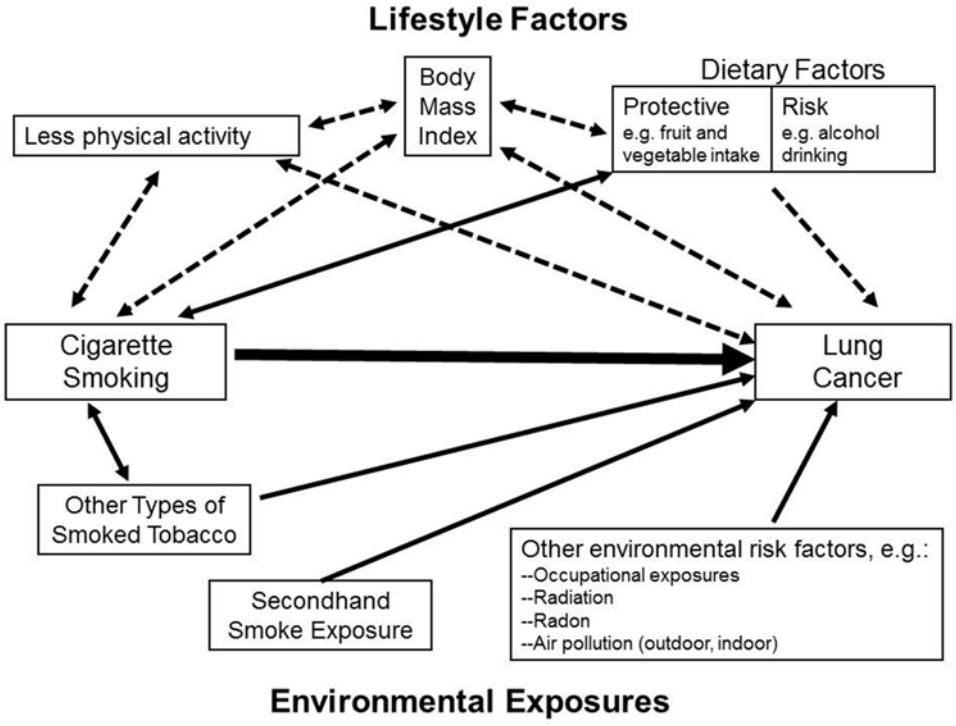

Fig. 8.2 A challenge to studying lifestyle factors such as physical activity and diet in relation to lung cancer risk is the complex interrelationships between these factors and cigarette smoking

Fruit Intake

In total, the epidemiologic evidence strongly points toward greater levels of fruit consumption being inversely associated with lung cancer risk. Based on a large and consistent body of data, the WCRF systematic review rated the evidence on this topic as "probable" that fruit consumption is associated with decreased risk of lung cancer [52]. Prospective cohort studies published since the 2007 WCRF report provide at least modest support for the premise that fruit consumption protects against lung cancer [53–55]. In the Shanghai Men's Health Study, any level of fruit consumption above the lowest fourth of total fruit intake was associated with a 24–25 % reduction in lung cancer risk [53]. In the European Prospective Investigation into Cancer and Nutrition (EPIC), the RR for the highest-versus-lowest fifth comparison was 0.80 (95 % CI 0.66–0.96, p-for-trend 0.01). In the NIH-AARP Diet and Health Study, the highest-versus-lowest fifth of total fruit consumption relative risk (RR) was 0.91 (p-for-trend 0.10) in men and 0.97 (p-for-trend 0.70) in women [55]. In the studies that stratified by smoking status, there was no clear pattern in the associations across the categories of never, former, and current smokers [54, 55]. In the EPIC study the results were stratified by histologic type, but no clear pattern emerged to suggest that fruit intake was clearly more strongly associated with specific histologic types of lung cancer than other histologic types [54].

In general, no clear pattern emerges when studies have examined specific fruits or classes of fruits. For example, in the studies cited above, berry intake was significantly associated with lower lung cancer risk in the EPIC Cohort [54] whereas in the Shanghai Men's Health Study a significant inverse association was observed for watermelon intake and a borderline association with citrus fruits [53].

Vegetable Intake

Paralleling the evidence for fruit consumption, the overall body of epidemiologic evidence suggests vegetable consumption is inversely associated with lung cancer risk. However, the results for a link between vegetable intake and lung cancer have been less consistent and the observed associations have been weaker than for fruit. Reflecting the more equivocal evidence that vegetable intake may exert protection against lung cancer, the WCRF report rated the overall evidence for vegetables as "limited—suggestive" [52].

Since the publication of the 2007 WCRF report, results of prospective cohort studies have been published in the same reports that catalogued results for fruit intake [53–55]. In the Shanghai Men's Health Study, any level of vegetable consumption above the lowest fourth of total vegetable intake was associated with a 10–12 % reduction in lung cancer risk that was not statistically significant [53]. In the European Prospective Investigation into Cancer and Nutrition (EPIC), the RR for the highest-versus-lowest fifth comparison was 0.96 (95 % CI 0.79–1.17, p-for-trend 0.58). In the NIH-AARP Diet and Health Study, the highest-versus-lowest fifth of total vegetable consumption relative risk (RR) was 0.93 (p-for-trend 0.08) in men and 1.05 (p-for-trend 0.23) in women [55]. The results of these three more recently published data from prospective cohort studies reinforce an overall body of evidence that demonstrates much less compelling evidence to support an inverse association between vegetable intake and lung cancer risk than which exists for fruit intake. This same observation was noted in a review limited to data from Japan [56].

In one study that stratified by smoking status a strong and statistically significant inverse dose–response trend was evident in former smokers [55], but this finding was not replicated in another study that stratified according to never, former, and current smokers [54]. In the EPIC study the results were stratified by histologic type and no clear pattern emerged to suggest a differential impact of vegetable intake by histologic type [54].

For specific classes of vegetables, such as cruciferous vegetables, the results have been more strongly and consistently associated with a reduced risk of lung cancer than for total vegetable intake. A systematic review and meta-analysis revealed a consistent body of evidence has been observed across studies indicating that cruciferous vegetable intake is inversely associated with lung cancer risk [57]. In studies published since the systematic review, the inverse association between cruciferous vegetable intake and lung cancer risk has persisted even when cigarette smoking has been carefully controlled for in the study design by matching [58, 59]. In the Shanghai Women's Health Study, a prospective cohort study, cruciferous vegetable intake was only marginally associated with lung cancer risk, with fully adjusted RRs across quartiles of 1.0, 0.81 (95 % CI 0.62–1.07), 1.00 (95 % CI 0.76–1.30), and 0.73 (95 % CI 0.54–1.00) [60]. As discussed below, the growing evidence of an inverse association between cruciferous vegetable intake and lung cancer risk has generated enthusiasm for isothiocyanates, bioactive phytochemicals abundant in cruciferous vegetables, as a promising chemopreventive agent.

Intake and Biomarkers of Micronutrients

The inverse associations observed between fruit and vegetable consumption and lung cancer risk spurred investigation into what the specific constituents may be that were linked to protection against lung cancer. Fruits and vegetables are the major dietary source of specific antioxidant micronutrients, and antioxidant micronutrients have been hypothesized to exert broad protection against malignancies by protecting DNA from the damaging effects of oxidative stress [50]. Two alternative strategies have been used to assess the potential role of micronutrients in lung cancer. One approach is to measure reported micronutrient intake using food-frequency questionnaires. A second approach is biomarker

based, measuring the circulating concentrations of micronutrients. When comparing the evidence provided by these two measurement approaches, food-frequency questionnaires provide a better average measure of micronutrient intake, whereas assaying circulating micronutrient concentrations provides a biological measure that is more proximal to the cellular level where the biologic effect is postulated to occur. However, circulating micronutrient concentrations will vary based on numerous factors including recent diet and thus are transient in nature and may therefore have limited relevance to the most biologically important exposure period. The strongest evidence for the biomarker approach is generated from prospective cohort studies, where blood is collected from a population that is initially cancer free and the population is then followed for the occurrence of lung cancer. For these reasons, these measurement approaches are best viewed as complementary.

The example of carotenoids exemplifies the complexities involved in attempting to determine the role of diet in the etiology of lung cancer. A systematic review of prospective studies of both dietary intake and prediagnostic blood concentrations indicated there was an inverse association between carotenoids and lung cancer [61]. For example, both dietary intake and circulating concentrations of total carotenoids were associated with 20–30 % lower risk of lung cancer in the highest-versus-lowest exposure categories [61]. In addition to these results for total carotenoids, prospective studies also showed that circulating concentrations of specific carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene) were consistently inversely associated with future lung cancer risk [61]. This body of evidence prompted the WCRF to rate foods containing carotenoids as "probable" protective factors for lung cancer [52]. However, it cannot be determined with certainty if the inverse association between carotenoids and lung cancer is directly due to carotenoid intake, or whether carotenoid intake merely serves as a marker of the intake of other protective substances or healthier dietary habits in general. As described in the section below on chemoprevention, this point is amplified by the fact that the results of large-scale randomized controlled trials conclusively demonstrated that β-carotene consumption is associated with increased risk of lung cancer in smokers [61].

The emphasis of this section on carotenoids is useful to illustrate the issues facing the study of micronutrients in relation to lung cancer in general. With respect to other micronutrients, for dietary intake the evidence is most abundant for vitamins A, C, and E. The evidence relating measures of retinol intake to lung cancer risk was rated by WCRF as providing "limited—suggestive" evidence that retinol is actually associated with increased risk of lung cancer [52]. The evidence for foods containing selenium was judged to be "limited—suggestive" of a protective association [52]. Studies of vitamin C have tended to consistently point toward an inverse association, but the evidence was judged insufficient for a conclusion by WCRF, as was the evidence for vitamin A, the B vitamins, and vitamin E/tocopherol [52].

With respect to updates of the evidence since the 2007 WCRF report, strong inverse associations were observed between serum B vitamin concentrations and subsequent lung cancer risk in the EPIC Cohort [62], particularly for vitamin B_6 and methionine. In the Melbourne Collaborative Cohort Study, dietary intake of riboflavin was inversely associated with lung cancer risk in current smokers (highest-versus-lowest RR 0.53 95 % CI 0.29–0.94) but not in never and former smokers; null associations were observed for the B vitamins or methionine [63]. In the Women's Health Initiative Cohort Study, a study of postmenopausal women, dietary vitamin D intake was not associated with lower lung cancer risk overall, but a statistically significant inverse association was observed in never smokers [64]. In the Shanghai Women's Health Study the RRs of lung cancer were 1.0 (referent), 0.87 (95 % CI 0.8–1.11), and 0.78 (95 % CI 0.58–1.07) according to the low, middle, and high thirds of dietary vitamin E intake (p-for-trend 0.12) [65]. These more recent results highlight the challenges to making strong inferences about the associations between many micronutrients and lung cancer. If associations are observed at all, they are not always statistically significant associations for the main results, and if associations are observed they may only emerge in specific population subgroups. Evidence of this nature makes it difficult to discern whether these associations are genuine or chance findings.

Phytochemicals

Phytochemicals are low molecular weight molecules produced by plants. Of the many classes of phytochemicals, those most commonly studied in relation to lung cancer include phytoestrogens, flavonoids, and glucosinoids.

The tumor promoting effects of steroid hormones can be blocked by phytoestrogens. Soya beans are a primary source of a specific class of phytoestrogens known as isoflavonoids. The relatively few studies to date of isoflavonoids in relation to lung cancer have not provided evidence of a link, and the WCRF report found the evidence too limited to reach a conclusion [52]. Data from the Shanghai Women's Health Study have since been published indicating a strong inverse association between soy food intake and lung cancer [66]. Yang et al. also carried out a meta-analysis of 7 case–control and 4 cohort studies of soy intake in relation to lung cancer and calculated a summary relative risk estimate of 0.83 (95 % CI 0.72–0.96) for the highest-versus-lowest category comparisons.

Flavonoids are polyphenolic compounds found in many foods derived from plants; flavonoids often exhibit potent antioxidant activity. Some fruits contain high levels of flavonoids, such as apples (quercetin) and white grapefruit (naringin). Flavonoid intake has been at least weakly associated with reduced risk of lung cancer in many, but not all, of the studies to date but the evidence was too limited for a conclusion to be drawn in the WCRF report. The evidence that flavonoid intake from food sources may be inversely associated with lung cancer risk continues to accrue [67].

Isothiocyanates are metabolites of the class of phytochemicals known as glucosinolates. Isothiocyanates could exert anticancer effects by blocking carcinogens via induction of phase II detoxification enzymes, such as glutathione S-transferase. Cruciferous vegetables contain high concentrations of glucosinolates, so that cruciferous vegetable intake is positively correlated with higher endogenous isothiocyanate concentrations. As with cruciferous vegetables, lung cancer risk is also consistently lower with higher intakes or urinary concentrations of isothiocyanates [68–70].

A postulated biologic relationship between isothiocyanates and a common polymorphism in the *GSTM1* gene provides an example of a potential gene–diet interaction relevant to lung carcinogenesis. A growing focus in cancer epidemiology is to characterize interindividual susceptibility to cancer by studying polymorphisms in genes involved in carcinogenic pathways, including how these genetic markers interact with environmental exposures to contribute to cancer risk. The role of glutathione S-transferase as a phase II detoxification enzyme has made a common polymorphism in the glutathione S-transferase M1 (*GSTM1*) gene of interest in relation to lung cancer. Compared to persons with the *GSTM1* present genotype, those with the *GSTM1* null genotype have a small but statistically significantly higher risk of lung cancer [71].

When isothiocyanates have been studied in combination with *GSTM1*, the decreased risk of lung cancer associated with isothiocyanates has been especially pronounced in persons with the *GSTM1* null genotype [57]. This association could represent the cancer blocking activity of isothiocyanates being allowed to play an enhanced role in *GSTM1* null individuals because they are not being metabolized as quickly as in those with the *GSTM1* present genotype. This example illustrates the potential interactions between genetic and dietary factors. Integrating genetic and epigenetic markers into the study of nutritional factors provides a mechanistically based approach that holds promise for advancing understanding of the complex role of diet in the etiology of lung cancer.

Fat

Evidence that dietary fat may facilitate tumor growth was reported as early as 1940. In case–control studies, total fat intake is consistently associated with lung cancer risk, with less consistent results for saturated fat, unsaturated fat, and cholesterol intake [72–75]. The prospective evidence shows a

different picture, with some cohort studies observing lung cancer risk to increase with total fat and saturated fat intake but not unsaturated fat and cholesterol, but the results of an important study that was a large, pooled cohort study found lung cancer risk was not strongly associated with fat (total, saturated or unsaturated) or cholesterol intake [76]. The evidence is equivocal, but the hint of associations in the direction of increased risk in some studies is reflected in the assessment of the overall evidence rating in the WCRF report that the evidence is "limited—suggestive" that total dietary fat is associated with increased lung cancer risk [52]. With respect to specific food sources of fats, the same level of evidence was applied to butter [52].

Body Mass Index

In contrast to the association seen for most types of cancer, prospective cohort studies consistently show a strong *inverse* association between body mass index (BMI) and lung cancer risk. These remarkably strong, consistent findings clearly demonstrate that leanness is statistically associated with lung cancer risk. The key remaining question is whether this association is genuine or whether it is indirect. Confounding by cigarette smoking is a viable explanation for these findings because cigarette smoking is strongly associated both with the risk of lung cancer and with leanness. The WCRF report thus rated the evidence as "limited—suggestive" that "low body fatness" is associated with increased risk of lung cancer [52].

However, the need to further test the hypothesis that leanness is a susceptibility factor for lung cancer is indicated by the results of studies in which this association persists even after careful control for cigarette smoking. As investigators continue to pursue this question further, the evidence continues to amass indicating that residual confounding by cigarette smoking may not completely explain away this association. Since the WCRF report, evidence from case–control [77, 78] and cohort studies [79–81] that have attempted to carefully control cigarette smoking still observe strong associations between leanness and lung cancer risk. Interestingly, hints that higher body mass index may also be associated with longer survival in lung cancer patients have also been observed [82, 83].

Beverages

Potential confounding by cigarette smoking recurs for the topic of beverage consumption. Many beverages, including alcohol, coffee, tea, and milk have been studied for a possible link to lung cancer [52]. The majority of studies of alcohol drinking in relation to lung cancer risk that have been adjusted for age and cigarette smoking have observed either null or weak associations [84, 85].

Some studies have observed heavy coffee consumption to be associated with an elevated risk of lung cancer after adjustment for cigarette smoking, but a host of case–control studies have generated findings that fluctuate around the null [52]. The issue of confounding between coffee drinking and other health behaviors, particularly cigarette smoking, has not been addressed adequately, indicating that much stronger evidence is needed for coffee drinking to be considered a risk factor for lung cancer. Despite numerous in vitro and in vivo studies that have observed potential tumor-inhibitory effects of tea [86], the epidemiologic evidence does not presently provide strong support for a link between tea drinking and lung cancer risk [87, 88].

The associations observed between milk drinking and lung cancer have varied considerably. For example, in a cohort followed up since childhood, milk drinking during childhood was significantly inversely associated with lung cancer risk, suggesting drinking milk was protective [89]. On the other hand, in a large prospective study in Sweden, lactose intolerance was associated with nearly a halving

in the risk of developing lung cancer, suggesting that avoidance of milk and dairy products led to a reduction in lung cancer risk [90]. The lack of data on cigarette smoking in both of these studies imposes a barrier to clear-cut inferences. Consistent with the equivocal nature of the evidence and concerns about confounding by cigarette smoking, the WCRF report did not provide evidence ratings for any of these beverages in relation to lung cancer risk [52].

Drinking water can be a route of exposure to environmental contaminants. This is exemplified by the clear increase in lung cancer risk associated with drinking water that is contaminated with high levels of arsenic [91]. Based on studies conducted in geographic regions where drinking water is contaminated with high concentrations of arsenic, the WCRF report rated the evidence as "convincing" that high concentrations of arsenic in drinking water is a risk factor for lung cancer [52].

Meat and Fish

Increased lung cancer risk has been observed to be associated with greater intakes of red meat and processed meat, but this evidence is counterbalanced by some null studies. The cooking method may play a role, as heterocyclic amines from cooked meat may contribute to an increased lung cancer risk. Based on the slight trending of the results toward increased risk, the WCRF report rated the evidence for both red meat intake and processed meat intake to be "limited—suggestive" of increased risk. The current evidence does not support a strong link between fish consumption and lung cancer; the WCRF report did not rate this evidence.

Since the WCRF report, large-scale prospective cohort studies such as the PLCO Study, the EPIC Study, and the NIH-AARP Diet and Health Study have published findings on the association of intake of red meat and processed meat on lung cancer risk [92–94]. None of the results from these studies showed strong associations, but the results from the NIH-AARP Diet and Health Study were statistically significant [94] whereas the results of the EPIC Study and the PLCO Study were not statistically significant [92, 93]. Even in the studies when the results were not statistically significant the associations tend to be in the direction of red meat and processed meat being associated with increased lung cancer risk. Thus, when the evidence is combined across studies in meta-analyses, the results show statistically significantly increased risk of lung cancer for consumption of red meat and processed meat [95, 96]. It remains to be clearly established whether or not red meat and processed meat genuinely contribute to the etiology of lung cancer.

Diet and Prevention: Chemoprevention Trials

The promising data from observational epidemiologic studies seen for fruit and vegetable consumption and for specific micronutrients led to interest in testing if antioxidant micronutrients delivered in concentrated form as dietary supplements could prevent against lung cancer. The two examples considered here are β-carotene, for which there was relatively strong a priori evidence for protection, and vitamin E (α-tocopherol), for which there was not strong a priori evidence for protection.

With respect to β-carotene, four large-scale, randomized, double-blind, placebo-controlled trials were undertaken to test the hypothesis that β-carotene supplementation protects against lung cancer [97–100]. Two of the trials were implemented in populations at high risk of lung cancer [97, 98] and two of the studies were in populations at average risk of lung cancer [99, 100]. All four studies indicated that β-carotene supplementation in later adulthood does not protect against lung cancer. To the contrary, β-carotene supplementation was associated with an increased risk of lung cancer among the

high-risk populations of heavy smokers in the ATBC Cancer Prevention Study [97] and smokers and asbestos-exposed workers in the CARET Study [98]. The WCRF thus rated this strong, consistent evidence from two randomized controlled trials as "convincing" that β-carotene increases lung cancer risk in current smokers. These experimental results thus not only failed to corroborate the evidence from observational studies, but also clearly demonstrated that β-carotene supplementation increased risk in groups at the highest risk of lung cancer.

In the two randomized controlled trials of β-carotene that comprised participants who were not at excess lung cancer risk, the Physicians Health Study and the Women's Health Study, neither trial showed evidence the β-carotene supplements protected against the development of lung cancer [99, 100]. In fact, in the Women's Health Study, more lung cancer cases were observed in the β-carotene arm ($n=30$) than the placebo arm ($n=21$) [100]. Considered in total, large-scale randomized, placebo-controlled trials have been carried out and provide very strong and consistent evidence that β-carotene supplementation increases the risk of lung cancer in populations at high risk of lung cancer and does not protect against lung cancer in more average-risk populations.

A substantial amount of evidence has amassed on the potential chemopreventive effect of vitamin E supplementation for lung cancer. This evidence has been generated mostly from multiple randomized trials of vitamin E supplementation whose primary endpoints were cardiovascular disease. In the Heart Outcomes Prevention Evaluation (HOPE) trial, the group randomly assigned to vitamin E had a significantly lower lung cancer incidence rate (1.4 %) than the placebo group (2.0 %) (RR = 0.72; 95 % CI, 0.53–0.98) [101]. In the ATBC study, α-tocopherol supplements had no impact on lung cancer risk (RR = 0.99; 95 % CI, 0.87–1.13) [97]. In the Women's Health Study, vitamin E supplementation did not lower lung cancer risk (RR = 1.09; 95 % CI, 0.83–1.44) [102]. There has also been interest in combining mixtures of antioxidant micronutrients. For example, the Medical Research Council/British Heart Foundation Heart Protection Study (HPS) was a randomized placebo-controlled trial to test antioxidant vitamin supplementation with vitamin E, vitamin C, and β-carotene. Compared with the placebo group, the lung cancer rate was slightly higher in the intervention arm (1.6 % vs. 1.4 %) [103].

In the HOPE Study, vitamin E supplements showed evidence of protecting against lung cancer, but when all these randomized trials are considered together, the combined results of multiple randomized controlled trials of vitamin E supplements are consistent with no effect on lung cancer risk.

Observational Versus Experimental Evidence

The evidence for vitamin E supplements to provide protection against lung cancer was not strongly supported by human data, but the epidemiologic evidence for β-carotene, as reviewed above, was robust. Thus, the discordance between the results of observational epidemiologic studies that consistently showed an inverse association between dietary intake and circulating concentrations of β-carotene and lung cancer risk and the results of the large-scale chemoprevention trials provoked considerable introspection about potential underlying explanations. Among the factors that have been considered include: (1) the protective effect may apply more to the earlier stages of carcinogenesis, so β-carotene was administered too late to impede lung carcinogenesis particularly in the trials that focused at those at high risk of lung cancer; (2) the supplemental doses administered were far higher than the normal dietary range; and (3) compounds present in fruits and vegetables other than β-carotene may protect against lung cancer. Clearly, fruits and vegetables comprise complex mixtures of antioxidants, phytochemicals, and other compounds that may each exert anticancer properties. This is not a comprehensive list, but still provides insights into the complexity involved in taking the findings from basic and epidemiological research to characterize the nutritional factors that influence lung carcinogenesis and translating this information into strategies to prevent lung cancer.

Conclusions

Key questions concerning the relationship between diet and lung cancer continue to progress toward resolution. As summarized in Table 8.2, several promising leads have emerged to suggest that nutritional factors could have a substantial impact on lung cancer risk in humans. For example, persons who eat more fruits and vegetables clearly have a lower risk of lung cancer than persons who consume less of these foods. In observational studies, the same holds true for intake of specific micronutrients, such as carotenoids. The specific constituents of fruits and vegetables that may confer protection are unknown. An important unanswered question is whether fruits and vegetables directly confer protection against lung cancer. An alternative explanation that is difficult to dismiss is that fruit and vegetable consumption is a marker of other differences between individuals who eat healthy and unhealthy diets that are leading to uncontrolled confounding. The association between fruit and vegetable consumption and lower risk of lung cancer has the potential to contribute to prevention.

In recent years, published epidemiologic studies have improved considerably in using methods to provide the strictest possible control for cigarette smoking, such as matching cases and controls in the study design, limiting the study population to never smokers, and carefully stratifying by smoking history in the analyses. Continued movement in this direction will help to resolve long-standing questions about dietary factors and lung cancer by addressing head-on the persistent concern about residual confounding by cigarette smoking.

Research that continues to provide fresh insights into the influence of diet and nutrition on the occurrence of lung cancer is critical to move the field forward, helping to define new strategies to prevent lung cancer. Increased mechanistic understanding about the complex interactions micronutrients play in the pathogenesis of malignancies may contribute to focusing on particular dietary components most relevant for study. As an example, various micronutrients have been shown to modify the action of enzymes responsible for methylating DNA and creating various histone modifications,

Table 8.2 Summary of World Cancer Research Fund evidence ratings for dietary factors and lung cancer

A.	Convincing evidence of decreased risk
	None
B.	Probable evidence of decreased risk
	Fruits
	Foods containing carotenoids
C.	Limited-suggestive evidence of decreased risk
	Non-starchy vegetables
	Foods containing selenium
	Selenium
	Foods containing quercetin
D.	Limited-suggestive evidence of increased risk
	Red meat
	Processed meat
	Total fat
	Butter
	Retinol
	Low body fatness
E.	Probable evidence of increased risk
	None
F.	Convincing increased risk
	Arsenic in drinking water
	Beta-carotene in smokers

the types of epigenetic modifications that have been demonstrated to be associated with the pathogenesis of lung cancer and other solid tumor malignancies. Additionally, a promising research strategy to more thoroughly investigate across food and nutrient categories is to study dietary patterns as opposed to individual dietary constituents. In this approach investigators are able to categorize overall diets according to whether they meet a predefined definition of "healthy eating" or "mixed dishes" pattern of eating [104, 105].

As progress is made in further understanding the role of diet and nutrition in lung cancer etiology, this progress should not obscure the fact that cigarette smoking is the predominant cause of lung cancer. Many important questions remain concerning the role of diet and nutrition in relation to lung cancer, but the primary way that the lung cancer epidemic will be controlled is to prevent the uptake of cigarette smoking among children and effectively assist addicted smokers to stop smoking cigarettes.

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Chapter 9
Epigenetics of Endocrine Tumors in Women and Dietary Prevention

Donato F. Romagnolo and Ornella I. Selmin

Key Points

- The vast majority of endocrine tumors in women are not linked to family history. This raises the question whether or not alterations of endocrine pathways by environmental and dietary factors contribute to the development of these types of tumors and the role of epigenetic mechanisms.
- The term epigenetics refers to changes in gene expression related to modifications in DNA CpG methylation, histone posttranslational modifications, chromatin remodeling factors, and noncoding RNAs. The sum of epigenetic changes that contribute to cancer development is defined as the cancer epigenome. Thus, understanding which epigenetic changes precede and/or accompany the transition from normal to cancer cell may provide new targets for prevention.
- In this chapter, we summarize recent findings related to epigenetic silencing of tumor suppressor genes in endocrine tissue with specific emphasis on those alterations that contribute to the development of breast, uterine, and ovarian cancers. We also discuss opportunities for epigenetic targeting of endocrine networks with dietary components. We highlight how selected dietary components [i.e., genistein, resveratrol, and (−)-epigallocatechin-3-gallate (EGCG)] and dietary patterns may offer opportunities for prevention of endocrine tumors in women. The epigenetic effects of these compounds on tumorigenesis are influenced by interactions with genotype, and timing and dose of exposure.
- We conclude that characterization of the early epigenetic events that control the transition from normal to tumor cells, and elucidation of epigenetic targets for specific dietary compounds and associations may advance nutrition prevention of endocrine tumors in women.

Keywords Epigenetics • Endocrine tumors in women • Diet • Prevention

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_9

Introduction

Inherited vs. Sporadic Tumors

Endocrine tumors, primarily breast, followed by uterine, ovarian, and cervical cancer, are frequent malignancies in women (Fig. 9.1). The development of these tumors can be linked to activation of oncogenes whose protein products contribute to stimulation of cancer processes such as cell proliferation, inflammation, invasion, angiogenesis, and metastasis; and/or inactivation of tumor suppressor genes which oppose the function of oncogenes and encode for proteins that inhibit cell proliferation, regulate DNA repair, and induce apoptosis. According to the Knudson "two-hits" hypothesis, hereditary cancers result from inherited gene mutations (first hit) in one copy of a cancer susceptibility gene (Fig. 9.2a). This is also referred to as haploinsufficiency when a single-copy loss in a tumor suppressor gene is sufficient for promotion of cancer. The second allele (second hit) is usually inactivated somatically (loss of heterozygosity) during growth and development [1]. Interestingly, only a small fraction (5–10 %) of cancers is linked to germline mutations of tumor suppressor genes and tends to occur early in life. Examples of tumor suppressor genes mutated in hereditary cancers are *Rb*, *p53*, *Apc*, *BRCA-1*, and *BRCA-2*. This scenario differs from that of sporadic tumors which represent the majority of tumors. They usually occur later in life in the context of other genetic and/or environmental insults [2] and when both alleles for a specific tumor suppressor gene are somatically inactivated. Therefore, understanding the mechanisms that lead to somatic inactivation of tumor suppressor genes provides rich opportunities for prevention of both hereditary and sporadic tumors including endocrine malignancies in women.

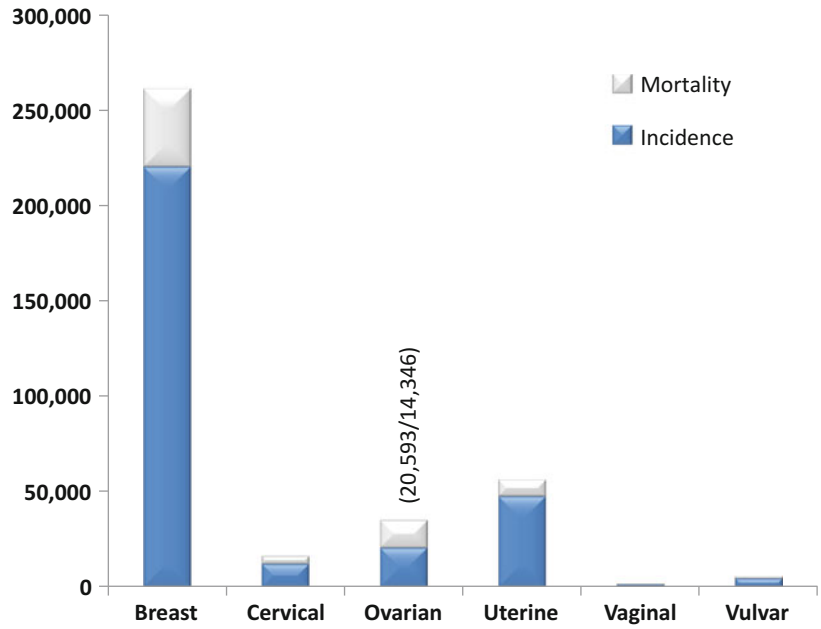

Fig. 9.1 Endocrine cancer statistics in women. Source: Centers for Diseases Control and Prevention (http://www.cdc.gov/cancer/dcpc/data/types.htm). Visited on 03/20/2015. Numbers in parenthesis indicate cancer incidence and mortality in the USA in 2011

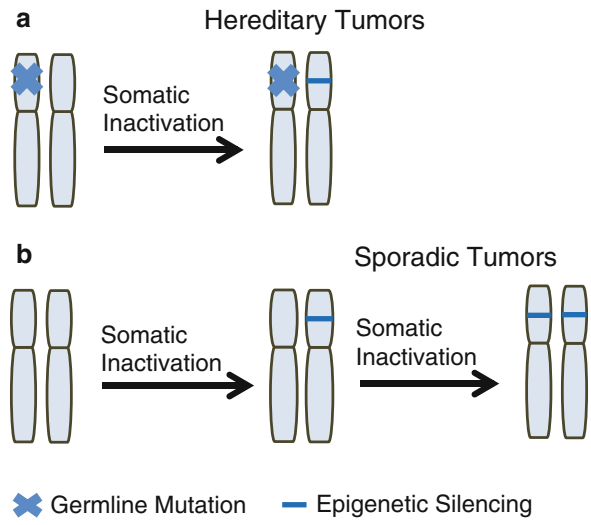

Fig. 9.2 Schematic epigenetic model of silencing of tumor suppressor genes contributing to hereditary and sporadic endocrine tumors in women. In (**a**) a germ-line mutation predisposes to hereditary endocrine tumors that develop after somatic inactivation via epigenetic repression of the second allele. In (**b**) Somatic inactivation via epigenetic silencing of both alleles of target tumor suppressor genes contributes to sporadic endocrine tumorigenesis

Role of Epigenetics in Hereditary and Sporadic Endocrine Tumorigenesis

A mechanism that contributes to loss of expression of tumor suppressor genes is epigenetics. This involves the inactivation of the wild-type allele for tumor suppressor genes in hereditary tumors or both wild-type alleles in sporadic tumors without changes in the DNA sequence (Fig. 9.2b). In keeping with the Knudson "two-hits" hypothesis of tumorigenesis, the inactivation of a given tumor suppressor gene may occur via a combination of mutational and epigenetic events. Epigenetics refers to changes in DNA CpG methylation, histone posttranslational modifications, chromatin remodeling factors, and noncoding RNAs. Epigenetic alterations may lead to the same phenotypic effect as loss-of-function mutations observed in hereditary tumors. Moreover, epigenetic changes may be transmitted to cell progenies and facilitate the clonal expansion of cells with cancer potential. The accumulation of epigenetic alterations in cancer cells is defined as the cancer epigenome [3]. Only a small percentage of endocrine tumors in women is linked to family history [4]. This raises the question whether or not disruption of the epigenome increase cancer risk in endocrine-responsive tissues [5]. Because epigenetic modifications are potentially reversible they offer exciting molecular targets for cancer prevention and treatment [6].

The objective of this chapter is to discuss how epigenetic changes contribute to silencing of tumor suppressor genes and impact on cellular processes in endocrine tissues in women. Also, we highlight opportunities for epigenetic targeting and prevention with bioactive food components (Fig. 9.3). Unfortunately, due to space limitations and to increase focus, we directed our attention to the impact of food components on changes in DNA methylation and histone modifications at target genes. Although not all pertinent literature could be cited, we refer the reader to in-depth reviews addressing the mechanisms of development of hereditary and sporadic endocrine-related cancers [7], and epigenetic regulation by microRNAs and bioactive food components for cancer prevention [8–11].

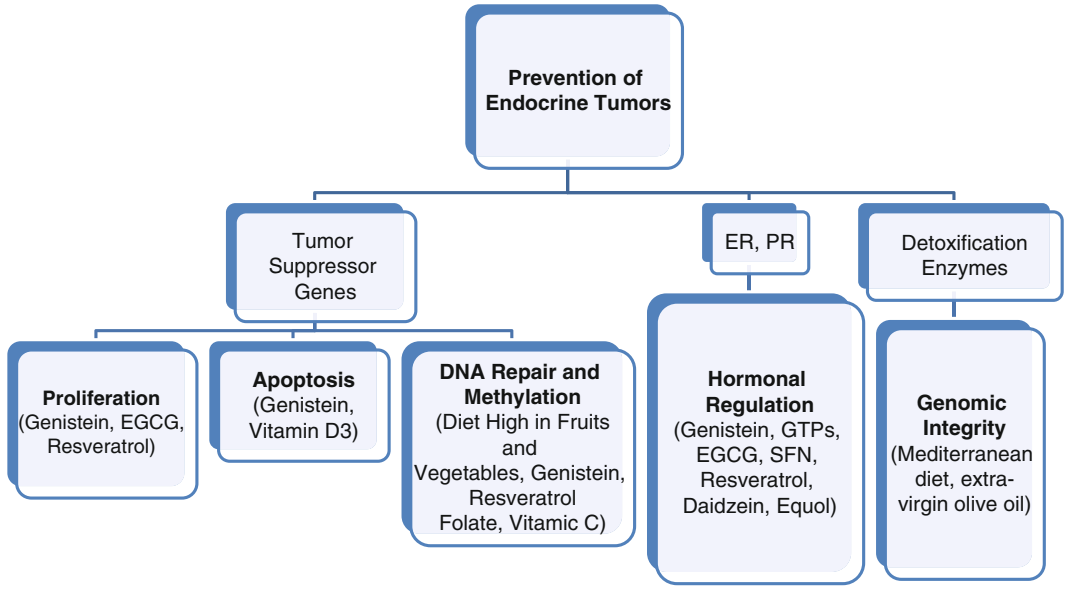

Fig. 9.3 Dietary prevention of endocrine tumors in women. Evidence suggests that selected dietary compounds and dietary patterns may prevent the development of endocrine tumors in women through modulation of tumor suppressor genes involved in regulation of proliferation, apoptosis, and DNA repair and methylation; nuclear receptors involved in hormonal regulation (i.e., estrogen and progesterone); and detoxification enzymes affecting telomeres and genomic integrity. *EGCG* (−)-epigallocatechin-3-gallate, *GTPs* green tea polyphenols, *SFN* sulforaphane, *ER* estrogen receptor, *PR* progesterone receptor

Epigenetic Targeting of Cellular Processes with Dietary Compounds

DNA Repair

The BRCA-1 protein plays a role in transcriptional control [12, 13] and repair of DNA damage [14]. Reduced BRCA-1 expression is found in both hereditary [15–17] and sporadic [18–24] breast and ovarian tumors. BRCA-1 promoter CpG methylation has been reported in ~10–85 % of sporadic breast cancers depending on type of tumors (lobular < ductal) [25, 26]. Loss of BRCA-1 is also linked to reduced expression of the estrogen receptor-α (ERα) [27]. BRCA-1-deficient breast tumors tend to be basal-like ERα-negative and often are also triple-negative (TNBC) with reduced expression of progesterone receptor (PR) and epidermal growth factor receptor-2 (HER2). Notably, hereditary BRCA-1 breast tumors (with one inherited copy of mutated BRCA-1) are refractory to endocrine therapy with tamoxifen [14, 28].

Epigenetic silencing of BRCA-1 via promoter CpG methylation has also been reported in ovarian tumors [29, 30]. Therefore, identifying the factors that contribute to epigenetic repression of BRCA-1 may provide new approaches for prevention strategies [31–33]. For example, in a French-Canadian cohort of women carrying BRCA-1 mutation the highest intake quintile of fruits and vegetables was found to be associated with a marked reduction (~80 %) in the incidence of breast cancer [34] (Table 9.1). These data suggested that great potential exists for prevention of breast tumorigenesis even in women carrying mutated BRCA-1.

The prepubertal exposure to the soy isoflavone genistein was reported to stimulate BRCA-1 expression [35], and protect against tumorigenesis [36]. Genistein may act as an inhibitor of DNA

Table 9.1 Examples of bioactive components that target epigenetically female endocrine tumors[a]

Dietary agent	Epigenetic effects	Reference
Higher quintile of fruits and vegetables	Reduction of breast cancer risk in BRCA-1 carriers	[34]
Genistein	Inhibition of DNMTs and reactivation of BRCA-1	[37, 39]
	Reactivation of RARβ, MGMT via CpG demethylation	[33]
	Reactivation ERα via CpG demethylation	[77]
	Decreased CpG methylation of RARβ2 and cyclin D2	[84]
	Decreased trimethylated marks at ERα and BRCA-1	[106]
	Decreased H3K9me3 and H3K27me3 with p21	[32]
	Reduced CpG methylation at ATM, APC, PTEN	[107]
Equol	Reduces CpG methylation at BRCA-1	[39]
Daidzein	Reduced CpG methylation at GSTP1	[108]
Resveratrol	Inhibition of BRCA-1 histone deacetylation	[48]
	Prevention of BRCA-1 CpG methylation	[49, 52]
	Induction of ERα CpG demethylation	[53]
	Reduced PTEN CpG methylation	[10]
	Repression of miR-21 and miR-155	[54, 55]
Folate	Increase familial breast cancer risk with 677C>T MTHFR	[59]
	Reduced p16 CpG methylation	[109]
	Increased PTEN, APC, RARβ2 CpG methylation	[110]
	Increased sporadic breast cancer risk with 1298A>C MTHFR	[111]
Lipotropes (methionine, choline, folate, B12)	Reduced HDAC-1 and mammary tumorigenesis	[112]
Vitamin C	Enhancement of Tet dioxygenase enzymes	[60]
EGCG	Reactivation of ERα and p16 via CpG demethylation	[45, 85]
SFN	Reactivation of ERα via CpG demethylation	[85]
Vitamin D3	Reduced CpG methylation at PTEN	[10]

[a]Abbreviations of food components: *EGCG* (−)-epigallocatechin-3-gallate, *GTPs* green tea polyphenols, *SFN* sulforaphane

methyltransferase (DNMT) activity [33, 37, 38] and be used for reactivation of the BRCA-1 expression via CpG demethylation [39] (Table 9.1). However, it remains unclear whether or not average intake of soy generates physiological concentrations of genistein sufficient to reactivate BRCA-1 expression. Human plasma levels of genistein range from 1.0 to 2.0 μMol/L in populations with average soy intake [40] or after supplementation with soy isoflavones [41]. It is also unclear whether or not supplemental genistein can cause harmful effects [42, 43]. For example, in preclinical models the postnatal exposure to genistein was reported to increase expression of estrogen-responsive genes, and the incidence and multiplicity of uterine leiomyomas [44]. Conversely, in rodent models prepubertal genistein was found to antagonize mammary tumorigenesis while inducing BRCA-1 expression [45, 46]. The latter effect could be attributed in part to repression of protumorigenic microRNA-21 (miR-21) [47].

The grape phytoalexin resveratrol has been shown to prevent repression of BRCA-1 transcription via inhibition of histone deacetylation and CpG hypermethylation [48, 49] at doses (1.0 μMol/L) approaching those measured (2.4 μMol/L) in human serum pharmacokinetic studies [50, 51] (Table 9.1). In rodent studies, the gestational pretreatment with resveratrol prevented the downregulation of BRCA-1 expression due to BRCA-1 promoter hypermethylation in mammary tissue of female offspring [52]. These effects of resveratrol on the BRCA-1 gene could be related in part to reactivation of ERα expression via demethylation of the ERα gene [53] as well as repression of oncogenic miR-21 [54] and miR-155 [55, 56].

The impact of folate nutrition on risk of endocrine tumors in women remains a subject of controversy. At doses higher than ~850 μg of dietary folate equivalents/day (DFE, 1 mg food folate = 0.6 mg of supplemental folic acid), folate was associated with increased risk of breast cancer [57]. These findings were in stark contrast with those of other reports documenting a reduction in the incidence of ERα-negative breast tumors in postmenopausal women who consumed ~1300 μg of DFE/day [58]. In addition to ERα, a factor that may impact the development of breast cancer is polymorphisms in genes that regulate folate metabolism. In BRCA1 mutation carriers, the single nucleotide polymorphism 677C>T in the methyl-tetrahydrofolate reductase (MTHFR) enzyme was associated with elevated risk of breast tumors. The MTHFR enzyme converts homocysteine to methionine, which supplies methyl groups for the DNMTs. Women with the MTHFR 677TT genotype had increased risk for developing breast (1.5-fold) and ovarian (0.6-fold) cancers compared to women with the 677CC genotype [59]. Clearly, interactions between BRCA-1 and MTHFR genotype and folate status appear to modulate breast and ovarian cancer risk.

A dietary compound that has been proposed to impact epigenetic regulation is vitamin C, which enhances the catalytic activity of the Ten eleven translocation (Tet) dioxygenase enzymes (Table 9.1). The latter induce the oxidation of 5-methylcytosine [60] leading to subsequent demethylation of gene promoters and upregulation of genes with cancer protective effects. In fact, knockout of Tet functions enhances the expression of genes that promote epithelial to mesenchymal transition (EMT), a process linked to breast cancer development and metastasis [61]. Therefore, through activation of Tet enzymes vitamin C may prevent epigenetically carcinogenesis in endocrine tissues.

Hormonal Regulation

The vast majority (~75 %) of breast tumors expresses the ERα and is associated with a better prognosis and treatment. This represents a paradox since the ERα mediates the effects of estrogens which stimulate the expression of proliferating factors and growth of ERα-positive breast cancer cells. On the other hand, removal of ovarian hormones via oophorectomy prevents mammary carcinogenesis in experimental animals, and treatment with antiestrogens (i.e., tamoxifen) and aromatase inhibitors are the gold standards for, respectively, adjuvant therapy of breast cancer in high risk pre- and postmenopausal women, and blockade of estrogen biosynthesis in postmenopausal breast cancer patients. However, tamoxifen exerts harmful estrogenic actions in both experimental animals (e.g., induces uterine weight and endometrial carcinomas in mice) and increases the incidence of blood clots and endometrial tumors in postmenopausal women (but not in premenopausal women) [62]. Nevertheless, tamoxifen remains the first-line medical treatment for ERα-positive breast tumors in both pre- and postmenopausal women [63]. Other synthetic antiestrogens (i.e., raloxifene) appear to be as effective as tamoxifen but they increase the incidence of noninvasive ductal carcinoma in situ (DCIS). Based on these observations, two important puzzles related to prevention of endocrine tumors arise. First, it would be important to ascertain under which hormonal conditions targeting of ERα exert stimulatory or inhibitory effects on proliferation of endocrine cells? Second, it should be determined if the long-term treatment with antiestrogens induces drug resistance and estrogenic effects. Solving these puzzles may provide the basis for developing prevention strategies based on dietary components alone or in combination with selective modulators that impact estrogen actions at the ERα.

One mechanism that may contribute to endocrine therapy resistance is loss of expression of ERα via aberrant CpG methylation of the ERα gene [64–66]. Loss of ERα expression due to DNA hypermethylation was reported in ~40 % of breast cancer cases [67, 68] and it appeared to increase in TNBCs [69]. Conversely, reactivation of the ERα gene through inhibition of DNMTs [70] and histone deacetylases (HDACs) [71, 72] was shown to restore sensitivity of breast cancer cells in culture to tamoxifen [73].

Interestingly, a dietary compound with therapeutic potentials against ER-negative breast tumors is the soy isoflavone genistein. It was found to reduce DNA methylation (after 10 days) in embryonic stem cells during differentiation [74] and epigenetically reactivate the expression of various genes including retinoic acid receptor (RAR)-β (RARβ) and O-6-methylguanine-DNA methyltransferase (MGMT) [75, 76] in breast cancer cells via reversal of hypermethylation, and of ERα in ERα-negative breast cancer cells [77]. The latter study used concentrations of genistein (25–50 μMol/L) in culture that are considerably higher than those found in humans (on average ~0.5–2.0 μMol/L with peaks of genistein reaching ~6.0 μMol/L) through routine consumption of soy products [78]. Supplemental genistein at levels (250 mg/kg diet) yielding concentrations (~2.5 μM) found in human serum [79] inhibited the growth of ERα-negative xenografts and prevented breast cancer development in a spontaneous mouse breast cancer model. In cell culture, the combination of genistein plus tamoxifen had synergistic effects on growth inhibition of ERα-positive breast cancer cells [80]. In contrast, it should be noted that other preclinical studies with supplemental genistein (≥250 mg/kg diet) yielding plasma levels of total genistein of ~0.4–3.5 μMol/L increased breast tumor growth [81]. Overall, preclinical and clinical dose- and developmental-based studies are needed to assess whether or not supplemental genistein or intake of soy products can be safely recommended for breast cancer prevention (reviewed in [82, 83]). This need is corroborated by evidence that in premenopausal women low-dose genistein (circulating plasma levels below 600 ng/mL) had no effect or decreased methylation of the RARβ2 and cyclin D2 in mammary intraductal specimens, whereas increased methylation of RARβ2 and cyclin D2 were seen with circulating genistein above 600 ng/mL [84].

Other dietary compounds found to reactivate ERα expression in ERα-negative breast cancer cells via ERα promoter demethylation include green tea polyphenols (GTPs), which are rich in catechins [i.e., (−)-epigallocatechin-3-gallate (EGCG)], and the cruciferous vegetables-derived compound, sulforaphane (SFN) [85]. EGCG contributes ~50 % of the GTPs found in green tea and possesses DNMT inhibitory properties [86, 87]. SFN is one of the isothiocyanates found in cruciferous vegetables and has HDACs inhibitory activities [88]. Both GTPs and SFN contribute to chromatin modifications at the ERα promoter. These include enrichment of transcriptionally active markers (i.e., acetylated H3K9), and reduction in the recruitment of the H3K9 methyltransferase, SUV39H1, which produces trimethyl-H3K9 (H3K9me3), a marker of inactive chromatin [3]. Taken together, these observations suggest that various dietary compounds (i.e., GTPs, SFN, resveratrol, and genistein) can reactivate ERα expression through various, likely combinatorial, mechanisms. Perhaps, the routine consumption of these food components may be a safer alternative to their supplementation at supraphysiological doses to maintain or achieve reactivation of ERα expression. Clearly, preclinical and human clinical studies are needed to establish the dose- and time-dependent effects of dietary compounds that target ERα expression and their interactions with ERα antagonists (i.e., tamoxifen) for breast cancer prevention.

A nuclear receptor involved in regulation of endocrine responses is the progesterone receptor (PR). In the uterine endometrium, PR activation by progesterone induces differentiation and inhibits proliferation. Loss of expression of PR is linked to the development of advanced endometrial type II serous or serous-like tumors of which 65–85 % are resistant to progestin therapies. Similarly, loss of PR expression has been documented in ~12 % of ER-negative breast cancers [89]. Knowledge of the mechanisms responsible for the loss of PR expression is lacking. Progesterone receptor promoter hypermethylation is one mechanism that contributes to silencing of the PR gene. For example, the combined treatment with the demethylating agent 5-aza-dC and the HDAC inhibitor LBH589 was found to remove repressor polycomb-2 proteins (i.e., EZH2, SUZ12) from the PR promoter and restore PR expression in type II endometrial cancer cells in culture. Moreover, the combination of the HDAC inhibitor trichostatin (TSA) and 5-aza-dC elevated PR expression in endometrial adenomyosis cells that had hypermethylated PR [90]. Currently, the treatment of choice for adenomyosis is hysterectomy and information on epigenetic regulation of the PR gene by food components is scarce. One study reported that nutrition-relevant concentrations of isoflavone mixtures (i.e., genistein, daidzein,

and equol) and coumestrol at physiological (1 μMol/L) concentrations enhanced PR expression in breast cancer cells [91]. Therefore, both preclinical and clinical studies are needed to establish whether or not food isoflavones can be safely adopted for reactivation of PR expression along with standard progestin treatments of advanced endometrial tumors, and possibly, of ER- and PR-negative breast cancers [92].

Proliferation and Apoptosis

Progression through the various phases of cell cycle is tightly controlled by networks of cyclins, cyclin-dependent kinase (cdk) inhibitors, and cell cycle checkpoints. Alterations in the relative expression of these factors compromise the fidelity of cell cycle division and are hallmarks of cancer. For example, aberrant promoter methylation at CpG islands in the p16INK4a, a cdk inhibitor of cyclin D1, is associated with overexpression of cyclin D1, an oncogene, in intraductal lesions of the breast [93]. Expression of cyclin D1 is under the transcriptional control of the ERα, whereas p16 blocks the activation of the cyclin D1 by cdk4/6. Therefore, the net effect of loss of p16 expression in ERα-positive breast cells under the influence of estrogens is the upregulation of cyclin D1 activity, which then leads to stimulation by the transcription factor E2F of expression of cyclin E and transition from G1 to the S phase of the cell cycle. This picture is further compromised in cells in which genes encoding for the retinoblastoma protein Rb, a binding partner or E2F, and p21, an inhibitor of cyclinD1/cdk4 and cyclin E/cdk2 complexes, are silenced. A compound that reactivates p16 expression is genistein [75]. The reactivating effects of genistein on the p16 promoter may contribute to its effects against mammary tumorigenesis [45]. Reversal of hypermethylation and reactivation of the p16INK4a gene has also been documented for EGCG (20 μMol/L). Since p16 promoter hypermethylation is associated with increased breast [94] and ovarian [95] cancer risk, genistein- and tea catechin-based strategies should be considered for the prevention of breast and other endocrine tumors in women.

Contrary to proliferation, apoptosis is a process that leads to cell death when cell damage compromises DNA replication and cell division. Induction of apoptosis is under the control of the intrinsic and extrinsic pathways. The latter is induced by the p53/p21 axis of tumor suppressor genes. Downregulation of p21 has been reported in various endocrine tumors, but unlike p53, which is mutated in about 50 % of cancers, p21 silencing is rarely linked to mutational inactivation. Studies with breast cancer patients found that the p21 gene was hypermethylated in 79 % of cancer cases in which p21 expression was downregulated [96]. Therefore, changes in the expression and methylation status of p21 and p16 may offer biomarkers of risk as well as efficacy for food components. For example, preclinical studies with genistein documented epigenetic reactivation of p21 and p16 via reduction in the association of the respective promoters with inactive chromatin markers H3K9me3 and H3K27me3; induction of apoptosis in breast cancer cells in culture; and inhibition of breast cancer development in mouse mammary xenografts [32].

Resveratrol along with vitamin D3 was effective in reducing promoter methylation of the phosphatase and tensin homologue (PTEN) tumors suppressor gene and expression of DNMT-1, while inducing the expression of p21, in ERα-positive breast cancer cells [10]. Via the ERα, estrogens activate the Ras/Raf/mitogen-activated protein kinase (MAPK)/activator protein-1 (AP-1) signaling pathway [97] which induces expression of DNMT-1 [98]. Conversely, PTEN is a repressor of AP-1. Therefore, activation of PTEN and p21 and inhibition of DNMT-1 expression by resveratrol and vitamin D3 would have the net effect of halting cell proliferation. These effects were as measurable as those induced by cancer preventing agents including adenosine analogues (i.e., 2-chloro-2′-deoxyadenosine, 2CdA), which inhibit DNA methylation. One possible implication of these data is that food components such as resveratrol and vitamin D3 could be combined with chemotherapy agents to improve efficacy of treatment against breast cancer.

Detoxification and Genomic Stability

Recent studies reported that inactivation of p16 and p21 pathways by hypermethylation was linked to telomere shortening in breast tumors, especially in the histological grades II and III [99]. Telomeres are repetitive DNA sequences at the ends of eukaryotic chromosomes that maintain genomic integrity and stability. Alteration of telomere length, also known as telomere crisis, in p16 and p21-deficient cells may contribute to carcinogenesis of the breast and other endocrine tumors. Thus, telomere shortening and both promoter hypermethylation of p16 and p21 might serve as biomarkers of risk when studying the effects of dietary patterns. For example, greater adherence to the Mediterranean diet was associated with longer telomeres in the Nurses' Health Study [100]. The protective effects of food components present in the Mediterranean diet on telomere length may be related to inhibition of oxidative stress and chronic inflammation. Foods normally found in the Mediterranean diet such as extra-virgin olive oil have been linked to activation of phase II enzymes such as glutathione S-transferase-P1 (GSTP1) [101] and reduction of mammary tumorigenesis compared to other dietary fats (i.e., corn oil rich in linoleic acid) [102]. It is interesting to highlight the fact that GSTP1 gene is hypermethylated in breast cancers and its hypermethylation has been linked to the pathogenesis of luminal A, luminal B, and HER2-enriched tumors. GSTP1 promoter hypermethylation was detected in one-third of breast tumor biopsies (74/215) and was associated with reduced GSTP1 expression [103]. Based on these observations, it has been proposed that loss of GSTP1 expression may result in greater DNA damage caused by estrogen metabolites in luminal progenitor cells since they are considered to have higher concentration of estrogens [104]. Conversely, EGCG and GTPs were reported to cause activation of expression of GSTP1 in endocrine-responsive cells thus offering new dietary tools for breast cancer prevention [105].

Conclusions

Despite considerable progress in early detection and treatment, endocrine tumors, primarily breast cancer, followed by uterine and ovarian cancer, remain frequent malignancies in women. Because these diseases are heterogeneous, discovering the molecular mechanisms that regulate tumor development may offer new targets for prevention and treatment. One such mechanism is epigenetics which contributes to dysregulation of genes involved in tumorigenesis. Interestingly, various dietary compounds (i.e., EGCG, resveratrol, genistein) and patterns (diet high in fruits and vegetables, Mediterranean diet) appear to affect the epigenetic machinery and show promise for the prevention of endocrine tumors in women. The anticancer effects of dietary compounds are influenced by interactions with genotype, and timing and dose of exposure. Nevertheless, because most of endocrine tumors in women are sporadic, dietary compounds alone or in combination with therapeutic drugs (i.e., antiestrogens) may offer rich opportunities for epigenetic prevention and treatment.

Acknowledgement This work was supported by grants from the Arizona Biomedical Research Commission (ADHS14-082995), US ARMY Medical Research and Materiel Command (BC134119), Soy Health Research Program (SHRP), and the Arizona Cancer Center Support Grant P30CA23074.

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Chapter 10
The Role of Nutrition and Diet in Prostate Cancer

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Key Points

- The more than 25-fold variation in prostate cancer incidence and 10-fold variation in prostate cancer mortality globally points to a role of lifestyle and dietary factors in the etiology of prostate cancer.
- Risk factor patterns differ markedly for potentially lethal and indolent disease, suggesting separate etiologies and distinct disease entities.
- Screening by prostate-specific antigen (PSA) has markedly impacted the results of studies of prostate cancer prevention. In the PSA era, overall incident prostate cancer mainly is indolent disease, and often reflects the propensity to be screened and biopsied.
- Studies must focus on cancers with lethal potential, and include long follow-up to accommodate the lead time induced by screening.
- Obesity is unrelated to total prostate cancer incidence, but is linked with an increased risk of prostate cancer-specific mortality.
- Tallness is associated with higher risk of prostate cancer. The association is more pronounced for aggressive disease, suggesting that early-life factors during puberty and adolescence, possibly related to nutritional status, influence prostate carcinogenesis.
- Growing evidence suggests that higher intake of tomatoes/lycopene, coffee, and fish are associated with a lower risk of prostate cancer, particularly for aggressive disease.
- Excess intake of calcium/diary and selenium should be avoided.
- The role of vitamin E on prostate cancer progression, particularly among smokers, requires further research.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_10

Keywords PSA screening • Obesity • Height • Tomatoes/lycopene • Selenium • Vitamin E • Coffee • Calcium/dairy • Vitamin D • Fish

Introduction

Globally, prostate cancer is the second most commonly diagnosed cancer and the most common cancer in males in 84 countries [1]. Although it occurs more frequently in the developed world, it has also become an emerging public health problem in developing countries. The majority of prostate cancers, detected by screening, are early-stage tumors with no symptoms and with a low risk of metastasis. However, prostate cancer is associated with significant impairments in quality of life [2], both from the disease itself and as a consequence of treatment, and is a major contributor of cancer death.

Tantalizing clues suggest that diet and lifestyle factors could play an important role in the prevention of prostate cancer incidence or progression. However, the influence of screening on population studies of prostate cancer needs to be recognized. Before screening with prostate-specific antigen (PSA) was widespread, a larger proportion of diagnosed prostate cancers had lethal potential and was diagnosed at advanced stage. However, in the PSA era, overall incident prostate cancer mainly is an indolent disease, and often reflects the propensity to be screened and biopsied. Studies must therefore focus on cancers with lethal potential, and include long follow-up periods to accommodate the lead time induced by screening. Moreover, risk factor patterns differ markedly for potentially lethal and indolent disease, suggesting separate etiologies and distinct disease entities.

Efforts to understand risk factors and predictors of more aggressive disease, defined by high grade, advanced stage or metastatic potential, are central in prostate cancer research, and represent an important public health challenge to reduce suffering from this disease. In this chapter, we will summarize the associations between diet and nutritional factors in relation to the primary and secondary prevention of prostate cancer, focusing on recent advances, and particularly for advanced diseases.

Global Burden of Prostate Cancer

Incidence

An estimated 1.1 million men worldwide were diagnosed with prostate cancer in 2012, accounting for 15 % of the cancers diagnosed in men, with almost 70 % of the cases occurring in more developed regions. Prostate cancer incidence varies more than 25-fold worldwide (Fig. 10.1) [3]. Among men in the USA, prostate cancer is the most frequently diagnosed cancer in men, with 60 % higher incidence rates in blacks than non-Hispanic whites [4]. Although differences in PSA screening may account largely for the global variation in incidence, geographic differences were apparent already in the era prior to PSA screening, highlighting a potential role of lifestyle and particularly dietary patterns to account for the variation in rates. Several countries have seen increasing incidence rates over time, including in Africa and Latin America, which cannot be attributed to greater screening intensity.

Mortality

Prostate cancer is the fifth leading cause of death from cancer globally in men with an estimated 307,000 deaths in 2012 [3]. There is less variation in mortality rates worldwide (~10-fold) than is

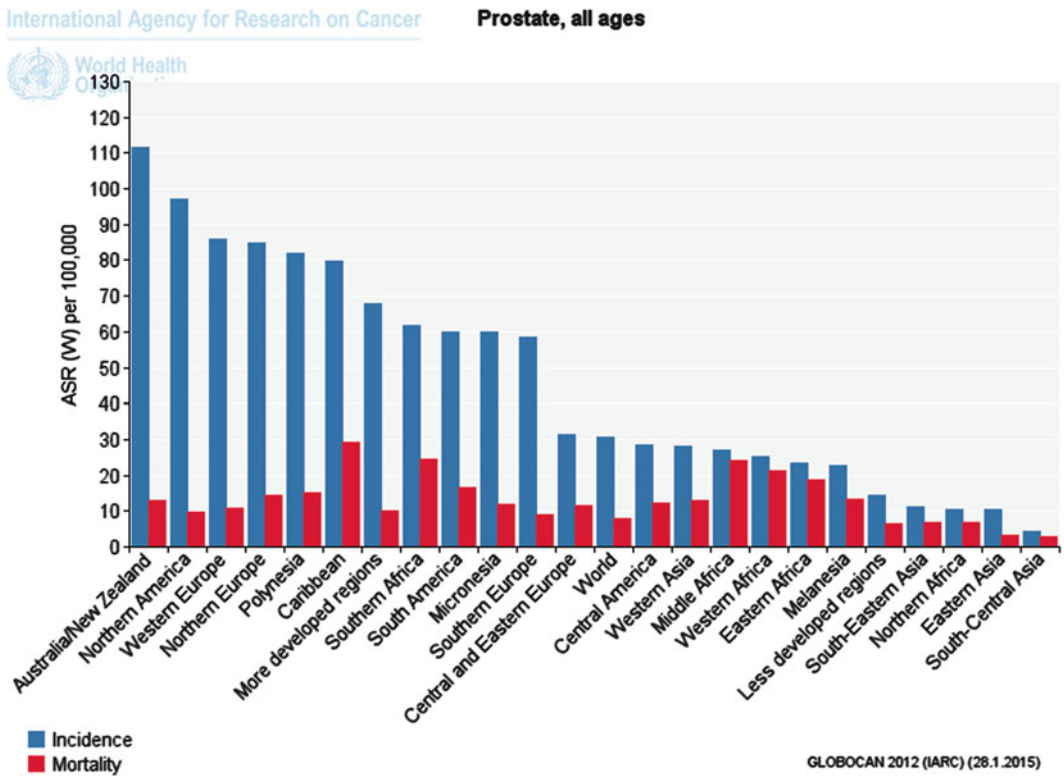

Fig. 10.1 Estimated cancer incidence and mortality (age-standardized rate [ASR] per 100,000) of prostate cancer worldwide, 2012. *Source*: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide. IARC CancerBase No. 11. IARC Press [3]

observed for incidence (Fig. 10.2). Rates of mortality are highest in countries in the Caribbean and among African-American men in the USA. During the past decades, prostate cancer mortality rates have shown declines in some countries, most notably in the USA since 1990s, which may be attributable in part to earlier detection through PSA screening and subsequent earlier treatment [5]. Notwithstanding the considerable mortality associated with this disease, most men die with and not from their cancer. Indeed, cardiovascular disease and other chronic diseases are responsible for more than three-quarters of deaths among men diagnosed with localized prostate cancer.

Influence of PSA Screening on Studies of Prostate Cancer Prevention

Current US guidelines regarding PSA screening vary and are a matter of significant debate, but the 2013 American Urological Association Guideline recommends screening between ages 55 and 69 years, during which men seem to gain the greatest benefit [6]. Although prostate cancer mortality has fallen after PSA screening was introduced, PSA screening with its long lead time of approximately 11 years [7] has led to widespread overdiagnosis and overtreatment of prostate cancer [8]. In a review of 19 autopsy studies, prostate cancer was found in 36 % of Caucasians and 51 % of African-American men aged 70–79, underlying the potential for widespread diagnosis of cases of prostate cancer that would have caused no clinical harm had they remained undetected [9]. As treatment is expensive and frequently has a significant impact on a man's urinary, sexual, and gastrointestinal quality of life [10],

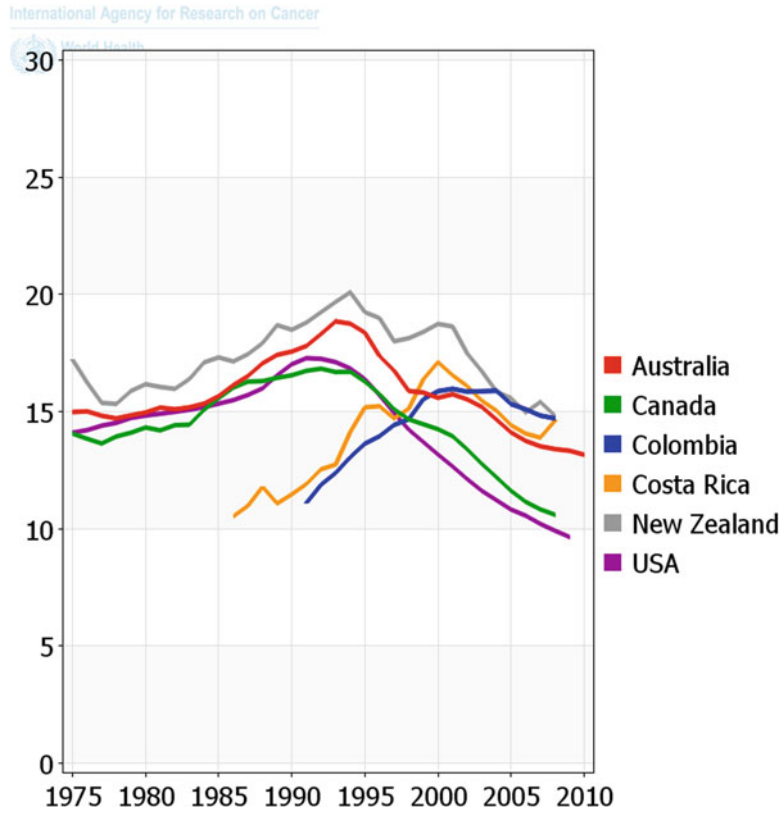

Fig. 10.2 Trends in mortality from prostate cancer in selected countries (age-standardized rate [ASR] per 100,000). *Source*: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide. IARC CancerBase No. 11. IARC Press [3]

and given the mixed findings from randomized trials of screening, the US Preventive Services Task Force in 2012 recommended against PSA-based screening for prostate cancer for men of all ages [11].

To fully understand the influence of PSA screening on the observed associations between specific dietary factors and prostate cancer risk, it is first important to recognize the differing pattern of risk factors for potentially lethal versus indolent disease, and separate etiologies may be involved. In the Health Professionals Follow-Up Study (HPFS) (Fig. 10.3), for example, height is strongly associated with higher risk of lethal but not indolent disease [12]; because height is uncorrelated with PSA screening behavior, this finding clearly demonstrates a biologic distinction between indolent cancers and cancers of potential. Other protective dietary factors including coffee [13], lycopene [14], and fish intake [15] have all been linked to lower risk of lethal disease, but not overall or indolent prostate cancer risk.

Before screening was introduced, potentially lethal cases could be identified as those with advanced stage (T3b or higher) at diagnosis; thus, pre-PSA cases were enriched with those of lethal potential, as compared with the distribution in screened populations, among which over 90 % of cases are well-differentiated tumors with low metastatic potential [16]. Therefore, epidemiologic studies of overall prostate cancer in the pre-PSA era tended to observe relative risk estimates closer to those found for lethal disease in contemporary studies.

Additionally, PSA screening may also be a potential confounder in epidemiological studies. Men who take part in regular screening practices, including PSA screening, tend also to take part in other healthy behaviors [17]. Thus, studies in the PSA era should account for PSA screening practices in their study design or data analysis.

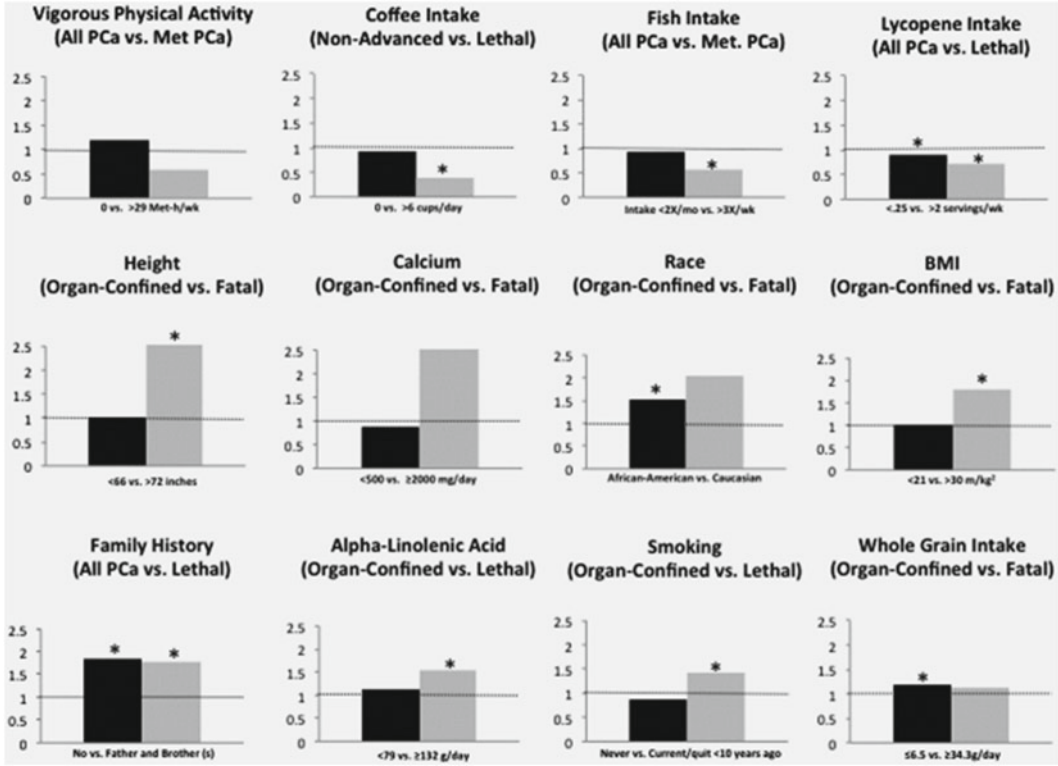

Fig. 10.3 Risk factors for indolent and lethal prostate cancers in the Health Professionals Follow-up Study. Multivariable RR for the highest versus reference category for selected variables from published results separately for total, incident, or organ-confined prostate cancer (*black*) and lethal/advanced/fatal prostate cancer (*grey*) in the most recent analyses of HPFS. From Jahn et al. [9]

For clinical trials focused on prostate cancer prevention, follow-up time must be very long to have enough numbers of lethal prostate cancer, due to the long lead time (i.e., 8–12 years) of PSA-detected cases. However, even trials of this length will have only modest power to detect any effect of the intervention on clinically significant, prostate cancers of lethal potential since men can live many years beyond the time of clinical detection. Indeed, in the most recent report from the Swedish trial of surgical intervention versus watchful waiting, findings for a marked benefit of surgery for prostate-specific and total mortality emerged clearly only after 15 years of follow-up [18].

Dietary and Nutrition Factors

Energy Balance

Obesity

Findings on obesity, which may reflect a positive energy imbalance, and total prostate cancer risk have been mixed, with most studies suggesting null associations. However, obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after

diagnosis. A meta-analysis of six cohort studies of initially cancer-free men showed a significant 15 % increase (95 % CI 1.06–1.25) in the risk of fatal prostate cancer for each 5 kg m^{-2} increase in BMI [19]. Similarly, among men with prostate cancer, a 5 kg m^{-2} increase in BMI was associated with a 20 % (95 % CI: 0.99–1.46) increased risk of prostate cancer-specific mortality.

Some evidence suggests that abdominal obesity is associated with more advanced disease. In the European Prospective Investigation into Cancer and Nutrition (EPIC), waist circumference (RR per 5 cm: 1.06; 95 % CI: 1.01–1.10) and waist-to-hip ratio (RR per 0.1 unit: 1.21; 95 % CI: 1.04–1.39) were positively associated with diagnosis of more advanced prostate cancer [20]. Waist circumference was significantly associated with more aggressive disease in the Melbourne Collaborative Cohort Study [21], but it was not associated with advanced stage or high-grade disease in the HPFS [22]. Because of the high correlation between BMI and measures of abdominal obesity, it is difficult to separate their effects.

Overweight and obesity in childhood and adolescence may impact sex hormone levels during periods of growth and development thus may be important for later prostate cancer risk. Some studies support a more pronounced association between childhood obesity and advanced prostate cancer [22, 23]; however, other studies found no association [24].

Height

Tallness has been used as a surrogate of high exposure to growth hormones, which are partially influenced by energy balance to the extent that a relative restriction of energy could lead to shorter stature. A meta-analysis of 58 studies suggests that height is positively associated with prostate cancer risk (RR per 10 cm: 1.06; 95 % CI: 1.03–1.09), with a stronger effect for prospective studies of more advanced/aggressive cancers (RR per 10 cm: 1.12; 95 % CI: 1.05–1.19) [25], suggesting that factors during puberty and adolescence, possibly related to nutritional status, influence prostate carcinogenesis.

Antioxidants

Lycopene and Tomato-Based Products

Evidence suggests that higher intake of tomatoes, particularly cooked tomatoes, or lycopene, a carotenoid with well-documented antioxidant effects, were associated with lower risk of prostate cancer [26]; however, findings have been inconsistent. A meta-analysis that included studies up to 2003 suggests that high consumption of tomato products was associated with lower risk of prostate cancer (RR for top vs. bottom quintile: 0.89; 95 % CI 0.80–1.00) [27]. For a high intake of cooked tomato products, which are more bioavailable sources of lycopene than raw tomatoes, the corresponding RR was 0.81 (95 % CI 0.71–0.92).

Two subsequent cohort studies, including dietary studies in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Screening Trial [28] and the Prostate Cancer Prevention Trial (PCPT) [29], have not reported significant inverse associations between dietary lycopene and prostate cancer risk. However, a large portion of prostate cancer cases were indolent as diagnosed by initial PSA screening. Previous studies suggest that the association between lycopene intake and prostate cancer risk was weaker for end points enriched with indolent cancers, and stronger for lethal prostate cancer than for total prostate cancer. Indeed, in the PLCO analysis, greater consumption of spaghetti/tomato sauce and pizza was associated with nonsignificant decreased risk of advanced disease (RR 0.81; 95 % CI: 0.57–1.16 and RR 0.79; 95 % CI: 0.56–1.10, respectively), suggesting a possible stronger role for lycopene in advanced disease [30].

In the most recent analysis from the HPFS, dietary lycopene intake was inversely associated with total prostate cancer (RR for top vs. bottom quintile: 0.91; 95 % CI: 0.84–1.00) and more strongly with lethal prostate cancer (RR 0.72; 95 % CI: 0.56–0.94) [14]. Moreover, the associations for total prostate cancer were stronger for cancers diagnosed prior to the introduction of PSA screening than cancers in the PSA era. To further reduce the influence of PSA screening, the authors restricted to men who had at least one negative PSA test, and a strong inverse association was observed for lethal prostate cancer with baseline lycopene intake (RR 0.48; 95 % CI: 0.30–0.78). The analysis also suggested that remote rather than more recent intake was important.

The majority of serum or plasma-based studies of lycopene and total prostate cancer have found protective associations for high lycopene levels, with corresponding summary relative risks of 0.55 (95 % CI 0.32–0.94) for case–control studies and 0.78 (95 % CI 0.61–1.00) for cohort studies [27]. In a recent nested case–control study within the PCPT [31], no association was reported for serum lycopene and prostate cancer risk, but incidentally diagnosed prostate cancer cases by end-of-study biopsies were analyzed alongside prostate cases diagnosed clinically (rising PSA, development of nodule, symptoms). A reanalysis that included only cancers diagnosed clinically showed a significant inverse association between high serum lycopene and prostate cancer [30]. Several other subsequent serum lycopene studies reported nonsignificant inverse associations [32–35] or no association [36] with risk of total prostate cancer. However, these studies were conducted in the post-PSA era likely enriched with indolent cancers. In a large case–control study within the EPIC cohort involving 966 total cases and 205 advanced stage cases of prostate cancer, plasma lycopene was significantly associated with inverse risk of advanced prostate cancer (RR for top vs. bottom quintile 0.40; 95 % CI: 0.19–0.88), but not total prostate cancer [36].

Hypothesized mechanisms include reduction in cellular oxidative stress [37] and high levels of reactive oxygen species compared to normal cells [38], leading to potential prostate cancer progression. Antioxidants may lower risk particularly for advanced prostate cancer by quenching free radicals and thus ameliorating damage from consequences of chronic inflammation and also by downregulating tumor angiogenesis [39]. In an analysis of tissue markers among 570 prostate cancers in the HPFS, higher lycopene intake was associated with tumors that displayed less angiogenic potential [14].

Selenium

The trace element selenium is not an antioxidant per se, but plays an important role as an essential element for the antioxidant enzyme glutathione peroxidase as well as other selenoproteins involved in exerting antitumor effects, including apoptosis and inhibition of cellular proliferation [40]. Because selenium contents in specific foods vary as a function of the selenium content of the soil, epidemiological studies of selenium require biological sampling, primarily measuring levels in blood or toenails.

The strongest evidence for selenium initially came from a secondary analysis in the Nutritional Prevention of Cancer (NPC) Trial, in which selenium supplementation (200 μg/day of selenium in 0.5-g high-selenium yeast) was associated with a 63 % reduction in prostate cancer risk [41]. However, with additional follow-up time, the protective effect of selenium supplementation appeared to be limited to those with low PSA levels at baseline or low selenium levels [42].

Findings from subsequent observational studies on both serum/plasma and supplemental selenium are mixed. In a meta-analysis including studies up to 2010, a decreased risk of total prostate cancer was observed with plasma/serum concentrations of 135 ng/mL up to the upper range investigated (170 ng/mL), and the association with advanced prostate cancer was more pronounced [43]. At 135 and 170 ng/mL, the estimated RRs for total prostate cancer were 0.85 (95 % CI: 0.74–0.97) and 0.60 (95 % CI: 0.45–0.81) compared to 60 ng/mL, respectively, and 0.75 (95 % CI: 0.65–0.86) and 0.50 (95 % CI: 0.36–0.68) for advanced prostate cancer.

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) reported no effect of selenium supplementation (200 μg/day from L-selenomethionine) on prostate cancer incidence (RR 1.09; 95 % CI 0.93–1.27) [44]. However, SELECT participants had adequate levels of selenium at baseline (median serum selenium levels of 135 ng/mL vs. 113 ng/mL in NPC), and a large proportion of the detected cancers may have been indolent cases (99 % of case patients in the selenium arm were localized T1/T2 cancers). An updated report from SELECT showed that selenium supplementation (combined selenium only and selenium + vitamin E arms) increased the risk of high-grade prostate cancer among men with higher selenium status (RR 1.91; 95 % CI 1.20–3.05) but not among those with lower baseline levels (<60th percentile of toenail selenium) [45]. The HPFS followed 4459 men initially diagnosed with nonmetastatic prostate cancer, and found that men who consumed 140 μg/day or more of supplemental selenium had a 2.60-fold (95 % CI 1.44–4.70) greater risk of prostate cancer mortality compared with nonusers [46].

It is thus suspected that a U-shaped relation between selenium supplementation and cancer may exist whereby persons with low selenium status benefit from supplementation because of increased expression of selenoenzymes, thereby increasing antioxidant protection; persons with somewhat higher levels have maximum antioxidant protection but may benefit from supplementation because of upregulation of apoptosis; and persons with high excess levels may be vulnerable to adverse effects [46]. Future studies exploring dose–response curve by stage of disease will provide more insights.

Vitamin E

Vitamin E refers to a group of ten lipid-soluble compounds that include both tocopherols and tocotrienols. The antioxidant properties of vitamin E include its ability to reduce DNA damage and inhibit malignant cellular transformation [47, 48]. α-Tocopherol is the predominant form of vitamin E in plasma.

Secondary results of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study showed a 32 % reduction in prostate cancer risk among men assigned to α-tocopherol supplementation (20 mg/day) compared to placebo [49], motivating two trials on vitamin E supplementation and risk of prostate cancer. The initial report of SELECT trial based on an average of 5.5 years of treatment, found a nonsignificant suggestion of increased prostate cancer risk among men receiving 400 IU/day of α-tocopherol [50]. With additional follow-up, the vitamin E group, but not the vitamin E plus selenium group, was found to have a significant increase in prostate cancer risk (RR: 1.17; 99 % CI: 1.04–1.36) [44]. The Physicians Health Study II also found no effect on the incidence of prostate cancer [51]. Of note is that all men in the ATBC trial were current smokers, whereas the prevalence of smoking in the other two studies was quite low. In addition, in the ATBC, prostate cancers were diagnosed outside the context of PSA screening, and thus, were generally aggressive, in contrast, SELECT and PHS II trials were performed in the PSA screening era.

Observational studies of vitamin E and prostate cancer risk generally pointed towards no overall association, but inverse associations for advanced cancers and among smokers [34, 52–58]. In the VITamins And Lifestyle study, a cohort study specifically designed to examine supplement use and future cancer risk, a 10-year average intake of supplemental vitamin E was associated with a reduced risk for advanced (regionally invasive or distant metastatic) (RR: 0.43; 95 % CI: 0.19–1.0 for 10-year average intake ≥400 IU /day vs. non-use) but not for total prostate cancer [58]. A nested case–control study of the PLCO found that higher serum α-tocopherol was associated with significantly lower prostate cancer risk (highest vs. lowest quintile RR 0.63; 95 % CI 0.44–0.92), but only in current and recently former smokers [59]. A recent meta-analysis of nine nested case–control studies found an inverse relationship between blood α-tocopherol levels and prostate cancer risk among all patients studied (RR for highest vs. lowest category 0.79; 95 % CI: 0.68–0.91) [60]. However, analyses stratified by smoking status, stage or grade, as well as dose–response meta-analysis were not conducted.

Coffee

A meta-analysis including 12 case–control and 9 cohort studies up to June 2013 suggests a modest inverse association (RR for highest vs. lowest category 0.91, 95 % CI 0.86–0.97) between coffee intake and risk of total prostate cancer [61]. The study found stronger associations with advanced prostate cancer (RR 0.82; 95 % CI 0.69–0.96) and fatal disease (RR 0.64; 95 % CI 0.47–0.80), indicating coffee may be more involved in prostate cancer progression.

Several potential mechanisms by which coffee could be associated with a lower risk of lethal prostate cancer have been proposed. Coffee is rich in biologically active compounds including caffeine, minerals, and numerous phytochemicals. In observational and animal studies, long-term coffee drinking has been associated with improved glucose metabolism and insulin secretion [62]. In the HPFS, men who consumed six or more cups of coffee per day had a 60 % lower risk of lethal prostate cancer (RR: 0.40; 95 % CI: 0.22–0.75) compared to nondrinkers [13]; intriguingly, the inverse association with lethal cancer was similar for men who drank either regular or decaffeinated coffee, suggesting that caffeine is not underlying the link. Other potential mechanism include that coffee is a potent antioxidant [63, 64] and intake may be associated with levels of different sex steroid hormones [65].

Calcium, Dairy Products, and Vitamin D

A meta-analysis including studies up to April 2014 suggests that dietary calcium was associated with an increased total prostate cancer risk (RR per 400 mg/day: 1.05; 95 % CI: 1.02–1.09) [66]. Total calcium and dairy calcium intakes, but not nondairy calcium or supplemental calcium intakes, were also positively associated with total prostate cancer risk. Supplemental calcium was associated with increased risk of fatal prostate cancer (RR: 1.50; 95 % CI: 1.13–1.99), although only two studies were included.

Dairy products, common dietary sources of calcium and animal fat, in some settings supplemented with vitamin D, have also been associated with risk of total prostate cancer, with summary RRs of 1.07 (95 % CI: 1.02–1.12) for total dairy products (per 400 g/day), 1.03 (95 % CI: 1.00–1.07) for total milk (per 200 g/day), 1.06 (95 % CI: 1.01–1.11) for low-fat milk (per 200 g/day), and 1.09 (95 % CI: 1.02–1.18) for cheese (per 50 g/day) in the same meta-analysis [66].

The weight of epidemiological evidence suggests no overall association between dietary, supplemental, and circulating vitamin D and prostate cancer incidence; however, some studies indicated vitamin D may play role in prostate cancer progression [67–70]. A nested case–control study including 1260 prostate cancer patients in the HPFS suggests that higher 25(OH)D levels were associated with a 57 % reduction in the risk of lethal prostate cancer (RR for top vs. bottom quartile 0.43; 95 % CI: 0.24–0.76) [70]. Genetic variants related to lower 25(OH)D levels were identified to be associated with risk of aggressive prostate cancer, however replications are needed [70, 71].

The correlation between dairy foods and these nutrients create challenges in trying to disentangle the independent effects of dairy, calcium, and vitamin D on prostate carcinogenesis. In studies that simultaneously consider dairy intake and calcium, relative risk estimates for dairy are attenuated compared to calcium [72–74]. One proposed mechanism involves calcium-sensing receptor (CaSR). Genetic variation across *CaSR* is associated with risk of lethal prostate cancer [75], and overexpression of CaSR has been associated with increased cell proliferation and bone metastasis [76]. A recent analysis of HPFS indicates another possibility. It is found that calcium intake was associated with total, lethal, and high-grade cancers prostate cancers but only at very high intakes and not independently of phosphorus intake [77]. In latency analysis, calcium and phosphorus had independent effects for different time periods between exposure and diagnosis. These findings merit further research.

Fat, Fatty Acids and Fish

A meta-analysis of 7 prospective studies found no significant associations between dietary intake of total, saturated, monounsaturated, and polyunsaturated fat and prostate cancer risk [78]. However, some but not all studies suggested saturated fat is associated with advanced prostate cancer [79, 80]. In the NIH-AARP, saturated fat intake, but not total, mono- or polyunsaturated fat, was related to increased risk of advanced prostate cancer (2930 advanced prostate cancer including 725 fatal cases) (RR for top vs. bottom quintile: 1.21; 95 % CI: 1.00–1.46) and fatal prostate cancer (RR 1.47; 95 % CI: 1.01–2.15) [81].

Findings on intake of the long-chain n-3 fatty acids, measured through dietary assessment and using biomarkers, are equivocal. In the HPFS, higher dietary intakes of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) were associated with lower total prostate cancer risk as well as advanced disease [82]. In the multiethnic cohort, a protective association of higher dietary intake of n-3 fatty acid intake was limited to men who were Latino or Caucasian [83]. In the Physicians' Health Study, an updated (1982–2000) analysis of these biomarkers measured in whole blood found a significant inverse association with for total prostate cancer, and a stronger association for aggressive disease [84]. An inverse association was confirmed in two population-based study [85, 81], but not in four other studies [86–89].

Studies up to 2009 suggests a null relationship between fish intake and total prostate cancer (RR: 1.01; 95 % CI: 0.90–1.14), but a significant inverse relation with prostate cancer-specific mortality (RR: 0.37; 95 % CI: 0.18–0.74) [90]. In contrast, a recent analysis from the SELECT trial reported positive associations of high plasma levels of long-chain ω-3 polyunsaturated fatty acids and risk of total and high-grade prostate cancer [91]. However, the majority of the 909 cases in this analysis had localized cancer, and only four were diagnosed with advanced-stage disease (T3). Even high-grade disease, defined as Gleason 4 + 3 or higher, is common on autopsy, and hence cannot serve as a surrogate for potentially lethal prostate cancer [92]. Because fish consumption is a characteristic of health-conscious behavior and is directly correlated with intensity of PSA screening [93], the modest apparent increase in risk of overall prostate cancer was likely due to more screening in men with higher fish consumption. Indeed, in the Physicians' Health Study, an apparent increase in overall prostate cancer associated with higher fish consumption was observed, but disappeared after adjustment for PSA screening [93].

Conclusion

Emerging evidence indicates that several dietary and lifestyle factors may alter prostate cancer incidence or progression. However, recommendations for specific changes in these factors for prostate cancer prevention should be considered in the context of promoting overall health among men.

For example, maintaining a healthy weight through prudent dietary choices in combination with exercise may impart substantial benefits for men, by reducing the risk of prostate cancer mortality, risk of other cancers, and other chronic diseases. Furthermore, encouraging men to increase intake of specific vegetables, such as tomato-based products, as well as coffee and fish may also have a positive benefit on reducing other cancers as well as other common diseases, such as cardiovascular disease [94–97]. Although the evidence for each of these factors for prostate cancer risk is not definitive, their overall health benefits are clear and it is not inconsequential that most men with prostate cancer will die of other chronic diseases rather than their cancer.

High intake of calcium and selenium from supplements may be deleterious for prostate cancer outcomes. In contrast, low calcium intakes could have detrimental effects on bone health, both as a

consequence of general aging [98] and for men with prostate cancer undergoing hormonal therapy [99]. However, little evidence suggests that such high intakes as 1500 mg/day, which have been associated with greater risk of advanced prostate cancer, are beneficial for general health in middle-aged to elderly men.

For researchers, it is important to recognize that in the PSA screening era, studies of total prostate cancer incidence are of limited value, due to the high prevalence of indolent cancers and the fact that incidence largely reflects the propensity for PSA screening intensity and having a biopsy. In addition, based on existing evidence, dietary factors in middle-aged and elderly men, if at all relevant, probably act largely on progression of localized cancers to lethal forms rather than on initiation. The potential influence of such progression factors may be completely missed if the end point is localized prostate cancer.

In addition, the positive association between height and prostate cancer, particular with advanced diseases, suggests that earlier-life diet and other lifestyle factors that occur during childhood and adolescence when the prostate is still maturing, may be as, or more, important for prostate cancer risk; however, these topics are largely unexplored [100]. Expanding research to early-life exposure may provide more insights to the etiology of this heterogeneous disease and also inform cancer prevention strategies, whether to shift the focus of some interventions in mid- to late adulthood to interventions that start in earlier stages of the life course and whether to use information on risk factors throughout the life course to guide prevention counseling.

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Chapter 11
Dietary Supplements and Cancer Risk: Epidemiologic Research and Recommendations

Rebecca L. Sedjo, Marian L. Neuhouser, and Cheryl L. Rock

Key Points

- Over one-half of all Americans use dietary supplements. Thousands of supplements of multiple combinations of vitamins, minerals, and herbs are available for purchase, but the most commonly used supplements are multivitamins (both with and without minerals) and single supplements of vitamin C and calcium (with or without vitamin D).
- Dietary supplements can provide a large proportion of total micronutrient intake for many consumers.
- Individuals who smoke should not use β-carotene supplements, as they have been shown to increase lung cancer risk among smokers.
- Vitamin E is not recommended for the prevention of cancer.
- Cancer benefits or harms associated with dietary supplements of vitamin C, calcium, and vitamin D are inconclusive.

Keywords Dietary supplements • Vitamin • Mineral • Cancer incidence • Beta-carotene • Vitamin E • Selenium • Multivitamin • Vitamin C • Calcium • Vitamin D • Folic acid

Introduction

Millions of Americans use dietary supplements [1, 2]. Data from national nutrition surveys suggest that between 49 and 52 % of all American adults use dietary supplements on a regular basis [1, 3, 4] with substantially higher use among certain population subgroups such as cancer survivors [5–9]. One reason for the high prevalence of use is the 1994 passage of Public Law 103-417, the Dietary

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_11

Supplement and Health Education Act (DSHEA) [10]. This legislation discontinued the premarket safety evaluations for ingredients used in supplements, placed the burden of proof of product safety on the United States (US) Food and Drug Administration (FDA) instead of the manufacturers, and permitted limited nutrition support statements without prior approval from the FDA [10]. These regulations resulted in an exponential increase in the number and variety of dietary supplements available for over-the-counter purchase, including those that consumers believe may prevent chronic diseases such as cancer [11–13]. The most common reasons US adults report using supplements is to improve or maintain overall health [4].

The medical community and the public are conflicted about the efficacy of dietary supplements in cancer prevention, although after numerous trials that have not demonstrated benefits (discussed in this chapter), the totality of the evidence to date suggests that cancer risk is unaffected by dietary supplement use. Randomized controlled trials (RCTs) of supplements have yielded some entirely unexpected findings including possible harm rather than benefit. For example, β-carotene, for which observational studies consistently linked higher dietary intake with reduced risk for lung cancer, was found to increase the incidence of lung cancer in two large clinical trials [14, 15]. Selenium, which was hypothesized to reduce the risk of non-melanomatous skin cancers among persons with previous basal cell or squamous cell carcinomas, had no effect on basal cell cancers, increased the risk of squamous and total nonmelanoma skin cancers and reduced the risk of several other cancers [16]. Supplementation with selenium alone, vitamin E alone, selenium + vitamin E, or placebo was subsequently tested in a large clinical trial for the primary prevention of prostate cancer, in which beneficial effects on incidence were not observed [17] (www.crab.org/select). These results are juxtaposed with a 2002 report claiming that most Americans do not obtain sufficient vitamins from the diet to prevent chronic disease, including certain cancers, and advised that all adults use a daily multivitamin supplement [18, 19]. Conversely, a National Institutes of Health (NIH) State-of-the-Science Panel concluded that current evidence, especially results from RCTs, does not support the usefulness of dietary supplements for cancer prevention [20]. Similarly, the guidelines from the American Cancer Society do not recommend dietary supplements for cancer prevention with the exception of calcium for the possible reduction colorectal cancer [21]. Updated guidelines on dietary reference intakes for calcium and vitamin D were established in 2011; however, these guidelines were not based on cancer outcomes as these data were inconclusive at that time [22]. Based in part on a systematic evidence review conducted by the Kaiser Permanente Research Affiliates Evidence-Based Practice Center [23], the US Preventive Services Task Force (USPSTF) in 2013 released recommendations against the use of β-carotene or vitamin E supplementation for the reduction of cancer or cardiovascular disease incidence [24]. Furthermore, the USPSTF report stated that there was not enough data at this time to determine the benefits and harms associated with multivitamins and other single or paired supplements besides β-carotene or vitamin E for the prevention of cancer or cardiovascular disease [24]. These opposing views from the medical community and unexpected results from intervention studies underscore the need for a synthesis of the research literature on supplements and cancer prevention.

This review describes epidemiologic evidence of dietary supplement use with cancer risk. As an introduction, we provide a definition of dietary supplements and briefly review potential biological mechanisms whereby dietary supplements could prevent cancer. We also present methodological considerations important for understanding epidemiologic studies on supplement use and cancer risk. The majority of the review is devoted to synthesizing results from studies that have provided data on supplement use and cancer risk. We then discuss issues relevant to this research, with emphasis on problems in assessment of supplement use and potential confounding factors. Lastly, we provide information on new studies in this area and give our recommendations about the use of dietary supplements to prevent cancer.

Definition of Dietary Supplements in the United States

Dietary supplements as defined by the Dietary Supplement and Health Education Act (DSHEA) are vitamins, minerals, herbs or other botanicals and amino acids which are sold as capsules, gelcaps, powders, or liquids and are intended to supplement the diet through increased dietary intake [10]. These categories are not mutually exclusive as many supplements contain mixtures of ten or more micronutrients, herbs, and other potentially bioactive compounds. Highly fortified food products and items intended as the only component of a meal (e.g., meal replacement beverages) are not classified as dietary supplements.

Hypothesized Mechanisms of Effect

There is evidence that a dietary pattern that emphasizes plant foods, which are generally good sources of micronutrients and bioactive food components, is associated with lower risk of human cancers [25–29]. A comprehensive review of diet and cancer concluded that the current evidence demonstrates a possible protective effect of vegetable consumption, and less definitively, fruit consumption, against almost all major cancers [25]. The mechanisms underlying these associations are complex and likely involve numerous compounds and multiple biochemical pathways [30, 31]. Included among potential agents from plant foods are a variety of vitamins (e.g., vitamin C, vitamin E, folate) and minerals (e.g., calcium, selenium) as well as a myriad of bioactive compounds such as carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) and flavonoids (e.g., quercetin, naringenin). Whether dietary supplements containing micronutrients and bioactive compounds found in plant foods would be effective chemopreventive agents has been of considerable public health interest. A particularly important point is that the bioavailability and pharmacokinetics of many bioactive food components from supplements has not been completely characterized; for some nutrients the bioavailability is increased from supplements, while for others it is decreased [32, 33]. For example, the bioavailability of folic acid is greater in supplement form, compared to natural food forms (i.e., fruits, vegetables) [32–34], but there is no difference in bioavailability of the forms of supplemental calcium (i.e., calcium citrate vs. calcium carbonate) although calcium citrate contains half the elemental calcium as calcium carbonate so twice the number of pills must be taken to reach the same intake of elemental calcium [35, 36].

Laboratory studies provide evidence for mechanisms whereby micronutrients commonly found in dietary supplements could prevent cancer. Much attention has focused on nutrients and bioactive food components with antioxidant properties: carotenoids, vitamin C, vitamin E, and selenium [17, 37–42]. There are many potentially relevant functions for antioxidants, including protection of cell membranes and DNA from oxidative damage, scavenging and reduction of N-nitroso compounds, and serving as cofactors or structural components for enzymes whose substrates are reactive nitrogen or reactive oxygen species [37, 42–46]. Vitamin E is a nonspecific chain-breaking antioxidant while vitamin C is a strong intracellular and extracellular antioxidant via its electron donor capabilities [45]. Selenium is a structural component of the glutathione peroxidases, which comprise both the intracellular and extracellular antioxidant defense systems [47].

Micronutrients have preventive properties apart from their potential antioxidant capabilities. Vitamin A (i.e., retinol) plays a role in the differentiation of normal epithelial cells and the maintenance of intercellular communication through gap junctions, thus repressing the processes leading to abnormal cell replication [48, 49]. The retinoic acid receptors, RAR and RXR, regulate gene expression of numerous enzymes and proteins and retinoids up-regulate the synthesis of natural killer cells and cytokines involved in the inflammatory response [50]. Carotenoids exhibit antioxidant activity in vitro; however, laboratory evidence strongly suggests that the retinoid-like activities of the carotenoid metabolites are the more important mechanisms by which they may inhibit the progression of carcinogenesis

in the human biological system [51, 52]. Similar to the effects of retinoids, carotenoids influence cell growth regulation, including the inhibition of growth and malignant transformation and the promotion of apoptosis in transformed cells [49, 53–56]. Vitamin C enhances the immune response and connective tissue integrity primarily via its role as a cofactor or co-substrate for enzymes involved in biosynthesis of collagen, catecholamines, and mixed function oxidases [47]. Vitamin E has strong antiproliferative effects on cultured human tumor cells, possibly mediated by its influence on important cell signaling pathways including TGF-β, c-Jun, and the mitogen-activated protein kinase signaling pathway [57]. Folic acid is an important water-soluble B vitamin that primarily functions as a methyl donor. Folate may be related to cancer risk because inadequate amounts of the vitamin may increase hypomethylation of DNA, with subsequent loss of the normal controls on gene expression [58, 59]. Low folate status may also impair DNA repair capacity, a noted risk factor for human cancers [60]. Calcium may influence colon cancer risk by binding bile acids [61] or by regulating colorectal epithelial cell proliferation [62]. Some evidence has suggested that vitamin D reduces risk of colon, breast, prostate, and other cancers by its crucial role in maintaining calcium homeostasis, but perhaps more importantly by regulation of gene transcription [63], inhibiting epithelial cell proliferation and enhancing apoptosis and cellular differentiation [22, 64–66]. Furthermore, vitamin D has also exhibited anti-angiogenic properties as well as anti-inflammatory properties that maybe important for the prevention of carcinogenesis [22]. Selenium may block the clonal expansion of early malignant cells by modulation of cell cycle proteins and apoptotic proteins, in addition to its antioxidant functions [67, 68]. Conversely, iron may increase risk of cancer because it enhances the growth of transformed cells and acts as a prooxidant, thereby increasing carcinogenic DNA changes and general oxidative stress [69]. Improved understanding of cancer biology will be needed for identifying the cellular and molecular processes that can be affected by vitamin and mineral supplementation.

Prevalence of Supplement Use in the USA

In the USA, consumption of dietary supplements is widespread and has been stable for the past decade [1, 4]. In National Health and Nutrition Examination Survey (NHANES) 2007–2010, 49 % of adults reported taking a dietary supplement in the past month [4]. In multivariate analysis, women (versus men), non-Hispanic whites (versus non-Hispanic blacks or Mexican-Americans), and those with a higher level of education, healthy body mass index, and higher level of physical activity were associated with a greater likelihood of reporting use of dietary supplements [1, 2, 4]. Importantly, many consumers take multiple supplements either alone or in combination with prescription or over-the-counter medications [3, 70]. For example, in a large cohort study of dietary supplement use and cancer risk in Western Washington State, 32 % of the 76,072 cohort participants reported daily use of five or more supplements over the previous 10 years [71]. It is important to note, though, that usage patterns among a group of habitual supplement users may differ from the general population. In NHANES 2005–2008, 34.3 % of adults used a dietary supplement and a prescription drug concomitantly [70]. Dietary supplements comprise a substantial portion of Americans' out-of-pocket medical expenditures estimated at $28.1 billion in 2010 [72].

Objectives of This Review

This review presents observational data from cohort studies and experimental data from clinical trials in adults on the use of dietary supplements and cancer risk. Data from RCTs are summarized for β-carotene, vitamin E, and selenium whereas data from both prospective studies and RCTs are reviewed for multivitamins, vitamin C, vitamin D, and calcium with a limited review of folic acid.

Issues in Interpreting Published Studies

Micronutrient Intakes from Foods and Supplements Differ Markedly

Dietary supplements can provide a large proportion of total intakes of some micronutrients, and therefore the variability in total micronutrient intake attributable to supplements can overwhelm that from foods. For example, in the Women's Health Initiative (WHI) [73], supplement users obtained from 50 to 70 % of their total vitamin A, vitamin C, and vitamin E from supplements, and the median dose from supplements was generally greater than that from foods (Table 11.1) [74]. Additional data from WHI show that antioxidant supplement use increased over time such that compared to 1993–1994, the odds of using single supplements of vitamin C and vitamin E in 1998 were 1.37 and 2.10, respectively [75]. These results were remarkably similar to results among men enrolled in a large chemoprevention trial for the primary prevention of prostate cancer. Forty-four percent of the men enrolled in the trial reported use of a multivitamin on a regular basis and approximately one-third used high-dose single supplements of vitamin C or vitamin E. Among supplement users, nutrient intake from supplements contributed to about half of total β-carotene and folate intakes and approximately 60 % of vitamins A, C, D and 90 % of vitamin E intakes (Fig. 11.1) [76]. These data illustrate the point that for many nutrients (such as vitamin E), the dose available from supplements (typically 200–1000 mg) is many times larger than can possibly be obtained from foods (about 8–10 mg). Therefore, in many observational studies of cancer risk, the highest levels of intake of many micronutrients could only be obtained from supplements. Because many of the earlier published studies did not present findings separately for nutrients from foods vs. nutrients from supplements, these studies did not assess supplements alone and were not included. Studies published within the last 15 years have more consistently presented results separately for nutrients from food vs. supplements.

An additional important point with regard to micronutrients from food vs. supplements is that the chemical isomers in supplements may differ from those in food. This is particularly important for vitamin E. The natural isomer most abundant in nature (RRR-α-tocopherol) is preferentially retained, compared to synthetic α-tocopherol, which consists of eight diastereoisomers (DL-α-tocopherol) [77]. Although the principal vitamin E isomer in foods is γ-tocopherol, which may be more effective at trapping reactive nitrogen species in cell culture studies [78], it is found in only very small concentrations in the human biological system.

Table 11.1 Vitamin and mineral supplement use among 16,747 participants in the Women's Health Initiative (WHI) [74]

Nutrient	Taking supplement containing nutrient (%)	Intake from supplements among supplement users (median)	Intake from foods (median)	Supplement intake as % of total intake among supplement users (mean)	Supplement intake as % of total intake among all participants (mean)
Retinol, mcg	43.5	2250	439	53.6	35.2
β carotene, mcg	42.6	4500	3264	37.1	24.3
Vitamin C, mg	53.1	200	95	51.4	33.7
Vitamin E, mg	53.2	30	7	71.3	46.7
Folate, mcg	44.0	400	245	41.1	26.9
Calcium, mg	51.5	500	672	30.9	20.3
Iron, mg	37.0	18	13	33.4	21.9
Selenium, mcg	32.5	20	89	11.3	7.4

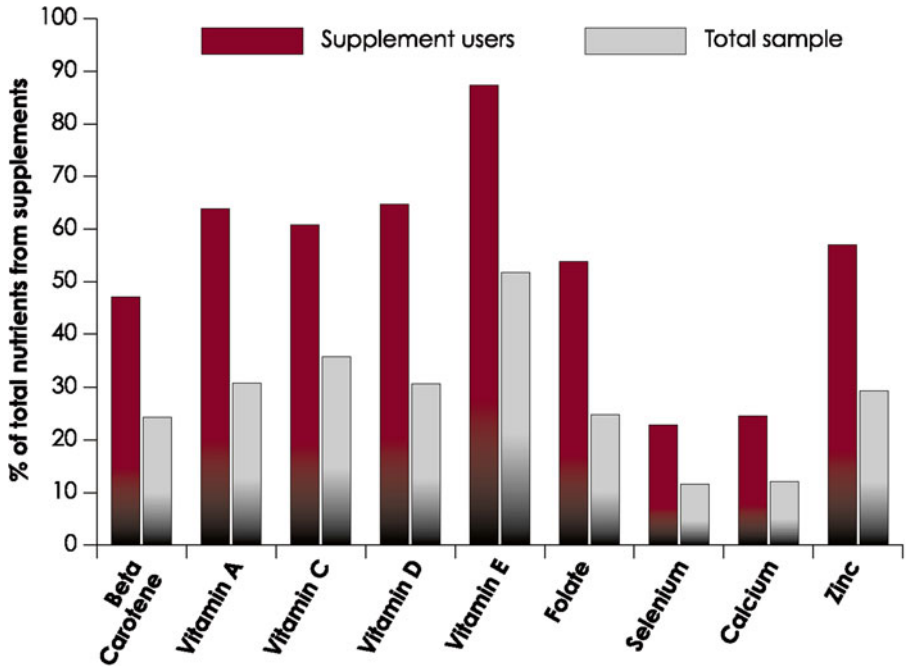

Fig. 11.1 Nutrient intake among supplement users ($n = 9263$) and complete sample ($n = 15{,}387$) of men enrolled in The Prostate Cancer Prevention trial [76]

The Effects of Micronutrients in Multivitamins Cannot Be Isolated

Findings from investigations that measure multivitamin use are a particular analytical challenge because one cannot isolate the potential effect of the micronutrient of interest (e.g., vitamin A, vitamin D) from all the other vitamins and minerals in the supplement. To investigate micronutrients from supplements, study samples must have sufficient numbers of participants using single supplements (e.g., a capsule containing only vitamin A) to separate out the effects of these micronutrients from multivitamin use. We suggest that findings on micronutrients that are seldom taken as single supplements (e.g., vitamin A, thiamin, zinc) almost certainly reflect the use of multivitamins and are thus confounded by other constituents. Therefore, we present observational studies on multivitamins and the following individual micronutrients: vitamin C and calcium. These micronutrients represent the most commonly used single supplements [1, 4, 71], and therefore it is at least plausible that studies of these micronutrients had enough users to isolate their effect from that of multivitamins. We also included prospective data on vitamin D supplements; however, the use of individual supplement is much lower and therefore there is the risk that their effects may be confounded by other multivitamins. There are insufficient epidemiologic data addressing herbal supplements and cancer risk to be included in this review.

Issues of Study Design as Related to Research on Vitamin Supplements

Below we briefly describe the two epidemiologic research study designs included in this review, with additional comments on their strengths and weaknesses when used to study associations of dietary supplements use with cancer risk.

Randomized Controlled Trials

In RCTs, considered the strongest design, the investigators allocate the exposure (i.e., the supplement) at random. Participants are then followed over a period of time to assess the occurrence of a specified disease outcome. Assuming the sample is sufficiently large, the experimental design of an RCT provides a very high degree of assurance about the validity of the results because both known and unknown characteristics of the intervention and control groups are assumed (or likely to be) identical, thereby eliminating the biases of observational studies. However, RCTs are too costly to conduct multiple trials for different types of cancers, nutrients, doses, combinations, or for long periods of time. The latter point is particularly important because carcinogenesis is a process that takes place over a period of many years; so short-term trials often do not yield definitive information [79]. In addition, in many trials, the participants are selected to be at high risk for the cancer of interest (e.g., smokers in studies of lung cancer), [14, 80], limiting the generalizability of the findings. Finally, prevention trials in humans cannot test an agent with known risk.

Cohort Studies

In cohort studies, the supplement use of a group of disease-free participants is measured, ideally over time. This group is then followed to assess the occurrence of multiple disease outcomes. Cohorts are attractive for studies of supplement use because they can assess the effects of many types, doses, and combinations of supplements, with multiple cancer outcomes. Their primary limitation is that the exposure is self-selected, so that investigators must measure and control for factors (such as diet, exercise, or smoking) likely to confound supplement-cancer associations. In addition, some cohort studies have inadequate statistical power to test associations of supplement use with cancer, because supplement use was relatively rare when these cohorts were established [81], although many cohorts have updated their dietary assessment strategies to include assessment of supplement use [71, 82, 83]. A case–control design used within a cohort study (i.e., nested case–control study) has the advantage of minimizing selection bias and avoiding recall bias as the supplement use is measured prior to disease outcome. In nested case–control studies, all cases from a specific cohort are selected and then a subsample of those without the disease outcome. The groups are then compared to see whether supplement use varies by disease status.

Review of Studies on Vitamin Supplements and Cancer Risk

Methods

To examine the role of supplement use, we included only studies that presented findings on multivitamins or nutrients from supplements, separate from food. Studies on total intake of nutrients (diet plus supplements) are not presented because it is not possible in such studies to separate effects of other bioactive compounds present in foods from those of the specific micronutrients of interest. Hereafter, we use the term “dietary supplement” to include both vitamin and mineral supplements, and we use the term “multivitamin” to refer to a one-a-day type multivitamins, which typically also contain minerals.

Below we summarize the published literature on vitamin supplements and cancer risk, organized by supplement type and study design. Results are grouped by supplement type because an evaluation of which vitamin supplements are associated with cancer at all sites is important for the development

of public health recommendations for the prevention of cancer as a whole. MEDLINE® database of the National Library of Medicine was used to identify epidemiologic studies on dietary supplement use and cancer risk. We limited our review in several ways. Because few studies presented findings on vitamin supplements before 1985, we searched the database from 1980 onward (up to Oct 2014). Only studies that utilized a RCT design or cohort design including nested case–control studies were included. Case–control and cross-sectional studies were excluded to avoid possible recall bias of supplement exposure or selection bias due to the selection of a control group. Only manuscripts with at least 50 cancer endpoints (cases or deaths) in adults were included. Studies from cancer-free adult population or at-risk populations were included, whereas those studies conducted in cancer survivors were not included. Studies of precancerous conditions (e.g., colorectal adenomas or in situ lesions) were not included. We present data on RCTs of β-carotene, vitamin E, and selenium because the unexpected results from these trials have strongly influenced public health recommendations for these nutrients. Next, we summarize data on multivitamins and other commonly used single supplements: vitamin C, vitamin D, and calcium. Further, due to the interest in folic acid and the potential that heavy folate supplement use may increase, rather than decrease, cancer risk, we include a short section on folic acid. As noted above, because many other vitamins and minerals (e.g., vitamin A, zinc) are generally only obtained from multivitamin pills, we do not present results for those individual nutrients.

Randomized, Controlled Trials of β-Carotene

Table 11.2 gives results from six RCTs that have examined either supplemental β-carotene alone or β-carotene combined with other supplemental nutrients [14, 15, 84–87]. These trials were motivated by observational epidemiology, animal experiments, and mechanistic studies of carcinogenesis. There were fairly consistent findings of protective associations for fruit and vegetable consumption (the dominant dietary sources of carotenoids), total carotenoid intake, and serum carotenoid concentration on cancer incidence, in particular for cancer of the lung. However, the intervention trials of β-carotene supplements, either alone or combined with other agents, did not support protective effects for incident cancer, cancer mortality, or total mortality. In two studies of persons at high risk of lung cancer, due either to smoking or asbestos exposure [The Beta-Carotene and Retinol Efficacy Trial (CARET) and The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC)], β-carotene significantly increased lung cancer incidence by 20 and 30 % [14, 15] while another trial, the Women's Antioxidant Cardiovascular Study, conducted in women at high risk for cardiovascular disease reported a similar although nonstatistically significant increased lung cancer risk associated with β-carotene supplementation [87]. In a trial examining skin cancer among those at risk for skin cancer, there was no effect of β-carotene on any type of skin cancer or cancer mortality overall [84, 88]. In trials conducted among low risk populations, one in male physicians and another among female health professionals, no effect of β-carotene on lung cancer incidence [85] or total cancer incidence and mortality [86] was reported. In CARET, β-carotene also significantly increased lung cancer mortality and cardiovascular disease mortality [15].

CARET and ATBC continued follow-up with study participants for several years to collect cancer and mortality endpoints [89–92]. ATBC reported that even after the cessation of the β-carotene intervention, the relative risk for mortality in the β-carotene arm remained elevated for up to 6 years during the post-trial follow-up. The majority of the excess deaths was due to cardiovascular events [89]. In terms of cancer incidence in the ATBC follow-up period, there were no statistically significant differences in cancer incidence between β-carotene recipients and non-recipients at any site with the exception of a late effect of β-carotene being associated with an 88 % increased risk of colorectal cancer [89]. With follow-up of 18 years, there was no long-term associations between β-carotene and cancer

Table 11.2 Randomized controlled trials of β-carotene and risk of cancer

Study name (ref)	Agent	Endpoint(s)	Cases	Relative risk for supplementation
Skin Cancer	50 mg β-carotene	Cancer mortality		
Prevention Study [84, 88]		All sites	82	0.8 (0.5–1.3)[a]
		Incidence		
		Nonmelanoma skin cancer	1952	1.0 (0.9–1.2)[a]
		Basal cell	651	1.0 (0.9–1.2)[a]
		Squamous cell	132	1.2 (0.9–1.7)[a]
The Alpha-Tocopherol, Beta-Carotene Study	20 mg β-carotene	Cancer mortality		
Intervention period results [14]		Lung	564	1.2 (ns)[b]
		Other	552	1.0 (ns)[b]
		Incidence		
		Lung	876	1.2 (1.0–1.4)
		Four other sites	126–250	No associations
The Alpha-Tocopherol, Beta-Carotene Study	N/A	Incidence		
Post-Intervention Follow-Up [89]		Lung		
		1993–1996	498	1.17 (0.98–1.39)
		1996–1999	539	0.97 (0.82–1.15)
		Colorectal		
		1993–1996	92	1.06 (0.70–1.60)
		1996–1999	113	1.88 (0.82–1.15)
		Five other sites		
		1993–1996, 1996–1999	52–395	No associations
The Alpha-Tocopherol, Beta-Carotene Study	N/A	Cancer mortality		
Post-Intervention Follow-Up after 18 years [91]		Lung	2671	1.05 (0.97–1.13)[a]
		Prostate	529	1.20 (1.01–1.42)[a]
		Other	2355	1.01 (0.94–1.10)[a]
		Incidence		
		Lung	2881	1.04 (0.96–1.11)[a]
		Colorectal	676	0.97 (0.84–1.13)[a]
Physicians' Health Study [85]	50 mg β-carotene on alternate days	Cancer mortality		
		All sites	766	1.02 (0.89–1.18)[a]
		Incidence	2316	0.99 (0.91–1.07)[a]
Beta Carotene and Retinol Efficacy Trial or CARET [15]	30 mg β-carotene and 25,000 IU vitamin A	Cancer mortality		
		Lung cancer	254	1.5 (1.1–2.0)
		Incidence		
		Lung	388	1.3 (1.0–1.6)
		Prostate	300	No associations
		Other cancers	730	No associations
Women's Health Study [86]	50 mg β-carotene on alternate days	Cancer mortality		
		All sites	59	1.11 (0.67–1.85)
		Cancer Incidence		
		All sites	747	1.03 (0.89–1.18)

(continued)

Table 11.2 (continued)

Study name (ref)	Agent	Endpoint(s)	Cases	Relative risk for supplementation
The Women's Antioxidant Cardiovascular Study (WACS) [87]	50 mg β-carotene on alternate days	Cancer mortality	176	0.84 (0.62–1.13)[a]
		Cancer incidence		
		All sites	624	1.00 (0.85–1.17)[a]
		Breast	257	1.01 (0.79–1.30)[a]
		Lung	79	1.26 (0.80–1.99)[a]
		Uterine	50	1.27 (0.73–2.23)[a]
		Four other sites	20–44	No associations

[a]Adjusted relative risk, see original studies for details

[b]Nonsignificant

incidence of lung, prostate, urinary tract, kidney, stomach, colorectal, pancreas or all-cause mortality [91]. In CARET, there was no protective association for persons taking the CARET study vitamins [92]. One of the most striking results was that fruit and vegetable consumption provided protection against lung cancer risk among these heavy smokers, but only among those taking the placebo. In essence, the high-dose β-carotene + retinol supplements had the apparent effect of negating any benefit from a high fruit and vegetable diet [92]. Interestingly, among CARET trial participants who used another dietary supplement, mostly multivitamins, there was an increased risk of aggressive prostate cancer risk that ceases when the study supplements were discontinued [93].

The fairly consistent negative or null findings from these clinical trials of supplemental β-carotene are perplexing, given the strong and consistent protective effects of dietary and serum carotenoids found in observational studies [25, 26, 94–97]. However, convincing evidence now exists that β-carotene supplements is contraindicated in smokers or by others at risk for lung cancer via occupational exposures to lung carcinogens [25]. Based on the evidence of harm in at-risk populations and a lack of effect in the general population, the USPSTF recommends against the use of β-carotene supplements in healthy populations [24].

Randomized, Controlled Trials of Vitamin E

Over the past several decades numerous randomized, controlled trials have been conducted to evaluate the potential benefit of vitamin E supplementation on various cancer outcomes (Table 11.3). In the ATBC Study (discussed above), male smokers were assigned to receive 50 mg/day dl-α-tocopherol with or without β-carotene, and no effects on cancer incidence or mortality were observed in association with vitamin E supplementation [14]. However, an unexpected finding in this trial was a statistically significant 30 % reduced risk for prostate cancer in the vitamin E arm of the trial [98]. With post-intervention follow-up, this association became attenuated and was no longer significant [89, 91]. Nevertheless, this study raised considerable interest in the possible reduced risk of prostate cancer with vitamin E supplementation, and two additional trials were conducted in men to specifically evaluate prostate cancer outcomes, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) and The Physicians' Health Study II (PHSII). The SELECT trial was designed to test selenium and/or vitamin E (400 mg/day dl-α-tocopherol) on prostate cancer incidence in >35,000 men enrolled at >400 sites in the USA [99]. In 2008, the active treatment (supplementation) in this trial was halted because the supplements, taken alone or together for a median of 5.5 years did not prevent prostate cancer and it was evident that an effect was unlikely to be achieved even with continuation of treatment [100]. Further, there were two findings that caused some concern, although these findings were not statistically significant. Slightly more cases of prostate cancer in men taking only vitamin E

Table 11.3 Randomized controlled trials of vitamin E and risk of cancer

Study name (ref)	Agent	Endpoint(s)	Cases	Relative risk for supplementation
The Alpha-Tocopherol, Beta-Carotene Study	50 mg/day dl-α-tocopherol	Cancer mortality		
Intervention period results [14, 89]		Lung	564	1.0 (ns)[a]
		Other	552	1.1 (ns)[a]
		Incidence		
		Lung	895	0.99 (0.87–1.12)
		Prostate	249	0.66 (0.52–0.86)
		Five other sites	89–169	No associations
The Alpha-Tocopherol, Beta-Carotene Study	N/A	Incidence		
Post-Intervention Follow-Up [89]		Lung		
		1993–1996	498	0.92 (0.77–1.09)
		1996–1999	539	1.14 (0.96–1.35)
		Prostate		
		1993–1996	277	0.89 (0.70–1.12)
		1996–1999	395	0.88 (0.72–1.07)
		All Other Sites		
		1993–1996, 1996–1999	52–126	No associations
The Alpha-Tocopherol, Beta-Carotene Study		Cancer mortality		
Post-Intervention Follow-Up after 18 years [91]		Lung	2671	0.99 (0.92–1.07)[b]
		Prostate	529	0.84 (0.70–0.99)[b]
		Other	2355	1.03 (0.95–1.12)[b]
		Incidence		
		Lung	2881	1.01 (0.94–1.09)[b]
		Prostate	2321	0.97 (0.89–1.05)[b]
Physicians' Health Study II [103]	400 IU α-tocopherol on alternate days	Cancer mortality		
		All sites	523	1.13 (0.95–1.34)[b]
		Prostate	76	1.01 (0.64–1.58)[b]
		Seven other sites	11–87	No associations
		Incidence		
		All sites	1943	1.04 (0.95–1.13)[b]
		Prostate	1008	0.97 (0.85–109)[b]
		Seven other sites	55–162	No associations
Physicians' Health Study II-Post Intervention Follow-Up [104]	N/A	Cancer mortality	859	1.11 (0.97–1.27)[b]
		Incidence		
		All sites	2669	1.02 (0.95–1.10)[b]
		Prostate	1373	0.99 (0.89–1.10)[b]
		Seven other sites	79–2402	No associations
The Women's Health Study [105]	600 IU α-tocopherol on alternate days	Cancer mortality		
		All sites	583	1.12 (0.95–1.32)[b]
		Cancer incidence		
		All sites	2865	1.01 (0.94–1.08)[b]
		Breast	1230	1.00 (0.90–1.12)[b]
		Lung	205	1.09 (0.83–1.44)[b]
		Colon	214	1.00 (0.77–1.31)[b]

(continued)

Table 11.3 (continued)

Study name (ref)	Agent	Endpoint(s)	Cases	Relative risk for supplementation
The Women's Antioxidant Cardiovascular Study (WACS) [87]	600 IU α-tocopherol on alternate days	Cancer mortality	176	0.87 (0.65–1.17)[b]
		Cancer incidence		
		All sites	624	0.93 (0.79–1.09)[b]
		Breast	257	0.98 (0.77–1.25)[b]
		Lung	74	1.25 (0.79–1.97)[b]
		Uterine	50	0.67 (0.38–1.18)[b]
		Four other sites	20–45	No associations[b]
Health Outcomes Prevention Evaluation (HOPE)/HOPE-The Ongoing Outcomes (HOPE TOO) [106]	400 IU α-tocopherol	Cancer mortality	334	0.88 (0.71–1.09)
		Cancer incidence	1138	0.94 (0.84–1.06)
The Selenium and Vitamin E Cancer Prevention Trial (SELECT) [100, 101]	400 IU all rac-tocopherol acetate	Cancer mortality	231[c]	0.84 (0.60–1.18)
		Incidence		
		Total	1680[c]	1.03 (0.91–1.17)
		Prostate	889[c]	1.13 (0.95–1.35)
		Two other sites	126–134[c]	No associations
The Selenium and Vitamin E Cancer Prevention Trial (SELECT)-Post Intervention Follow-UP [101]	N/A	Incidence		
		Total	2298[c]	1.07 (0.96–1.19)
		Prostate	1149[c]	1.17 (1.004–1.36)
		Two other sites	160–196[c]	No associations

[a]Nonsignificant
[b]Adjusted relative risk, see original studies for details
[c]The actual significant level is not given in the original paper [14]. Compared to placebo, α-tocopherol reduces the risk of prostate cancer by 36 % (RR = 0.64, 95 % CI 0.44–0.94) [88]. Includes participants randomized to vitamin E alone or placebo.

supplements and slightly more cases of diabetes in men taking only selenium supplements were reported [100]. As compared to placebo, vitamin E supplementation did not increase the risk for cancer incidence or cancer mortality at the time the study was stopped. With additional post-intervention follow-up, the 13 % increased risk of prostate cancer (HR = 1.13, 95 % CI 0.95–1.35) associated with vitamin E supplementation observed when the study was stopped became significant (HR = 1.17, 95 % CI 1.004–1.36) [101]. The PHSII was designed as a RCT using a factorial design of vitamin E, vitamin C, β-carotene, multivitamins, or placebo among ≥14,000 US male physicians who were 50 years of age or older [102]. In response to the ATBC trial findings, the vitamin E arm was designed specifically to evaluate the potential long-term effects on prostate cancer as well as other cancers. While the majority of men included in this study had not been diagnosed with a previous cancer, this trial did allow men with a history of cancer to be enrolled. Findings from this trial found no association between prostate cancer and vitamin E supplementation during a mean of 8 years [103]. An additional 2.8 years of mean follow-up did not alter these results [104].

The randomized placebo-controlled trials of vitamin E conducted among women or both men and women combined have also reported null findings in regards to cancer outcomes. In the Women's Health Study, 39,876 women ≥45 years old were prescribed vitamin E supplements (600 IU every other day RRR-α-tocopherol with or without aspirin and followed for an average of 10.1 years) [105]. No effects on cancer incidence or overall mortality were observed in association with vitamin E supplementation. In the Women's Antioxidant Cardiovascular Study (discussed above), women at high risk for cardiovascular disease were randomized to receive vitamin C, vitamin E (600 IU α-tocopherol every other day), β-carotene, or placebo [87]. There were no associations with vitamin E supplementation and either cancer incidence or cancer mortality, taken individually or in combination with the other study supplements. In the Heart Outcomes Prevention Evaluation (HOPE) and HOPE-The Ongoing Outcomes (HOPE-TOO) studies, cancer incidence and cancer mortality were

examined as primary outcomes in a target group of 9541 and 7030 individuals, respectively [106]. Study participants were ≥55 years of age with vascular disease or diabetes mellitus, randomized to vitamin E supplementation (400 IU/day RRR-α-tocopherol) or placebo. There were no differences in cancer incidence and cancer deaths across the two study arms, during a median duration of follow-up of 7 years.

Despite initial optimism about the benefits of vitamin E, the results from the RCTs do not support a reduction in any cancer or cardiovascular outcomes association with vitamin E supplementation [23]. Based on adequate evidence demonstrating a lack of effect for both cancer and cardiovascular disease in the general population, the USPSTF recommends against the use of vitamin E supplements in healthy populations [24].

Randomized, Controlled Trials of Selenium

The Nutritional Prevention of Cancer (NPC) Trial was designed to test whether selenium supplementation would prevent recurrence of nonmelanoma skin cancers among persons living in parts of the US where soil selenium levels are low [107]. Compared to those in the placebo group there was a 25 % reduction in total cancer incidence and a 40 % reduction in cancer mortality. The selenium supplementation reduced prostate and colon cancers by about half, but it statistically significantly increased squamous cell and total nonmelanoma skin cancer by 25 % and 17 %, respectively, compared to placebo [16, 107, 108].

Findings from the NPC generated considerable clinical and scientific interest in selenium's cancer preventive properties [109, 110], which resulted in part to the development of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) (described above). This trial was designed to test the effects of selenium (200 μg/day L-selenomethionine) and/or vitamin E (400 mg/day dl-α-tocopherol) on prostate cancer incidence in men [99]. It was ended early after a median of 5.5 years at which time there was no association between selenium and prostate cancer [100]. Of concern when the trial was stopped was the nonsignificant increased risk of diabetes among those men randomized to take selenium supplements. With additional post-intervention follow-up, there was no long-term association between selenium supplementation and the risk of prostate cancer or diabetes [101]. However, among men with high baseline selenium as measured in toenail samples, there was an increased risk of high-grade prostate cancer but not low-grade prostate cancer associated with selenium supplementation [111]. No associations have been observed with selenium and other secondary cancer outcomes including all cancer causes, lung, colorectal [100, 101, 112], and bladder cancers [112]. In another trial conducted among 699 men at high risk for prostate cancer, there was no effect of selenium supplementation (200 μg or 400 μg/day) on the incidence of prostate cancer over a median of 36 months; however, concerns have been raised that some prostate cancers may have been present but missed at baseline [113].

Multivitamins

Multivitamins are the most commonly used dietary supplement in the US and elsewhere [1, 4, 71, 114]. Numerous prospective studies [115–144] and five RCTs have investigated the role of multivitamin use with cancer site-specific incidence or mortality [102, 145–148]. While only a few studies have examined all cancers sites, results are not particularly consistent or promising.

Large cohorts with considerable long-term follow-up have examined the associations between self-reported nutrient intake including multivitamin supplements and various prospectively collected cancer outcomes. In the VITamin And Lifestyle (VITAL) cohort of 77,719 subjects living in Washington

State, self-reported multivitamin use over the 10 year period prior to baseline was not associated with 5-year total cancer mortality [115]. Similarly, in the Multiethnic Cohort study of US participants, multivitamin intake was not associated with either total cancer incidence or site-specific cancers of the lung, colorectal, prostate or breast with 11 years of follow-up [116]. In Women's Health Initiative (WHI) cohort of 161,808 postmenopausal women who were followed for 8 years, on average, use of multivitamins neither increased nor decreased risk of eight cancers (invasive breast, colon, endometrium, lung, kidney, ovary, stomach, and bladder) [117]. The WHI study of multivitamins and cancer risk is one of the largest to date and endpoints were ascertained via medical records with physician review, which increases the likelihood the results and conclusions are valid.

The potential effect of multivitamins on individual cancer sites common to both men and women has been evaluated in prospective studies. Multivitamins were shown to have no association with lung cancer risk in three large cohort study [41, 116, 117] and a modest increased risk for women but not men in a pooled analysis from seven prospective studies [39]. Of the observational studies that examined multivitamin and colorectal cancer, none reported an association although four out of six studies of colon cancer found nonsignificant reduced risks [117–122]. Additionally, five large cohort studies reported reduced risk of colon cancer, but only for long-term use of multivitamins (>10 years) [123–127]. Two recent meta-analyses reported 8–12 % colorectal risk reduction with multivitamin supplement use [128, 129]. Among prospective cohort studies examining multivitamin use and cancers of the esophageal and stomach [117, 130, 131], there were no significant associations. Of the studies of bladder cancer, all of them reported no association [117, 132, 133]. Two studies of non-Hodgkin's lymphoma found a near doubling in risk for women who used multivitamins over a 10 year period, but no association for men [82]. One study of liver cancer conducted in China reported a statistically significant increased risk associated with multivitamin use among men but not women, although hepatitis B or C infection was not measured thus there is the potential for confounding [134]. No associations were observed for melanoma [135] or hematologic malignancies [136] among men and women aged 50–76 years living in Washington State, USA.

Many studies have also investigated the potential role of multivitamins among gender-specific cancers. The studies of breast cancer risk have been inconsistent: several large cohort studies found a relative risk near the null value of 1.0 [105, 116, 117, 124, 137, 138] and one of these studies suggested a modest effect modification by alcohol intake [105]. Only one cohort study, conducted among US female nurses, reported a weak, nonsignificant inverse association of multivitamin use with breast cancer risk when the supplements were used for 5–9 years [124]. A study conducted among Swedish women observed an increased risk of breast cancer incidence associated with multivitamin use [139]. A meta-analysis of five cohort studies reported a nonstatistically significant reduction in breast cancer risk [140]. Additionally, in women the use of multivitamins neither increased nor decreased the risk of endometrium or ovary cancer [117, 141]. Among men, two studies reported increased risk of prostate cancer among users of multivitamins [142, 143], particularly increased risk for advanced or fatal prostate cancer [142]. A recent meta-analysis that included five studies of multivitamin use reported a null finding for prostate cancer [144].

In addition to the prospective data, five RCTs of multivitamins have been completed among the general population [102, 145–148]. The first trial was conducted in a resource-poor area of China where much of the population had nutritionally inadequate diets [145, 149]. This trial focused on cancers of the upper digestive tract using four combinations of nutrients that included retinol + zinc, riboflavin + niacin, vitamin C + molybdenum, and β-carotene + vitamin E + selenium. Supplementation with a combination of β-carotene, selenium, and vitamin E resulted in a reduction in cancer rates (RR 0.87, 95 % CI 0.75, 1.00), especially stomach cancer (RR 0.79, 95 % CI 0.64, 0.99), in the general population of Linxian County, China [149]. However, there was no association with lung cancer mortality [145] and no association with incidence of other cancers of the upper digestive tract [149] with any of the nutrient combinations. After 20 years of post-intervention follow-up, no long-term differences were observed in total or site-specific cancer mortality [150]. In another trial conducted in

Linxian, China, supplementation with high-dose multivitamins with minerals had no effect on total, esophageal or stomach cancer incidence or mortality among persons with esophageal dysplasia [146]. However, findings from the trials conducted in China may not be generalizable to well-nourished populations. A British trial in 20,536 adults investigated whether a combined supplement of β-carotene, vitamin C, and vitamin E could reduce the incidence of cardiovascular disease. Cancer was a secondary endpoint of this trial; there were no statistically significant differences in cancer incidence or mortality at any cancer site between the treatment groups [148]. In the Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX), supplementation with a combination of nutritional doses of vitamin C, vitamin E, β-carotene, selenium, and zinc (versus placebo) was not associated with total cancer incidence among 13,017 French adults after 7.5 years; however, there was a significant 31 % reduction in total cancer risk in men only [151]. The investigators suggested that this differential finding may be attributed to lower baseline status, especially for β-carotene, in the men enrolled in that trial. In men with a normal level of prostate-specific antigen (PSA) participating in that trial, vitamin supplementation was associated with a significant reduction in the incidence of prostate cancer (HR 0.52, 95 % CI 0.29, 0.92) [152]. Furthermore, an additional subset analysis of the SU.VI.MAX results revealed that the antioxidant supplement increased the risk of skin cancer, especially melanoma in women, but not men [153]. With an additional 5 years of post-intervention follow-up, there was no longer a beneficial effect observed for total cancer incidence or cancer mortality [154] or a detrimental effect for melanoma in either men or women [135]. The fourth trial is the Physicians' Health Study II (PHSII) which tested a standard multivitamin (containing 32 micronutrients) vs. placebo on various health outcomes, including cancer, among 14,641 male physicians aged 50 years and older [102]. After a median of 11.2 years, there was a modest reduction in total cancer incidence and nonsignificant reduction in cancer mortality between those taking the multivitamin as compared to those taking the placebo [147]. Cancer site-specific analysis revealed an 8 % reduction in total epithelial cell cancers with multivitamin supplementation but not with eight other cancer sites (prostate, colorectal, lung, bladder, pancreatic, lymphoma, leukemia, and melanoma) [147]. A recent meta-analysis of nine RCTs on multivitamin supplements comprising 69,600 participants reported no association between multivitamin supplementation and cancer mortality [155].

Overall studies of multivitamins are suggestive but not conclusive for a protective association for total cancer incidence. The evidence does not support protective associations for cancers of the upper digestive tract (esophageal and stomach), breast, prostate, lung, bladder, endometrium, ovary, or melanoma. Evidence is not consistent for associations with colorectal cancer and there may be increased risks of non-Hodgkin's lymphoma in women. An Executive Summary from the 2006 NIH Conference "Multivitamin/Mineral Supplements and Chronic Disease Prevention" noted that further research is necessary before it can be concluded that multivitamins are unequivocally harmful or beneficial in relation to cancer prevention [20]. Guidelines from the American Cancer Society do not recommend multivitamin supplements for cancer prevention [21]. This conclusion is consistent with the 2014 USPSTF recommendation stating that the current evidence is insufficient to assess either support of oppose the use of multivitamin supplements for the prevention of cancer or cardiovascular disease [24].

Vitamin C

Many cohort studies have reported associations of supplemental vitamin C with cancer incidence or mortality [38, 39, 41, 115, 124, 130, 131, 133, 134, 137, 156–171]. Five studies examined all cancer sites combined, and none found either large or statistically significant associations [115, 156–159]. One study reported a nonsignificant reduced risk of stomach cancer mortality with supplemental vitamin C use [130]. Six studies of breast cancer found no statistically significant associations or consistent trends [124, 137, 158, 160–162]. Of the four studies that examined lung cancer, none were

statistically significant [39, 41, 158, 163]. Of the three studies that examined colon cancer, no association was observed; two found non-statistically significant reduced risks [158, 164], and one a non-statistically significant reduced risk for women only [165]. Results from the six studies that examined bladder cancer were inconsistent. Three large cohort studies reported no association between vitamin C and bladder cancer [131, 133, 166] although there was a nonsignificant 27 % reduced risk observed with 10 or more years of vitamin C supplementation [131]. One study conducted in a retirement community found a statistically significant 40 % reduced bladder cancer risk for men only [158]. One cohort study reported a nonsignificant 25 % increased risk for bladder cancer mortality associated with use of vitamin C supplements [167]. The single study of liver cancer conducted in China reported a statistically significant increased risk associated with use of supplemental vitamin C among men but not women, although confounding is possible since hepatitis B and C infections were not measured [134]. Studies of basal cell carcinoma [38, 168], prostate cancer [169], hematologic malignancies [170], pancreatic cancer [171], and non-Hodgkin's lymphoma [82] did not find any statistically significant associations with vitamin C use.

Only two RCTs, the PHSII and the Women's Antioxidant Cardiovascular Study (discussed above) evaluated vitamin C (500 mg/day) [87, 103]. The PHSII was designed to evaluate vitamin C or placebo as well as other vitamins (discussed above) among US male physicians [103]. After an average of 8 years of treatment, there was no effect of vitamin C on total cancer incidence or total cancer mortality as compared to placebo. Furthermore, there was no effect of vitamin C on cancers of the prostate, colorectal, lung, bladder, pancreas, lymphoma, leukemia, or skin. Analysis that included an additional ~3 years of post-intervention follow-up reported similar findings to the original report [104]. The second trial, the Women's Antioxidant Cardiovascular Study, also evaluated vitamin C (500 mg/day) [87] among women at high risk for cardiovascular disease. After an average of ~9 years of treatment, there was no effect of vitamin C on cancer mortality, total cancer, or cancers of the breast, colorectal, lung, pancreas, uterine, ovary, or non-Hodgkin lymphoma.

Studies of vitamin C do not support a protective effect for total, breast, colorectal, prostate, or lung cancers.

Calcium and Vitamin D

Calcium, vitamin D, calcium combined with vitamin D, and cancer incidence has been examined in numerous studies, both observational studies [62, 118, 119, 134–136, 172–187] and clinical trials [79, 188–192]. Studies have examined vitamin D alone, calcium alone, or calcium + vitamin D while others investigate calcium or vitamin D from other types of supplements; thus, investigating the exposure from calcium or vitamin D separately or together is extremely difficult.

Numerous prospective studies have examined the relationships between calcium alone, vitamin D alone, calcium + vitamin D supplements, and various cancer outcomes. In the WHI cohort study, null finding were observed between calcium only, vitamin D only, or calcium and vitamin D combined and the risk of total invasive cancer [193]. As noted by the authors, there was considerable contamination of the calcium and vitamin D group combined such that over half of participants who were taking either individual calcium or vitamin D supplements at baseline were taking a calcium combined with vitamin D supplement after 3 years of follow-up. Of the studies that examined the relationship between calcium supplements and colon or colorectal cancer, three found a statistically significant reduced risk [119, 172, 173], two found non-statistically significant ~30 % reduced risks [62, 118], and two reported no association [174, 175]. Of the studies that have examined the relationship between vitamin D supplements and colorectal cancer, one found an inverse association among women [62], one reported a reduction in men but not in women [173], and the other found no association [174]. A

single report for rectal cancer found a near statistically significant 24 % reduced risk with calcium supplements but no association with vitamin D supplements [176]. In the WHI cohort study, there was a no significant association between colorectal cancer and calcium only, and a nonsignificant reduction with vitamin D supplements or calcium plus vitamin D supplements [193]. The data on prostate cancer are inconsistent; several cohort studies have reported statistically significant increased risk with calcium supplementation [177–179]. Caution is warranted in the interpretations of these findings due to the exceptionally wide confidence intervals in one study [179] and the small number of cases in the highest quintile of calcium intake in the other [178]. Results from two large cohort studies did not reveal any significant associations (either protective or harmful) between calcium supplements [180, 181] or vitamin D supplements [180] and prostate cancer risk. Calcium, vitamin D, and calcium + vitamin D supplements have been of interest to many clinicians and researchers in relation to breast cancer risk. However, results from large cohorts of postmenopausal women reveal no associations of supplements of calcium, vitamin D, or calcium + vitamin D with breast cancer risk [182–185, 193]. There were no associations between vitamin D supplements with cancers of the lung [186, 187], bladder cancer [133], hematologic malignancies [136], melanoma [135], or basal cell carcinoma [168]. There were no associations of calcium supplementation with cancers of the liver [134, 187] and lung [186].

RCTs of individual calcium or vitamin D supplements have been evaluated among three completed [188–190] and one ongoing trial [191]. Secondary analysis of a small randomized trial of 1200 mg, two times per day, of calcium to prevented colorectal adenomas did not reveal any significant associations (either protective or harmful) for prostate cancer risk after a mean of ~10 years [190]. A randomized placebo controlled trial of oral vitamin D_3 (100,000 IU) every 4 months (versus placebo) was conducted to determine the effect of fractures among ~2700 adult men and women aged 65–85 years of age [188]. Secondary analyses from this trial reported no association between vitamin D and total cancer incidence (RR = 1.09, 95 % CI 0.86–1.36) or cancer mortality (RR = 0.86, 95 % CI 0.61–1.20) after adjustment. The Randomized Placebo-Controlled trial of Vitamin D_3 and/or Calcium (RECORD) was conducted to evaluate the effect of daily supplementation with vitamin D_3 (800 IU), calcium (1000 mg), both, or placebo [189]. Among over 5000 adults with pervious fragility fractures who were 70 years of age or older, vitamin D or calcium supplementation was not significantly associated with cancer incidence or mortality. Given compliance with the supplementations was 67 % at 12-months and 63 % at 24-months, post-hoc analysis to adjust for compliance was conducted and found not to change the conclusions. In a meta-analysis of RCTs of calcium supplementation only, trial-level data from seven trials reported no association with total cancer incidence, colorectal cancer, breast cancer or total cancer mortality, and a reduced risk of prostate cancer over 4 years [194]. Of these seven studies reviewed, patient-level data were available from four studies and confirmed the findings of no association with total cancer incidence, breast cancer and cancer mortality, but there was a nonsignificant reduction in prostate cancer risk [194]. In a meta-analysis that included two studies [189, 195], vitamin D supplementation did not influence breast cancer risk [195]. Currently, a large RCT is ongoing that will test whether vitamin D_3 (2000 IU/day) or marine omega-3 fatty acid supplementation reduces the risk of total cancer incidence as well as site-specific cancers, the VITamin D and OmegA-3 TriaL (VITAL) [191]. This trial goal is to randomized ~20,000 men aged 50 years or older and women aged 55 years and older with 5 years of follow-up. Results from this trial are anticipated be available in 2017 and will help to clarify the effect of vitamin D on cancer outcomes.

Two RCTs have reported on the effect of supplementation of calcium combined with vitamin D [79, 192]. In a RCT of calcium, calcium + vitamin D, or placebo conducted in 1180 postmenopausal women, total cancer incidence at all common cancer sites was significantly lower among the women taking calcium + vitamin D, but the number of cases was so small that inferences about the effectiveness are unclear [192]. A similar magnitude of association was observed in those participants randomized to the calcium only arm; however, the association was not statistically significant. In the Women's

Health Initiative clinical trial of calcium + vitamin D supplementation (WHI CaD, 1000 mg calcium carbonate and 400 IU vitamin D_3 daily) or placebo, the secondary outcome of colon cancer incidence did not vary between the intervention and placebo groups among ~36,000 randomized postmenopausal women after ~7 years [79]. Furthermore, no associations of calcium or calcium + vitamin D were observed for breast cancer risk [196]. Total cancer incidence, cancer mortality, or site-specific cancers of the breast, skin (melanoma), colon, endometrium, ovary, non-Hodgkin Lymphoma, or lung were not associated with calcium + vitamin D supplementation within this trial [197]. In a post hoc subgroup analysis, an interaction was observed based on personal vitamin D or calcium supplements use at baseline; there was a reduction in risk for total cancers and breast cancer with the intervention among those women who were not taking vitamin D or calcium supplements at baseline [198]. Since breast cancer was not a primary or secondary outcome, Chlebowski and colleagues have urged for caution in the interpretation of the findings [199]. Subsequent post hoc analysis revealed similar results with the suggestion of a reduction in breast cancer and invasive cancer among those women who were not taking personal supplements at baseline [200]. In another post hoc analysis from this trial, calcium + vitamin D supplementation did not reduce the risk of nonmelanoma skin cancer or melanoma although there was a reduction in risk of melanoma among women with a family history of nonmelanoma skin cancer but this was based on very small numbers of cases [201]. With an additional 5 years of postintervention follow-up, calcium + vitamin D supplementation did not reduce colorectal cancer or invasive cancer risk but exploratory analysis showed a reduced risk of in situ breast cancer [202]. It is worth noting that postmenopausal women enrolled in the WHI CaD trial were recruited from a dietary modification trial and a hormone therapy trial and were allowed to consume personal calcium and vitamin D supplements. All WHI analyses adjusted for trial assignment.

Studies of calcium supplementation (with or without vitamin D) and cancer are limited. There is modest evidence for a protective association of calcium supplementation with colon cancer risk in observational studies, but these findings are not confirmed by RCTs. A few prospective studies have noted increased risk of prostate cancer when calcium supplements are used, but the findings are inconsistent. There is tremendous scientific interest in vitamin D and cancer risk [203]. However, it is difficult to separate the effects of vitamin D supplementation as often it is used in combination with calcium. Results on vitamin D supplementation from the RECORD trial have not demonstrated a protective effect against cancer among women; however, the primary outcomes for this trial were not specific to cancer. Thus, it is not possible at this time to draw any conclusions about vitamin D as a single supplement and overall cancer risk. However, more information will become available when the VITAL RCT is complete.

Folic Acid

Epidemiologic studies have demonstrated an inverse association of folic acid supplementation via single supplements or with that obtained from multivitamins with reduced risk of colon cancer, or a precursor of colon cancer—colorectal adenomas [122, 123]. While the work presented in this chapter is primarily focused on cancer endpoints, it is worthwhile noting that numerous studies have been published on the associations of supplemental folic acid with surrogate endpoint biomarkers including aberrant crypt foci, DNA methylation, and dysplasias [60, 204]. While many of these studies initially appeared promising with regards to folic acid supplementation and cancer risk reduction, more recently it has become apparent that caution must be applied to these findings [205, 206]. In 2007, results were published from a double-blinded, placebo-controlled trial of 1 mg folic acid/day (with or without 325 mg/day aspirin) or placebo (with or without 325 mg/day aspirin), the Aspirin/Folate Polyp Prevention Study (AFPPS), where the primary trial endpoint was recurrence of

colorectal adenomas, a known risk factor for colon cancer [207]. The folic acid supplements did not reduce the risk of recurrence of colorectal adenoma; rather in participant follow-up it was noted that the folic acid supplementation was associated with a more than twofold increased risk of three or more adenomas [207]. Subsequent sub-analysis found that baseline folate status measured in diet or in circulation did not modify the association [208]. Additionally, a 2.6-fold increased prostate cancer risk was observed in those participants who received folic acid as compared to those who received placebo [209].

Several meta-analyses pooling data from RCTs of folic supplementation on adenoma recurrence and/or other outcomes such as cardiovascular disease have been conducted to evaluate the effect of folic acid supplementation on cancer outcomes. Two meta-analyses of RCTs of folic acid supplementation among participants with a prior adenoma reported no association with the incidence of adenomas recurrence [210, 211]. Subsequently, a meta-analysis by Vollset and colleagues was conducted to evaluate folic acid supplement (median daily dose of 2 mg alone or in combination with other vitamins) or placebo [212]. This analysis included the three trials included in one of previous meta-analysis [211] as well as 10 trials conducted among participants at high risk for cardiovascular disease to evaluate cancer endpoints among ~49,600 participants [212]. Findings from this meta-analysis revealed no association between folic acid supplementation and overall cancer incidence or site-specific cancers of the large intestine, prostate, lung, breast, or other sites after ~5 years [212]. Similarly a meta-analysis by Qin and colleagues of 13 trials of ~49,400 participants reported no effect of folic acid supplementation on risk of total cancer incidence, colorectal cancer, prostate cancer, lung cancer, breast cancer, or hematological malignancies; however, a 53 % reduction in melanoma risk was observed [213]. Despite a similar approach in identifying trials and the inclusion of only data from the supplement period, Vollset and colleagues' null finding for melanoma risk was based on more than three times as many melanoma cases as the analysis conducted by Qin and colleagues [212, 213]. Two other meta-analyses that included fewer RCTs reported increased total cancer incidence with folic acid supplementation [214, 215], but only one was significant [215]. Additional research is clearly needed before recommendations can be made about folic acid and cancer prevention [206, 216, 217].

Discussion

The results from this review indicate that the associations of dietary supplement use with cancer risk are complex and generally not supportive of benefit. The strongest findings are from the RCTs that indicate β-carotene can increase incidence of lung cancer in smokers and a lack of benefit associated with vitamin E supplementation. No supplements appear to be definitively related to cancer risks of the most common cancers—breast, prostate, lung, or colorectal. Notably, when subgroup analysis is conducted with consideration of the dietary intake of micronutrients or baseline nutritional status, supplementation appears more likely to confer benefits for only some subsets rather than null effects or increased risk among those with suboptimal status. However, in the published study of multivitamin use from the Women's Health Initiative, stratified analyses by fruit and vegetable intake did not support the notion that those with poor diets may receive benefit from multivitamins [117].

There are a number of methodological problems in much of the epidemiologic research on dietary supplement use. These limitations are important to consider before drawing conclusions from this review. Here we discuss three important issues: (1) measurement error in assessment of supplement use, (2) importance of a time-integrated measure, and (3) supplement use as a marker for behaviors that may alter cancer risk.

Measurement Error in Assessment of Supplement Use

Much recent research supports the notion that systematic bias in dietary self-report is a common problem in observational studies [218, 219]. Because individuals have a strong tendency to underreport their dietary intake, any observed associations with disease outcomes often become attenuated or even distorted [218, 220]. Even less is known about the measurement properties of instruments used to assess dietary supplement use and the extent to which supplement use is misreported. Epidemiologic studies typically use personal interviews or self-administered questionnaires to obtain information on three to five general classes of multiple-vitamins and on single supplements, the dose of single supplements, and sometimes frequency and/or duration of use. Considerable effort has been expended towards validating dietary supplement collection instruments [33, 221–225].

In 1998, results were published from a validation study comparing supplement data collected in a telephone interview and from a brief self-administered questionnaire with data derived from a detailed in-person interview and transcription of the labels of supplement bottles (i.e., a gold standard) among adult supplement users in Washington State ($n=104$). Correlation coefficients comparing average daily supplemental vitamin and mineral intake from the interview or questionnaire to the gold standard ranged from 0.8 for vitamin C to 0.1 for iron [223]. These results suggest that commonly used epidemiologic methods of assessing supplement use may incorporate significant amounts of error in estimates of some nutrients. The effect of this type of nondifferential measurement error is to attenuate measures of association, which could obscure many significant associations of supplement use with cancer.

In 2003, a validity study of a very extensive and detailed 24-page instrument of dietary supplement use was compared to an array of nutritional biomarkers. In a sample of 220 adults aged 50–74 years there were modest correlations of self-reported use of supplemental intake with serum concentrations of vitamin C ($r=0.29$) and β-carotene ($r=0.31$) and a very good correlation with serum vitamin E ($r=0.69$) [222]. The high correlation with serum vitamin E might suggest that this self-administered supplement questionnaire was quite accurate with the lower correlation for the other biomarkers due to other influences on those serum measures. Still, there are limitations with these self-reported measures. Until recently, few reliable databases existed that included reliable ingredient information for the thousands of dietary supplements available for purchase in the USA [33, 226]. The USDA's Nutrient Data Laboratory, the Office of Dietary Supplements (ODS), and the National Center for Health Statistics initiated the development of a Dietary Supplement Ingredient Database (DSID) [227]. This database, which was originally released in 2009 and then updated in 2012, includes analytic data for common vitamin and mineral preparations, as opposed to simply the label ingredient information, and will greatly improve the ability to accurately assess nutrient exposure from dietary supplements [226–228].

Importance of a Time-Integrated Measure of Supplement Use

Investigators studying diet and chronic diseases usually want to measure an individual's long-term nutrient intake because the induction and latent periods for these diseases are often many years. However, many studies only asked participants about their current use of vitamin and mineral supplements, or only obtained information about supplement use at one point in time. Potential sources of variability in supplement use over time include changes in (1) the type of multivitamin used, (2) number of years the supplement was taken, (3) formulations of multivitamins or dose of single supplements, and (4) frequency of taking supplements.

Investigators in Washington conducted a mailed survey to examine the relationship between current and long-term (10 year) supplement use ($n=325$ adults) [225]. Estimates of current daily intakes for supplemental micronutrients were roughly twice that of average daily intake over the past 10

years. Correlations between current intake and long-term intake from supplements alone were 0.77, 0.75, and 0.65 for vitamins C, E, and calcium, respectively [225]. This type of measurement error may also have contributed to many of the null associations in this review.

Supplement Use as a Marker for Cancer-Related Behavior

Observational studies on supplement use can be compromised by confounding because supplement use is strongly related to other factors that affect cancer risk. Supplement users are more likely than nonusers to be female, non-Hispanic white, better educated, affluent, nonsmokers, light drinkers, and to consume diets lower in fat and higher in fiber and some micronutrients [1, 2, 4, 11, 229–231]. However, potential confounding variables include those specific to cancer risk such as preventive screening, use of potentially chemopreventive agents, and diet-related attitudes and behavior [232, 233].

Demographic and health-related characteristics of high-dose supplement users were assessed as part of a cohort study of dietary supplement users and cancer risk in western Washington. Among women, those who had a mammogram in the previous 2 years were 60 % more likely to be users of calcium, 50 % more likely to use multivitamins, and 20 % and 40 % more likely to use vitamins C and E, respectively, than women who did not have a mammogram. Among men, those who had a PSA test within the previous 2 years were about 1.5 times more likely to be users of vitamin E, 40 % more likely to be users of multivitamins and vitamin C than men who did not get a PSA test [71, 233]. For both men and women, there was a strong positive association between having a sigmoidoscopy, using NSAIDs and using multivitamins and single supplements. High-dose supplement users were statistically significantly more likely to be of normal weight, be nonsmokers, exercise regularly, and eat five or more servings fruits and vegetables per day [71]. These results are similar to previous results from a random-digit-dial survey to monitor cancer risk behavior in adults in Washington State ($n=1449$) [233].

These relationships could confound observational studies of supplement use and cancer risk in complex ways. For example, female supplement users were more likely to have had a mammogram, which is associated with increased diagnosis of breast cancer. Thus, supplement users could appear to have a higher incidence of breast cancer. However, since early diagnosis of breast cancer by mammogram reduces mortality, supplement users could appear to have lower breast cancer mortality. Health beliefs influence cancer risk through behavior such as diet and exercise. For example, in a previous prospective study, it was reported that belief in a connection between diet and cancer was a statistically significant predictor of changes to more healthful diets over time [234]. In cohort studies, the increasing healthfulness of supplements users' diets and other health practices over time could result in a spurious positive association between supplement use and chronic disease.

In theory, control in analyses for demographic characteristics and health-related behavior adjusts for these confounding factors. However, absence of residual confounding cannot be assured, especially if important confounding factors are unknown, assessed with error, not assessed at all, or not included in the analyses. Therefore, many of the observational studies of supplement use and cancer risk may actually be assessing healthy behaviors in general; it is very hard to disentangle these exposures [230, 233, 235, 236].

Future Research

Despite the large number of studies reviewed, published studies to date on dietary supplements and cancer risk are far from definitive. Research on dietary supplements must continue in order to inform public health recommendations for this common health behavior. The NIH supports research on dietary supplements through the ODS and the National Center for Complementary and Alternative

Medicine (NCAAM). The 1994 DSHEA legislation mandated the creation of the ODS [10]. The ODS supports research, sponsors workshops and consensus conferences and disseminates information about dietary supplements to researchers, clinicians, and consumers. The ODS can be accessed at http://dietary-supplements.info.nih.gov/. The website contains links to several of the dietary supplement consensus conferences, supplements information guides and summaries of recent research.

Several large projects funded by the NIH have been reported. Specifically with regard to supplements, the WHI CaD tested, among other outcomes such as fracture risk, whether a combined dose of calcium and vitamin D would reduce the incidence of colorectal or breast cancer in postmenopausal women, but no associations were reported for either colorectal or breast cancer outcome [79, 196] although the breast cancer [198, 200] or invasive cancer [200] risks may be modified by baseline nutritional status. The SELECT ended early due to the slight increased risk of diabetes and prostate cancer [100]. Vitamin E supplementation did not increase the risk for cancer incidence or cancer mortality at the time the study was stopped [100]. The PHSII was designed as a RCT using a factorial design of vitamin E, vitamin C, β-carotene, multivitamins, or placebo among ≥14,000 US male physicians who were 50 years of age or older [102]. In response to the ATBC trial findings, the vitamin E arm was designed specifically to evaluate the potential long-term effects on prostate cancer as well as other cancers. While the majority of men included in this study had not been diagnosed with a previous cancer, this trial did allow men with a history of cancer to be enrolled. Findings from this trial found no association between prostate cancer and vitamin E supplementation during a mean of 8 years [103]. An additional 2.8 years of mean follow-up did not alter these results [104]. The PHSII is a randomized trial of β-carotene, vitamin E, vitamin C, and multivitamins among healthy, male physicians to test whether these supplements will reduce the incidence of total and prostate cancers, as well as cardiovascular disease and eye diseases [102]. Results from this trial reported a modest reduction in cancer mortality and in total epithelial cell cancer but no association with eight other cancers among those taking a standard multivitamin [147]. No association was observed between prostate cancer and vitamin E supplementation [103, 104] or between vitamin C and total cancer mortality, total cancer incidence, or eight other cancer sites [103]. These and other investigations have provided important data on specific dietary supplements in relation to cancer risk that will be useful to both scientists and clinicians.

Conclusion

Health professionals are confronted regularly with questions regarding the usefulness of supplements for cancer prevention. Physicians must remain informed about research showing efficacy, harm or no effect of dietary supplements in relation to cancer prevention.

Most supplement users believe that these products improve their health. A study using NHANES data from 2007 to 2010 on motivations and beliefs of supplement users ($n = 11{,}956$) reported that supplement users consume multivitamins to improve their "overall health" (48 %), calcium for "bone health" or "healthy joints and prevention of arthritis" (74 %), vitamin C to "boost immune system, prevents colds" (45 %), and vitamin D for "bone health" (38 %) [4]. Twenty-three percent of supplements were taken based on advice from their healthcare professional [4].

Given the large numbers of Americans taking dietary supplements, we believe that it is important to formulate recommendations regarding their use. The following recommendations regarding dietary supplements and cancer risk are consistent with the literature and are appropriate based on current knowledge:

- Results of RCTs clearly indicate that cigarette smokers, or other individuals at high risk for lung cancer, should not take β-carotene supplements. Healthy adults are unlikely to receive any benefit from β-carotene supplementation.
- Results from RCTs indicate that healthy adults are unlikely to receive any benefit from vitamin E supplementation.

- A daily multivitamin and mineral supplement is likely neither harmful nor beneficial. If a multivitamin is used, doses should generally not exceed the %Daily Value without specific clinical evidence suggesting the need to restore adequate status and medical monitoring for adverse effects. This recommendation concurs with the USPSTF recommendation on multivitamin use [24].
- Limited data suggest that vitamin C supplementation provides no reduction in the risk of cancers, particularly those of the GI tract or the bladder.
- There is evidence from observational studies that calcium supplements may reduce risk of colon cancer. However, results from the Women's Health Initiative randomized trial did not support the observational data [87]. Given the evidence that calcium and vitamin D might prevent age-related fractures, use of calcium and vitamin D supplements may be prudent for many Americans, particularly those with inadequate calcium and/or vitamin D intake from dietary sources [22, 236], but for bone health rather than cancer risk reduction.
- The evidence on vitamin D is inconsistent to evaluate the role of vitamin D with cancer outcomes. The 2011 Institute of Medicine report on Dietary Reference Intakes for Calcium and Vitamin D was based on bone health outcomes since the evidence for cancer outcomes was currently limited at the time of the report [22]. In addition to cutaneous exposure to sunlight, many Americans obtain vitamin D either from milk products or multivitamins. At this point in time it is difficult to disentangle the potential associations for vitamin D from that of calcium intake. Results from the large VITamin D and OmegA-3 TriaL [189] will help to clarify the role of vitamin D in cancer prevention.
- There is conflicting evidence demonstrating that folic acid can both increase and decrease colon cancer risk [123, 205, 207, 209, 216]. Limitations of the currently published studies are that many of the outcomes are intermediate endpoints (e.g., adenomas). Further research is needed in this area.
- There is little epidemiologic research on other vitamins (e.g., vitamin A, thiamin, riboflavin, vitamin B_6, and vitamin B_{12}), minerals (e.g., chromium, copper, magnesium, iron, zinc) and/or herbal supplements and cancer risk reduction. Additionally, due to the risk of toxicity, high-dose supplementation of vitamin A, particularly for women of childbearing potential, cannot be recommended [237]. Given the possibility that iron increases cancer risk [69, 238], large doses of this mineral are not advised. No recommendations are possible regarding other minerals.

Americans need a strong message that there are many bioactive compounds in foods, especially in fruits and vegetables, which likely play an important role in the prevention of cancer and other diseases [25]. Dietary supplements cannot replace the benefits obtained from eating a diet high in fruit and vegetables, nor can they reverse the potential risk associated with eating an unhealthy diet. Finally, healthcare providers should always ask patients about use of dietary supplements. As noted in this report, many Americans use multiple supplements on a regular basis [5, 71]. Clinicians must be aware of several issues, including the potential for supplement-drug interactions, high monetary expenditures for supplements, and the possibility for patients to replace (or substitute) important healthy behaviors, such as achieving and maintaining a healthy weight, engaging in physical activity, eating a diet high in fruit and vegetables or ceasing to smoke, with a dietary supplement pill. However, published evidence suggests that users of dietary supplements adhere to healthy lifestyle behaviors [4, 232]. Additional research will provide clinicians with information that may be useful in formulating public health recommendations about dietary supplements and cancer prevention.

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Chapter 12
Nonnutritive Components in Foods and Cancer Risk

Lauren E. Conlon and John W. Erdman Jr.

Key Points

- Cancer is now thought to be primarily influenced by factors other than hereditary traits, including the environment, physical activity, and dietary habits.
- Specific nonnutritive food components, termed "bioactives," have been associated with the decreased risk of several cancers. These bioactives include carotenoids, polyphenols, indoles, isothiocyanates, and organosulfur compounds, among others.
- Additionally, nonnutritive components from herbs, tea, and spices have shown inverse associations with risks for certain cancers.
- Research continues to provide evidence suggesting that nonnutritive bioactive components play critical roles in cancer processes including proliferation, apoptosis, differentiation, cellular and hormonal signaling, cell-cycle regulation, invasive potential, and induction/inhibition of detoxification/bioactivation enzymes.

Keywords Cancer • Bioactive • Carotenoid • Lycopene • Catechin • Indole-3-carbinol • Glucosinolate • Isothiocyanate • Sulforaphane • Organosulfur • Sulfhydryl

Introduction

Cancer is a complex and multifaceted disease. Once thought to be primarily influenced by hereditary factors alone, research now suggests only 5–10 % of cancer cases can be attributed solely to hereditary causes [1]. The remaining 90–95 % of cancer cases are thought to be primarily influenced by environmental factors including epigenetic alterations and dietary habits [1]. It has been hypothesized that dietary patterns may account for 60 % of all cancer cases in women and 40 % of cancer cases in men [2]. While significant, the risk for cancer development depends on the entire diet, type of cancer, other environmental factors, and genetic profile of the individual.

While the association between total fruits and vegetables and cancer incidences is weak, specific types of fruits and vegetables have been inversely associated with cancer incidences [3, 4]. Yet, an overwhelming majority of Americans do not meet their daily recommendations for fruit and vegetable consumption, especially orange and green leafy vegetables [5]. In 2000, Serdula et al. reported men and women living in the USA consumed an average of 3.3 and 3.7 servings of fruits and vegetables

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_12

per day, respectively, much lower than the former 5-A-Day program recommended by the USDA [6, 7]. More recently, in 2010, NHANES data indicated that over 90 % of all sex-age groups had intakes below the USDA's MyPlate recommendations for several food groups including total fruits and vegetables [5]. While the number of servings of fruits and vegetables depends on several societal factors including cost and availability, and several personal factors such as age, education, and race, there is unquestionable evidence that the majority of the US population consumes less fruits and vegetables than the USDA public health recommendations. The epidemiological associations between specific fruits and vegetables and cancer incidences warrants further investigation of individual foods or their nonnutritive dietary components as modulators of the cancer process.

Specific components of fruits and vegetables found to have anticancer capabilities include carotenoids, flavonoids, flavanols, indoles, isothiocyanates, glucosinolates, sulfhydryls, and vitamins including vitamin C and vitamin E. These components likely have multiple and overlapping mechanisms of action including modulation of detoxification enzymes, cell-cycle dysregulation, antioxidant and anti-inflammatory properties, inhibition of proliferation and angiogenesis, induction of apoptosis, and hormone signaling, among others.

The micronutrient and nonessential nutrient constituents of foods are largely attributed as modulators of cancer risk. The term "bioactive" has been coined for nonessential food components, many of which have been demonstrated to modulate metabolic processes and result in improved health. Additionally, the emergence of "functional foods" and "nutraceuticals," foods containing bioactive compounds that may provide health benefits, has continued to captivate the interests of scientists, legislators, and consumers worldwide [8]. While there are no legal definitions for functional foods or nutraceuticals, the increasing popularity of foods that provide benefits beyond their nutritional value indicates increased awareness and public interest in nutrition and health.

The associations between dietary habits and cancer incidences depend on many food processing and meal preparation factors. The method of cooking and presence of other meal constituents (dietary fat) can greatly affect the bioavailability of many of the food components discussed in this chapter. Some bioactive components have poor bioavailability when synthesized or in their purified form, and have increased bioavailability when ingested in their naturally occurring state. Additionally, the dose, timing, and stability of compounds may complicate comparisons between studies and conclusions about bioactives and cancers. Synergistic and antagonistic interactions between food components and their matrices further complicate studies aiming to determine whether single food components impact cancer prevention. The need to better understand the interaction between dietary components and the environment is necessary to determine dietary recommendations for specific populations to minimize cancer risk.

In this chapter, we will discuss the limited number of nonnutritive food components with the most evidence for chemopreventative properties. Carotenoids are found in a variety of fruits and vegetables and have been extensively investigated. Cinnamic acid is a polyphenol found in cinnamon oil and used in commercial flavorings. Flavonoids are widely present in fruits, teas, cocoa, and soybeans. Quercetin is a common flavonoid found in apples and onions. Isoflavones from soybeans have been the center of debate for breast cancer risk but they appear to have protective roles in other hormone-related cancers. Indoles and isothiocyanates can be formed from precursors in cruciferous vegetables and are potent inducers of detoxification enzymes. *Allium* foods, including garlic, onion, and leeks, contain sulfur compounds that may have health benefits. Table 12.1 lists some compounds and their common food sources.

Carotenoids

Carotenoids are responsible for the yellow, orange, and red pigments found in a variety of fruits and vegetables. The structure of carotenoids is based on a C40 isoprenoid backbone that may be cyclic or acyclic and have polar groups. Carotenoids containing at least one oxygen atom are classified as

Table 12.1 Potential anticarcinogenic compounds in fruits and vegetables

Compound	Primary food sources
Carotenoids	Fruits and vegetables
Lycopene	Tomato, guava, watermelon
Beta-carotene	Carrots
Polyphenols	Fruits, tea, soy
Cinnamic acid	Cinnamon
Tangeretin	Citrus
Nobiletin	Citrus
Quercetin	Apples, onion
Catechins	Tea
Naringenin	Grapefruit
Hesperidin	Grapefruit
Isoflavones	Soybeans
Indoles	Cruciferous vegetables
Indole-3-carbinol	Broccoli, cabbage, kale
Isothiocyanates	Cruciferous vegetables
Sulforaphane	Broccoli, cabbage, kale
Organosulfur compounds	Garlic, onion, leeks

xanthophylls, while carotenes are characterized by a hydrocarbon structure. While over 600 carotenoids have been identified in nature, only 60 or so appear to be consumed in the diet.

Interest in the structure and function of carotenoids stems from epidemiological evidence supporting the protective effects of carotenoid-rich fruits and vegetables against many chronic and degenerative diseases including cardiovascular disease, age-related macular degeneration, and some cancers [9–11]. Some of the protective effects of carotenoids have been attributed to their ability to serve as antioxidants and quench singlet oxygen [12]. However, their antioxidant capacity alone is not likely the sole reason for their effectiveness. Additional anticancer mechanisms including modulation of growth factor and signaling pathways, alteration of cell-cycle dynamics, and inhibition of the invasive ability of cancer cells have been investigated as functions of carotenoids [13–15].

Lycopene, the carotenoid responsible for the red color of tomatoes, watermelon, and guava, has been investigated for its effects against several types of cancer including prostate, gastric, pancreatic, and bladder cancers [16, 17]. Since lycopene is not converted to vitamin A, it and its metabolites are widely thought to have other functions in the body such as antioxidant, anti-inflammatory, and modulate cell and hormonal signaling [15]. In 1995, Giovannucci et al. reported that intake of tomatoes and tomato products was inversely associated with prostate cancer risk [9]. Since then, there have been numerous studies examining the effects of lycopene and tomato products on prostate cancer risk. Most recently, a high lycopene intake was shown to be inversely associated with total prostate cancer and more strongly with lethal prostate cancer in men taking part in the Health Professionals Follow-up Study [18]. Lycopene has been demonstrated to modulate growth factor signaling, cell-cycle programming, angiogenesis, and apoptosis in vitro and in vivo. Lycopene has been reported to reduce insulin-like growth factor-1 (IGF-1) levels and increase levels of insulin-like growth factor binding proteins (IGFBPs) in breast and lung cancer cells suggesting a reduction in the proliferation and survival signals of cancer cells [19]. In prostate cancer cells, lycopene inhibited the activation of IGF-1R through inhibition of IGF-1 stimulation and increased IGFBP3 expression [20]. Furthermore, lycopene has been shown to alter signaling involved in migration and invasion of tumor cells. Studies using human umbilical vascular endothelial cells (HUVEC) suggest inhibition of migration and tube formation with lycopene [21]. Lycopene given at high doses inhibited tumor growth and circulating

levels of VEGF in xenograft mouse models transplanted with prostate or hepatocarcinoma cells suggesting a reduction in the angiogenic potential of the tumor cells by lycopene [22]. Additionally, lycopene has been shown in several studies to induce cell-cycle arrest in human hepatoma cells, MCF-7 breast cancer cells, and LNCaP prostate cancer cells [19, 22, 23]. The evidence for lycopene's effect on cancer cells in vitro has been studied extensively, and while these studies have provided promising mechanisms of lycopene's action, in vivo studies are necessary to confirm these findings.

In rodent models, lycopene and tomato have been shown to be protective against prostate [24–27] and lung cancers [28]. Lycopene provided at 50 ppm daily significantly reduced the incidence of lung cancer in male B6C3f1 mice [28]. Our laboratory has examined the effect of lycopene and tomato products on prostate carcinogenesis and have shown a reduction in carcinogenesis with tomato powder feeding in NMU-testosterone-treated rats and in the transgenic adenocarcinoma of the mouse prostate (TRAMP) models [26, 29]. Moreover, tomato powder and lycopene feeding reduced expression of genes involved in androgen metabolism and signaling pathways including 5-alpha reductase 1, 5-alpha reductase-2, Pxn, and SREBF1 in TRAMP mice [30]. Furthermore, tomato powder reduced genes associated with stem cell features while lycopene significantly reduced expression of genes associated with neuroendocrine phenotypes suggesting that lycopene and tomato powder may exert their protective effects on prostate cancer by altering testosterone-regulated genes early in carcinogenesis [30].

In breast and liver cancer studies, lycopene resulted in mixed findings. Lycopene reduced migration, invasion, and proliferation in human hepatoma cell lines [31–33] and reduced the incidence of pre-neoplastic lesions in the liver-specific carcinogen diethylnitrosamine (DEN) rat model [31, 34]. However, lycopene did not affect the incidence of spontaneous liver tumors in Long-Evans Cinnamon rats [35]. The primary outcomes for these studies were pre-neoplastic hepatic lesions that can develop into liver tumors [36]. The effect of lycopene on liver tumor development and progression in animal models remains to be determined. Lycopene has also been investigated for its effects on breast cancer in vivo. Nagasawa et al. reported significant inhibition by lycopene of spontaneous mammary tumor developments using the dimethylbenz(a)anthracene (DMBA) model [37]. In a study by Sharoni et al., intraperitoneal injections twice weekly of 10 mg/kg BW tomato carotenoid mixture inhibited tumor multiplicity, but not tumor incidence in the DMBA rat model [38]. However, in the NMU model of breast cancer, lycopene fed to rats at 250 and 500 ppm had no effect on mammary tumor growth [39]. The latter results are supported by cohort studies that showed no association between the intake of fruits and vegetables and breast cancer risk [40–42].

Prostatectomy patients consuming a 15 mg of a lycopene supplement for 3 weeks saw a decrease in serum PSA when compared to the control group [43]. In another study, men consuming tomato sauce prior to prostatectomy had decreased serum PSA, decreased DNA oxidative damage, and increased prostatic lycopene levels [44]. In contrast, results from the Prostate Cancer Prevention Trial found no association of lycopene with prostate cancer risk [45]. Furthermore, a study of men with recurrent prostate cancer consuming a mixed diet of 25 mg of lycopene a day for 4 weeks resulted in no difference in serum PSA compared to the control group [46]. The conflicting results from these small clinical intervention trials suggest that evidence for lycopene's effect in preventing prostate cancer in humans is still needed.

Due to differences between cell types, animal models, timing of treatment, dosage, and bioavailability of lycopene consumed, results concerning lycopene's effect on cancer in cell, animal, and human trials have been mixed. The timing of the intervention, stage in carcinogenesis, and dose of lycopene are variables that lead to inconsistent results concerning the effects of lycopene on cancer risk. Nonetheless, preclinical data, human data, and results from epidemiology suggest an antitumorigenic activity of lycopene [47].

Beta-carotene, a 40-carbon tetraterpene is distinguished by the presence of two cyclic, unsubstituted beta rings. Beta-carotene can be cleaved symmetrically by beta-carotene 15, 15′-monooxygenase to form two molecules of retinal. Each molecule of retinal can be further converted to retinol and retinoic acid.

Beta-carotene, found in deep yellow, orange, and dark green fruits and vegetables such as carrots, peaches, spinach, and broccoli, has been investigated in several cancer types including breast, prostate, and neuroblastoma. Its consumption has been shown to reduce tumor differentiation and modify cancer stem cell markers in a xenograft model of neuroblastoma [48]. Beta-carotene consumption has also been inversely associated with breast cancer risk in Chinese women and has been found to induce cell-cycle arrest and apoptosis in breast cancer cells in vitro [49, 50]. There have been mixed results concerning beta-carotene and prostate cancer. One prospective and three case–control studies have observed a protective effect of beta-carotene [51–54], while other case–control studies have observed no effect of beta-carotene on prostate cancer [55–57]. Further studies are needed to understand the effect of beta-carotene in prostate cancer.

Beta-carotene is perhaps best known for its impact on lung cancer risk. While epidemiological evidence suggests consumption of beta-carotene-rich fruits and vegetables are associated with a lower risk of lung cancer [58], three large intervention trials using beta-carotene have resulted in no effect or were associated with an increased risk of lung cancer [59]. Results from the Carotene and Retinol Efficacy Trial (CARET) and Alpha-Tocopherol, Beta-Carotene Trial (ATBC) suggested an increased risk of lung cancer in smokers consuming beta-carotene, and no protective effect of beta-carotene supplementation in nonsmokers [59, 60]. No protective effect of beta-carotene on lung cancer was observed in the Physician's Health Study [60]. The results of these studies suggest that while there are no adverse effects of beta-carotene from foods, beta-carotene supplements enhance the risk of lung cancer in smokers.

Other carotenoids that have been examined for their anticarcinogenic properties include beta-cryptoxanthin, alpha-carotene, lutein, and zeaxanthin. In two case–control studies, beta-cryptoxanthin and alpha-carotene were shown to be inversely correlated with colon and breast cancer risk in Chinese adults [49, 61]. Lutein and zeaxanthin had no effect on colon cancer risk, whereas both were inversely associated with breast cancer risk [49]. In PC-3 prostate cancer cells, lutein treatment resulted in decreased proliferation and survival-associated gene expression [62]. Future animal studies and clinical trials are necessary to determine the potential protective effect of these carotenoids and cancer risk.

While cell culture, animal, human, and epidemiology studies have described a decreased risk of prostate cancer by lycopene and/or tomato powder consumption, the American Institute for Cancer Research (AICR) has recently classified lycopene as "limited-no-conclusion" for its effect in prostate cancer based on mixed results from human clinical trials and epidemiology [63]. Further studies in humans are needed to understand the effects observed in cell and animal models. While consumed as a supplement, beta-carotene may increase lung cancer risk in smoking individuals. However, there is no evidence that shows beta-carotene consumption from foods as having pro-carcinogenic effects and, in fact, may have anticarcinogenic properties.

Polyphenols

Polyphenols are a structural class of organic compounds characterized by the presence of many phenol structural units. Many polyphenols are found as complex mixtures in foods including fruits, vegetables, tea, red wine, chocolate, coffee, olives, and herbs. There are four primary classes of polyphenols, phenolic acid, flavonoids, flavonoids, and lignans [64]. Polyphenols have widely been investigated for their effects on cancers; however, we will only focus on a few in this chapter.

Phenolic acids including cinnamic acid have been investigated for its protective effects in vitro. Cinnamic acid is a component of cinnamon oil and is used commercially in flavorings [65]. It has been shown to have antitumor properties among several cancers including human lung adenocarcinoma [66] and human melanoma cells [67]. In lung adenocarcinoma cells, cinnamic acid reduced invasive properties and signaling in vitro [66]. Furthermore, cinnamic acid induced apoptosis in

melanoma cells and inhibited proliferation in Caco-2 cells [67, 68]. It must be emphasized that concentrations of cinnamic acid necessary to bring about antitumorigenic effects in vitro are supra-physiological (2–8 mM), thus their true physiological importance remains to be determined in vivo.

Flavonoids

Flavonoids are a group of over 6000 organic molecules found in plants. The estimated average flavonoid intake in the USA in people older than 19 years is 189 mg/day [69]. The diverse reported biological functions of flavonoids include antioxidative, antiallergic, antiviral, and anti-inflammatory properties [70]. Additionally, they have been investigated for their health benefits in cardiovascular disease, stroke, diabetes, and some cancers [70]. Recently, considerable attention has been focused on examining the effect of flavonoids on cancer processes. Their ability to inhibit cell-cycle progression and proliferation, induce apoptosis and detoxification enzymes, and reduce oxidative stress make them attractive targets to investigate for chemoprevention [70].

Flavonoids are classified on the basis of substitution on one or more rings. There are six subclasses of flavonoids including flavones, flavonols, flavonones, isoflavones, anthocyanidins, and flavanols. Table 12.2 lists some food sources of flavonoid compounds within each class that have been studied for their anticarcinogenic properties.

Flavones

Tangeretin and nobiletin are O-methylated flavones found in citrus peels. They have been investigated for their effects on cancers in vitro and in vivo including gastric, colon, breast, prostate, melanoma, and lung cancer [71–74]. Inhibition of proliferation, induction of apoptosis, and interference with the cell-cycle are mechanisms by which tangeretin and nobiletin are hypothesized to exhibit anticancer effects. Nobiletin suppressed the proliferation of A549 lung cancer cells in vitro and inhibited xenografted lung tumor growth in nude mice [74]. In colon and breast cancer cells, tangeretin and nobiletin have been shown to induce G1 cell-cycle arrest [72]. Furthermore, 500 ppm nobiletin and auraptene a day reduced prostate cancer severity in a transgenic rat model of prostate cancer [75]. Though tangeretin and nobiletin have been demonstrated to suppress cancer progression in vitro and in vivo, their combined use with traditional medicines may interfere with their effectiveness. Bracke et al. showed

Table 12.2 Classes of flavonoids and common food sources

Class	Primary food sources
Flavones	
Tangeretin	Citrus
Nobiletin	Citrus
Flavonols	
Quercetin	Fruits, vegetables, cereal grains
Catechins	Tea
Flavonones	
Naringenin	Grapefruit
Isoflavones	
Genistein	Soybeans
Daidzein	Soybeans

that tangeretin and tamoxifen separately exhibited similar inhibitory effects on growth and invasiveness in human mammary cells in vitro, but when the two were combined, interference with the effectiveness of tamoxifen was observed [76]. Furthermore, in a xenograft model, only tamoxifen was effective in inhibition of tumor growth when provided in the drinking water suggesting differences between absorption of the two compounds [76, 77]. This demonstrates the need to thoroughly evaluate alternative cancer therapies with their effects on traditional medicine.

Another flavone, apigenin, is found in celery and parsley and has been investigated for its effects against cancers including breast, colon, skin, and prostate [78, 79]. Prior to carcinogenic insult, apigenin exposure resulted in protection against skin and colon cancer in mice [78, 80]. In human breast cancer cells [81] and four lines of human prostate cancer cells [82], apigenin was shown to inhibit proliferation and induce apoptosis. Results from in vivo studies suggest a very poor bioavailability of these pure compounds because of their poor solubility in water and organic solvents [79]. Apigenin from foods is commonly found as β-glycoside conjugates which increases its bioavailability compared to its pure form. Flavones are rapidly metabolized by UDP-glucuronosyltransferases and sulfotransferases in the gut which glucuronidate the aglycone compound and results in transfer to the blood, bile, or urine.

Collectively, flavones have been demonstrated to have several anticancer mechanisms including interfering with cell-cycle regulation, estrogenic/antiestrogenic activity, and regulation of cellular signaling. While their potential negative interactions with traditional medicine and poor bioavailability of purified compounds require further investigation into their effectiveness, there is a large body of cell and animal evidence supporting the potential of tangeretin, nobiletin, and apigenin as chemopreventive compounds.

Flavonols

Quercetin, one of the most common flavonols, can be found in foods such as tea, broccoli, kale, and apples and has been implicated in cancer protection. Quercetin is considered an excellent free-radical scavenging antioxidant and has nearly four times the antioxidant capacity of vitamin C in apples [83, 84]. Its effects on cell-cycle, apoptosis, and proliferation have been widely demonstrated in cancer cell lines. Quercetin has been shown to modulate several targets in the cell-cycle including p21, cyclin B, p27, and other cyclin-dependent kinases (CDKs) [83]. Specifically, quercetin has been shown to induce cell-cycle arrest at the G1 phase through induction of p21 and reduction of retinoblastoma (Rb) expression in human breast carcinoma cell lines [85]. Similarly, in human lung cancer cell lines, quercetin induced cell-cycle arrest at G2/M phase by increasing expression of proteins such as cyclin B and Wee1 [86]. The many mechanisms by which quercetin has been reported to induce apoptosis renders this molecule an interesting tool in the field of oncology. Quercetin can induce apoptosis by reducing MMP expression which, in turn, promotes the activation of caspases-3, -8, and -9 [87, 88]. Additionally, quercetin can trigger apoptosis though the generation of ROS and subsequent activation of AMPKA1 and ASKL which activate p38 and caspases [89] and can enhance TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis, through the expression of death receptor-5 [90]. Interestingly, quercetin has been shown to induce apoptosis and inhibit proliferation in cancer cells, but has little to no effect on non-transformed cells at concentrations effective in cancer cells [91]. These mechanistic findings, while promising, need to be further investigated in animal models and humans.

Quercetin has been effective in animal models of cancer. In a benzo(a)pyrene-induced lung tumor mouse model, quercetin reduced the tumor burden and increased the activity of antioxidant enzymes including superoxide dismutase [92]. Similarly, the administration of quercetin prior to exposure of azoxymethane reduced aberrant crypt foci and pre-neoplastic lesions in rat colons [93]. Quercetin in

combination with resveratrol and catechins was able to reduce distal metastases to liver and bone in nude mice though upregulation of FOXO1 and NFκBIα which activate apoptosis and inhibit NFκB activity [94]. While these studies begin to demonstrate mechanisms investigated in cell culture, the route of administration of quercetin in these and many other studies is intraperitoneally. Difficulties with pure quercetin bioavailability exist because of the poor solubility of quercetin in water and organic solvents. Quercetin is mostly found as glycoside conjugates in foods and is extensively metabolized upon ingestion. Quercetin glucosides are hydrolyzed by enterobacteria which generate quercetin aglycones that can be directly absorbed or further metabolized to the methylated, sulfonylated, and glucuronidated forms by enteric transferases [83]. Therefore, it is important that studies investigating quercetin utilize bioavailable forms. In human studies, quercetin from onions has been shown to have better bioavailability than pure quercetin [79]. Quercetin supplemented in the diet combined with tea polyphenols provided in the drinking water of a xenograft mouse model of prostate cancer resulted in inhibition of tumor growth after 6 weeks [95]. Quercetin remains one of the most commonly consumed polyphenols in the USA. While its effects on cancer cells have been promising, animal and human clinical trials remain necessary to confirm the cell culture findings in vivo.

Tea is the most widely consumed beverage after water per capita in the world [96]. Tea is primarily consumed in China, Japan, and a few countries in the Middle East and Northern Africa, but it has been gaining popularity in other parts of the world. Green tea is manufactured from the fresh leaf preventing the oxidation of polyphenolic compounds, while black tea is fermented to ensure a high degree of enzymatically catalyzed oxidation of polyphenolic components [96]. Fresh tea leaves are rich in catechin polyphenols which may constitute up to 30–42 % of the dry leaf weight [97]. Other polyphenols include flavonols and their glycosides, chlorogenic acid, and theogallin. Oxidation causes the formation of various quinones that condense to form bisflavanols, theaflavins, and thearubigens which are responsible for the taste and color of black tea [96].

Although inconsistent, consumption of tea has been associated with a risk reduction in certain cancers [97–103]. In particular, there may be benefits for cancers of the digestive tract including esophageal and colon cancer, although consumption levels of tea needed for protection are very high [98]. It should be noted that exaggerated intakes may create side effects in some individuals. Surprisingly, Lu et al. suggested tea consumption was associated with an increased risk of bladder cancer [104]. Differences between studies may be due to confounding factors such as smoking, alcohol consumption, differences in tea characteristics and preparation, and individual differences in metabolism of tea components [97].

Generally, tea polyphenols are poorly bioavailable and undergo extensive metabolism by the colonic microbiota [97, 105, 106]. Since the bioactivity of the metabolites are not yet well understood and may be less effective than the parent compound, cautious interpretation of in vitro studies using supra-physiological levels of parent compounds should be taken. The major polyphenols in green tea include (−)-epigallocatechin-3-gallate ((−)-EGCG), (−)-epigallocatechin ((−)-EGC), (−)-epicatechin-3-gallate ((−)-ECG), and (−)-epicatechin ((−)-EC). Of these, (−)-EGCG is the most abundant and has been the most extensively investigated tea polyphenol for cancer prevention.

A growing number of animal studies have demonstrated green tea's effectiveness in reducing the risk of skin, liver, and lung cancers. The effect of green tea on skin carcinogenesis has been extensively investigated. Oral administration of 6 mg of tea solids/mL decreased the incidence of skin tumor formation in nude mice exposed to UVB light twice a week [107]. Interestingly, decaffeinated tea was less effective than fully caffeinated green tea [107]. In regards to liver cancer, pure (−)-EGCG was effective in reducing the incidence of spontaneous hepatoma in C3H/HeNCr mice by 27 % compared to the control mice [108]. Furthermore, mice given a green tea infusion prior to chemically induced liver carcinogenesis (DEN followed by PCP) and throughout the experimental period had a 50 % reduction of hepatoma compared to the control conditions [109]. Studies examining tea's effect in animal models of lung cancer have yielded promising results. In the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK)-induced mouse model of lung cancer,

560 ppm (−)-EGCG in drinking water significantly decreased lung tumor multiplicity after 13 weeks [110]. Mice given 1.25 % green tea in drinking water and treated with DEN for 8 weeks had decreased formation of lung tumors and a reduction in liver tumors compared to the control group [111].

Based on cell and animal trials, tea polyphenols are thought to affect several steps in cancer development and progression including inhibiting tumor growth, angiogenesis, and metastasis through regulation of the cell-cycle and apoptosis. Potential mechanistic targets include p53 [112], VEGF [113, 114], NFκB [115], and the Ras-MAP kinase pathway [116]. In SKH-1 mice given 0.6 % green tea for 2 weeks and exposed to UV light, there was upregulation of p53 in apoptotic positive cells [117]. Other studies have observed that (−)-EGCG induced and stabilized p53 expression [112, 118]. (−)-EGCG has also been shown to affect invasion and metastasis in vitro and in vivo. In human breast cancer cells, (−)-EGCG reduced VEGF secretion and expression thereby limiting the cells' ability to generate new vasculature [113]. Furthermore, mice injected with HT29 colon cancer cells and then treated intraperitoneally with 1.5 mg of (−)-EGCG or (−)-EC had a 61 % reduction in tumor volume and 55 % reduction in tumor weight as well as increased apoptosis and reduction of tumor vessels [114]. In prostate cancer, TRAMP mice treated with 0.1 % GTP (w/v) in drinking water exhibited no metastasis to lung, liver, or bone compared to the control mice which had metastasis incidences of 65 %, 40 %, and 25 %, respectively, to those tissues suggesting an inhibitory effect of (−)-GTP on invasion and metastases of primary prostate tumors [119].

Tea catechins have also been shown to induce phase I enzymes and inhibit CYP1A1 and CYP1B1 bioactivating enzymes to promote degradation of carcinogenic compounds [120–122]. Additionally, different preparations of tea have been shown to inhibit nitrosamine formation and their bioactivation [123]. A small human clinical trial found that tea supplementation was effective at reducing fecal nitrite, thereby sequestering it and making it unavailable for nitrosamine formation [124]. While the results of cell culture and animal studies have provided evidence for green tea polyphenols' effectiveness against several cancers, the results from human clinical trials and epidemiology have been mixed.

Epidemiological evidence regarding the association between green tea consumption and cancer incidence has mostly shown a positive effect of green tea and inhibition of cancers. Specifically, a reduction in cancers of the digestive tract has been associated with green tea consumption [125]. A case control study from China observed green tea consumption to be associated with a lower risk of stomach cancer [126]. A separate case–control study in China confirmed the previous study and observed an inverse association between green tea consumption and gastritis and stomach cancer risk [127]. The risk for esophageal cancer in women has also been inversely associated with green tea consumption in Japan and China [128].

Although the epidemiology evidence is promising for certain cancers and tea consumption, breast and pancreatic cancers have had inconsistent results. An inverse association with tea consumption and pancreatic cancer risk was observed in two case–control studies in China and Japan [129, 130]. However, another case–control study from Japan demonstrated that drinking more than five cups of green tea/day was associated with an increased risk of developing pancreatic cancer [131]. Case–control studies investigating breast, prostate, and ovarian cancer risk have shown inverse associations with green tea consumption in Asian populations [132–134]. Additionally, a case–control study from Japan suggested black or green tea consumption may be associated with an increased risk of bladder cancer [104]. While the evidence from epidemiology concerning tea polyphenols and cancer risk are mixed, evidence from human clinical trials can provide additional information regarding possible mechanisms of action.

In 71 % of human rectal biopsies from subjects consuming varying levels of green tea powder, tea was shown to reduce the colorectal cancer biomarker, prostaglandin E2 (PGE2) 4 h after consumption [135]. In a clinical trial in smokers with oral leukoplakia, 3 g of an oral tea mixture reduced oral lesions significantly in the tea group compared to the control group [136]. Pathologically, there was a reduction in proliferating cell nuclear antigen (PCNA) in the oral mucosa nuclei in the tea-treated group suggesting a potential mechanism of tea polyphenol protection [136]. The result of a Phase II

trial of cancer patients with androgen-independent metastatic prostate cancer instructed to consume 6 g of green tea powder/day delayed the progression in only a single patient suggesting that green tea polyphenols may not be effective in reducing the progression of late stage and aggressive prostate cancer [137].

Tea flavonols have been extensively investigated for their effects in cancer chemoprevention. While the mechanistic cell culture data have shown promising avenues for chemoprevention of tea catechins such as inhibiting angiogenesis, decreasing formation of nitrosamines, and altering the cell-cycle, dose and bioavailability of the compounds used must be considered when translating findings to human and epidemiological studies. Differences in tea preparations, genetic variations, lifestyle factors, and discrepancies concerning amount consumed can add to the variability observed within the results.

Flavanones

Naringenin, a flavanone found in grapefruit, oranges, and tomatoes, has been shown to induce phase II enzymes which stimulate the detoxification of carcinogens and their elimination from the body. However, the interactions between naringenin and other compounds found in grapefruit juice which alter and downregulate CYP3A4, represents an important and potentially problematic drug–nutrient interaction [138, 139]. CYP3A4 is the largest sub-family of CYP enzymes found in the gastrointestinal tract and liver and is responsible for the metabolism and activation of various toxic and carcinogenic products [138]. Consumption of grapefruit juice inhibits CYP3A4 within 30 min and impairs the metabolism of certain drugs including calcium channel blockers, felodipine, and verapamil [138]. It will become increasingly important, as fruit and vegetable consumption hopefully increases, to understand the impact of bioactive components on drug metabolism.

Isoflavones

There has been considerable evidence supporting the ability of isoflavones found in soybeans and soy products to alter the cancer process [140]. Isoflavone intake has long been believed to contribute to the differences in world cancer incidences, especially regarding cancers of the breast and prostate. A 2009 meta-analysis of case–control and cohort studies suggested a decreased risk for prostate cancer by soy consumption (RR/OR of 0.74) [141]. Interestingly, the risk reduction was lost when the analyses were limited to fermented soy foods (RR/OR 1.02), suggesting that the protective effect observed was due to intake of unfermented soy products [141]. Epidemiology studies concerning breast cancer indicate that the protective effect of soy intake on breast cancer risk is modest and suggests that timing of soy intake is a critical factor in determining response [142–144].

Soybeans are the major dietary source of the isoflavones, genistein, daidzein, and glycitein. The major isoflavone found in soybeans, genistein, has been largely studied for anticarcinogenic properties in hormone-related cancers due to its molecular composition's similarity with 17β-estradiol (E2). Although isoflavones are considered weak estrogens, they can act as estrogen antagonists or agonists depending on the target tissue, species examined, and amount consumed. Evidence now supports the ability of isoflavones to influence sex hormone metabolism and activity, alter intracellular enzymes and protein synthesis, and modulate cellular signaling through growth factors, proliferation, and differentiation [145, 146].

Concern about possible increased breast cancer risks upon isoflavone exposure is based, to an extent, on research that showed that genistein or genistin stimulated estrogen-dependent MCF-7 tumor growth in athymic ovariectomized mice with estradiol levels comparable to menopausal

women [147–151]. Furthermore, several studies have indicated isoflavone-mediated growth in estrogen-sensitive cancer cells [147, 152, 153]. However, results from human clinical trials investigating soy interventions on breast tissue biomarkers have resulted in conflicting messages [146]. Consumption of 37.5 g of soy protein (reported 75 mg isoflavones) per day for 5 months resulted in a stimulatory effect in premenopausal females characterized by increased breast fluid secretion, elevated plasma estradiol, and the appearance of hyperplastic epithelial cells [154]. In contrast, Cheng et al. observed no effect of 60 mg of isoflavones over the course of 3 months on breast cell proliferation or expression of hormone receptors in healthy postmenopausal women [155]. Furthermore, a randomized, double-blinded, and placebo-controlled 2-year clinical trial of 406 postmenopausal women receiving 80 or 120 mg isoflavones/day found that isoflavone supplements did not modify breast density, which when increased, correlates with an increased risk of breast cancer [156]. This study suggested that isoflavones do not act like hormone replacement medications [156]. Timing of isoflavone exposure is considered a critical factor in the risk for breast cancer with exposure earlier in life considered more protective than exposure later in life [156]. However, more research is needed concerning the effects of soy supplementation on breast cancer risk as it may be possible that some women may be at risk when exposed to high doses of genistein supplements.

Isoflavones have been demonstrated to bind estrogen receptors alpha and beta (ERα and ERβ) to a weaker extent than estradiol; however, their actions through ERs are of significance [156]. Genistein and daidzein preferentially bind ERβ, which is thought to be responsible for counteracting the proliferative effects of ERα and promoting differentiation in a tissue-specific manner [157–159]. Because ERα and ERβ are expressed in different ratios depending on the tissue type, the effect of isoflavones may impact carcinogenesis in organs differently. In cell culture, genistein induced a concentration-dependent increase in proliferation when cells mainly express ERα [153]. However, in the presence of ERβ, genistein and estradiol no longer induced proliferation supporting the importance of the ERα:ERβ ratio with respect to proliferation [153].

While there is a large focus on soy isoflavones and breast cancer risk, evidence suggests that isoflavones may play a key role in reducing the risk for prostate cancer in men. Evidence from epidemiology suggests men from Asian countries have a lower risk of developing prostate cancer than their Western counterparts [160]. Interestingly, the ability to convert daidzein into its metabolite, equol, is also a characteristic of Asian populations. The ability to produce equol is an important feature of men and women who consume soy products on a regular basis. It is estimated that 80 % of Asian populations have the microbiota ability to produce equol compared to 25 % of individuals in North America and Europe [161]. This difference in equol production has been hypothesized as one possible explanation between the differences in cancer incidences geographically. Equol has been shown in vitro and in vivo to bind 5α-dihydroxytestosterone (DHT) without binding the androgen receptor, thereby sequestering DHT from binding to the androgen receptor and modulating prostatic growth [162]. Equol administration to rats decreased prostate size and serum DHT levels without altering testosterone, estradiol, or luteinizing hormone levels [162]. Additionally, both equol and genistein have been shown to bind ERβ with high affinity [161]. And equol has antioxidant, antiproliferative, and anti-inflammatory properties greater than those of daidzein [161]. Therefore, future studies describing the differences between prostate cancer incidences and risk should not only examine soy consumption, but should also examine the individual's capacity for microbial metabolism of isoflavones, particularly the daidzein to equol conversion.

There is considerable evidence suggesting that isoflavones can modulate selected enzymes including CYP1A1 [163]. Genistein has also been shown to inhibit protein tyrosine kinases, attenuate both growth factor- and cytokine-stimulated proliferation in normal and neoplastic cells, and may inhibit cell growth by modulating transforming growth factor (TGF) beta-1 signaling pathways [164]. Angiogenesis plays a key role in cancer metastases and studies have shown that soy products and genistein inhibit endothelial cells proliferation and downregulate mRNA levels of vascular endothelial growth factor receptor-1 (VEGFR1) and VEGFR2 at pharmacological concentrations of 5–150 μM

[160, 165]. Tumors from LNCaP cells in athymic mice had reduced growth, less vascular volume, and reduced microvessel density in mice fed the soy diet than mice on the control diet [166]. Furthermore, soy isoflavones were able to reduce the pro-angiogenic cytokine interleukin-8 (IL-8) in PC-3 cells [167]. IL-8 is directly correlated with the Gleason score and pathologic tumor stage and could distinguish organ-confined from non-confined prostate tumors [168]. In LNCaP, PC-3, and DU-145 prostate cell lines, genistein and daidzein downregulated a set of genes necessary for angiogenesis including endothelial growth factor (ECGF1), fibroblast growth factor 1 (FGF1), IL-1β, IL-6, IL-8, platelet/endothelial cell adhesion molecule (CD31), and CXCL10 [167, 169]. These data and others support the antiangiogenic properties of soy isoflavones in prostate cancer. Further investigation of these effects in cell culture needs to be investigated in human clinical trials and animal models.

Indoles

Indolylmethyl glucosinolate glucobrassicin is present in cruciferous vegetables such as cabbage, broccoli, and Brussels sprouts and is hydrolyzed during ingestion to several idole metabolites, including indole-3-carbinol (I3C). I3C is unstable in the acidic environment of the stomach and undergoes self-condensation reactions to form [3, 2-b]carbazole (ICZ) and diindolylmethane (DIM) as well as other trimers and oligomers. DIM is the major intermediate formed in vitro and in vivo. Preclinical studies indicate that I3C has preventative activity in cancers of the breast, stomach, prostate, lung, liver, and colon [170]. Many studies have used large quantities of dietary I3C (0.5–3 %), however providing as little as 56 ppm has been shown to be sufficient to alter enzymes involved in carcinogen bioactivation [171]. Protection likely depends on the indole species and tissue examined. Mechanisms by which indoles may modify carcinogenesis include endogenous metabolism of estrogens, stimulation of apoptosis, downregulation of proliferation, and augmenting cellular defenses against genotoxic chemicals [170].

I3C and DIM have been studied extensively as inducers of phase I cytochrome P450 (CYP) 1A1, CYP 1A2, and CYP 3A4 enzymes. These enzymes generally lead to detoxification, but are also involved in the bioactivation of carcinogens. DIM has also been shown to decrease CYP1B1, 4-hydroxylation of estradiol, and decrease 4-OH-estrone [172]. The ability of I3C and DIM to modulate estrogen metabolism through CYP-mediated oxidation is thought to be responsible for protection against breast and cervical cancers. DIM-mediated inhibition of CYP3A1 resulted in increased capacities of hepatic microsomes to metabolize 17β-estradiol (E2) and estrone (E1) to less estrogenic 16-OH-estrone derivatives, thus interfering in processes involved in estrogen-responsive cancers [170, 172]. Moreover, I3C has been shown to significantly repress E2-activated signaling in a dose-dependent manner in vitro and downregulate expression of estrogen responsive genes, pS2 and cathepsin-D [173]. Currently, an oral DIM supplement is being tested in a phase III human clinical trial for cervical dysplasia as a result of several studies demonstrating that I3C and DIM can cause growth arrest and apoptosis of cervical cancer cells and reduce cervical intraepithelial neoplasia and its progression to cervical cancer [174–176]. In addition to its effect on estrogen metabolism, I3C exposure may alter the formation of genotoxic intermediates involved in carcinogenesis. This response may be a result of phase I and phase II enzyme modulation. I3C has been shown to decrease the binding of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinolone (IQ) to mammary tissue [177] which are both activated enzymatically by CYP1A1 and CYP1A2. Furthermore, Stressor et al. demonstrated that DIM inhibits CYP-mediated metabolism of hepatocarcinogen-aflatoxin B1 [178]. Collectively, these studies appear to indicate an enhancement of detoxification enzymes by I3C and DIM; however, the balance between increase and decrease in activation depends on indole, dose, and target tissue.

Interestingly, DIM has been shown to induce selective apoptosis in cancerous cells [179–183], but not in normal cells [178]. This has been shown in CRL2221 prostate epithelial cells, normal human

keratinocytes, and human pancreatic ductal epithelial cells among others [178]. Mechanistically, DIM has been shown to induce mitochondrial membrane potential and cytochrome C release which results in the activation of caspases-3 and -9, and poly (ADP-ribose) polymerase [178]. Induction of apoptosis by DIM treatment has been observed in androgen-sensitive and androgen-independent prostate cancer cells. In androgen-sensitive LNCaP cells, DIM-induced apoptosis was associated with p53 stabilization and downregulation of NFκB expression resulting in the decreased expression of Bcl-2 [176, 184, 185]. Downregulation of the anti-apoptotic Bcl-2 protein and the enhancement of pro-apoptotic Bax expression after DIM treatment supports DIM as a potential anticancer agent [178, 186].

DIM's effect on apoptosis and proliferation has been demonstrated, in part, through modulation of the cell-cycle. In breast, ovary, prostate, colon, and thyroid tissues, DIM has been shown to cause cell-cycle arrest in the G1 phase [187–190]. Inhibition of cyclin-dependent kinase 2 (CDK2) by the increase in expression of CDK-inhibitor, p21, by DIM or I3C treatment arrests cells in the G phase and prevents them from entering the S phase. In addition, DIM treatment causes a reduction in levels of cyclin A and Dl, CDK4, CDC2, and CDC25C phosphate resulting in a restriction of cell-cycle progression [187]. Antiproliferative activity of DIM in follicular thyroid cancer cells was also found to be mediated by G1 arrest and subsequent induction of apoptosis [187]. Furthermore, DIM and I3C have been demonstrated to inhibit the phosphorylation of Rb through CDK2 and CDK4 [175, 191]. The actions of DIM and I3C on the cell-cycle provides mechanistic evidence for their effects on proliferation and apoptosis.

Indole consumption does not always result in a beneficial effect on carcinogenesis. I3C treatment in a multiorgan tumorgenesis animal model resulted in a fourfold increase in GST-P foci volume in the liver, no difference in tumor multiplicity in the mammary tissue, and a 40 % decrease in aberrant crypt foci in the colon [192]. In a different study, I3C enhanced colon tumorgenesis through induction of AHH activity in rats [193]. Evidence suggests the tumor-promoting effect of indoles may be a result of indole treatment after carcinogen exposure in animal models [194, 195]. In cultured rat hepatocytes, ICZ downregulated gap junction signaling by activation of the Ah receptor and/or CYP1 activity suggesting a mechanism for the tumor-promoting effects of indole metabolites in hepatocytes [196]. These studies suggest further investigation of the antagonistic and synergistic effects of indole compounds and biological mechanisms involved in carcinogenesis.

Generally, the consumption of cruciferous vegetables has been associated with protective effects against cancers. The ability to modulate estrogen signaling, cell-cycle progression, and induce detoxification enzymes have been reported by indoles. However, reports regarding the pro-carcinogenic effects of indoles on certain types of cancers must be evaluated and further investigated before widespread recommendations about indole consumption are made to the public.

Isothiocyanates

Cruciferous vegetables such as broccoli, Brussels sprouts, mustard, and cabbage are rich in S-β-thioglucoside *N*-hydroxysulfates also known as glucosinolates. In the plant matrix, glucosinolates are accompanied by β-thioglucoside enzymes known as myrosinases which are physically separated from their glucosinolate substrate in the intact plant matrix. Upon matrix destruction, such as chewing, myrosinase comes into contact with its substrate and rapid hydrolysis produces highly reactive compounds known as isothiocyanates (ITC). Myrosinase has been demonstrated to be extremely heat-labile and cooking of broccoli has been shown to drastically reduce the bioavailability of ITC in humans [197, 198]. The formation of ITC has been attributed, in part, for the major chemopreventive benefit of cruciferous vegetable consumption. Sulforaphane, phenethyl isothiocyanate (PEITC), and benzyl isothiocyanate (BITC) have received much of the attention regarding chemoprevention and have been implicated in inhibiting carcinogenesis in a variety of tissues including liver, lung, breast, intestine, stomach, colon, prostate, and esophagus [199–203].

Many animal studies have demonstrated the effect of ITC in chemically induced, xenograft, and transgenic models of carcinogenesis. In the TRAMP model of prostate cancer, mice were orally gavaged with 6 μmol of sulforaphane three times a week or fed a sulforaphane-rich broccoli sprout diet. Both groups had significantly reduced prostate carcinogenesis and pulmonary metastases compared to the control [204, 205]. In mice xenografted with human colon cancer cells, 400 μmol of sulforaphane/day injected subcutaneously decreased tumor weight by 70 % compared to the control [206]. 7.5 μmol of sulforaphane/day fed to mice treated with benzo[a]pyrene inhibited the development of stomach cancer [207] and gavage of sulforaphane and PEITC (20 or 50 μmol/day) reduced the formation of azoxymethane-induced colonic aberrant crypt foci [208]. These and other studies propelled the interest in ITCs as potent chemopreventative compounds. Further animal and cell culture studies have aimed to elucidate possible mechanisms of protection.

Similarly to the indoles, ITCs are able to modulate the activity of phase I detoxification enzymes. Sulforaphane and PEITC have been shown to have duality in the activation and deactivation of phase I enzymes [209, 210]. PEITC induces CYP1A1 and CYP1A2, but inhibits CYP3A4 and CYP2A3 [209]. Phase II enzymes play an important role as transferases in the detoxification of xenobiotics and carcinogens. ITC induce phase II enzymes such as epoxide hydrolase, ferritin, glutathione-S-transferase (GST), heme oxygenase-1 (HO-1), UDP-glucuronosyltransferase (UGT), and NAD(P)H:quinone oxidoreductase 1 (NQO1) [202, 210, 211]. Sulforaphane increased the activity of NQO1 and GST in murine Hepa1c1c7 cells, and upregulated the expression of GSTA1/2 and GSTP1 in rat hepatocytes [212–214]. Furthermore, in vivo studies have confirmed the in vitro findings. In rats given sulforaphane orally, GST and NQO1 enzyme activity was increased compared to the control in the prostate, stomach, duodenum, bladder, liver, colon, and pancreas [212, 213, 215].

Sulforaphane, BITC, and PEITC induce phase II enzymes through transcription by the induction of the antioxidant response element (ARE). Genes that contain this enhancer sequence are regulated by transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 is found bound in the cytoplasm of cells to Kelch-like ECH-associated protein 1 (Keap1). Release of Nrf2 from Keap1 results in the ARE-driven gene expression of phase II enzymes. Sulforaphane can disrupt the Nrf2-Keap1 complex through reactions between the thiol groups of Keap1 [202]. PEITC induced the expression of ARE-mediated phase II enzymes differently than sulforaphane. PEITC increased the phosphorylation of JNK1/2 and ERK1/2 which directly phosphorylates the glutathione S-transferase-Nrf2 protein and releases Nrf2 from Keap1 where it is then free to translocate to the nucleus [202]. Activation of Nrf2 through different mechanisms by ITCs confers protection against carcinogens and is therefore an important feature of ITC. The upregulation of phase II detoxification enzymes can also confer tumor protection against cytotoxic anticancer drugs, so drug therapies that induce the Keap1-Nrf2 signaling pathway are administered in pulsed doses instead of continuous doses to avoid constant activation of this pathway.

Mechanisms by which ITC have been proposed as chemoprotective include modulation of the cell-cycle, induction of apoptosis, and inhibition of angiogenesis. Sulforaphane has been shown to inhibit the cell-cycle at multiple stages of progression. Cell-cycle arrest by sulforaphane treatment has been observed for colon, breast, prostate, and bladder cells in both the G1 and G2/M phase block [210, 211]. The main type of phase arrest induced by sulforaphane is the G2/M phase and is thought to be due to mechanisms including a reduction in protein levels of cyclin B1, CDC25B, and CDC25C [216]. Sulforaphane also causes cell-cycle arrest in the G1 phase due to inhibition of CDK4 activity by increased expression of p21 and decreased expression of cyclin D1 [217]. Evidence describing the roles of ITC in apoptosis and proliferation are extensive. PEITC has been demonstrated to inhibit Akt, a component of Ras signaling, in several different cancer types [218–220]. Ras activation by oncogenes is a common method of sustaining proliferation exploited by cancer cells [209]. Additionally, conjugates of PEITC with N-acetyl cysteine (NAC) inhibit the phosphorylation of Rb, thereby leading to cell-cycle arrest [221]. Additionally, ITC have been shown to generate ROS to cause mitochondrial modification of proteins like Bcl-2, and Bax which cause cytochrome C release into the cytosol and

subsequent apoptosis [209]. Furthermore, PEITC has been shown to induce apoptosis through Fas-mediated apoptosis and induction of death receptors in oral and cervical cancer cells [222–224]. Interestingly, sulforaphane-mediated apoptosis is independent from p53. Treatment of HT29 colon cancer cells with sulforaphane does not change p53 levels [225].

ITCs are promising compounds for inhibiting angiogenesis and the epithelial to mesenchymal transition (EMT). Sulforaphane reduced the in vitro formation of microcapillaries and inhibited VEGF expression and its receptor KDR/flk-1 in human microvascular endothelial cells [226]. PEITC was also shown to inhibit VEGF expression in vitro, but the exact mechanism is unclear [227]. Lastly, ITCs decrease metastases in cell culture and animal studies. Sulforaphane reduced lung metastases induced by metastatic melanoma cells in mice [228]. PEITC significantly reduced the migration of luciferase breast cancer cells to the brain in athymic mice [229]. Furthermore, BITC-mediated inhibition of EMT resulted in upregulation of E-cadherin and downregulation of vimentin and fibronectin in breast cancer cells [230]. While promising, additional studies are needed in order to determine the exact mechanisms by which ITCs inhibit angiogenesis and EMT.

Organosulfur Compounds

Garlic, onions, and leeks represent the major *Allium* foods consumed worldwide. Compared to other foods, about 0.35 % of garlic's fresh weight or 1 % of its dry weight is contributed by sulfur [231, 232]. A variety of sulfur-containing compounds can be found in garlic including thiosulfinates, dithiins, and ajoenes [233, 234]. The use of garlic for medicinal purposes dates back to the year 1550 BC, and it is one of the best-selling herbal products on the market [235]. There is limited evidence for garlic and cancer prevention. A 2009 meta-analysis of 19 human studies reported no credible evidence to support a relationship between garlic intake and a reduced risk of gastric, breast, lung, or endometrial cancers, and indicated there was very limited evidence to support the relationship between garlic consumption and colon, prostate, esophageal, larynx, oral, and renal cancer risk [236]. Results from epidemiology have also been mixed. In China, a high-garlic diet (20 g/day) reduced the mortality of stomach cancer by threefold compared to people consuming less than 1 g/day [237, 238]. Another Chinese study found an inverse association with the consumption of 10 g of *Allium* vegetables and prostate cancer risk [239], while studies in Greece and the Netherlands showed no effect of garlic on breast cancer risk [240, 241]. The results from epidemiological trials should be considered estimates as information on the form, quantity, and preparation of garlic consumed is not often adequate. Although the epidemiology evidence is mixed and human clinical data are limited, at best, laboratory studies in vitro and in vivo have elucidated potential anticancer mechanisms of *Allium* compounds.

Animal studies have shown that garlic and its associated components suppress breast, colon, uterine, oral, esophageal skin, and lung cancers [242–245]. Mechanisms proposed include the blockage of nitrosamine formation, free radical scavenging, suppressed bioactivation of carcinogens, and a reduction in proliferation and apoptosis. Garlic appears to play a key role in blocking DNA alkylation, a critical step for nitrosamine carcinogenesis, through CYP2E1 and/or the formation of nitrosothiols [246]. Furthermore, garlic and its allyl sulfur compounds effectively suppress DNA adduct formation in rodents and cell culture suggesting multiple mechanisms by which garlic and its components may be chemopreventative [247, 248]. Because garlic and its components alter metabolic activation of carcinogens, it is likely that phase I and phase II enzymes are involved [246], however little change in CYP1A1, 1A2, 2B1, and 3A4 have been observed [249–251]. Induction of NQO1 and GST has been suggested as possible mechanisms of protection by garlic [252–255]. Feeding garlic powder to rats increased GST activity [254, 255]. Garlic and its components are also known to block cell-cycle in the G2/M stage, stimulate apoptosis, and inhibit proliferation [256–258]. Collectively, the effect of garlic on cancer risk is moderate, at best, in individuals consuming high quantities of garlic. Side effects of

high garlic consumption include breath and body odors as well as esophageal and abdominal pain and increased blood coagulation times [259]. Therefore high intakes of garlic may interfere with drug metabolism and efficacy, especially aspirin [260]. Nonetheless, garlic and its compounds remain of interest as potential modulators of risk factors for cancer.

Conclusions

Dietary habits are now believed to play a critical role in an individual's risk for cancer. The intake of some specific fruits and vegetables containing nonnutritive bioactive components are inversely associated with the risk for many cancers. Research continues to provide evidence suggesting that carotenoids, polyphenols, indoles, and isothiocyanates play important roles in cancer processes including proliferation, apoptosis, differentiation, cellular and hormonal signaling, cell-cycle regulation, invasive potential, and induction/inhibition of detoxification/bioactivation enzymes. While possible mechanisms of action have been elucidated for some of the bioactive components, it is likely that these components exert their effects through multiple mechanisms. Similarly, the synergistic/antagonistic effects between multiple bioactives and with other nutrients in the diet will be important to determine as we consume mixed diets containing foods with several nonnutritive bioactive components. Furthermore, the interactions between dietary bioactives and traditional medicines must be fully understood before recommendations to the public can be made about specific nonnutritive components and health outcomes. There are several factors that influence the efficacy of these nonnutritive bioactive components including dose, form, bioavailability, and experimental model investigated. While dietary habits are only one modifier of cancer risk, evidence from epidemiological studies suggests that the Western population should alter their lifestyle to increase their consumption of plant-based foods to reduce their risk for cancer. The interaction between genetics and diet will provide further elucidation of the true importance of bioactives and their potential effects on cancer processes. As technology improves, the idea of personalized medicine and nutrition intervention to improve health outcomes may come closer to a reality.

Recommendations

It is fundamentally important that we have an understanding of the role of nonnutritive bioactive components in cancer processes. Understanding the specific molecular targets and tissues where bioactive components can be most effective beyond traditional medicine is key to developing and improving recommendations to the public. Future studies should aim to investigate interactions between nonnutritive bioactives from foods, spices, herbs, and tea and their relationship with genetic makeup and with other dietary components to provide an individualized approach to cancer and nutrition. Furthermore, carefully designed human clinical trials will help our understanding of the mechanisms whereby bioactives impact healthy and cancerous populations.

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Part III
Cardiovascular Disease Prevention

Chapter 13
Diet Quality and Cardiovascular Disease Prevention

Janice L. Atkins and S. Goya Wannamethee

Key Points

- In recent years the focus of dietary research has shifted from single nutrients and food items to overall diet quality and dietary patterns.
- Two main approaches have been developed to assess diet quality: hypothesis-driven (a priori) approaches which generate diet scores and indexes and data-driven (a posteriori) approaches such as factor analysis and cluster analysis.
- Epidemiological evidence shows that high diet quality (assessed using both diet scores/indexes and data-driven approaches) is associated with reduced cardiovascular risk.
- Strong evidence from randomized controlled trials and prospective cohorts have found protective effects of adherence to healthy diet scores, including the Mediterranean diet and the Dietary Approaches to Stop Hypertension diet, on cardiovascular risk factors and incidence of cardiovascular disease.
- Studies using data-driven dietary patterns have frequently identified Healthy/Prudent and Unhealthy/Western dietary patterns, with adherence to the former diet generally associated with reduced risk of cardiovascular disease.

Keywords Cardiovascular disease • Cluster analysis • Dietary pattern • Diet index • Diet quality • Diet score • Factor analysis

Introduction

Cardiovascular disease (CVD) is the biggest cause of death and disability worldwide [1, 2]. CVD is largely preventable and much focus of prevention efforts has turned to promoting healthy lifestyle behaviors, including a healthy diet which is well established in reducing the risk of CVD [1, 3–5]. A recent American Heart Association statement identified a healthy varied diet as an essential behavior for ideal cardiovascular health [6]. Historically, studies investigating the associations between diet and CVD have focused on single foods items or specific dietary nutrients. However, this approach has several limitations; it does not take into account the fact that foods are eaten in combination, interactions and synergies between nutrients are likely to exist, the effects of single nutrients may be too small to detect, and single nutrient analysis may be confounded by the effects of overall dietary patterns [7–9]. In recent years the focus of nutritional epidemiology has shifted towards examining overall dietary patterns to reflect the complex and

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_13

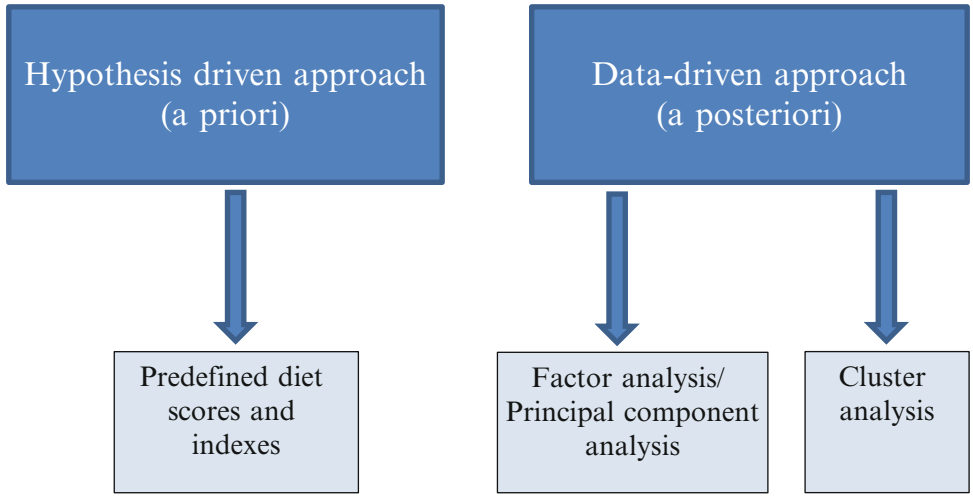

Fig. 13.1 Methods to derive dietary patterns. Adapted from Schulze et al. [8]

multidimensional nature of diets consumed in the population, to examine the cumulative effects of the consumption of various foods/nutrients and to reflect real world dietary preferences.

Two main approaches have been developed to assess diet quality: (1) Hypothesis-oriented or theoretically defined approaches which are a priori in nature, since they use available scientific evidence to generate predefined dietary scores or indexes based on dietary recommendations or guidelines and (2) Data-driven or exploratory approaches which are a posteriori in nature, since dietary patterns are derived from the available data based on factor analysis, such as principal component analysis, or cluster analysis (Fig. 13.1) [8, 10].

This chapter will review the current evidence from prospective cohort studies and randomized controlled trials (RCTs) on the associations between diet quality, measured using *a priori* and *a posteriori* approaches, and the risk of CVD.

A Priori-Defined Dietary Patterns and CVD Prevention

A variety of diverse *a priori*-defined dietary scores and indexes have been developed to assess overall diet quality, based on adherence to healthy diet patterns or adherence to national or international dietary guidelines [4, 9, 11, 12]. Evidence of the relationship between some of the most commonly used scores and indexes and CVD will be presented here.

Mediterranean Diet Score

A 'Mediterranean diet' reflects the dietary patterns characteristic of several olive growing countries in the Mediterranean Basin in the early 1960s including Greece, southern Italy, and Spain [13]. It was first defined by Ancel Keys in the Seven Countries Study who observed lower incidence of CVD in some Mediterranean countries and hypothesized this was due to the dietary habits of these populations [14]. The traditional Mediterranean diet is characterized by an abundant consumption of olive oil as the main source of dietary lipids, a high consumption of fruit, vegetables, legumes, cereals, and nuts, a moderate to low consumption of fish, dairy, and wine (consumed with meals), and a low consumption of meat and meat products [13] (Fig. 13.2). The Mediterranean Diet Score (MDS) is one of

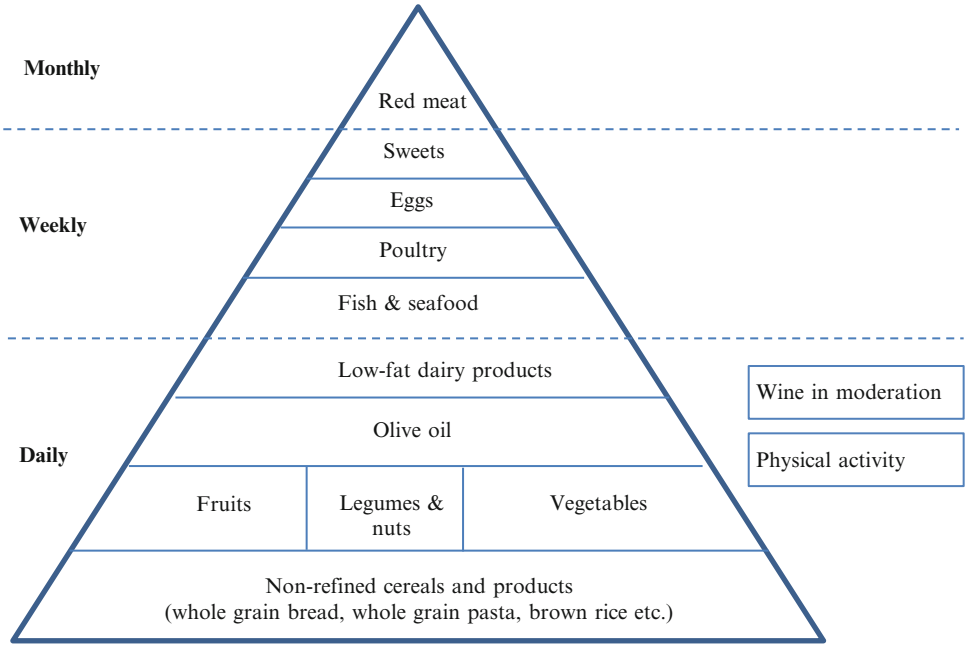

Fig. 13.2 Mediterranean diet pyramid (Adapted from the Supreme Scientific Health Council, Ministry of Health and Welfare Greece [25])

Table 13.1 Mediterranean Diet Score (MDS) components and scoring[a]

Component	Score = 0	Score = 1
Vegetables	< Median intake	≥ Median intake
Legumes	< Median intake	≥ Median intake
Fruit and nuts	< Median intake	≥ Median intake
Cereals	< Median intake	≥ Median intake
Fish and seafood	< Median intake	≥ Median intake
Monounsaturated/saturated lipids ratio	< Median intake	≥ Median intake
Meat and meat products	≥ Median intake	< Median intake
Dairy products	≥ Median intake	< Median intake
Ethanol	Men: <10 g/day or >50 g/day	Men: 10–50 g/day
	Women: <5 g/day or >25 g/day	Women: 5–25 g/day
Total score	0	9

[a]MDS components and scoring as used by Trichopoulou et al. [15, 16]

the most commonly researched predefined dietary patterns and is based on adherence to a combination of food items characteristic of a Mediterranean-style diet. The MDS was first developed by Trichopoulou et al. in 1995 [15] and later revised to include fish intake [16]. The MDS ranges from 0 (minimal adherence) to 9 (maximal adherence). Further details of the components of the MDS and its scoring can be found in Table 13.1.

Since the original MDS was defined, several modified versions have been used [17], but regardless of the slight variations in scores, the association with CVD has shown consistent beneficial results across studies. Numerous prospective cohort studies, based in European and North American adult populations, have shown consistent protective effects of adherence to a Mediterranean diet on the risk

of CVD. A systematic review and meta-analysis carried out by Sofi et al. in 2010, which pooled data from 18 cohorts (including more than two million subjects and 50,000 deaths or incident cases), showed that a two-point increase in the MDS was associated with a 10 % reduction in CVD incidence and mortality [pooled relative risk (RR): 0.90, 95 % confidence interval (CI): 0.87–0.93] [18]. This was followed up by Martinez-Gonzalez et al. in 2014, which included seven more recent prospective studies, including separate estimates for both men and women, and showed highly consistent results. A two-point increase in the MDS was associated with a 13 % relative reduction in the incidence of CVD events (pooled RR: 0.87, 95 % CI: 0.85–0.90) [19]. There is also evidence that a Mediterranean-style diet is effective in reducing the risk of CVD in older adult populations. A recent review in elderly cohorts (aged 65 years or older) identified 20 studies assessing the relationship between the Mediterranean diet and cardiovascular disease, and found that such a diet had benefits on incidence of myocardial infarction (MI), cardiovascular mortality, and cardiovascular risk factors [20]. A recent systematic review assessed association between a Mediterranean diet and cardiovascular risk factors and suggested that possible causal mechanisms underlying the protective effects of a Mediterranean diet on CVD include improvements in blood lipid profile, and a reduction of blood pressure, insulin resistance, and inflammatory markers [21].

The strongest evidence of a causal association between adherence to a Mediterranean diet and the prevention of CVD comes from two RCTs. The Lyon Diet Heart Study, a randomized secondary prevention trial in over 600 French survivors of a first MI, compared an intervention of a Mediterranean diet to a control group receiving standard dietary advice [22]. Interim analysis after 27 months showed a 76 % reduction in major coronary events in the Mediterranean diet group [22] and this protective effect was maintained up to 4 years after the first MI [23]. In a large multicenter randomized primary prevention trial in Spain (PREDIMED), over 7000 individuals at high cardiovascular risk were allocated to one of three diets: a Mediterranean diet supplemented with mixed nuts, a Mediterranean diet supplemented with extra-virgin olive oil, and a control group receiving advice to reduce dietary fat [24]. The risk of major cardiovascular events was reduced by 30 % in the Mediterranean diet and olive oil group and by 28 % in the Mediterranean diet and nuts group at 4.8 years of follow-up, at which point the trial was stopped on the basis of these results. A recent review of the evidence of an association between adherence to a Mediterranean diet and the risk of CVD used meta-analysis to combine the results from these two aforementioned trials, showing a pooled CVD risk reduction of 38 % after intervention with a Mediterranean diet (RR: 0.62, 95 % CI 0.40–0.85) [19].

Dietary Approaches to Stop Hypertension

The Dietary Approaches to Stop Hypertension (DASH) diet is well established in the prevention and control of hypertension. This dietary pattern is rich in fruits, vegetables, and low-fat dairy products, includes whole grains, legumes, fish, poultry, and nuts, and is limited in sugar-sweetened foods, red meat, and added fats. Further details of the components of DASH can be found in Table 13.2. RCTS have shown the DASH dietary pattern to lower systolic blood pressure (SBP) and diastolic blood pressure (DBP) by 5.5 and 3.0 mmHg, respectively [26]. As well as decreasing SBP and DBP, RCTs have also shown DASH improves other CVD risk factors. A meta-analysis of RCTs found that an intervention with the DASH diet resulted in significant reductions in total cholesterol (−0.20 mmol/L, 95 % CI: −0.31, −0.10) and low density lipoprotein cholesterol (−0.10 mmol/L, 95 % CI −0.20, −0.01) [27].

In addition to examining cardiovascular risk factors, many studies have also assessed the associations between adherence to the DASH dietary pattern and the incidence of CVD. A recent systematic review identified six such prospective cohort studies and pooled analysis showed that a DASH-style diet significantly reduced the risk of CVD, coronary heart disease (CHD), stroke, and heart failure by 20 %, 21 %, 19 %, and 29 %, respectively [28].

Table 13.2 Dietary Approaches to Stop Hypertension (DASH) dietary pattern components[a]

Component	Servings
Whole grains	6–8 Servings/day
Vegetables	4–5 Servings/day
Fruits	4–5 Servings/day
Fat-free or low-fat milk products	2–3 Servings/day
Lean meats, poultry, and fish	<6 oz/day
Nuts, seeds, and legumes	4–5 Servings/day
Fats and oils	2–3 Servings/day
Sweets and added sugar	≤5 Servings/day

[a]Adapted from Lichtenstein et al. [29]

Table 13.3 Alternative Healthy Eating Index (AHEI) components and scoring[a]

Component	Score = 0[b]	Score = 10[b]
Vegetables (servings/day)	0	5
Fruit (servings/day)	0	4
Nuts and soya protein (servings/day)	0	1
Ratio of white to red meat	0	4
Cereal fibre (g/day)	0	15
trans Fat (% of energy)	≥4	≤0.5
Ratio of polyunsaturated fatty acids to saturated fatty acids	≤0.1	≥1
Duration of multivitamin use[c]	<5 years	≥5 years
Alcohol (servings/day)	Men: 0 or >3.5	Men: 1.5–2.5
	Women: 0 or >2.5	Women: 0.5–1.5
Total score	2.5	87.5

[a]AHEI components and scoring as used by McCullough et al. [31]
[b]Minimum score is 0. Maximum score is 10. Intermediate intakes are scored proportionately between 0 and 10
[c]For multivitamins, the minimum score is 2.5 and the maximum score is 7.5

Healthy Eating Index

The Healthy Eating Index (HEI) was originally proposed by the United States Department of Agriculture to measure adherence to Dietary Guidelines for Americans and the Food Guide Pyramid [30]. The HEI is a 10-component system made up of five food groups (grains, fruit, vegetable, milk, and meat), four nutrients [total fat, saturated fatty acids (SFA), cholesterol, and sodium], and a measure of diet variety, with a total possible 100-point score. McCullough et al. developed a modified version of this score, the Alternative Healthy Eating Index (AHEI). The AHEI was designed to assess intake of food groups and macronutrient sources associated with reduced chronic disease risk, and compared to the HEI it distinguishes quality within food groups and acknowledges the health benefits of unsaturated oils [31]. The AHEI consists of nine components with a possible score from 2.5 to 87.5. Further details of the components of the AHEI and its scoring can be found in Table 13.3.

A study in two large American cohorts prospectively compared the two scores and found that AHEI was better at predicting CVD risk than the original HEI. Men from the Health Professionals' Follow-up Study and women from the Nurses' Health Study, with AHEI scores in the top compared to the bottom quintile had a 39 % and a 28 % reduction in CVD risk, respectively [32]. Similarly, in the Whitehall II study, British adults in the top compared to the bottom tertile of AHEI score showed a 42 % reduction in the risk of CVD mortality after controlling for potential confounders [33].

Table 13.4 Healthy Diet Indicator (HDI) components and scoring criteria[a]

Component	Score = 0	Score = 1
Saturated fatty acids (% energy)	>10	0–10
Polyunsaturated fatty acids (% energy)	<3 and >7	3–7
Protein (% energy)	<10 and >15	10–15
Complex carbohydrates (% energy)	<50 and >70	50–70
Dietary fibre (g/day)	<27 and >40	27–40
Fruits and vegetables (g/day)	<400	>400
Pulses, nuts, seeds (g/day)	<30	>30
Monosaccharides and disaccharides (% energy)	>10	0–10
Cholesterol (mg/day)	>300	0–300
Total score	0	9

[a]HDI components and scoring as used by Huijbregts et al. [34]

Healthy Diet Indicator

The Healthy Diet Indicator (HDI) is another *a priori*-defined dietary score which was developed by Huijbregts et al. and is based on adherence to World Health Organization dietary guidelines for the intake of nutrients and food components [3, 34]. The HDI consists of nine components (SFA; polyunsaturated fatty acids [PUFA]; protein; complex carbohydrates; dietary fibre; fruit and vegetables; pulses, nuts, and seeds; monosaccharides and disaccharides; and cholesterol), each scoring one if the dietary guideline is met and zero otherwise, resulting in a total score range from 0 to 9. Further details of the components of the HDI and its scoring can be found in Table 13.4. The HDI has been shown to be inversely associated with cardiovascular mortality risk in older European men, with an 18 % risk reduction in the group with the highest HDI score [34]. Similarly, associations have also been found in Eastern European populations, with a 10 % reduction in CVD mortality risk and a 15 % reduction in CHD mortality risk per one standard deviation increase in HDI score [35].

However, associations of the HDI with CVD risk have been inconsistent across studies. Findings from elderly male cohorts in Sweden and Britain have shown no association between HDI and CVD mortality [36, 37] and in Dutch adults from the European Prospective Investigation into Cancer and Nutrition (EPIC-NL), no association was found between HDI and CVD incidence [38].

Other A Priori-Defined Dietary Patterns

In addition to some of the most commonly used scores and indexes mentioned above, there are other less widely used dietary scores which have been developed, and in some cases tailored for specific populations or countries or designed to evaluate prevention efforts for specific diseases [12].

It has been suggested that recall of usual dietary behaviors may be less prone to recall errors than specific types or amounts of food consumed and an alternative approach to assessing healthy dietary patterns is the dietary behavior score (DBS). The DBS is based on the usual consumption related to recommended dietary behavior, including consumption of fruit, vegetables, whole grains, low-fat dairy, and low-fat meats. In the American Association of Retired Persons Diet and Health Study, participants in the highest quintile of the DBS, compared to the lowest had a 23–30 % lower risk of CHD mortality [39].

A Posteriori-Defined Dietary Patterns and CVD Prevention

Many studies have used data-driven or exploratory approaches to assess overall diet quality and the two predominant approaches are factor analysis, such as principal component analysis, and cluster analysis [4, 9, 40]. Factor analysis or principal component analysis identifies foods that are frequently consumed together and aggregates food items or groups on the basis of the degree of correlation with one another [8, 10]. Cluster analysis derives dietary patterns based on differences in dietary intakes between individuals who are separated into mutually exclusive groups [7, 10]. In factor analysis, individuals are scored based on their degree of adherence to each derived dietary pattern, whereas in cluster analysis individuals are assigned to one cluster only. Typical dietary patterns derived by such methods tend to include healthy or prudent and unhealthy or Western style patterns.

In the Nurses' Health Study, factor analysis identified two major dietary patterns—prudent (characterized by higher intakes of fruits, vegetables, legumes, fish, poultry, and whole grains) and Western (characterized by higher intakes of red and processed meats, sweets and desserts, French fries, and refined grains) [41]. The prudent diet score was associated with a reduced risk of CHD (quintile 5 vs. quintile; 1 RR: 0.76, 95 % CI: 0.60–0.98) and the Western diet was associated with an increased risk of CHD (quintile 5 vs. quintile; 1 RR: 1.46, 95 % CI: 1.07–1.99). Similarly, the EPIC-NL study used principal component analysis to identify a prudent pattern (high intakes of fish, high-fibre products, raw vegetables, and wine) and a Western pattern (high consumption of French fries, fast food, low-fibre products, other alcoholic drinks and soft drinks with sugar) and found that the prudent pattern was associated with a reduced risk of CHD (hazard ratio (HR) for extreme quartiles: 0.87, 95 % CI: 0.75–1.00) and stroke (HR: 0.68, 95 % CI: 0.53–0.88), but found no association with the Western dietary pattern [42].

The PREDIMED RCT identified two major baseline dietary patterns using factor analysis based on 34 predefined food groups—a Western dietary pattern (rich in red and processed meats, alcohol, refined grains, and whole dairy products) and a Mediterranean-type dietary pattern (MDP) [43]. Higher adherence to the MDP was associated with a lower CVD risk (adjusted HR for fourth vs. first quartile: 0.52, 95 % CI: 0.36–0.74) but the Western pattern was not significantly associated with CVD risk.

In the Whitehall II study, cluster analysis identified four dietary patterns at baseline: unhealthy (white bread, processed meat, fries, and full-cream milk), sweet (white bread, biscuits, cakes, processed meat, and high-fat dairy products), Mediterranean-like (fruit, vegetables, rice, pasta, and wine), and healthy (fruit, vegetables, whole-meal bread, low-fat dairy, and little alcohol) [44]. Compared with the unhealthy cluster, the healthy cluster was associated with a reduced risk of CHD mortality (HR: 0.71, 95 % CI: 0.51–0.98) after adjustment for confounders. However the other dietary patterns were not associated with CHD risk. Cluster analysis was also used in the EPIC-NL study, which identified a prudent dietary pattern and a Western pattern, similar to the patterns identified in this cohort using principal component analysis analysis mentioned above [42]. Individuals in the prudent cluster showed a reduced risk of CHD (HR: 0.91, 95 % CI: 0.82–1.00) and stroke (HR: 0.79, 95 % CI: 0.67–0.94) compared to those in the Western cluster [42].

Caveats of Dietary Pattern Analysis

It is important to take into account some general limitations of deriving dietary patterns to analyse the association of diet quality with health outcomes. Dietary patterns are population specific and likely to vary according to sex, ethnicity, culture, and socio-economic status, so it is difficult to make informative comparisons across studies [7]. Also, measurement error exists in the assessment of all dietary data, with problems surrounding recall bias, social desirability in reporting, and issues of under- or

over-reporting of total energy intake [9, 45]. In addition, diet quality may strongly relate to other behavioral risk factors for disease that may not be fully accounted for in studies by adjustment for measured confounders.

In particular, there are some limitations of using *a priori* methods to assess diet quality in relation to CVD risk that should be taken into account [10]. Firstly diet quality scores or indexes may be culturally or regionally specific so may not be universally applicable. Scores may also be dependent on the selected underlying dietary guidelines, which are related to morbidity and mortality risk generally but not specifically to CVD risk. Adding together equally weighed dietary components implies that each component is equally important to CVD risk, which may not be the case. Also, scores which dichotomize components do not take into account the full range of consumed foods, so using scoring ranges may be preferable to simple cut-offs [11].

Using data-driven approaches to generate dietary patterns has the advantage of not making any prior assumptions but uses the existing data to characterize total diet, meaning that results can be meaningful, interpretable and can show some reproducibility across populations [10]. However, *a posteriori* methods of deriving dietary patterns, including factor analysis and cluster analysis, do have some considerations. Data are limited on the validity of these methods, and subjectivity may be introduced when grouping dietary variables, making analytic choices about statistical methods and in selecting final dietary patterns to use [7, 10, 40].

Conclusions

CVD is the biggest cause of death and disability worldwide and diet is well established in reducing the risk of CVD. In recent years the focus of dietary research has shifted from single nutrients and food items to overall diet quality, assessed by hypothesis-driven, *a priori*, approaches which generate diet scores and indexes, and data-driven, *a posteriori*, approaches such as factor analysis and cluster analysis. Epidemiological evidence shows that high diet quality is associated with reduced risk of CVD. Adherence to healthy dietary patterns, identified from either diet scores and indexes or factor and cluster analysis, has tended to show an inverse association with CVD risk but the magnitude of protective effects has varied across studies. Consistent evidence from prospective studies and RCTs has provided strong evidence for an inverse association between adherence to a Mediterranean diet or a DASH diet and reduced CVD risk. Such dietary patterns may therefore be a relatively low cost tool for public health nutrition interventions for the prevention of CVD.

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Chapter 14
n-3 and *n*-6 Fatty Acids Reduce Risk for Cardiovascular Disease

William S. Harris

Key Points

- Both families of polyunsaturated fatty acids (PUFAs), omega-6 and omega-3, have cardioprotective properties.
- The benefits of these PUFAs on cardiovascular disease (CVD) risk arise via mechanisms that do not necessarily involve reductions in classic risk factors like LDL-cholesterol or blood pressure.
- The principal omega-6 fatty acid in the diet is linoleic acid; the best sources are (most) plant oils.
- The principal omega-3 fatty acid in the diet is alpha-linolenic acid (from certain plant oils), but its effects on CVD risk are less clear than those of the longer-chain omega-3 fatty acids (from marine oils and seafoods).
- PUFAs should be viewed as important components of the life-long diet to slow the progress of CVD rather than short-term interventions to treat preexisting CVD.

Keywords Fish oil • *n*-3 fatty acids • Eicosapentaenoic acid • Docosahexaenoic acid • Linoleic acid • *n*-6 fatty acids • Arachidonic acid • Cardiovascular disease • Biomarkers

Introduction

Fish oils are one of the most popular nutritional supplements on the market today. Reports that the *n*-3 fatty acids (FA) contained in fish oils, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may benefit patients with diseases from Alzheimer disease to Zellweger syndrome have driven the demand. Although provocative, much of the evidence is circumstantial and indirect, making firm conclusions elusive, even for coronary heart disease (CHD), the one malady for which there had been no doubt about the benefits of fish oil. The other family of polyunsaturated fatty acids, the *n*-6 family, also has had a role to play in CHD prevention, although for this family, the current controversy is not so much whether it is beneficial or not, but indeed, if it is actually harmful.

After defining the chemical structures of these fatty acids and their nomenclature, this review will then discuss each class in turn, focusing first on dietary (or encapsulated) sources of the fatty acids, next on their relations with CHD risk and then summarizing current recommendations. It will conclude with a consideration of the utility of measuring *n*-3 and *n*-6 fatty acid blood levels as prognostic markers of risk for CHD.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_14

Definitions and Structures

Chemically, *n*-3 and *n*-6 FA are long chains of carbon atoms (18–22) with 3–6 double bonds in the chain. The essential FA families are shown in Fig. 14.1, along with the structures of the major *n*-6 FA and food sources of each. They are all known as polyunsaturated fatty acids (PUFA) because they have >1 double bond, and are called omega-3 (or omega-6) FA because the first double bond is on the third (or sixth) carbon atom from the end of the molecule (the *omega* carbon, ω being the last letter of the Greek alphabet). They are also known as *n*-3 (where the *n* refers to *nth* carbon in the chain). When referring to the *n*-3 FA, the terms *long-chain* (≥20 carbon atoms) and *short-chain* (<20 carbon atoms) refer to EPA/DHA and to alpha-linolenic acid (ALA), the plant *n*-3 FA, respectively. For the *n*-6 FA, arachidonic acid would be considered long chain and linoleic acid short chain. (Note that outside of the context of the essential fatty acids, "short chain" fatty acids are typically <6 carbons in length, "medium chain" fatty acids are from about 6–12 carbons long, "long chain" would be 14–20 carbons long, and "very long chain" 22 or more carbons.)

n-3 Fatty Acids

Sources of n-*3 Fatty Acids*

Foods

Both EPA and DHA are found almost exclusively in seafoods (Table 14.1) because these FA are originally synthesized by single-cell organisms at the base of the ocean food chain. Fish do not synthesize *n*-3 FA any more efficiently than do humans. Fish consume *n*-3 FA in their diets and have a nutritional

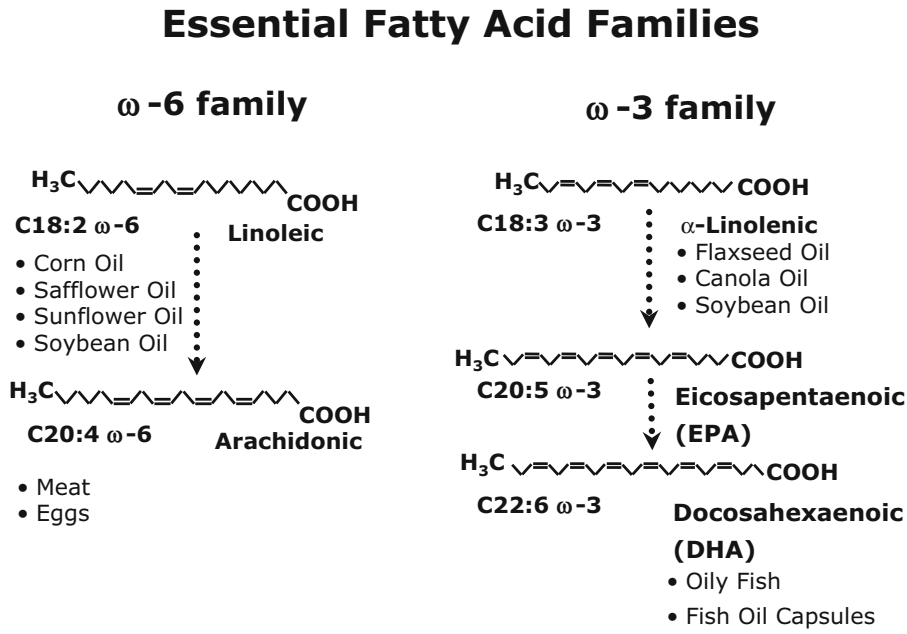

Fig. 14.1 Essential fatty acid families and food sources

need for them just as humans do. Generally speaking, the oilier the fish, the more *n*-3 FA present. Note that just because a fish lives in cold water does not necessarily mean that it is a good source of *n*-3 FA. Salmon and cod can both be found in the North Atlantic, but fillets of the former are rich in EPA and DHA while fillets of the latter contain very little. Cod, of course, store large amounts of *n*-3 FA-rich oil in their livers whereas salmon do not.

Owing to the increasing demand for fish and fish oils, fish stocks have come under increasing pressure, and the need for novel sources of EPA and DHA for use in fish farming is growing. According to the *n*-3 trade group, Global Organization for EPA and DHA, in 2010 <4 % of global fish catch and <20 % of fish oil production was destined for the manufacture of supplements and *n*-3 drugs; the vast majority of fish oil goes to feeding farmed fish. One approach to meeting the demand for *n*-3 FA without harvesting fish has been the development of new products that, when consumed, raise tissue EPA levels through internal bioconversion. One such product is a genetically engineered variety of soybean oil containing the *n*-3 FA stearidonic acid (C18:4ω−3). This FA is the first product in the pathway from ALA to EPA and is catalyzed by the rate-limiting enzyme, delta-6 desaturase. Consuming stearidonic acid-enriched soybean oil has been shown to significantly raise levels of EPA in blood and tissues in humans [1]. This oil could, in theory, provide virtually unlimited amounts of *n*-3 FA for the human diet [2], and, similar to when the fortification of salt with iodine reduced the incidence of goiter, adding effective *n*-3 FA precursors to commonly consumed foods could remediate the chronically low *n*-3 FA levels so prevalent in western populations.

Capsules

Fish oil capsules have become one of the most popular dietary supplements in the USA. A wide variety of supplements are available with differing levels of total *n*-3 FA and differing ratios of EPA to DHA (Table 14.2). Higher concentrations (or larger capsules) clearly reduce the number of pills that need to be consumed to achieve a target intake of EPA + DHA. For vegan and vegetarians, there are now encapsulated products available that are derived from nonanimal sources (e.g., algae) [3].

There are four chemical forms in which fish oil products are generally available: triglycerides, ethyl esters, phospholipids, and free acids. FA are esterified in the first three to glycerol, to ethanol, or to a phosphatidic acid residue (e.g., phosphatidyl choline, or lecithin), respectively. The free acid (nonesterified) form is primarily a pharmaceutical agent. Most fish oil supplements are in the triglyceride form. This is the least expensive form to produce because it simply requires the concentration and purification of rendered fish oil (usually from anchovy, sardine, and mackerel). The most common of these contain 180 mg of EPA and 120 mg of DHA per 1000 mg of fish oil in capsule form, i.e., 30 % EPA + DHA. Ethyl ester forms are more concentrated than the triglyceride forms, and because they require more processing, they are often more expensive. Both Lovaza and Vascepa, which are pharmaceutical agents, are ethyl ester forms. Supplements that provide >35 % EPA + DHA and do not indicate their form on the label are most likely ethyl esters. Concentrated products that are in the triglyceride form will most likely be labeled as such. The phospholipid form, at present confined to krill oil products, are least concentrated in EPA + DHA. Some products are krill oil—triglyceride blends to increase the amount of EPA + DHA on the label. Salmon oils can contain triglycerides only or a blend with ethyl esters (again, to achieve higher EPA + DHA concentrations), but this information may not appear on the label. Finally, cod liver oil products are only very rarely sold with additional ethyl esters, so one can safely assume that they are what they claim to be.

There are also *n*-3 FA capsules that contain oils derived from non-fish sources to serve the vegetarian population. The oils are derived from specific strains of single-celled algae. These are typically DHA-only products, but recently, algae-derived EPA products have become available. Again,

Table 14.1 Approximate levels of EPA + DHA in fish, and corresponding amounts required to provide approximately 1 g/day of EPA + DHA[a]

	EPA	DHA	EPA + DHA	
	mg/3 oz (85 g) serving			Amount providing 1 g EPA + DHA
Salmon, Atlantic (farmed)	587	1238	1825	1.6
Herring, Atlantic	773	939	1712	1.8
Salmon, Atlantic (wild)	349	1215	1564	1.9
Tuna, Bluefin	309	970	1279	2.3
Salmon (chum)	460	778	1238	2.4
Herring (pickled)	717	464	1181	2.5
Salmon, Coho (farmed)	347	740	1087	2.8
Mackerel (canned)	369	677	1046	2.9
Salmon, Coho (wild)	341	559	900	3.3
Oysters (steamed)	523	327	850	3.5
Sardines (canned in oil)	402	433	835	3.6
Swordfish	108	656	764	3.9
Rainbow Trout (farmed)	220	524	744	4.0
Tuna, Albacore (White) (canned in water)	198	535	733	4.1
Sockeye Salmon	228	445	673	4.5
Sea Bass	175	473	648	4.6
Salmon (pink)	185	339	524	5.7
Crab, Dungeness	357	144	501	6.0
Alaskan Pollock	73	360	433	6.9
Crab, King	251	100	351	8.5
Walleye	93	245	338	8.9
Flat Fish (flounder/sole)	143	112	255	11.8
Tuna, Light (canned in water)	40	190	230	13.0
Halibut	68	132	200	15.0
Lobster, Northern (steamed)	99	66	165	18.2
Clams (canned)	60	90	150	20.0
Scallops (steamed)	61	88	149	20.1
Crab, Blue	86	57	143	21.0
Haddock	43	93	136	22.1
Cod	3	131	134	22.4
Mahi-Mahi (dolphin fish)	22	96	118	25.4
Tilapia	4	111	115	26.1
Tuna, Yellowfin	13	89	102	29.4
Shrimp	43	44	87	34.5
Catfish (farmed)	17	59	76	39.5
Orange Roughy	5	21	26	115.4

[a]Values are typically based on fish cooked with dry heat (i.e., baked) and are derived from the USDA Nutrient Data Laboratory [88]. Amounts of EPA + DHA are estimated because they vary markedly with season, the fish's diet, age, stage of life, and storage, as well as cooking methods

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *USDA* United States Department of Agriculture

because of the greater cost of production, these products are more expensive than fish oil-based supplements. Finally, encapsulated (and liquid oil) products providing ALA are available, usually from flax, chia, or hemp seed oils. ALA is considered to be the essential *n*-3 FA in the diet because it can be converted, albeit quite inefficiently (<5 %, often <1 %) [4], into the longer chain metabolites which—although they play important roles in metabolism—are not in themselves dietary essentials.

Table 14.2 EPA and DHA content in selected products (per softgel)

Product	Oil/cap	EPA	DHA	EPA + DHA	EPA + DHA
Fish oils	mg	mg	mg	mg	%
Nature Made krill oil	1000	50	24	74	7
Nature's Bounty krill oil	1000	57	45	102	10
Source Naturals Neptune krill oil	1000	150	90	240	24
Schiff MegaRed krill oil	300	50	24	74	25
Nature Made fish oil	1000	130	120	250	25
Onemia *n*-3 phospholipids	500	54	86	140	28
Spring Valley Omega-3 fish oil	1000	180	120	300	30
Eye Omega advantage	1000	230	230	460	46
Carlsons Super Omega-3 Gems fish oil	1000	300	200	500	50
Carlsons Very Finest fish oil	1000	300	200	500	50
Nordic Naturals Ultimate Omega	1000	325	225	550	55
GNC Triple Strength fish oil 1500	1500	540	360	900	60
Omegor Vitality	1000	420	210	630	63
Source Naturals Ultra Potency fish oil	1250	450	340	790	63
Nature's Bounty fish oil triple strength	1400	647	253	900	64
Omegavia	1500	780	260	1040	69
VitalOils 1000	1200	250	750	1000	83
Minami CardiO-3	1000	635	194	829	83
Non-fish (plant)-Derived Products					
Ovega-3 (algae oil)	2000	130	320	450	23
Deva Vegan Omega-3 (algae oil)	500	130	70	200	40
New Harvest Omega-3 (yeast oil)	1200	600	0	600	50
Pharmaceutical Products					
Epanova[a]	1000	550	200	750	75
Lovaza[a]	1000	465	375	840	84
Vascepa[a]	1000	960	0	960	96
Liquid Oils	Oil/tsp				
Carlson's Very Finest fish oil	4500	800	500	1300	29
Nordic Naturals Liquid fish oil	4500	825	550	1375	31
Barlene's Fresh Catch	4500	880	585	1465	33
Cardiotabs Omega-3	4500	610	1570	2180	48
Carlson's Medomega fish oil	4500	1200	1200	2400	53
Stronger Faster Healthier SO3	4500	2209	990	3199	71

[a]EPA and DHA are present in these products as ethyl esters, which have a higher molecular weight than free EPA and DHA. For comparison with Epanova (FFA form), Lovaza contains 425 mg of free EPA and 345 mg of free DHA, or 770 mg per capsule (77 %). Vascepa contains 876 mg of free EPA per capsule (88 %). *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

n-*3 Fatty Acids and CHD*

During the last 40 years, there have been numerous epidemiologic, observational, experimental, and randomized controlled trials (RCTs) examining the effects of the marine *n*-3 FA on CHD risk. The reader is referred to several comprehensive reviews [5–11]. As regards ALA, there are some data suggesting it also may have CHD benefits [12], but whether they are completely independent of their conversion to EPA + DHA is not clear. The evidence relating to the marine *n*-3 fatty acids and CHD risk from RCTs and epidemiological investigations will be considered below.

Randomized Trials

The major RCTs with *n*-3 FA that examine effects on clinical CHD end points are summarized in Table 14.3. Among these trials, four would be considered positive, five neutral, and one negative. Such heterogeneity invites meta-analyses [13], with no less than five being published in 2012 alone [14–18].

The meta-analysis that received the most media attention was that of Rizos et al. [14] because of the authors' conclusion that *n*-3 FA supplementation was not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction (MI), or stroke. This apparently counter intuitive finding was primarily the result of the statistical approach these authors took (for detailed reviews see [13, 19]). Normally in meta-analyses, which summarize a large body of published literature examining a specified hypothesis, 95 % confidence intervals are employed to declare statistical (or not) significance. The most egregious error in Rizos et al. [14] was their decision to raise the statistical bar from the usual 95 % to a 99.4 % confidence interval. They declared that, in order for an effect to be declared statistically significant, the *P* value had to be <0.006 instead of the standard <0.05. Hence, even though Rizos et al. [14] reported that fish oil use was associated with a 9 % reduction in cardiac death ($P = 0.01$), the authors chose to consider this finding as nonsignificant. This decision turned a positive into a negative, and, in so doing, engendered immense confusion that continues to impact physician (especially) views on the use of *n*-3 FA for the prevention of CHD. Continued use of 1 g of EPA + DHA per day in RCTs will likely continue to produce the same results. In that regard, two ongoing RCTs are of particular interest as they are using much higher doses. The Reduction of Cardiovascular Events with EPA—Intervention Trial (REDUCE-IT [20]) is evaluating supplementation with 4 g/day of EPA ethyl esters (icosapent ethyl) in 8000 patients at high risk for CHD who are on a background of statins. Another trial, A Phase III, double-blind, long-term outcomes study to asses Statin Residual Risk Reduction with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH, Clinicaltrial.gov identifier NCT02104817) will test supplementation with 4 g of EPA + DHA given in a free fatty acid form in a similar population. The results of both REDUCE-IT and STRENGTH are expected to be available between 2017 and 2019.

Epidemiology

As important as RCTs are in the adjudication of benefit/harm of (typically) pharmaceutical agents, their use in nutrition research is problematic for a number of reasons (see [19]). While prospective cohort studies also have significant limitations, the relations between nutrient intakes (or, better, nutrient biomarker levels) and disease outcomes should be considered complimentary and equally important as RCT data (see Discussion in [21]). There have been at least 16 cohort studies that have used dietary estimates of EPA + DHA intake as the exposure marker for CHD end points, and 13 studies that have used circulating EPA + DHA levels. These have been included in a major meta-analysis in 2014 by Chowdhury et al. [22]. In this analysis, higher intakes of long chain *n*-3 FA from 16 studies were associated with a 13 % (95 % CI, 3–22 %) reduction in CHD events, and this class of FA was the only one linked to a lower risk of events. Consistent with this, higher circulating levels of these two *n*-3 FA (and levels of docosapentaenoic acid, DPA) were significantly associated with reduced risk for CHD events (Table 14.4). This indicates that, in the long run and in more "natural" settings outside of clinical trials, *n*-3 FA are consistently and directly associated with better cardiac health.

Mechanisms of Action of n-*3 Fatty Acids*

Detailed reviews of *n*-3 FA mechanisms of action, both at the cellular and at the systemic levels, have been recently published [7, 23, 24]. Unlike drugs that typically inhibit or activate a specific enzyme

Table 14.3 The 10 largest (>2000 Participants) *n*-3 fatty acid randomized controlled trials assessing cardiovascular outcomes

Trial, year	Population	Interventions Compared	Duration (yrs)	End points	RR (95 % CI)
DART 1989 [89]	2033 men with recent MI (mean 41 days)	2 servings/week fatty fish (or fish oil capsules) versus other dietary advice	2	IHD events	0.84 (0.66–1.07)
				Total deaths	0.71 (0.54–0.93)
GISSI-P 1999 [90]	11,324 men with recent MI (≤3 months)	Usual care plus 882 mg/day EPA + DHA, vitamin E, both or neither	3.5	Major CV events	0.90 (0.82–0.99)
				Nonfatal events	0.98 (0.83–1.15)
				Cardiac deaths	0.78 (0.65–0.92)
				Sudden deaths	0.74 (0.58–0.93)
DART 2 2003 [91]	3114 men with angina	2 servings/week fatty fish (or fish oil capsules) versus other dietary advice	3–9	Cardiac deaths	1.26 (1.00–1.58)
				Sudden deaths	1.54 (1.06–2.23)
JELIS 2007 [42]	18,645 patients with total cholesterol ≥6.5 mmol/L (with and without CHD history)	1.8 g/day EPA versus usual care	4.6	Coronary events	0.81 (0.69–0.95)
				Nonfatal events	0.81 (0.68–0.96)
				Coronary deaths	0.94 (0.57–1.56)
				Sudden deaths	1.06 (0.55–2.07)
GISSI-HF 2008 [92]	6975 patients with heart failure	840 mg/day EPA + DHA versus placebo (not defined)	3.9	Total death	0.91 (0.83–0.99)
				Death or hospitalization for CVD	0.94 (0.89–099)
OMEGA 2010 [93]	3851 patients with recent MI (≤2 weeks)	840 mg/day EPA + DHA versus placebo (olive oil)	1	Major CV events	1.21 (0.96–1.52)
				Sudden deaths	0.95 (0.56–1.60)
Alpha-Omega 2010 [94]	4837 patients with history of MI (median 3.7 years)	376 mg/day EPA + DHA versus placebo margarine and ALA (1.9 g/day) groups combined	3.4	Major CV events	(0.87–1.17)
				CHD deaths	0.95 (0.68–1.32)
SU.FOL. OM3 2010 [95]	2501 patients with recent coronary or cerebral ischemic event (median 101 days)	600 mg/day EPA + DHA versus placebo (not defined) and B vitamin groups combined	4.2	Major CV events	1.08 (0.79–1.47)
				CHD deaths	Not reported
ORIGIN 2012 [96]	12,536 patients with diabetes, IGT, or IFG, most with CVD	840 mg EPA + DHA versus olive oil placebo	6.2	CVD deaths	0.98 (0.87–1.10)
				Major CV events	1.01 (0.93–1.10)
Risk and Prevention 2013 [97]	12,513 patients at increased risk for CHD but without MI	850 mg EPA + DHA versus olive oil placebo	5.0	Death, nonfatal or stroke	0.98 (0.88–1.08)
				CVD death	1.02 (0.82–1.30)

CHD coronary heart disease, *CV* cardiovascular, *DART* Diet and Reinfarction Trial, *HDL* high density lipoprotein, *HDL-C* HDL cholesterol, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *IHD* ischemic heart disease, *LDL* low density lipoprotein, *LDL-C* LDL cholesterol, *MI* myocardial infarction, *NR* not reported, *ORIGIN* Outcome Reduction with Initial Glargine Intervention, *RCT* randomized control trial, *SU.FOL.OM3* Supplementation en Folates et Omega-3

or receptor, *n*-3 FA alter biology in a more global and fundamental manner and can affect many molecular pathways (Fig. 14.2).

n-3 FA are incorporated as structural components into the phospholipids of the cell membrane, and therefore increase membrane (especially lipid raft) fluidity. This incorporation can nonspecifically impact cellular function by altering the activity of membrane-associated receptors, ion channels, transporters, and enzymes. In addition, *n*-3 FA serve as natural substrates for cyclooxygenases, lipoxygenases, and cytochrome P450 mono-oxygenases [25–28], and thereby generate hundreds of molecular species, including prostaglandins, leukotrienes, epoxides, resolvins, protectins, and maresins. These molecules produce favorable changes in a wide range of cellular activities important to cardiovascular function (e.g., vasodilation and vasoconstriction, cellular adhesion process, inflammatory responses, and platelet aggregation). Additionally, *n*-3 FA (or eicosanoids [20-carbon metabolites] and docosanoids

Table 14.4 Relations between circulating fatty acid levels and CHD events

Fatty acid	Number of studies	Relative risk (95 % CI)
Alpha-linolenic	8	0.93 (0.83–1.03)
Eicosapentaenoic (EPA)	13	0.78 (0.65–0.94)
Docosahexaenoic (DHA)	13	0.79 (0.63–0.93)
EPA + DHA	13	0.75 (0.62–0.89)
Docosapentaenoic *n*-3	4	0.64 (0.47–0.89)
Linoleic	10	0.99 (0.77–1.28)
Arachidonic	10	0.83 (0.74–0.92)

Taken from Chowdhury et al. [22]

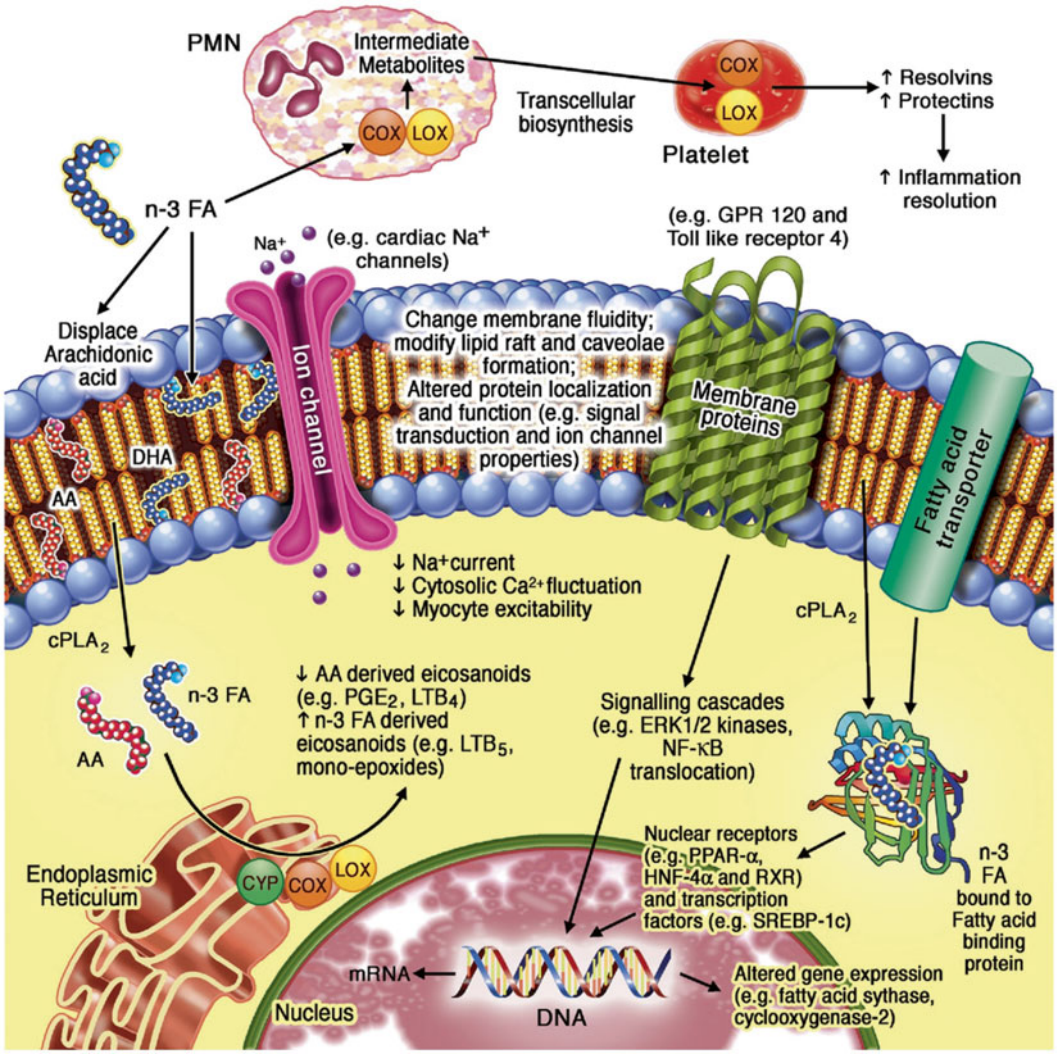

Fig. 14.2 *n*-3 Fatty acids modulate multiple molecular pathways that together contribute to their physiological effects. Reprinted from *Journal of the American College of Cardiology*, 58/20, Mozaffarian D, Wu JH. *n*-3 Fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. 2047–2067, ©2011 [7], with permission from Elsevier. *AA* arachidonic acid, *COX* cyclooxygenase, *cPLA*$_2$, cytosolic phospholipase A2, *CYP450* cytochrome P450, *DHA* docosahexaenoic acid, *ERK* extracellular signal-regulated kinase, *FA* fatty acids, *GPR* G-protein-coupled receptor, *HNF-4α* hepatic nuclear factor-4 alpha, *LOX* lipoxygenase, *LTB* leukotriene B, *mRNA* messenger RNA, *NF-κB* *n*-3, omega; nuclear factor-kappa B, *PGE*$_2$ prostaglandin E2, *PMN* polymorphonuclear leukocyte, *PPAR-α* peroxisome proliferator-activated receptor-alpha, *RXR* retinoid X receptors, *SREBP-1c* sterol regulatory element binding protein-1c

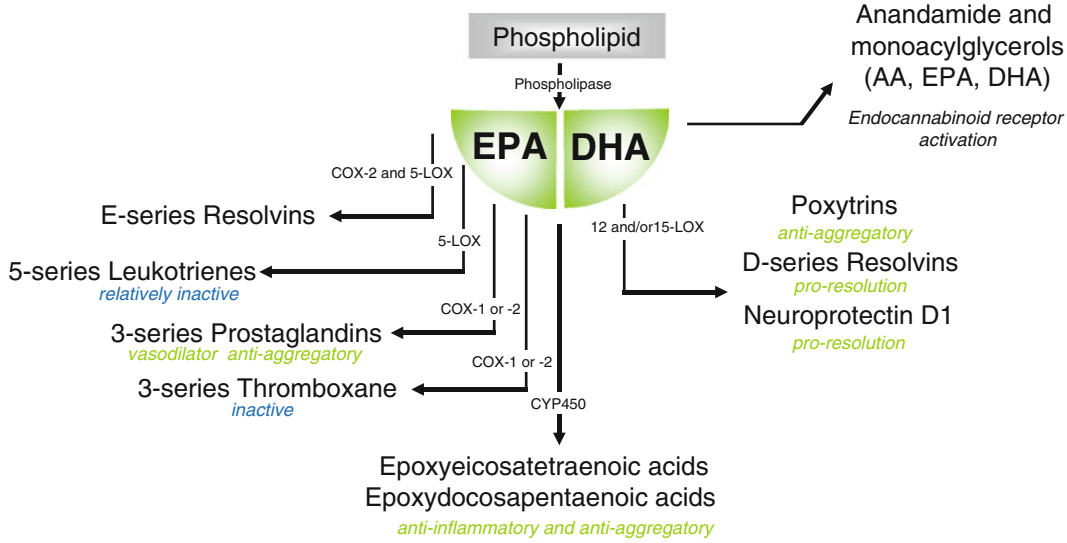

Fig. 14.3 Overview of lipid mediators derived from eicosapentaenoic acid (*EPA*) and docosahexaenoic acid (*DHA*). Modified from [98]. Reprinted with permission

[22-carbon metabolites] produced from them; see below) serve as ligands for nuclear transcription factors that control genes, such as peroxisome proliferator-activated receptors α and γ, which, when activated, regulate expression of genes for FA uptake and metabolism [29]. Together, and in ways not precisely understood, these basic mechanisms influence several factors important in the development of CHD [23], including reductions in serum triglyceride levels [30], blood pressure [31], platelet aggregation [32], heart rate [33], susceptibility to ventricular fibrillation (in some settings) [34], and plaque vulnerability [35, 36] along with improvements in endothelial function [37]. There appear to be no effects on atrial fibrillation [38], even for patients in the post-cardiac surgery setting [39].

One general mechanism noted above includes the generation from *n*-3 FA of eicosanoids and docosanoids. These pathways are illustrated in somewhat greater detail in Fig. 14.3. The potential interactions of the metabolites shown here—both individually and in concert with each other and the similar suite of *n*-6 FA (see below)—is, at present, virtually impossible to predict, and thus much work remains to be done to fully understand the mechanisms by which these fatty acids influence metabolism, physiology, and risk for CHD.

Recommended Intakes

The American Heart Association (AHA) Nutrition Committee published a scientific statement regarding *n*-3 FA and heart disease and stroke [40]. The statement included three recommendations: For patients with diagnosed CHD, the AHA recommended about 1 g of EPA + DHA per day. Although "oily fish" was the recommended source (Table 14.1), the AHA nevertheless acknowledged that many people either cannot or will not eat sufficient fish to meet this target. Accordingly, for these individuals, an EPA + DHA supplement (Table 14.2) could be considered in consultation with their physicians. For patients without known heart disease, the AHA recommended ≥2 meals of oily fish per week and the inclusion of oils and foods rich in ALA. Note that this intake of fish would provide 400–500 mg of EPA + DHA per day on average. For patients with elevated serum triglyceride levels, the AHA recommended a higher dose of EPA + DHA, 2–4 g/day. This dosing would, of course, be done only under medical supervision and with capsules. The author feels that this recommendation should be revised to 3–4 g/day.

In addition, in 2010, the AHA published recommendations for *n*-3 FA supplementation specifically for women [41] that included the use of fish or fish oil supplements (e.g., EPA at 1800 mg/day), for women with high cholesterol or triglycerides, whether primary or secondary prevention. (The suggested dose was that used in the JELIS study in which the majority of participants were women [42].) Whether or not the AHA will, after considering the full spectrum of relevant data, revise any of the dose recommendations remains to be seen. The author would argue against a revision at this point.

The 2010 Dietary Guidelines for Americans recommends consumption of ≥250 mg of EPA + DHA daily for the primary prevention of CHD [43]. Although not as high a dose as others have recommended (e.g., American Dietetic Association and Dietitians of Canada [44] and the International Society for the Study of Fatty Acids and Lipids [45], both of which recommend 500 mg/day), this was the first time that any recommendation whatsoever for EPA + DHA was included in US dietary guidelines, so it is a step in the right direction.

n-6 Fatty Acids

Dietary Sources

Except for a few "omega-3.6.9" products which purport to provide "all of the essential omega's" (for which this author sees no value), there are no *n*-6 fatty acid supplements, these FA being completely provided in foods. The richest sources are vegetable oils (Table 14.5).

Table 14.5 Fatty acid composition of selected edible fats and oils (taken from Academy of Nutrition and Dietetics [53])

Lipid	Quantity	SFA	8:0	10:0	12:0	14:0	16:0	18:0	MUFA	18:1	PUFA	18:2	18:3	EPA DPA DHA	ARA	TFA
Avocado oil	1 Tbsp	11.9	0.0	0.0	0.0	0.0	11.3	0.7	72.6	69.9	13.9	12.9	1.0	0.0	0.0	0.0
Beef tallow	1 Tbsp	46.8	0.0	0.0	0.9	3.5	23.5	17.8	39.3	33.9	3.8	2.9	0.6	0.0	0.0	0.0
Butter[i]	1 Tbsp	53.6	5.1	2.6	2.7	7.8	22.6	10.4	22.0	20.8	3.2	2.9	0.4	0.0	0.0	0.0
Canola oil	1 Tbsp	7.6	0.0	0.0	0.0	0.0	4.4	6.8	65.1	63.5	29.0	19.6	9.4	0.0	0.0	0.4
Coconut oil	1 Tbsp	11.8	1.0	0.8	6.1	2.3	1.1	0.4	0.8	0.8	0.2	0.2	0.0	0.0	0.0	0.0
Com oil	1 Tbsp	12.9	0.0	0.0	0.0	0.0	10.6	1.8	27.6	27.4	54.7	53.5	1.2	0.0	0.0	0.3
Flaxseed oil	1 Tbsp	9.0	0.0	0.0	0.0	0.1	5.1	3.4	18.5	18.3	67.9	14.3	53.4	0.0	0.0	0.1
Grapeseed oil	1 Tbsp	9.6	0.0	0.0	0.0	0.1	6.7	2.7	16.1	15.8	69.9	69.6	0.1	0.0	0.0	–
Lard	1 Tbsp	36.9	0.0	0.1	0.2	1.3	22.4	12.7	42.5	38.8	10.5	9.6	1.0	0.0	0.0	0.0
Olive oil	1 Tbsp	13.7	0.0	0.0	0.0	0.0	11.2	1.9	72.4	70.7	10.4	9.7	0.7	0.0	0.0	–
Palm oil	1 Tbsp	49.3	0.0	0.0	0.1	1.0	43.5	4.3	37.0	36.6	9.3	9.1	0.2	0.0	0.0	–
Palm kernel oil	1 Tbsp	81.5	3.3	3.7	47.1	16.4	8.1	2.8	11.4	11.4	1.6	1.6	0.0	0.0	0.0	–
Rice bran	1 Tbsp	19.7	0.0	0.0	0.0	0.7	16.9	1.6	39.3	39.1	35.0	33.4	1.6	0.0	0.0	–
Salmon oil	1 Tbsp	19.9	–	–	–	3.3	9.9	4.3	29.0	17.0	40.3	1.5	1.0	31.3	0.7	–
Soybean oil	1 Tbsp	15.7	0.0	0.0	0.0	0.0	10.4	4.4	22.8	22.6	57.7	51.0	7.1	0.0	0.0	0.5

Values as a percent of total fatty acids. For conversion to mass, one tablespoon is considered to contain 13.6 g of fatty acids

Abbreviations: SFA, saturated fatty acids (FA); MUFA, monounsaturated FAs; PUFA, polyunsaturated FAs; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid (n-3); DHA, docosahexaenoic acid; ARA, arachidonic acid; TFA, trans FAs

n-*6 Fatty Acids and CHD*

In 2009, the American Heart Association (AHA) published a Science Advisory that examined the evidence relating the intake of *n*-6 FA (primarily linoleic acid) and CHD [46]. The impetus for this statement was the growing belief among the public (fueled by the writings of a few scientists [47–52]) that this family of fatty acids (primarily arachidonic acid, a metabolic product of linoleic acid) causes "inflammation." Beginning in the 1960s, when vegetable oils rich in linoleic acid were starting to show their cholesterol-lowering properties, recommendations from virtually all health groups endorsed the substitution of liquid oils for solid fats (rich in saturated fatty acids). Hence, PUFA, like linoleic acid—which is by far the most prevalent PUFA in the human diet—were viewed as heart healthy. Anti-*n*-6 proponents have been challenging this view over the last decade, hence the AHA's interest in re-examining the issue. The AHA committee concluded after its review that

> Aggregate data from randomized trials, case-control and cohort studies, and long-term animal feeding experiments indicate that the consumption of at least 5 % to 10 % of energy from omega-6 PUFAs reduces the risk of CHD relative to lower intakes. The data also suggest that higher intakes appear to be safe and may be even more beneficial (as part of a low–saturated-fat, low-cholesterol diet) [46].

The AHA's position is echoed by other international health organizations [53–56]. As with the *n*-3 FA, the relations between linoleic acid and CHD have been examined using both RCT and prospective cohort designs. These will be discussed below.

Randomized Trials

There have, of course, been several RCTs testing the hypothesis that the substitution of saturated FA with PUFA (from vegetable oils) will reduce risk for CHD. Depending on which meta-analysis one attends to, this hypothesis has been supported [57] or no significant relationship has been seen [22]. In no meta-analysis has an adverse risk relationship been observed. Ramsden et al. [58] have raised the concern that these studies cannot strictly be applied to linoleic acid since in several studies small increases in ALA accompanied the large increase in linoleic acid (i.e., studies using soybean oil, where the LA: ALA ratio is about 7:1), and thus one cannot unambiguously attribute the benefit to LA. While strictly correct, the view that small increases in ALA (for which there are some data suggesting CHD benefit [12]) more than compensate for the purported adverse effects of LA on CHD risk stretches credulity. Much more likely is the consensus view that both classes of long-chain PUFA are heart-healthy [59].

Epidemiology

The most comprehensive analysis of the association between the dietary intake of linoleic acid and CHD events was published in 2014 by Farvid et al. [60]. Utilizing data from both published and unpublished studies (via direct investigator contact), Farvid et al. included 13 cohort studies involving about 310,000 individuals with over 12,000 CHD events and about 5900 CHD deaths. Intakes of LA were estimated by a variety of dietary questionnaires, and follow-up ranged from 5 to 30 years. Comparing the highest to the lowest intake groups, risk for CHD events was lower [47] by 15 % and for CHD death by 21 %, both statistically significant. Viewed another way, risk for events was *increased* by 18 % and death by 27 % in the lowest intake group compared to the highest. Relations with CHD have also been assessed by examining associations between baseline *n*-6 FA levels in blood/plasma and clinical outcomes. In the Chowdhury meta-analysis [22], circulating levels of LA were not related to subsequent risk for CHD either beneficially or adversely. Interestingly, however, arachidonic acid levels (the putative direct precursor to inflammatory biomarkers) were significantly *inversely* related to CHD risk. The most recent report comparing circulating levels of *n*-6 fatty acids

and CHD risk came from the Cardiovascular Health Study [61]. Here, Wu et al. found significant inverse relations between plasma phospholipid linoleic acid levels and total and CHD mortality, with risk in the highest quintile being 13 % less than that in the lowest quintile. There were no relations between risk for mortality and other *n*-6 fatty acids, and the greatest benefit was observed in those with the highest *n*-6 and *n*-3 fatty acid levels.

n-*6 Fatty Acids Mechanism of Action*

The LDL-cholesterol lowering effects of high-PUFA diets is well known, and its mechanism (based on animal work summarized by Nicolosi [62]) appears to be an increase in LDL clearance secondary to upregulation of the LDL receptor. Hence, one way in which linoleic acid reduces risk for CHD is via LDL-lowering. Inflammation also plays a role in the development of atherosclerosis [63], hence if nutrient X increases "inflammation," then it would increase risk for CHD. Since arachidonic acid is a metabolic product of linoleic acid, and the former gives rise to at least some pro-inflammatory cytokines and eicosanoids, then it was a short step to the conclusion that linoleic acid may increase risk for heart disease. However logical it may appear on the surface, the evidence does not support the view that linoleic acid (or even oral arachidonic acid) exacerbates "inflammatory status"[64–67], much less increases risk for CHD. At least one of the reasons for this could be that arachidonic acid is the precursor for a variety of both pro- *and* anti-inflammatory molecules, not to mention nitrated metabolites of both LA and ARA that can also have beneficial effects (Fig. 14.4). These data indicate that one pillar of the anti-*n*-6 position—that higher AA levels mean higher risk—is invalid.

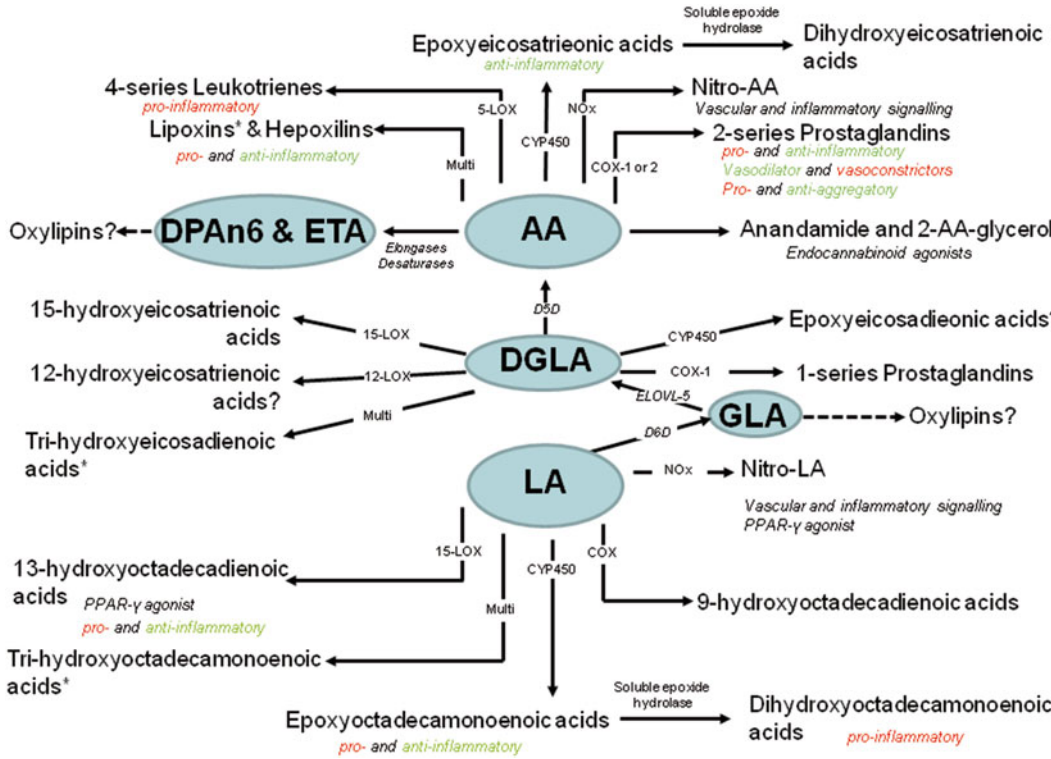

Fig. 14.4 Overview of the known and potential metabolites of *n*-6 FA [99]

Recommended Intakes of n-*6 Fatty Acids*

The American Heart Association (AHA) Nutrition Committee recommends between 5 and 10 % of energy come from *n*-6 fatty acids (essentially linoleic acid), as does the Academy of Dietetics and Nutrition [53].

Monitoring Blood Fatty Acid Status

A variety of clinical laboratories in the USA are now (in 2014) offering fatty acid tests (in plasma, RBC, and/or whole blood). Since there is no consensus on what levels are "healthy" for linoleic acid, and since arachidonic acid levels are highly resistant to changes in linoleic acid intake [68], most have focused on *n*-3 fatty acid levels. Fatty acids can be measured in a variety of sample types [69], but red blood cell (RBC) membranes may be the best for assessing in vivo tissue *n*-3 fatty acid status. In 2004, the author (along with C. von Schacky) proposed that the Omega-3 Index (which is the EPA + DHA content of RBC membranes expressed as a percent of total fatty acids) as a new risk factor of mortality risk from CHD [70]. It has since been validated as a biomarker of tissue *n*-3 FA status [69, 71]. The individual's blood level reflects longer-term *n*-3 FA status just as measurement of the glycosylated hemoglobin (HbA_{1c}) level does for an individual's glucose. The within-person variability of the Omega-3 Index is one-fourth that of the EPA + DHA level in plasma [72], and RBC *n*-3 FA levels are unaffected by an acute dose of fish oil, unlike plasma levels [73]. The Omega-3 Index has been shown to be an independent risk predictor for cardiac disease [74–78], cellular aging [79], and cognitive dysfunction [80, 81]. A low Omega-3 Index is also a marker for increased inflammatory status [82]. Although an Omega-3 Index >8 % has been proposed as optimal for cardioprotection [83], the US average is approximately 4–5 % [84], with only approximately 10 % of the population having an index of >8% [85]. The Omega-3 Index increases across the life span, with average values in 30-year-old persons being approximately 4 % and in the 70-year-olds, 5.1 % [85]. Since the response of the Omega-3 Index to fish oil supplementation can vary markedly [86], titrating to a cardioprotective target level (instead of simply recommending a certain intake of *n*-3 fatty acids) is a more rational approach for patient care. Indeed, the variability in response to *n*-3 fatty acid treatment may explain, in part, the neutral results in several recent *n*-3 RCTs [87]. At present, there is no agreement on an internationally standardized method for assessing *n*-3 fatty acid status.

Conclusion

Both of the long-chain *n*-3 and *n*-6 fatty acid families are cardioprotective. Consumption of at least 250 mg of EPA + DHA per day and between 5 and 10 % of energy as linoleic acid appears to be the optimal combination for reducing risk for CHD.

Acknowledgement *Conflict of Interest Statement* William S. Harris, PhD, is the owner of OmegaQuant Analytics, LLC.

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Chapter 15
Trans Fatty Acids: A Summary of the Evidence Relating Consumption to Cardiovascular Outcomes and the Efficacy of Prevention Policy to Reduce Levels in the Food Supply

Shauna M. Downs, Sarah Loehr, and Jason H.Y. Wu

Key Points

- Partially hydrogenated oils are the main dietary source of trans fatty acids. They entered the food supply in the early 1900s and quickly became a key ingredient in processed foods given their long shelf life and low cost.
- Trans fatty acid consumption from partially hydrogenated oils adversely affect lipid metabolism, and has been linked with an increased risk of coronary heart disease. In addition, there is evidence to suggest that high intakes may be associated with visceral adiposity, insulin resistance, and type 2 diabetes.
- In order to reduce intakes of trans fatty acids, countries, cities, and states worldwide have adopted trans fat labeling and bans. Accumulating evidence suggests these initiatives have resulted in the gradual reduction of trans fat levels in processed foods, which have coincided with reductions in intakes.
- In June 2015, the United States Food and Drug Administration ruled to revoke the "generally recognized as safe" status to partially hydrogenated oils which, once implemented, will act as a pseudo ban in the country.
- Despite ongoing policy efforts to lower trans fat levels in the food supply, global intakes of trans fatty acids remain high, and exceeds recommended upper limits of intake in many countries. Further implementation of policies and continued monitoring is needed to ensure progress towards elimination of harmful partially hydrogenated oil derived trans fatty acids from the food supply.

Keywords Trans fat • Dietary fat • Cardiovascular disease • Trans fat policy

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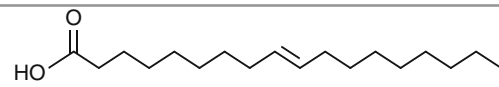
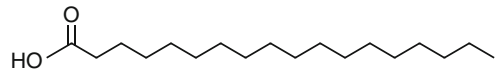
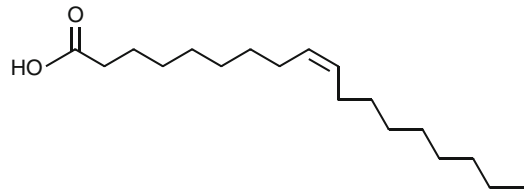
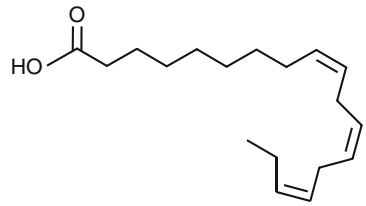
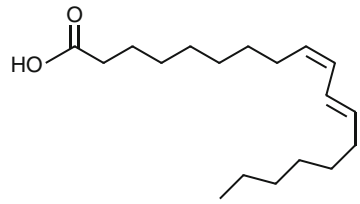
A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_15

Introduction

Trans fatty acids (TFA) are unsaturated fats with at least one double bond in the *trans* configuration [1]. The *trans* formation (carbon chain extends from opposite sides of the double bond) differs from the *cis* formation (carbon chain extends from the same sides of the double bond) in that rather than having a kink in the shape of the molecule (such as that observed in mono- and polyunsaturated fats) it creates a more linear shape resembling a saturated fatty acid (SFA) chain (Table 15.1) [2]. The properties of TFA are also similar to SFA in that it is semisolid at room temperature, whereas unsaturated fats (in the *cis* formation) are liquid at room temperature [1]. In addition to the physical consistency, TFAs are less reactive, which makes them more stable against oxidation [3]. This has important implications for its use in processed foods—products that contain TFA have an extended shelf life and can withstand repeated heating or frying. It is also cheap making it a desirable choice for use in processed foods [4, 5].

There are two main types of TFAs—naturally occurring TFA and those that are industrially produced. Ruminant animals (cows, sheep, goats, etc.) produce naturally occurring TFAs (rTFA) through the biohydrogenation process in the rumen. Industrially produced TFAs (iTFA) are produced by adding hydrogen gas to edible oil (typically vegetable oils) containing unsaturated fatty acids, heating it and combining it with a nickel catalyst. This process transforms the unsaturated fat molecule into TFAs,

Table 15.1 The biochemical structures of the different fatty acids and their nomenclature

Name of fatty acid	Type of fatty acid	Biochemical structure	Nomenclature[a]
Trans-oleic acid[b]	Trans fatty acid		t9-18:1
Stearic acid	Saturated fatty acid		18:0
Cis-oleic acid	Monounsaturated fatty acid		c9-18:1
Alpha linolenic acid	Polyunsaturated fatty acid		c9,12,15–18:3
Conjugated linoleic acid[c]	Conjugated unsaturated fatty acid		c9,t11-18:2

[a]The nomenclature specifies the placement of the double bond (in the trans or cis formation and the carbon number in which the double bond appears counting from the carboxylic acid end of the molecule) followed by the number of carbons in the chain: the number of double bonds

[b]Also referred to as elaidic acid

[c]Note there are several other isomers of conjugated linoleic acid, some of which have *cis–cis* conjugated double bonds. The most common isomer is presented in this table (c9,t11-18:2)

resulting in a fat with a harder consistency and higher melting point. An edible oil can be partially hydrogenated through this process, producing partially hydrogenated oils (PHOs) containing iTFAs, or it can be fully hydrogenated, which converts all unsaturated fats to saturated fats in the oil [6].

PHOs are the major dietary source of iTFAs and their predominant iTFA is elaidic acid (t9-18:1, Table 15.1). PHOs have typically been synthesized from soybean oil, cottonseed, or, more recently, palm oil to produce a more solid fat such as a margarine or shortening, which can be used in processed foods. PHOs are used in baked goods, popcorn, dried soups, fried snacks, crackers, and fast foods [7].

The two primary rTFA trans isomers produced during the biohydrogenation process are *trans-vaccenic* acid (t11-18:1), and *cis*-9, *trans*-11 conjugated linoleic acid (CLA; c9,t11-18:2) [8, 9]. These rTFAs are produced from unsaturated fatty acids, mainly linoleic acid and α-linolenic acid. Trans-vaccenic acid is the predominant trans isomer in ruminant fats (50–80 % of total rTFA) and is a major precursor to CLA [9], but it is also present in hydrogenated plant oils, contributing an estimated 13–17 % of the *trans*-vaccenic acid found in the diet [10]. While up to 60 % of total fat in PHOs can be comprised of iTFA [11], dairy, beef, and lamb contain a relatively low proportion of rTFA (2–5 % of total fatty acid in dairy and 3–9 % in beef and lamb) [12, 13].

History of TFA

iTFAs entered the food supply in the early 1900s [6]. A German chemist, Wilhelm Normann, discovered the process of partial hydrogenation, which converted inexpensive liquid vegetable oils into a vegetable fat with a similar consistency to butter [14]. This discovery later led to the inventors receiving the Nobel Prize in 1912 [14]. Because these cheaper products, with a longer shelf life, mimicked the traditional cooking fats of European and North American cuisines (e.g., lard or butter), it quickly entered the food supplies of many countries [14]. By 1911 Crisco shortening appeared on the store shelves in the USA and was marketed as an economical alternative to butter [15]. In addition, a free cookbook containing 615 Crisco recipes was circulated to American housewives—its use became popular almost immediately [15]. PHOs such as Crisco quickly became the poor man's butter and largely replaced it in times of economic uncertainty such as World War I and II [16].

In the 1960s, evidence began to emerge from the Seven Countries study led by Ancel Keys that SFA consumption may increase cardiovascular disease (CVD) risk [17]. As evidence for the unfavorable effects of SFA relative to polyunsaturated fatty acids (PUFA) on CVD lipid risk factors emerged, there was a shift in consumer demand for fats from animal to non-animal sources [18]. Between the 1960s and 1990s there was a move in the USA to cook with margarines rather than butter, and many of those margarines were PHOs [18]. However, over this time experimental and metabolic studies began to accumulate that identified harmful metabolic effects of iTFA [19], which was further supported by large observational studies that showed higher iTFA consumption to be robustly associated with elevated coronary heart disease (CHD) risk [20].

Industrially Produced Trans Fatty Acids and Cardiovascular Disease

The majority of the research conducted to date has focused on the effects of iTFA rather than rTFA on cardiovascular outcomes. For that reason, rTFA is discussed below in a separate section. The effects of iTFA intake on intermediate risk factors of CVD have been examined in animal experimental, cross-sectional, and controlled metabolic studies. While unfavorable effects of iTFA on serum lipid levels are well established, emerging evidence suggests additional adverse influence of higher iTFA intakes on lipoprotein metabolism, inflammation, endothelial function, and adiposity.

Serum Lipids and Lipoproteins

Research examining the effects of iTFA on total serum cholesterol began in the 1960s. This early work demonstrated that iTFA consumption increased total serum cholesterol; however, these early studies did not examine the effects of TFA on the distribution of the different lipoproteins. In 1990, Mensink and Katan performed one of the first randomized controlled trials (RTC) in this area [19]. In this pivotal study, they compared the effects of consuming 10 % of total energy from either *cis* oleic acid (a monounsaturated fatty acid, MUFA), the *trans* isomer of oleic acid, or SFA on serum lipoprotein concentrations in 59 healthy men and women. Participants consumed each of the three diets for a 3-week period. Both the diets enriched with iTFA and SFA increased low-density lipoprotein cholesterol (LDL-C) compared to the high oleic acid diet [19]. However, while the diets high in oleic acid and SFA increased high-density lipoprotein cholesterol (HDL-C) concentrations, consumption of the diet high in iTFA reduced HDL-C levels [19]. Therefore, while the high SFA diet resulted in an increase in total serum cholesterol, the high iTFA diet resulted in an undesirable change in the total cholesterol/HDL-C ratio [19], which has been shown to be a better predictor of CHD risk than total or individual lipoprotein cholesterol levels [21, 22]. Subsequent trials have reported similar findings. In a meta-analysis of 8 controlled trials, the isocaloric replacement of SFA or *cis* unsaturated fatty acids with iTFA increased levels of LDL-C, reduced HDL-C, and increased the ratio of total cholesterol/HDL-C [23]. It was estimated that the isocaloric replacement of 1 % of energy from iTFA with a 1:1:1 mixture of carbohydrate, MUFA, and PUFA would reduce total cholesterol/HDL-C ratio by 0.04 [23], an effect nearly twice as large as similar replacement of 1 % of energy from SFA.

The mechanisms involved in dietary iTFA induced alterations in serum lipoproteins are not well understood; however, a number of different pathways have been implicated. Although the production rate of LDL apolipoprotein B-100 (apoB-100) and HDL apolipoprotein A-1 (apoA-1) appears to remain constant, consumption of iTFA appears to decrease rates of LDL apoB-100 catabolism and increase rates of HDL apoA-1 catabolism, contributing to the greater pool size of LDL-C and reduced HDL-C [24]. Furthermore, there is evidence to suggest that altered LDL and HDL-C concentration may be due to iTFA induced increase in cholesteryl ester transfer protein activity, which facilitates the transfer of cholesterol esters from HDL to LDL and very low-density lipoproteins (VLDL) [23, 25].

In addition to changes in LDL-C and HDL-C, TFA intake has been reported to have other important adverse effects on serum lipids. Several studies have demonstrated that the consumption of iTFA increases fasting triglyceride concentrations [26–29]. Additionally, results from a meta-analysis of 8 RCTs indicated that the consumption of iTFA raises lipoprotein (Lp)(a) levels [29], another risk factor for CHD [30]. Lastly, dietary iTFA intake has also been reported to significantly reduce the LDL particle size, resulting in an increased distribution of small, dense LDL particles [31], which are more atherogenic than larger, more buoyant particles [32, 33].

Systemic Inflammation

Systemic inflammation is a risk factor for various health conditions including CVD [34]. Several observational studies have identified positive associations between dietary TFA intake and pro-inflammatory biomarkers [34–37]. For example, in middle age (mean 61 year, range: 44–70 year), generally healthy women, TFA intakes were positively associated with tumor necrosis factor (TNF) receptors 1 and 2 independent of other known factors that might influence systemic inflammation including age, body mass index (BMI), smoking, physical activity, medication use, alcohol consumption, and other dietary factors [34]. When results were further adjusted for concentrations of LDL-C, HDL-C, TG, and Lp(a), the magnitude of the associations were partly attenuated (~25 %); however,

it remained significant suggesting that the observed associations were only partly mediated by the effects of TFA on lipids [34]. TFA intakes were not associated with concentrations of other systemic inflammatory biomarkers (interleukin 6 (IL-6) or C-reactive protein (CRP)) in the overall group; however, a positive association was found between TFA intake and both IL-6 and CRP in women with a higher BMI [34]. These findings suggest that dietary TFA may induce an inflammatory response, particularly in those who are overweight or obese.

Despite demonstrated associations between TFA intake and inflammation from observational studies, the evidence from controlled trials has been mixed. While some feeding trials reported that higher iTFA consumption increased inflammatory markers including TNF alpha [38, 39], TNF receptor 1 [40], IL-6 [38, 39], and CRP [41, 42], other experimental studies reported no differences in inflammation markers [43–45]. Potential reasons for the inconsistent findings include differences in studied populations, level of iTFA consumed, and the duration of intervention—additional well-controlled intervention studies are needed to elucidate the effect of iTFA on inflammatory pathways. Nevertheless, the potential of iTFA to induce inflammation has been further supported by animal and cellular studies that have also provided insight into the possible pro-inflammatory molecular mechanisms of iTFA. For example, iTFA intake causes chronic inflammation and hepatic damage in animal models [46, 47], which could be due to increased activation of nuclear factor-kB (NF-kB)—a critical upstream regulator of many genes which participate in the inflammatory response [48, 49].

Endothelial Dysfunction

In short-term intervention trials, iTFA intake causes endothelial dysfunction, which is a risk factor for CVD in both healthy populations and those with pre-existing CVD [50–53]. A study of 21 healthy men demonstrated that a test meal high in iTFA had similar acute adverse effects on brachial arterial flow-mediated vasodilation (FMD), a direct measure of endothelial dysfunction, when compared to a test meal high in similar amounts of SFA [54]. However, over a 4-week period, consumption of a high iTFA diet (9.2 % of energy) resulted in a greater impairment in FMD compared to a diet composed of equivalent calories from SFA in healthy men and women (29 % reduction of FMD with the iTFA diet as compared to the SFA diet) [54]. Concentrations of E-selectin, another marker of endothelial dysfunction, were also significantly higher after consuming a high iTFA as compared to SFA diet (8 % of daily energy from iTFA or SFA) in a 5-week trial of 50 healthy men [55]. In an observational study, TFA intake estimated in 730 women was positively associated with E-selectin, vascular cell adhesion molecule (sVCAM-1) and soluble cell adhesion molecules—all markers of endothelial dysfunction [36]. Compared to the lowest quintile of TFA intake (mean TFA intake 0.9 % of energy), those in the highest quintile (mean TFA intake 2.1 % of energy) had significantly higher plasma concentrations of E-selectin, vascular cell adhesion protein-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1) [36]. These findings are consistent with metabolic trials, and suggest that TFA could have adverse effects on endothelial function even at relatively low intake levels.

Adiposity

Obesity is a major risk factor for CVD [56]. Increased adiposity, particularly increased visceral fat, is especially harmful [57]. Emerging evidence suggests that weight gain and fat accretion, especially in the midsection, may be adversely affected by iTFA consumption. Results from two large prospective cohort studies indicated that dietary intake of TFA was positively associated with waist circumference in men [58] and weight gain in women [59]. Among overweight women, there was a 1 kg (2.3 lbs)

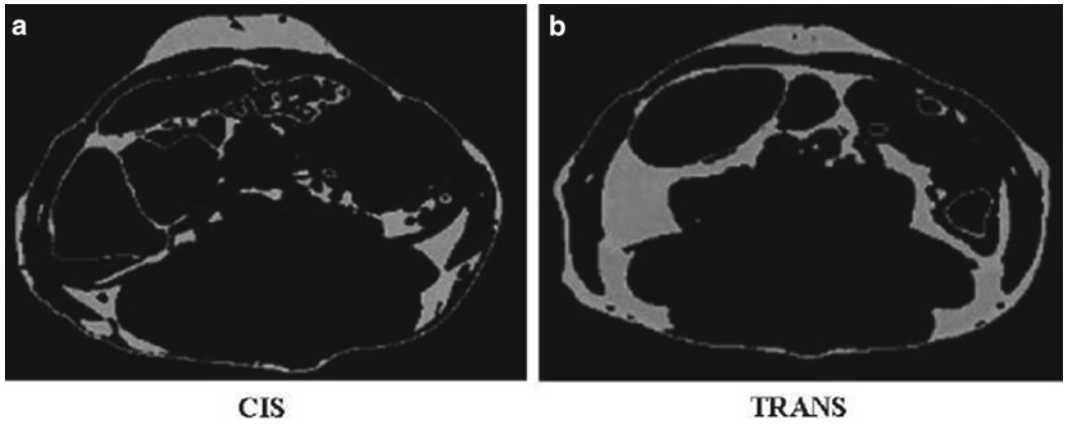


Fig. 15.1 A CT scan of abdominal fat accumulation in monkeys fed cis (A) and trans (B) monounsaturated fat matched on total body weight. The CT scan was performed at the end of the 6-year study where *green* monkeys were fed an isocaloric diet containing 8 % of total energy from either iTFA or MUFA. The scan depicts higher fat volumes (*light grey* area of scan) in the abdomen in monkeys fed a diet high in TFA. *Source*: Kavanagh et al. [62]

weight gain for every 1 % increase in energy from TFA [59]. Additionally, it was reported that the content of TFA in adipose tissue, a reflection of dietary fatty acid intake in the medium- and long-term, was positively associated with BMI, skinfold thickness, waist circumference, and weight gain in both men and women [60, 61]. However, given that these studies were observational, it is possible that individuals consuming higher levels of TFA also engaged in other unhealthy behaviors leading to changes in body composition.

Although observational evidence suggests an association between TFA intake and adverse abdominal fat deposition and weight gain, long-term RCTs are required to help determine causation. However, due to ethical limitations these types of studies cannot be performed in humans. Nevertheless, limited animal experiments also suggest iTFA intake may have adverse effects on adipose distribution. In a 6-year RCT, green monkeys were fed an isocaloric diet containing 8 % of total energy from either iTFA or MUFA (a partially hydrogenated soybean oil and an equivalent oleic acid-enriched fatty acid blend, respectively) [62]. iTFA fed monkeys had marked increases in intra-abdominal fat deposition as compared to the monkeys in the control group [62]. Figure 15.1 depicts a CT scan of the visceral adiposity of monkeys, matched for total body weight, fed the isocaloric *cis* and *trans* monounsaturated fat diet. This marked increase in visceral adiposity has important implications, given that visceral adiposity has been associated with insulin resistance, increased risk of diabetes and CVD as well as the metabolic syndrome [63]. However, the mechanisms by which iTFA consumption may lead to increased abdominal fat deposition are not well understood and require further elucidation.

The Effects of Industrially Produced Trans Fatty Acid Consumption on Health Outcomes

Coronary Heart Disease

iTFA consumption has consistently been shown to relate to higher CHD risk in prospective cohort studies. A meta-analysis of prospective cohort studies including nearly 140,000 participants estimated that a 2 % increase in energy from TFA was associated with a 23 % increase in CHD [20]. Similarly,

a more recent meta-analysis that included 155,270 participants and 4662 coronary events from 5 prospective cohort studies reported a 16 % (confidence intervals: 6–27 %) increase in the risk of CHD events in participants in the top as compared to the bottom third of baseline dietary TFA intakes [64]. The concordance between adverse effects of iTFA on multiple physiologic risk factors and its robust association with elevated CHD risk in prospective observational studies underpins current national and international dietary guideline recommendations to reduce dietary iTFA [65–67]. In particular, replacing dietary iTFA with polyunsaturated fatty acids (PUFA) will likely lead to the greatest reduction in CHD risk [65, 68].

Stroke

Although a consistent and strong relationship between iTFA consumption and CHD has been observed, less is known about its impact on stroke. The association between TFA intake and stroke has been examined in several large cohort studies and the findings have been mixed. The Health Professionals Follow-up (men aged 40–75 years) and the Nurse's Health studies (women aged 34–59 years) found no association between TFA intake and ischemic stroke; however, there was an inverse association with TFA intake and parenchymal hemorrhagic stroke in the latter cohort [69, 70]. Three other cohorts—the Women's Health Initiative, the Cardiovascular Health Study, and the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort—all found positive associations between TFA intake and stroke [71–74], although elevated risk was only observed among men but not in women in the REGARDS study [72]. In the absence of consistent evidence from observational studies, and a lack of experimental studies, the effects of iTFA consumption on stroke remain unclear.

Insulin Resistance and Type 2 Diabetes

Animal models indicate that consumption of iTFA (ranging from 12 weeks to 6 years) can alter phospholipid fatty acid composition, reduce membrane fluidity, worsen muscle and adipocyte insulin sensitivity, and impair glucose disposal [62, 75–77]. However, only a limited number of human trials have assessed the effects of iTFA intake on markers of glucose-insulin homeostasis and these have produced mixed findings. Studies have typically been small ($n = 14$–63), of limited duration (most often between 4 and 6 weeks), and mostly did not utilize optimal assessment of insulin resistance (i.e., hyperinsulinemic-euglycemic clamp) [27, 34], which may each contribute to the conflicting findings. A recent meta-analysis that included a total of 208 subjects found no significant effect of iTFA intake on insulin sensitivity or hyperglycemia [27]. Similarly, mixed results have been reported in observational studies. While the Nurses' Health Study, which followed 84,941 women over a 16-year period, found a positive relationship between TFA intake and type 2 diabetes (T2D) [78], others did not [79, 80]. Participants in the latter two studies had lower intakes of TFA (0.7–2.0 % daily energy intake and 2.2–5.2 g TFA/day, respectively) as compared to Nurses' Health Study (1.3–2.9 % daily energy) and the smaller range of intake may have reduced the likelihood of detecting an association. Overall, the causal effect of iTFA consumption on insulin resistance and risk of T2D remains speculative. Further research is needed to better understand if iTFA intakes increase insulin resistance and which populations may be most at risk.

Ruminant Trans Fatty Acids and Cardiovascular Disease

As described previously, the major TFA found in ruminants (*trans*-VA and CLA) differ from the main structural isomer in iTFA (elaidic acid). A growing number of clinical and observational studies have assessed the metabolic effects of rTFA and their relationship to CHD risk, the findings of which are summarized below.

Effect on Cardiovascular Risk Factors

Unlike industrially produced TFA, rTFA (particularly CLA) has been shown to exert beneficial effects on several cardiovascular risk factors in a number of animal experimental models, including reducing atherosclerotic lesions [81–84], triglycerides [85–89], total cholesterol [84–90], LDL-C [85, 88–90], VLDL-C [85, 90, 91], and ApoB [85, 88]. Because of the potentially different health properties associated with consumption of rTFA, there have been some discrepancies in the way in which TFAs are defined by countries worldwide, with some excluding CLA from the definition (Box 15.1). It has also led—despite a lack of robust evidence of its benefits in humans—to the development of CLA supplements that are marketed as promoting weight loss, immune function, and other health benefits.

rTFA and CVD Risk: Experimental Studies

Although animal models have found rTFA consumption to have beneficial effects on CVD risk factors, in the majority of randomized feeding studies low to moderate levels of rTFA (1.5–2.9 % of daily energy, equating to 3.6–6.8 g/day) had no significant effect on total cholesterol, LDL-C, HDL-C, total-cholesterol/HDL-C ratio, or triglycerides, compared to control diets [93–102]. Notably, a few trials that administered intervention diets with high levels of rTFA (above 10 g/day, equivalent to >3.6 % of total daily energy intake) found significant adverse effects of rTFA on lipid risk factors including increased total cholesterol, increased LDL-C, decreased HDL-C, and increased total/HDL-C [93, 103, 104]. The results of many of these metabolic studies should be interpreted with caution due to weaknesses in study design, including small sample sizes, insufficient statistical power, short duration of intervention (between 3 and 5 weeks) and inadequate control of diets which may have resulted in important differences in overall fatty acid composition of the treatment diets, and not just differences in levels rTFA [105]. Overall, limited evidence to date suggests that at high levels of consumption, rTFA may have qualitatively similar effects on CVD lipid risk factors as iTFA from PHOs in humans. Additional well-designed RTC are needed to address physiologic effects of rTFA across a wide range of intake.

Box 15.1. Defining Trans Fatty Acids

The Codex Alimentarius excludes CLA from the TFA definition based on the evidence—which consists mostly of preclinical studies—that suggests there may be health benefits in terms of weight management and cancer prevention associated with its intake [8]. This has led to inconsistencies in the definition of TFA from one country to the next, which has implications for labeling policies. For example, the definition used for TFA in Canada does not include CLA; however, other countries such as Chile, Colombia, and Jamaica do not make this exclusion [92].

rTFA and CVD Risk: Observational Studies

In contrast to iTFA, higher consumption of rTFA was generally not associated with CHD risk in prospective observational studies. Indeed, a 2011 meta-analysis of 4 prospective cohort studies (93,627 participants, 1463 CHD events) found that intake of rTFA (increments ranging from 0.5 to 1.9 g/day) was not significantly associated with risk of CHD (RR = 0.92 (0.76–1.11); $P = 0.36$) [106]. One possible explanation for the lack of association of rTFA with CHD risk is the generally very low level of rTFA intake in the study populations (<1 % energy) [105]. Based on metabolic studies (see above), such a low level of rTFA intake may not have clinically significant effects on CVD lipid risk factors and hence would be predicted to have limited influence on CHD risk. Alternatively, the narrow range of rTFA intake in these populations may reduce the power of observational studies to detect an association. It is also possible that other beneficial nutrients (e.g., vitamins and minerals) in major sources of rTFA such as dairy could offset adverse effects of rTFA [107]. Given that for most populations around the world rTFA intakes are substantially less than 2 g (<1 % of energy) per day [108–111], current epidemiologic evidence suggests this subclass of TFA is unlikely to significantly influence the risk of CHD. However, additional studies are needed to assess the relationship between rTFA consumption and risk of other cardiometabolic diseases.

Recommendations on Limiting TFA Consumption

Given the plethora of adverse metabolic effects and robust evidence for association with CHD risk of iTFA from PHOs, dietary guidelines worldwide have consistently recommended limiting iTFA consumption [112]. For example, the World Health Organization (WHO) and the Institute of Medicine recommend that iTFAs should be limited as far as possible and should not exceed 1 % of total energy intakes (i.e., approximately 2 g/day) [6, 67]. rTFA intake is already very low in most populations and, at these low levels, do not appear to demonstrate adverse associations with CHD risk. Therefore, in order to ensure that intakes do not exceed these recommendations, the WHO has focused on the elimination of PHOs from the global food supply and has identified its removal as a "best-buy" (an affordable, feasible, and cost-effective intervention) for addressing noncommunicable diseases in low- and middle-income countries (LMICs) [67, 113]. Importantly, the WHO also recommends replacing TFA with unsaturated (rather than saturated) fats in order to achieve the best cardiovascular health outcomes [67].

Monitoring TFA Levels in the Food Supply

iTFAs may come from both packaged foods (e.g., purchased from supermarkets) and pre-prepared products (e.g., restaurant and take-out meals). Given the tremendous number of food manufacturers globally, and the constant introduction of new, as well as reformulations of existing food products, monitoring TFA levels is a challenging yet vital task to ensuring that population intakes of TFA do not exceed the WHO recommendations and to ensure that PHOs are being phased out of the food supply.

Levels of iTFA in specific food categories can vary markedly based on brand, or even for products from the same brand but sold in different countries, making it important for brand-specific nutrition composition data to be available globally. For example, a study that examined the iTFA levels in foods between 2004 and 2006 found that the iTFA content of frying oil was markedly different within the same chain restaurants in different countries [114]. In McDonald's outlets in the USA, South Africa and Peru frying oils contained between 23 and 28 % iTFA, in Oman it was 33 % in contrast to 1 % in

Denmark [114]. Thus, just because a company reformulates in one country to reduce iTFA, as was done in Denmark in accordance to the mandated iTFA limit (2 % of total fat from iTFA), it does not mean that they will reformulate worldwide.

Another challenge regarding monitoring iTFA levels in foods, which is faced by LMICs in particular, is the large informal food sectors. Although branded products dominate sales in high-income countries, this is not the case in many LMICs where unlabeled foods are often ubiquitous in the food supply. In India, the majority of processed food comes from the informal food sector and many of the products tend to contain iTFA [115]. Moreover, for those foods that do contain labels, there may be significant labeling inaccuracies [116, 117].

Global Responses to Reduce TFA Intakes

Trans Fat Bans

The impetus for product reformulation has been government regulations—or the threat of regulation in the absence of satisfactory voluntary action—combined with consumer demand for no/low TFA products. The main forms of regulations that have been put in place in countries worldwide have been iTFA limits (also referred to as iTFA bans), labeling policies, or voluntary measures aimed at promoting product reformulation. Table 15.2 lists the countries that have adopted mandatory iTFA policies worldwide. iTFA bans have been found to be the most effective at reducing the availability of iTFA in the food supply; however, all forms of regulation, including voluntary self-regulation, have helped to reduce the availability of iTFA in the food supply [118].

Table 15.2 Countries with mandatory TFA limits and labeling policies worldwide [92, 118]

Type of TFA policy	Countries
Mandatory TFA limits in foods	• Denmark
	• Switzerland
	• Austria
	• Hungary
	• Norway
	• Iceland
	• Argentina
	• Chile
Mandatory TFA labeling policy	• Canada
	• US
	• Brazil
	• Taiwan
	• Hong Kong
	• Singapore
	• Uruguay
	• Paraguay
	• Argentina
	• Peru
	• Ecuador
	• Chile
	• South Korea
	• Fiji

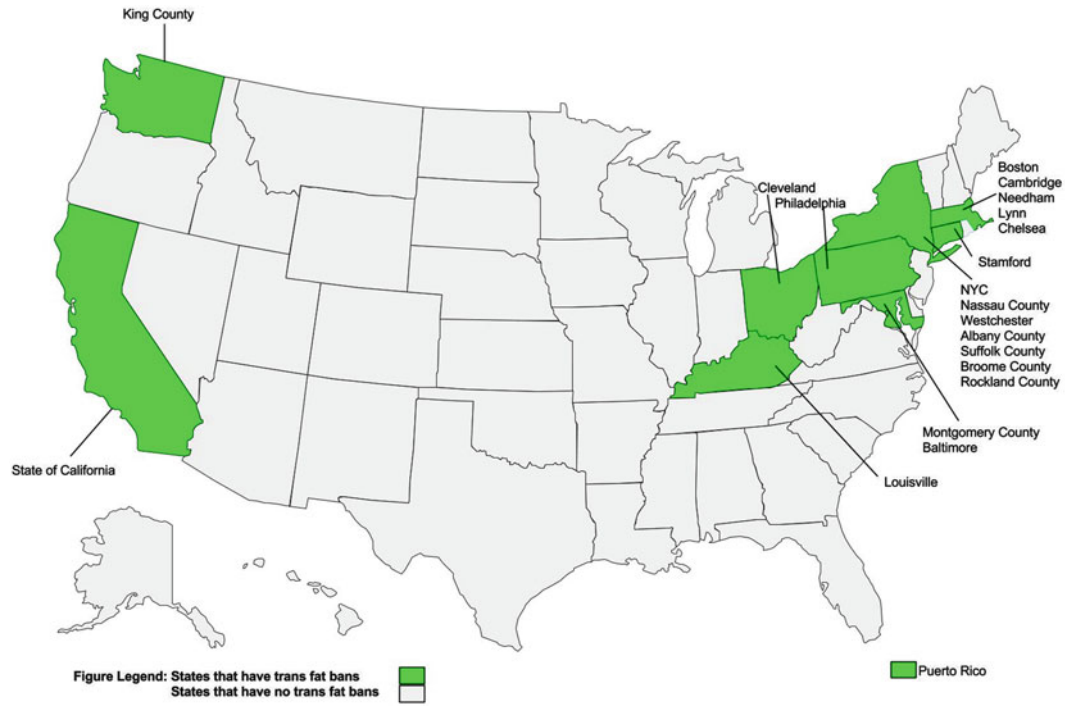


Fig. 15.2 The states, counties, and cities with TFA bans in restaurants and fast food outlets across the USA. States highlighted in *green* have a local trans fat ban in restaurants and fast food outlets in a city, county, or the entire state

Denmark was the first country to ban iTFA in 2003, paving the way for other countries, cities, and states to implement iTFA regulation [119]. Although coined a ban, the regulation is a iTFA limit of 2 % of total fat in fats and oils. The ban in Denmark virtually eliminated trans fat from the food supply, while European countries that have yet to introduce iTFA policies continue to have high levels of iTFA in the food supply, particularly in Eastern Europe [120]. Since 2003, seven countries worldwide, most of which are in Europe, have followed Denmark's lead and set mandatory iTFA limits (see Table 15.2) [118]. India has also recently proposed setting an upper limit of 5 % iTFA in PHOs but it has yet to be implemented [121].

In the USA, a more local approach to iTFA bans has been adopted in restaurants and fast food outlets. New York City was the first city in the USA to ban iTFA in restaurant and fast food outlets, which led other jurisdictions to adopt similar policy measures [122]. Figure 15.2 highlights the cities, counties, and states in which local bans have been enacted throughout the USA. These iTFA bans have successfully reduced the quantity of iTFA in the food supply and there has been very high compliance by food business operators with these regulations—reported compliance rates ranged between 81 and 99.5 % [118, 123, 124]. It has been estimated that the iTFA bans across New York state alone have likely led to a 4 % reduction in deaths attributable to CVD [122].

TFA Labeling

Labeling policies have been another approach adopted by countries to try to reduce TFA intakes. Canada and the USA were the first countries to enact mandatory TFA labeling in 2005 and 2006, respectively. In Canada, all packaged foods containing more than 0.2 g/100 g of TFA are required to

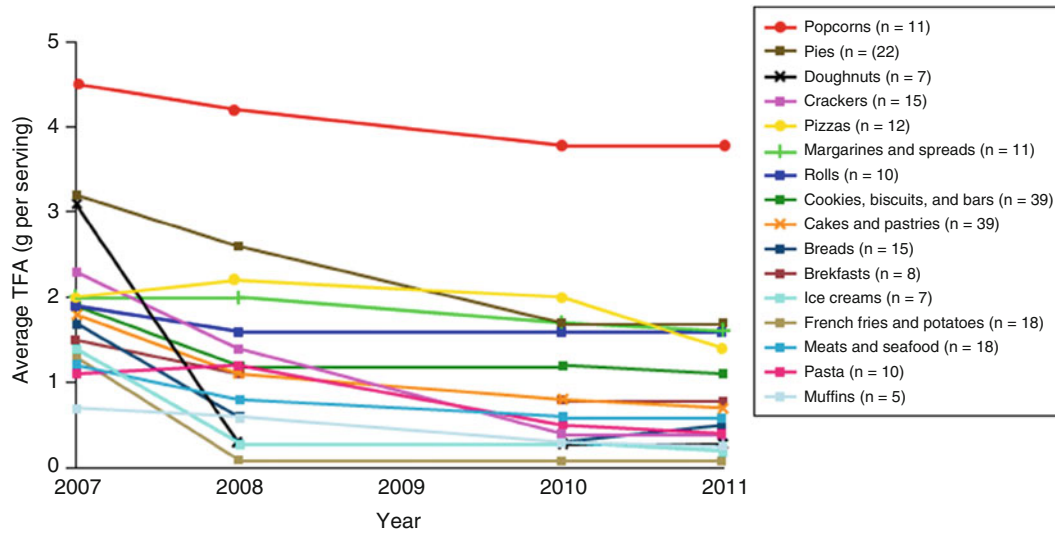


Fig. 15.3 Average TFA content from 2007 through 2011 of brand-name US supermarket food products that contained ≥0.5 g/serving TFA in 2007. Different colors represent the different food categories. Data were collected in 2007, 2008, 2010, and 2011. All products listing TFA as 0 g but that listed partially hydrogenated oils in the ingredients list were considered to still contain 0.25 g/serving of TFA. Source: Otite et al. [129]

label the quantity of TFA whereas in the USA the same is true of products containing 0.5 g/100 g—all products containing less than these respective quantities can be labeled as containing 0 g of TFA. This "loophole" in the US labeling regulation has led to criticism with the concern that an individual consuming multiple servings of products labeled as containing no trans fat could easily surpass the WHO/US dietary guidelines recommendations for TFA consumption. Nevertheless, the labeling approaches in both Canada and the USA coincided with significant changes in the iTFA levels of the food supply [118, 125, 126]. These reductions have been largely due to product reformulation by food industry.

In both Canada and the USA, consumers were becoming more and more aware that TFA consumption was bad for their health, resulting in an increased demand for TFA free foods [127, 128]. It is likely that this increasing awareness, together with the introduction of the mandatory TFA labeling, led the food industry to reformulate iTFA containing products. Figure 15.3 depicts the reductions in the average TFA content of different food categories (that contained 0.5 g/serving or more TFA in 2007) in US supermarkets from 2007 through 2011 [129]. Although there have been significant reductions in iTFA levels in most food categories, the pace of change has slowed in more recent years [129]. There was also substantial variability in the rate of reformulation between food categories as well as manufacturers—with some showing impressive progress while others did not change. As iTFA levels in foods decreased in the USA, so too did population intakes. Vesper et al. [130] found a 53 % decrease in plasma TFA levels (which is a biomarker of TFA consumption) of white non-Hispanics in the USA between 2001 (prior to TFA labeling) and 2009 (3 years post TFA labeling implementation) [130].

In Canada, there has also been significant progress towards product reformulation to reduce iTFA in foods since mandating TFA labeling. By 2009, three quarters of products had been reformulated to meet national level voluntary limits of 2 % of total fat from iTFA; however, products that exceeded that limit remained on store shelves [131]. The reduction in iTFA levels in foods likely led to reductions in TFA intakes as well. A study examining the TFA content of human breast milk samples in Canadian women ($n=639$) in 2009, 2010, and 2011 found a reduction from 2.7 % TFA content in 2009 to 1.9 % in 2011 [132].

Since Canada and the USA implemented mandatory labeling, other countries have followed. Labeling can be an effective way to promote product reformulation of iTFA containing products; however, it tends to be less effective than iTFA bans for several reasons. First, products containing iTFA will most likely remain on the market, as has been observed in the USA and Canada [126, 129]. If people consume those specific products in high quantities they can exceed the WHO recommended limits. Second, if iTFA containing products are cheaper it may lead price conscious consumers to purchase these products instead of their TFA free counterparts. Third, many of the products containing iTFA are pre-prepared foods that do not contain labels such as foods purchased in restaurants and fast food outlets. Lastly, for labeling regulation to be effective, the population must be both aware of TFA and be able to accurately interpret nutrition labels. In high-income countries, where literacy levels are high, it is likely to be a more effective means of reducing iTFA than in LMICs. Indeed, limited evidence suggests lack of efficacy of mandatory labeling on reducing iTFA in packaged foods in LMICs. For example, a study in Brazil found that after the mandatory labeling regulation was implemented, more than 80 % of the cookies and savory snacks sold still contained TFA, as well as 50 % of the cereal bars and chocolates (the proportion prior to the regulation being implemented was not reported) [133]. Consistently, another study in Brazil found that TFA levels in human breast milk samples taken before and after the regulation had been implemented were comparable, suggesting that the regulation may have had a negligible impact on TFA intakes [134].

Voluntary Approaches to TFA Reduction

A few countries have opted for a more voluntary approach to reducing iTFA in the food supply, with varying degrees of success. The most notable successes regarding voluntary approaches to iTFA reduction are Costa Rica and The Netherlands. Costa Rica was able to significantly reduce intakes after active engagement between the public health sector and the oil industry to voluntarily reduce iTFA levels in PHOs after they were identified as the main source of TFA in the diet [135, 136]. Between 1994 and 2006 the iTFA content in soybean oil fell from 20 to 1.5 %, stick margarine fell from 13 % to less than 1 % and the levels in industrial margarines (e.g., bakery shortening) and baked goods fell from 5 to 2 % [137]. Moreover, a cross-sectional sample of TFA intakes in adolescents in 1996 and 2006 found a reduction in intakes from 4.52 g/day (SD 0.74) to 2.8 g/day (SD 1.04) of TFA over the 10 year period [138].

The Netherlands also succeeded in reducing iTFA through voluntary measures (intakes are now below the WHO recommended limit) [139]. In response to the accumulating body of evidence linking iTFA consumption to CVD risk, Unilever (one of the largest food manufacturer in the world) removed iTFA from their products in The Netherlands [139]. Other producers followed Unilever's example and by 1996 the majority of margarines sold in The Netherlands only contained trace amounts of iTFA [139]. In order to address the remaining iTFA in the food supply—from fast food and bakery products—in 2004 the Product Board for Margarine, Fats and Oils, representing all trade and production companies in the Dutch edible oils and fats supply chain, set up a Task Force for Responsible Fatty Acid Composition. The Task Force then initiated a campaign to reduce the use of PHOs, in addition to saturated fats, as frying oils in restaurants. By 2005, 45 % of Dutch fast food outlets were using frying oils low in iTFA (<5 %) and high in unsaturated fat (>55 %). Dietary TFA intakes in the country dropped by 20 % with the voluntary TFA self-regulation [139].

In contrast to the examples of success in Costa Rica and The Netherlands there are also examples where voluntary approaches have failed. In NYC, a voluntary approach to limiting use of iTFA by food business operators was initially tried and it wasn't until it failed that mandatory measures were put in place. In the Americas, many large multinational companies signed a declaration to help reduce iTFA, but few provided data to show their progress towards this goal [136].

Product Reformulation: What Goes in When the TFA Comes Out?

There have been mixed approaches to product reformulation to reduce iTFA in foods, largely based on product categories. When industry reformulates products it needs to consider the availability and affordability of the replacement oil along with the organoleptic properties required such as the texture and mouth feel. Reformulating fried products to reduce iTFA has been more straightforward than bakery products, given that liquid oils (i.e., oils high in unsaturated fat) can easily be used; however, bakery products require fats that are semisolid making their product reformulation more challenging [140, 141]. There are several options for reformulating products that contain PHOs, which are dependent on the product's application. For frying applications, PHOs can be directly substituted with oils high in either saturated or unsaturated fats. iTFA free products that require a more solid fat, such as bakery products, can be reformulated using: (1) oils high in saturated fat (e.g., palm, butter or fully hydrogenated fats) or (2) no trans, lower saturated fat shortenings that blend hard fractions (e.g., palm stearin or interesterified fats) with unsaturated oils [140, 141]. Alternatively, a lower iTFA product (but one that still contains some iTFA) can be produced using a base oil high in saturated fat (e.g., palm oil) and hydrogenating this to a lesser degree than is normally done when producing PHOs [140, 141]. This creates a PHO that has less iTFA than when produced using a base oil high in unsaturated fats but the end product is very high in saturated fat. A more detailed description of the applications of different oils for product reformulation can be found elsewhere [141, 142].

The WHO recommends replacement of iTFA with unsaturated fats in order to maximize the health benefits of product reformulation. One of the concerns that has been raised regarding product reformulation to reduce iTFA has been that products will simply be reformulated using oils high in saturated fat (e.g., palm oil, and butter) and that it may increase total fat content of foods. However, to date reformulation has largely been accomplished without increasing total fat and with variable responses in terms of the replacement fat used [118, 143, 144]. Table 15.3 summarizes the studies that have looked at the fatty acid profile of foods before and after iTFA reduction interventions—fat content has either remained stable, or in some cases decreased, and saturated fat levels have only increased in specific product categories [118].

Although industry has reformulated products containing iTFA with unsaturated fats, there has been a recent move towards reformulating using palm oil, which is high in saturated fat [149]. Palm oil has become ubiquitous in the global food supply and it is currently being used in many ultra-processed foods. In order to ensure that industry does not simply reformulate iTFA containing products with palm oil, government incentives (e.g., regulation) alongside greater consumer demand for products that are both free of iTFA and low in saturated fat are needed. In addition, in order to make it easier for industry to reformulate using unsaturated fats, global supply chains of these oils need to be strengthened to ensure that these oils are both available and affordable for industry to use in product reformulation [141]. In the USA, after the introduction of mandatory iTFA labeling, supply chains for alternatives to PHOs were strengthened to enable industry to reformulate using healthier oils [143, 150]. This then enabled product reformulation using a variety of oils high in unsaturated fats including canola, sunflower, soybean, and others [143].

Global TFA Intakes

TFA intakes vary substantially worldwide. A recent study by the Nutrition and Chronic Diseases Expert Group, as part of the 2010 Global Burden of Disease (GBD) study, examined global dietary fat and oil intakes in 1990 and 2010 using 266 surveys representing 113 countries (82 % of the global population) [151].

Table 15.3 Average changes in the fatty acid composition of foods following TFA regulation

Policy intervention	Authors	TFA	SFA	MUFA and/or PUFA	Total fat[a]
Mandatory TFA labeling	Lee et al. [145]	↓	↑ Bakery products	↑ Restaurant food	↓
	Mozaffarian et al. [125]	↓	↑ Supermarket foods ↓ Restaurant foods	Not included	↓ (SFA+TFA)
	Van Camp et al. [146]	↓	↑ Bakery products	↑ Oils high in PUFA and MUFA in chips	NC
Mandatory TFA limits (bans)	Angell et al. [147]	↓	↓	Not included	↓ (SFA+TFA)
	Angell et al. [124]	↓	↑	Not included	↓ (SFA+TFA)
Mandatory TFA labeling + voluntary limits	Ricciuto et al. [4]	↓	NC	↓ MUFA ↑ PUFA	↓ (Significance not assessed)
	Ratnayake et al. [131]	↓	↓	↓	NC
	Ratnayake et al. [126]	↓	↑ Crackers, cookies, and garlic spreads & donuts	↑	NC
Voluntary TFA selfregulation	Temme et al. [148]	↓	NC	NC ↓ Decrease in biscuits	NC
	Stender et al. [144]	↓	↑ Popcorn and cakes/biscuits	↑	↑ (Significance not assessed)

Source: Adapted from Downs et al. [118]

[a]Change in total fat calculated by adding fatty acids when not reported by authors; in these cases, significance was not assessed. For studies that did not examine MUFA and PUFA, SFA+TFA changes are reported. *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids, *TFA trans* fatty acids, *NC* no change

Overall, global mean TFA consumption have remained relatively stable between 1990 and 2010 [151], despite strong evidence accumulating over this period to support its removal from the food supply. Nevertheless, there were shifts in consumption patterns within countries and specific regions. Consumption increased the most in North Africa/Middle East (0.4 % of energy) and in South Asia (0.3 % of energy), whereas intakes decreased in Southern Sub-Saharan Africa and Western Europe [151]. Promising reductions have also been achieved in several countries around the world, including Canada and the USA, as a result of labeling and local bans that have led to substantial reductions of PHO from the food supply [118].

Despite limited progress in reducing iTFA globally, it is also apparent that much remains to be done. The GBD study found a fivefold difference in TFA consumption globally in 2010, ranging from 0.2 to 6.5 % of energy intake [151], with the mean global TFA intake at 1.4 % of total energy intake which exceeds the WHO recommendations. Furthermore, less than 10 % of countries examined had mean intakes less than 0.5 % of total energy [151]. Even in countries like the USA, where there has been significant progress in terms of product reformulation intakes remain above recommendations [152]. Figure 15.4 provides an overview of the TFA intakes in adults over 20 years of age worldwide. The highest intakes were found in Egypt, Pakistan, Canada, Mexico, and Bahrain, whereas the lowest levels were found in island nations in the Caribbean (Barbados and Haiti), followed by countries in east Sub-Saharan Africa [151].

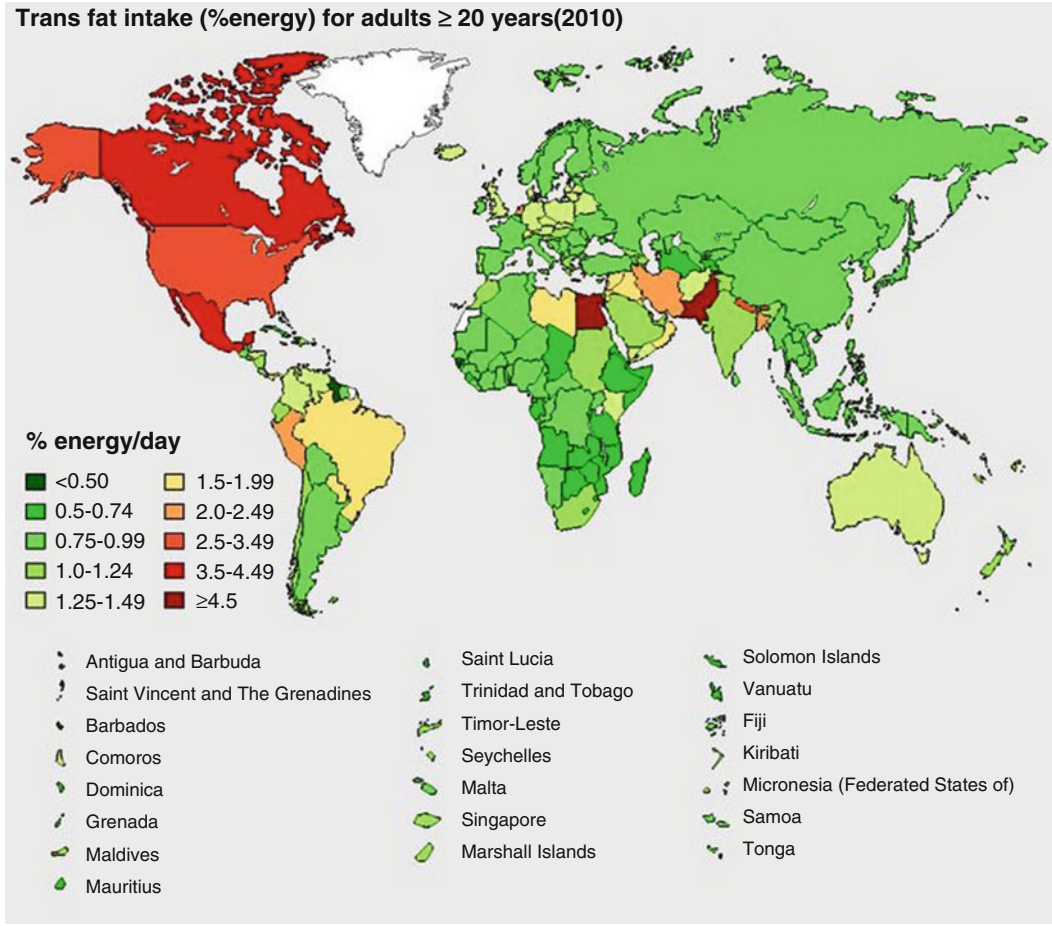


Fig. 15.4 Mean TFA consumption (% energy/day) in adults ≥ 20 years of age worldwide in 2010. Different colors depict the mean TFA consumption (*dark green* represents the lowest and dark red represents the highest consumption). Source: Micha et al. [151]

Differences in Subpopulations

Not only do TFA intakes vary widely across countries but they can also vary substantially by subpopulations including by age and socioeconomic status. Younger adults and adolescents have been found to have substantially higher intakes of TFAs than those who are older. This trend has been observed in many parts of the world, particularly in South Asia, high-income North America and Central and Tropical Latin America [151]. There is also evidence to suggest that TFA intakes may be higher in lower socioeconomic status groups [153].

There are several potential explanations for the disparities in TFA consumption by different socioeconomic and age groups. One reason relates to dietary patterns—for example, younger and more socioeconomically disadvantaged populations tend to have a higher consumption of unhealthy processed foods [154–156], which typically contain iTFA. Second, because PHOs are cheap, the products that contain them are often lower in price than those that are iTFA free [4, 5, 118]. For example, in Canada, after mandatory labeling of TFA was adopted some manufacturers continued

to sell their TFA containing margarines but also manufactured a TFA free version and sold it at a higher price [4]. Such a relationship is not exclusive to North America. A study conducted in Brazil also found that TFA free foods were more costly than those that contained TFA [133]. Lower income consumers are likely more sensitive to price differentials and thus more likely to purchase lower cost, higher TFA products. Finally, even in countries requiring mandatory labeling of TFA content in foods, consumers with lower nutrition knowledge and literacy are less likely to understand or use nutrition labels [157], and thus may continue to purchase products with high iTFA. Overall, these findings suggest that even in countries where the majority of products are TFA free—so long as products containing iTFA remain available—TFA consumption will likely remain a public health problem and contribute to health disparity due to higher consumption by lower socioeconomic groups. iTFA bans therefore remain the most effective and equitable approach to reduce TFA consumption.

Moving Forward with TFA Reduction

In the past, there have been calls for a global iTFA ban [158, 159]; however, mandating a global ban would require significant political buy-in and is unlikely to be feasible in the foreseeable future. However, other promising policy approaches to tackle iTFA reduction in the food supply are possible. In June 2015, the US Food and Drug Administration (FDA) ruled to remove the "generally recognized as safe" (GRAS) status from PHOs in the USA which will essentially act as a countrywide TFA ban [160]. Industry will have three years to comply with the regulation.

The FDA's decision to revoke the GRAS status from PHOs, means they will now be classified as a "food additive" and manufacturers will no longer be permitted to sell PHOs directly or use them as ingredients in food products [161]. This will apply to packaged foods as well as restaurant foods. This has important implications for iTFA use in processed foods—by defining PHOs as unsafe for consumption, the USA sends a strong message globally. This may facilitate fresh impetus for the Codex Alimentarius to redefine the terms for the use of PHOs. LMICs in particular, often look to Codex for guidance on issues of food safety and aim to comply with its standards [162]. There may be potential for Codex to follow a similar approach to that used by the USA and define PHOs as unsafe for consumption. If Codex were to take these steps it would essentially act as a pseudo global ban and prevent trans fat regulation from being considered a technical barrier to trade. Although this could be a feasible approach to establishing a global trans fat ban, vested interests are strong, as would be political opposition to such a measure [163]. In the meantime countries will need to continue to use their own policy tools to improve the quality of fat that is available, affordable, and acceptable in the food supply.

Conclusions

The evidence is clear that consuming iTFA from PHO causes metabolic disturbances that leads to higher risk of CHD, and their intake should be limited as much as possible. Reducing the availability of iTFA in the food supply is therefore one of the most straightforward and powerful interventions that will lead to substantial reductions in morbidity and mortality due to CHD; however, TFA intakes remain high in many countries worldwide. Countries need to continue to enact policies that limit iTFA availability and promote product reformulation by industry. Moreover, continued global monitoring of TFA levels in foods, as well as population intakes, is essential.

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Chapter 16
Nutrition Aspects of Stroke Prevention

Katherine Patton and Mandy L. Corrigan

Key Points

- Modifiable risk factors for preventing stroke include hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation, smoking, physical inactivity, obesity, drug abuse, alcohol use, and poor quality diet.
- Hypertension is the strongest modifiable risk factor for preventing stroke. Sixty percent of the population is classified as having prehypertension or with hypertension. Stroke is a major public health problem and prevention is key to the health of the US population and worldwide.
- Dietary Approaches to Stop Hypertension (DASH) Eating Plan has the ability to dramatically lower blood pressure.
- Physically active adults have a 25–30 % lower risk of stroke compared with adults that have the low activity levels. Additionally physical activity lowers blood pressure, and coupled with responsible nutritional intake, promotes weight loss or maintenance.

Keywords Stroke • Prevention • Nutrition • DASH diet • Sodium • Hypertension

Introduction

In the USA, stroke is the fourth leading cause of death [1] and the second highest cause for long-term disability in adults. Stroke has a significant cost burden on healthcare systems as the second leading cause of death in the world [2]. Over the past decade death from stoke worldwide has continued to rise from 5.6 million in 2000 to 6.7 million deaths in 2012 [2]. Stroke is a major public health problem with prevention as the center strategy to decrease these statistics. While we cannot modify age, gender, or ethnic background, it is disheartening that stroke continues to maintain a high mortality ranking since many other risk factors have modifiable or preventable components such as hypertension (HTN), diabetes mellitus, dyslipidemia, atrial fibrillation, smoking, physical inactivity, obesity, drug abuse, alcohol use, and diet. Within this chapter we will review the nutrition aspects of stroke prevention related to controllable risk factors and diet components.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_16

Role of Nutrition for Controllable Stroke Risk Factors

Hypertension

HTN is the single most important risk factor for stroke prevention. HTN is a modifiable risk factor for ischemic stroke. Stroke prevention focuses not only on those with HTN (defined as ≥140 mmHg/≥90 mmHg), but also targets a large population of adults with prehypertension (defined as 120–139 mmHg/80–89 mmHg) [3].

The prevalence of prehypertension in the USA from National Health and Nutrition Examination Survey (NHANES) is striking and nearly mirrors those with a formal HTN diagnosis. From 1999 to 2000 the prevalence of prehypertension in the USA was 31 % and HTN prevalence was 29 % [3, 4]. From 1999–2000 through 2007–2008, HTN prevalence has remained stable at 29 % [4, 5]. Targeting 60% of the US population (those with HTN and prehypertension) to lower blood pressure is not the only message to prevent stroke, but accompanied by many other nutrition prevention strategies.

HTN and prehypertension are intertwined with excess weight status. The NHANES data also revealed 64 % of those with prehypertension had overweight or obesity as the most prevalent stroke risk factor [3, 4]. When blood pressure values fall below the guidelines for antihypertensive pharmacological intervention, lifestyle modifications, including nutrition, take center stage. Nutrition factors involved with contributing to HTN include increased sodium/salt intake, decreased consumption of potassium, excessive weight status, and excessive alcohol intake. There is a dose responsive relationship between lower dietary consumption of sodium and improved blood pressure readings [6]. Specific diet guidelines are discussed at length later in this chapter.

Diabetes

Diabetes is an independent risk factor and doubles the risk of stroke [7]. Unfortunately 9.3 % of the US population has diabetes with nearly 29.1 million individuals therefore at higher risk of stroke than the general population [8]. The mainstay of treating diabetes is achieving and maintaining adequate glycemic control. Interestingly, from looking at stroke as a macrovascular complication of diabetes, is no stroke risk reduction with tight glycemic control in those with type 2 diabetes. Three main studies provide support for our understanding: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, Action in Diabetes and Vascular Disease-Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE), and Veterans Affairs Diabetes Trial (VADT) [9]. A 6 year follow-up of participants in the ADVANCE again showed no benefits on reducing any macrovascular compilations (including stroke) in those that were assigned to the intense glycemic control group (goal A1C < 6.5 %) [10].

Evidence is strong for reducing stroke risk through aggressive control of HTN for those with diabetes. The United Kingdom Prospective Diabetes Study Group (UKPDS) prospectively evaluated newly diagnosed patients with type 2 diabetes mellitus [11]. The tight blood pressure group achieved blood pressure average 144/82 mmHg and the control group with liberalized blood pressure control achieved a mean blood pressure of 154/87 mmHg [11]. In addition to the statistically significant improvement in mean blood pressure, there was also 44 % reduced stroke risk in the tight blood pressure control group [11]. Long-term follow-up if the UKPDS patients revealed tight blood pressure control must continue beyond the initial diagnosis phase and on into the future for any risk reduction to be maintained [12].

In addition to HTN, hyperlipidemia is another atherosclerotic risk factor that plagues patients with diabetes as part of the all too commonly seen metabolic syndrome (HTN, hyperlipidemia and obesity). The Heart Protection Study (HPS) showed addition of a statin in patients with HTN and

hyperlipidemia yielded a 24 % reduction in stroke [13]. The Collaborative Atrovastatin Diabetes Study (CARDS) enrolled patients with type 2 diabetes with LDL <160 mg/dL, and one additional 1 risk factor (HTN, smoking, etc.) but no history of cardiovascular disease. A 48 % reduction in stroke was seen with use of a statin [14].

In addition to a carbohydrate controlled diet, patients with diabetes benefit from control of HTN, weight loss (which will improve glycemic control and possibly prevention of microvascular complications), and a statin to reduce stroke risk. Prevention of stroke also focuses on identifying individuals with prediabetes to delay onset of diabetes. Thirty-seven percent of people in the USA have prediabetes and more than half of these individuals are over the age of 65 [8]. Exercise, diet, and weight loss may delay the progress to diabetes. As such, targeting patients with prediabetes for lifestyle changes is prudent as another approach to preventing stroke related morbidity and mortality.

Dyslipidemia

High total cholesterol has been found to be a risk for ischemic stroke in a number of studies, but not all. Cholesterol is synthesized by the human body and also enters the body exogenously (via consumption of animal products). Cholesterol is mainly composed of high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG). Genetic influence on cholesterol production lends to a strong pharmacologic focus of treatment in addition to physical activity and diet modification. In individuals with low HDL cholesterol, niacin may be considered in addition to drug therapy, but the effectiveness isn't well delineated. Niacin has been linked to myopathy and caution should be exercise with use [15, 16]. Additionally, large studies have shown inconsistent evidence about the role of elevated TGs and stroke risk. The body of evidence is weak and inconclusive since some investigators use fasting TG levels whereas others utilize non-fasting TGs. The stronger body of evidence with dyslipidemia and stroke risk surfaces in patients with diabetes mellitus as hyperlipidemia contributes to atherosclerosis.

Obesity

Obesity is frequently measured by a simple calculation of Body Mass Index (BMI) along with other methods, such as waist-to-hip ratio, and waist circumference, looking specifically at the location of excess adiposity tissue (see Tables 16.1 and 16.2) [17]. Body composition can be measured with imaging such as Dual Energy X-Ray Absorptiometry (DEXA), Computed Tomography, Bioelectrical Impedance Analysis (BIA), or Magnetic Resonance imaging (MRI), but BMI has been the steadfast tool since it is simple, reliable, noninvasive, and widely available.

Table 16.1 Body mass index (BMI)[a] classification [17]

Adult BMI	Interpretation
<18.5 kg/m^2	Underweight
18.5–24.9 kg/m^2	Normal weight status
25–29.9 kg/m^2	Overweight
≥30 kg/m^2	Obese
≥40 kg/m^2	Morbid obesity

[a]BMI = weight (kg)/height^2 (m)

Table 16.2 Waist circumference values indicating abdominal obesity[a] [17]

Gender	Waist circumference
Male	>40 in.
Female	>35 in.

[a]For use with BMI < 35 kg/m^2

BMI values in the range of 25–50 kg/m^2 showed a 40 % increase in stroke mortality with each 5 kg/m^2 increase in BMI, but BMI 15–24 kg/m^2 did not show any relationship with mortality [18]. The prevalence of obesity between 2009 and 2010 was 35.7 % among adults in the USA [19] and anticipated that obesity will continue to rise [20]. Obesity was highest among non-Hispanic African Americans, Mexican Americans, and Hispanics. There is of great concern especially as obesity rises, increases in HTN and diabetes mellitus are also anticipated [21].

There is an undeniable relationship between obesity, stroke, hypertension, heart disease, and diabetes mellitus. There is a clear link to decreasing hypertension with weight reduction, but it has been challenging to link decreased weight as a single factor to improved stroke prevention. The benefits of weight reduction are multifactorial and affect a variety of stroke risk factors including hypertension, dyslipidemia, and diabetes mellitus. Improved risk parameters may manifest as improvements in controlling other stroke risk factors rather than weight status alone. The American Heart Association/American Stroke Association recommends weight reduction to decrease blood pressure and the risk of stroke in individuals with a BMI in the overweight and obese categories [15].

Physical Activity

The benefits of physical activity are vast and important to the context of this chapter; show a clear reduction in the risk of stroke. Physically active adults have a 25–30 % lower risk of stroke compared with adults that have the low activity levels [22]. Public health messaging encourages moderate to vigorous intensity aerobic activity for at least 150 minutes per week distributed between at least 3 days. For example, brisk walking for 50 minutes three days per week [22].

Alcohol and Smoking

Alcohol is thought to have either a protective factor or risk factor for stroke, but the delineating factors are related to volume and frequency of consumption. A protective effect of alcohol consumption with low to moderate quantities is not supported by prospective randomized trials and the risk for hemorrhagic stroke increases with any quantity of alcohol ingestion [15]. Current public health messaging encourages limiting men to less than or equal to 2 drinks daily, 1 drink or less for women daily, and non-alcohol consumers are ***not*** encouraged to initiate alcoholic drinks [15].

Excessive alcohol consumption is discouraged for a variety of health reasons with a strong focus on cardiovascular consequences. The relationship between excess alcohol intake and stroke is related to HTN, cardiomyopathy, coagulation, atrial fibrillation, and reduced blood flow to the brain [15, 23, 24]. A large meta-analysis showed less than one drink per day had a decreased relative risk of stroke, but those with five or more drinks per day had an increased relative risk of stroke [23]. Of those in excess of five drinks per day, there was a linear association between consumption and stroke risk with the highest relative risk of hemorrhagic stroke [23].

The nutrition burden of excessive alcohol consumption is often overlooked. Alcohol contributes 7 cal/g, which is only second only to fat as one of the most concentrated caloric sources. Excessive alcohol intake can be associated with weight gain and obesity, which further compounds the risk for stroke.

There is a plethora of health data showing negative impact of smoking. In multiple large longitudinal studies, smoking nearly doubles the risk of stroke [25]. The recent decline in smoking rates among those in the USA may in part be due to legislative smoking regulations and heavy taxes. Regardless of the etiology of the shift, a positive benefit has shown decline ischemic and subarachnoid hemorrhage rates [21]. Support for smoking cessation (via behavioral interventions with or without the use of drug therapy) may also include nutritional and physical activity counseling to prevent unintentional weight gain.

Components of Diet to Prevent Stroke

Dietary intake plays a major role in contributing to the development of controllable risk factors like HTN, hyperlipidemia, diabetes mellitus, and obesity which increase risk for stroke; however dietary intake can also enact the opposite effect [15]. Food that we eat can provide functional benefits to prevent stroke. Functional foods not only provide the basic nutrients that the body needs, but also offer an additional health benefit by providing protective benefits. Plant based foods such as fruits, vegetables, whole grains, nuts and legumes are examples of functional foods. Nutrients found in animal sources have both protective benefits and some potentially harmful effects; therefore moderation of animal energy sources is key to preventing stroke. The 2010 Dietary Guidelines for Americans (DGA) and the American Stroke Association (ASA) endorse the Dietary Approaches to Stop Hypertension (DASH) Eating Plan to prevent stroke. The DASH diet is highly endorsed to prevent stroke because of its proven ability to dramatically lower blood pressure, which is a critical controllable risk factor in the prevention of stroke [26–28]. The DASH diet is rich in fruits, vegetables, fat-free or low-fat dairy products, and fiber which helps provide adequate intake of the minerals: potassium, magnesium, and calcium. The DASH diet is low in total fat, saturated fat, cholesterol, and sodium. Each component of the DASH diet has been studied individually and has unique properties that contribute to prevention of stroke.

Research supporting fruit and vegetable intake and its effect on stroke is strong [29–34]; however not enough Americans are consuming the recommended amount according to the 2010 DGA. The average daily intake of vegetables is 1.6 cups and 1 cup for fruit or fruit juice [35], compared to recommended 2–3 cups of vegetables and 1.5–2 cups of fruit [36]. The DASH diet recommends 4–5 servings of both fruit and vegetables daily [37]. Sources of fruit can be fresh, frozen, dried, canned in own juice or water, or in the form of juice. To reduce sodium intake the DASH diet recommends choosing fresh, frozen, and low-sodium or no-salt-added canned vegetables. Refer to Table 16.3 for serving sizes of fruit and vegetables. The potassium, magnesium, and fiber that fruits and vegetables provide are believed to be the catalyst for improved blood pressure and decreased risk of stroke.

In the first DASH study [38], subjects who not only ate more fruits and vegetables, but also consumed more fat-free or low-fat dairy products lowered their blood pressure more than subjects who only increased fruit and vegetable intake. Research also shows that there is an association between intake of milk and milk products and reduced risk of cardiovascular disease in adults [39]. The form of dairy products and their nutritional composition vary quite a bit. Full fat cheese and milk are high in saturated fat and cholesterol, plus mostly all forms of cheese are high in sodium. Saturated fat, cholesterol, and sodium intake are all associated with increased risk of stroke. According to the DGA 2010, most adults consume less than the recommended three cups of dairy products per day. Almost half of the dairy products Americans consume are from full fat cheese and 2 % or full fat milk are the

Table 16.3 2000 cal DASH eating plan [37]

Food group	Daily servings	Serving sizes	Examples and notes	Significance of each food group to DASH eating pattern
Grains[a]	6–8	1 Slice bread	Whole wheat bread, rolls, pasta, bagel, cereal, oatmeal, brown rice	Major sources of energy and fiber
		1 oz Dry cereal[b]		
		½ Cup cooked rice, pasta, or cereal		
Vegetables	4–5	1 Cup raw leafy vegetable	Broccoli, carrots, collards, green beans, green peas, kale, potatoes, tomatoes	Rich sources of potassium, magnesium, and fiber
		½ Cup cut-up raw or cooked vegetable		
		½ Cup vegetable juice		
Fruit	4–5	1 Medium fruit	Apples, raisins, apricots, bananas, dates, grapes, melons, peaches, pears	Important sources of potassium, magnesium, and fiber
		¼ Cup dried fruit		
		½ Cup fresh, frozen, or canned fruit		
		½ Cup fruit juice		
Fat-free or low-fat milk and milk products	2–3	1 Cup milk or yogurt	Fat-free (skim) or low-fat (1 %) milk, cheese, yogurt, or frozen yogurt	Major sources of calcium and protein
		1½ oz Cheese		
Lean meats, poultry, and fish	6 or less	1 oz Cooked meats, poultry, or fish	Select only lean; trim away visible fats; broil, roast, or poach; remove skin from poultry	Rich sources of protein and magnesium
		1 Egg[c]		
Nuts, seeds, and legumes	4–5 per week	1/3 or 1½ oz nuts	Almonds, hazelnuts, peanuts, walnuts, sunflower seeds, kidney beans, lentils, split peas	Rich sources of energy, magnesium, protein, and fiber
		2 Tbsp peanut butter		
		2 Tbsp or ½ oz seeds		
		½ Cup cooked legumes (dry beans and peas)		
Fats and oils[d]	2–3	1 Tsp soft margarine	Soft margarine, vegetable oil, low-fat mayonnaise, light salad dressing	The DASH study had 27 % of calories as fat, including fat in or added to foods
		1 Tsp vegetable oil		
		1 Tbsp mayonnaise		
		2 Tbsp salad dressing		
Sweets and added sugars	5 or less per week	1 Tbsp sugar	Fruit-flavored gelatin, fruit punch, hard candy, jelly, maple syrup	Sweets should be low in fat
		1 Tbsp jelly or jam		
		½ Cup sorbet, gelatin		
		1 Cup lemonade		

[a]Whole grains are recommended for most grain servings as a good source of fiber and nutrients
[b]Serving sizes vary between ½ cup and 1¼ cups, depending on cereal type. Check the product's Nutrition Facts Label
[c]Since eggs are high in cholesterol, limit egg yolk intake to no more than four per week; two egg whites have the same amount of protein content as 1 oz of meat
[d]Fat content changes serving amount for fats and oils. For example, 1 Tbsp of regular salad dressing equals 1 serving; 1 Tbsp of a low-fat dressing equals one-half serving; 1 Tbsp of a fat-free dressing equals zero servings

most consumed forms of milk; therefore most Americans are not reaping the potential benefits of fat-free and low-fat dairy products. By not consuming three cups of dairy products, Americans are also missing out on increasing their intake of potassium which is associated with reduced risk of stroke [40]. The DASH diet recommends 2–3 servings of fat-free or low-fat milk, yogurt, or cheese daily.

Refer to Table 16.3 for serving sizes of milk, yogurt, or cheese. The calcium and protein that dairy provides is thought elicit improved blood pressure.

A higher intake of protein from fish, poultry, and nuts is also associated with lower blood pressure [38]; however the majority of protein in the American diet is from meat, poultry, and eggs. The DASH diet suggests 6 oz or less of lean meat, poultry, and fish per day. Lean meat is considered any meat that has no more than 3 g of fat per ounce. All forms of skinless chicken and fish that are not fried are considered lean. Lean cuts of beef are Select or Choice grades with fat trimmed, such as ground round, sirloin, tenderloin; cubed, flank, porterhouse, and T-bone steak; chuck, rib and rump roast. Lean cuts of pork are ham, tenderloin, Canadian bacon, rib or loin chop, and rib or loin roast. Choose fresh meat, poultry, and fish instead of canned, smoked, cured, or processed versions to limit sodium intake.

Dietary intake of nuts, especially, peanuts, almonds, walnuts, pistachios, and hazelenuts, has been linked to reduced risk of stroke and cardiovascular risk factors [39, 41]. The DASH diet recommends 4–5 servings of nuts, seeds, and legumes per week. Refer to Table 16.3 for serving sizes of nuts, seeds, and legumes. Unsalted nuts and seeds are the best option. Soaking dried beans, lentils, and peas in water then cooking would provide less sodium than choosing canned beans.

Whole grains are another component of the diet that has been shown to reduce risk of cardiovascular disease [39], as well as improve risk factors for stroke. Intake of whole grains is associated with lower body weight, blood pressure, and cholesterol, as well as reduced risk of diabetes [39, 42]. A whole grain is a grain that has been kept intact to contain three parts, the bran, germ, and endosperm. Examples of whole grains include whole wheat flour, brown rice, wild rice, and oatmeal. Keeping grains whole supplies many B vitamins, minerals-iron, magnesium, selenium, and dietary fiber. The dietary fiber that whole grains offer is the nutrient that confers many benefits. Fiber is the component of plant based foods, like whole grains, fruits, and vegetables, that does not completely digest in the body. Whole grain starches that are high in fiber, promote satiety, which leads to less caloric intake [43, 44]. Soluble fiber in the form of beta-glucan and pectin help lower low density lipoprotein (LDL) cholesterol [45].

The DGA for Americans 2010 and the DASH Diet endorse choosing at least half of grains consumed come from whole grains. Whole grain intake among Americans is low [39]. Instead, Americans chose mostly refined grains [39]. A refined grain processed to remove the bran and germ which removes the fiber, iron, and many B vitamins. Refined grains are often enriched with B vitamins and iron, but lack fiber and have more solid fat, added sugar, and sodium [39]. The extra sodium and lack of fiber in refined grains have made them a poor choice when aiming to prevent stroke.

The DASH diet recommends 6–8 servings of grains per day. Refer to Table 16.3 for recommended serving sizes of a grain. The DASH diet encourages choosing ready to eat breakfast cereals that are lower in sodium, like shredded wheat. Whole grain rice, pasta, and hot cereal should be cooked without added salt. Instant flavored rice, pasta, and cereal mixes are discouraged on the DASH diet due to their higher sodium content.

Dietary fat can have both protective and detrimental impact on the body with respect to stroke. Fat that has been shown to have protective benefit are unsaturated fats, while saturated fat and *trans* fat are associated with raising LDL cholesterol [46]. High cholesterol is a risk factor for stroke; therefore minimizing dietary intake of saturated fat, *trans* fat, and cholesterol is endorsed by the DASH diet, DGA 2010, and the Therapeutic Lifestyle Change (TLC) diet [46]. Monounsaturated and polyunsaturated fats are types of unsaturated fats. When saturated and *trans* fat are replaced with mono and polyunsaturated fat, cholesterol levels are lower, which lowers risk of stroke [39, 46]. A monounsaturated fat rich diet, like the Mediterranean diet has been shown to decrease risk of cardiovascular disease including stroke [41]. Omega-3 fatty acids are a form of polyunsaturated fat that is associated with reduced risk of stroke [47].

The Institute of Medicine and TLC diet recommend total dietary fat make up no more than 20–35 % of daily calories. According to the DGA 2010, on average 34 % of total calories come from fat. Total fat on the DASH diet consist of 27 % of calories. The TLC diet recommends limiting calories from saturated fat to less than 7 % of total calories, while DASH eating plain contains 6 % of calories

from saturated fat. The average American consumes 11 % of calories from saturated fat. Saturated fat comes primarily from animal sources, for example, high fat cuts of beef, pork, veal, or lamb; solid fat on meat, skin on poultry, butter, lard, and fat found in full fat dairy products. Tropical oils, coconut, palm, and palm kernel are examples of plant based sources of saturated fat. The majority of saturated fat in the American diet comes from full fat cheese, pizza, desserts (cookies, cake, pie, pastries, ice cream, frozen yogurt, pudding), chicken and mixed chicken dishes, and pork (sausage, hot dogs, bacon, ribs) [39].

Trans fatty acid is a nonessential fat that is most commonly man made through food processing, however it is found naturally in some foods. A common source of *trans* fat is solid margarine and vegetable shortening. Manufacturers use *trans* fat as an ingredient in order to prolong the shelf life of the product. Examples of sources of added *trans* fat include pre-packaged baked goods, powdered coffee cream, and flavored liquid coffee cream. There is no recommend intake of *trans* fat since it is a nonessential fat. Increased intake of *trans* fat is associated with increased risk of cardiovascular disease; therefore it is best to avoid foods which contain *trans* fat or list any form of partially hydrogenated oil as an ingredient [39].

Monounsaturated fat comes from plant based sources such as olive and canola oil, avocado, olives, and are the primary source of fat found in most nuts. Polyunsaturated fat also comes from many plant based sources, including many oils: corn, safflower, sunflower, soybean, flaxseed, and nuts, especially walnuts. Omega-3 fatty acids are a form of polyunsaturated that is found in certain fish, such as salmon, mackerel, tuna, herring, anchovies, and sardines. Plant based sources of omega-3 fatty acids are walnuts, flaxseed, chia seed, and soybeans. The DGA 2010 recommends replacing saturated fat and *trans* fat with mono and polyunsaturated fat.

Fruit and milk products which have been mentioned earlier contain natural sources of sugar. Fruit contains the natural sugar fructose, along with dietary fiber, vitamins, and minerals. Milk products also contain natural sugar from lactose, plus contain protein, fat, vitamins, and minerals. Other sources of sugar in the diet are considered added sugar. Added sugars are sugars and syrups that have been added to foods during preparation. Examples of added sugar are white sugar, brown sugar, raw sugar, corn syrup, corn syrup solids, high fructose corn syrup, honey, and molasses. These sources of sugar provide calories, but no other nutritional value, yet Americans consume on average 16 % of calories from added sugar [39]. The main sources of added sugars in the American diet are soda, energy drinks, sports drinks, grain based desserts, sugar sweetened fruit drinks, dairy based desserts, and candy.

Excess intake of added sugar is associated with HTN, obesity, type 2 diabetes, and dyslipidemia [48]. Minimizing intake of added sugar plays a strong role in preventing risk factors associated with stroke. The DASH diet recommends 5 or fewer servings of sweets or added sugars per week. Refer to Table 16.3 for examples of servings. The American Heart Association endorses women limit added sugar to six teaspoons 100 cal per day and men limit to nine teaspoons (144 cal) per day [48]. One twelve ounce can of soda contains the daily recommend amount of added sugar from 140 to 150 cal from corn syrup; therefore it is very easy to consume excess calories from added sugar.

Sodium and potassium are both minerals our body requires. Sodium is responsible for maintaining fluid balance both in the blood and around cells, transmitting nerve impulses, and allowing muscles to work properly. Potassium is necessary to regulate heartbeat and allow muscle function. Since sodium attracts fluid to maintain fluid balance, excess dietary sodium intake will increase blood volume [49]. Research has shown that low blood potassium causes sodium retention. Increased blood volume puts stress on the heart to circulate more blood through blood vessels, which increases pressure in the arteries, therefore increasing blood pressure. A combination of excess sodium and inadequate potassium in the diet is a contributing factor of HTN. Since HTN is a major modifiable risk factor to prevent stroke, it is crucial that adequate amounts of dietary sodium and potassium are consumed.

The DGA 2010 recommends consuming less than 2300 mg of sodium for adults aged 18–50. The recommendation decreases to less than or equal to 1500 mg for adults 51 and older and adults of any age who are African American or have HTN, DM, or chronic kidney disease. Americans consume

3400 mg/day according to DGA 2010. Americans consume sodium in their diet through table salt and processed foods. Salt is used as a preservative in processed foods in order to retain moisture, improve flavor, and maintain the shelf life of the food. According to NHANES 2005–2006, the highest sources of sodium in the American diet, respectively, were yeast breads, chicken and chicken mixed dishes; pizza, pasta and pasta dishes; cold cuts; condiments; tortillas, burritos, tacos; sausage, hotdogs, bacon, ribs; and regular cheese [39].

The 2010 DGA emphasizes the importance of consuming an adequate intake of potassium daily in order to prevent retention of sodium and potential for elevated blood pressure. Potassium is one of the nutrients that is under consumed in the USA, likely because it is most abundant in fruits, vegetables, and milk, which most Americans consume inadequate amounts. The Adequate Intake (AI) for potassium is 4.7 g/day for adults. Fortunately, following the DASH diet limits sodium to 2300 mg and supplies 4.7 g/day potassium by endorsing intake of fresh fruits, vegetables, fat-free or low-fat milk and milk products, whole grains, lean proteins, nuts seeds, and legumes.

Conclusion

As the population in the USA ages, the incidence of stroke and the burden on health care costs are anticipated to continue to rise. Focusing efforts on primary stroke prevention for the many modifiable stroke risk factors and secondary stroke prevention strategies are key to best control the burdens of stroke. Many primary prevention strategies are tightly intertwined with medical, behavioral, and nutritional factors. Embracing the DGA and the DASH diet are key to nutrition related stroke prevention.

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Chapter 17
B Vitamins Influence Vascular Cognitive Impairment

Tammy M. Scott, Kristen E. D'Anci, and Irwin H. Rosenberg

Key Points

- Cognitive decline in aging is associated with small vessel abnormalities and nutritional status, notably with folate, vitamin B12 and with elevated homocysteine.
- Homocysteine levels can be lowered through nutritional supplementation with B vitamins including folic acid, vitamin B12, and vitamin B6.
- Blood levels of folate are more strongly associated with cognitive function.
- Intervention trials with B vitamins have shown mixed findings with respect to cognitive performance.
- Supplementation with adequate doses of B vitamins appears to be effective in preventing cognitive decline in individuals with low nutrient intake and status.

Keywords Aging • Alzheimer's disease • B vitamins • Cerebrovascular disease • Cognition • Dementia • Folate • Folic acid • Vitamin B6 • Vitamin B12

Introduction

As the number of elderly in the USA and globally continues to increase, age-related neurological disorders, such as Alzheimer's disease and vascular dementia, are a growing concern. The loss of memory, emotional changes, and impairments in general cognitive functioning frequently result in social isolation, loss of independence, and institutionalization. However, cognitive decline is not an inevitable consequence of growing old. Indeed, although some forms of cognitive disorders may have a genetic component, cognitive decline is also influenced by nutritional factors and may be secondary to nutritionally mediated conditions such as diabetes or vascular disease. As such, there is a strong need to identify modifiable nutritional factors that regulate the proper maintenance of brain function to facilitate healthy aging.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_17

The relationship between diet and vascular disease is well established. The nutritional foundation of cardiovascular disease prevention is a diet high in fruits and vegetables and fiber and low in saturated fat. One of the prevailing risk factors for cardio- and cerebrovascular disease is elevated blood levels of homocysteine. Plasma homocysteine may be considered a functional indicator of B vitamin status, including that of folate and vitamin B12 and, to a lesser extent, vitamin B6. High plasma homocysteine concentrations can be largely attributed to inadequate status of these vitamins [1]. Data from several laboratories indicate that plasma homocysteine increases with age independent of vitamin status and that hyperhomocysteinemia is highly prevalent in the elderly. Several studies have shown consistent and strong relationships between homocysteine concentration, heart disease, and other vascular outcomes including cerebrovascular disease [2, 3].

Vascular Cognitive Impairment and Dementia

Individuals at high risk for vascular disease are also at greater risk for cognitive decline [4]. Vascular cognitive impairment ranges in severity from subtle neuropsychological deficits to frank dementia and frequently coexists with and possibly contributes to other neurodegenerative conditions such as Alzheimer's disease [5]. Where memory loss is often the first clinical indication of Alzheimer's disease, loss of executive function—the cognitive domain that includes planning, cognitive flexibility, and inhibition of inappropriate actions—may be a marker for vascular cognitive impairment or dementia [6]. Epidemiological studies have shown that while case-mortality of stroke has decreased over the past three decades, the rate of stroke has not changed significantly over time, and the risk of dementia after stroke has increased substantially [7]. Better understanding of potentially modifiable risk factors for vascular disease can aid in developing long-term intervention strategies for controlling or preventing the cognitive dysfunction attributable to large and small vessel cerebrovascular disease [4].

There is some evidence that small vessel disease is the most common cause of vascular cognitive impairment [8]. The presence of silent brain infarcts on MRI increases the risk of dementia and predicts a steeper decline in cognitive function [9]. White matter hyperintensities (WMHI), which are also thought to primarily reflect small vessel disease, have been shown to be associated with cognitive impairment [8], although this finding is not always consistent [10]. Mild to moderate elevation of plasma homocysteine concentration has been implicated as a risk factor for cardiovascular disease [11], stroke [12], dementia [13], and cognitive impairment [14–16]. While research had initially focused on the relationship between homocysteine, atherosclerosis, and large vessel disease [12], some have suggested that homocysteine is a greater risk factor for small vessel disease as opposed to other subtypes of stroke [16–19]. Not all studies, however, have been able to confirm this finding [20]. While the mechanisms underlying small vessel disease are not entirely clear, chronic endothelial dysfunction may play a role. Homocysteine has been found to be toxic to the endothelium both in vitro [21] and in vivo [18], giving biological plausibility for a role as an independent risk factor. In a study by Pavlovic and colleagues [22] homocysteine levels were strongly associated with cognitive and functional status and the severity of WMHI in patients with cerebral small vessel disease.

B Vitamins, Homocysteine, and Brain Function

Possible biochemical interpretations of the putative effects of low B vitamin status and high homocysteine on cognitive decline can be made on the basis of one-carbon metabolism (Fig. 17.1). Folate serves as a carrier of one-carbon groups for the methylation cycle. In this cycle, methionine with its available

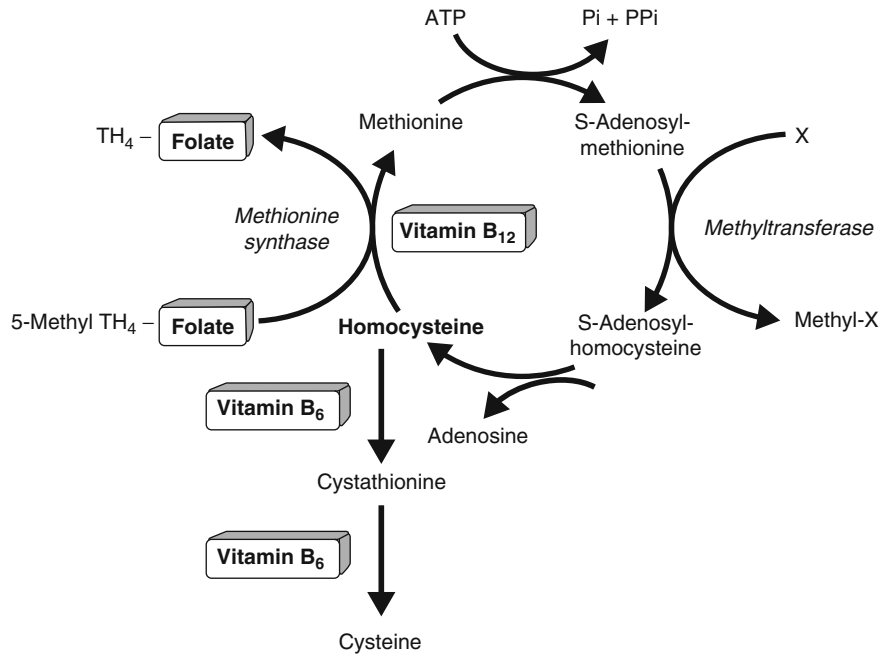

Fig. 17.1 Use of *S*-adenosylmethionine as the methyl donor for biological methylation reactions results in the formation of *S*-adenosylhomocysteine. Homocysteine is formed from the hydrolysis of *S*-adenosylhomocysteine. Homocysteine may be remethylated to form methionine by a folate-dependent reaction that is catalyzed by methionine synthase, a vitamin B12-dependent enzyme. Alternately, homocysteine may be metabolized to cysteine in reactions catalyzed by two vitamin B6-dependent enzymes (Adapted from [27])

methyl group is activated by adenosine triphosphate to form *S*-adenosylmethionine, which is the universal methyl donor in a multitude of methyl transfer reactions including many that are of vital importance to central nervous function. Through the transfer of its methyl group, *S*-adenosylmethionine is converted to *S*-adenosylhomocysteine, which is hydrolyzed to homocysteine. Homocysteine can regenerate methionine for an additional methylation cycle by acquiring a new methyl group from methyl tetrahydrofolate in a reaction that is catalyzed in all tissues by vitamin B12-requiring methionine synthase [23]. Excess intracellular homocysteine can also be removed from the methylation pathway by conversion to cystathionine in the *trans*-sulfuration pathway or through export into circulation [23]. It has been proposed that cognitive impairment in the elderly is due in part to vasotoxic effects of homocysteine and/or to impaired methylation reactions in brain tissue [24]. While it has yet to be determined if hyperhomocysteinemia is a cause of vascular disease or indicative of some other physiological change leading to vascular damage, current data demonstrate that homocysteine is strongly associated with cognitive dysfunction in aging [14, 25, 26].

B Vitamin Deficiency and Cognitive Impairment

It has long been known that severe deficiency of vitamins such as niacin, vitamin B12, and thiamine causes cognitive impairment [2] and that replacement of deficient nutrients can prevent or ameliorate those forms of cognitive impairment that are caused by deficiency (i.e., [28]). While more severe vitamin deficiencies or congenital defects are not common in the USA, these milder subclinical B

vitamin deficiencies are prevalent in the elderly [14, 29, 30]. Decreased intestinal absorption and poor appetite contribute to these subclinical deficiencies. Studies suggest that even moderately low or subclinical levels of B vitamins are associated with cognitive impairment, dementia, and other psychiatric disorders [31–33].

Extensive research supports the hypothesis that B vitamin deficiencies moderate cognitive brain functioning through effects on cerebrovascular health, DNA synthesis, and neurotransmitter metabolism [2, 34]. Vitamin B12 and folate are closely linked in the methylation process, such that a B12 deficiency can lead to a secondary folate deficiency through a decrease in the retention of folate. It is proposed that low levels of B12 are linked with peripheral neuropathy and subacute combined degeneration of the spinal cord, whereas folate may have more of a role in cognition and mood, perhaps through effects on serotonin, dopamine, and noradrenergic systems [34, 35].

Dietary B Vitamins and Cognition

In a seminal study, healthy, independently living elderly individuals with subclinical malnutrition (i.e., low dietary intake of protein and selected vitamins including vitamin B12) scored lower on tests of verbal memory and nonverbal abstract reasoning than did their peers with normal intake [36]. However, subsequent studies have yielded conflicting results with respect to nutrient intake and cognitive function. In a prospective cohort study [37] examining the relation between nutritional intake and daily functioning, dietary intakes were not associated with a change in functional decline over a 6-month period in nursing home residents. In a retrospective case–control study [38] comparing patients with Alzheimer's disease with healthy controls, cases and controls were asked to recall their past food consumption using a food-frequency questionnaire during three age periods: 20–39, 40–59, and 60 or more years of age. It was found that those with Alzheimer's disease had lower mean dietary intakes of vitamin B6 and folate than controls in the over-60 age group, but not in younger age groups. However, there was no relationship between either folate intake and homocysteine levels or homocysteine levels and cognitive status. Finally, adding to the complexity of these findings, some research [39] shows a slower decline in cognitive test performance over a 6-year period in subjects with high vitamin B12 intake but faster decline among subjects with a high folate intake (>400 μg/day) from either food sources or supplements.

B Vitamin Blood Level Associations with Cognition and Dementia

Poor B vitamin status and/or high homocysteine is associated with poorer cognitive performance [15, 40–43]. Previous studies have found that patients with dementia, especially those with Alzheimer's disease, have lower serum concentrations of B vitamins [14, 44, 45]; moreover, serum levels of these micronutrients are associated with the severity of the disease [46]. Several cross-sectional studies have found that patients with Alzheimer's dementia had significantly higher levels of serum total homocysteine than did age-matched hospitalized controls [34, 47] and healthy community-dwelling elderly individuals [47, 48]. In a longitudinal study, higher levels of homocysteine in Alzheimer's patients were associated with greater progression of hippocampal atrophy as measured by medial temporal lobe thickness, as well as a similar trend in Mini Mental State Evaluation score decline [49]. More recent studies have found inconsistent results with respect to the predictive value of high homocysteine or low B vitamin status and cognitive performance in the elderly [50, 51].

Vitamin B12

A number of studies have investigated a potential correlation between serum vitamin B12 levels and cognitive function or diagnosis of several types of dementia and cognitive impairment [52]. Most of these studies have focused on Alzheimer's disease. Based on longitudinal studies, serum vitamin B12 levels did not affect the risk of developing Alzheimer's disease or dementia. The existing evidence from studies that implemented a cognitive function assessment instrument did not support any correlation between serum vitamin B12 levels and cognitive function. Among cross-sectional studies, there was a tendency for vitamin B12 serum levels to be lower in patients with Alzheimer's disease or other types of dementia, which in certain studies reached statistical significance. However, this trend was not consistent. Finally, an inverse relationship between vitamin B12 levels and duration of Alzheimer's disease has been reported. In general, evidence from longitudinal cohort and case–control studies suggests that there is no significant association between blood concentrations or the dietary intake of vitamin B12 and cognitive test performance or the progression of Alzheimer's disease. Although some studies reported higher vitamin B12 blood concentrations to be associated with better cognitive test performance, no consistent pattern of association with a particular cognitive domain has been reported.

Folate

With respect to folate, Miller and colleagues [53] showed that blood folate levels are predictive of homocysteine levels. Elevated homocysteine levels, in turn, are associated with poorer performance on several cognitive tasks. In this study, however, there was no clear relationship between folate status and cognitive performance. Teunissen and colleagues [54] found that serum levels of homocysteine were negatively correlated with verbal learning and memory at baseline testing only, whereas higher serum folate levels were associated with better delayed recall performance. Elevated homocysteine was associated with poorer functioning on several cognitive tasks looking at immediate recall, attention, and performance during a 6-year follow-up period and there were no further associations with folate status. Morris and colleagues [42, 55] reported similar findings with respect to folate and recall. Folate status was positively correlated with recall performance. Moreover, elevated levels of homocysteine were associated with poor recall. When looking at folate and homocysteine levels, low folate levels in combination with higher levels of homocysteine (above the 80th percentile) were associated with significantly poorer performance than low folate or lower homocysteine levels alone [55].

Recent research shows an association with folate levels and mild cognitive impairment (MCI) and some forms of dementia. In these studies, people with the lowest serum levels of folate were at greater risk for Alzheimer's disease, MCI, and dementia. Snowdon and coworkers [46] examined atrophy in the brains of deceased nuns with Alzheimer's disease and compared these data with serum folate levels that had been determined earlier in the nuns' lives. They found an inverse relationship between folate status and severity of atrophy. This effect was seen even in participants without significant atherosclerosis or brain infarcts, suggesting further that the role of folate is not limited to its relationship with homocysteine and homocysteine's putative vascular effects. While there is abundant evidence to indicate an association between folate status and the development or progression of cognitive decline or dementia, a causative role is not clear-cut. On the one hand, some data suggest that folate inadequacy precedes onset of cognitive impairment or dementia. On the other hand, there is evidence to suggest that dietary intakes of folate are lower in people with Alzheimer's disease. In contrast to the data on vitamin B12, the majority of studies evaluating blood folate concentrations reported a positive association between low folate levels and poor cognitive test performance.

Fortification with Folic Acid and Cerebrovascular Effects

A number of countries have instituted mandatory folic acid fortification of wheat flour and cereal grains, with the aim of reducing neural tube defects in developing fetuses. Fortification has not been implemented in many countries, particularly in Europe, due in part to concerns about the potential for high levels of folic acid intake to exacerbate the neurological consequences of a vitamin B12 deficiency. Indeed, some of the research discussed in this chapter suggests that high levels of folate or folic acid in people with low vitamin B12 status have negative effects on memory and cognition. However, in the USA, since the initiation of fortification, positive effects have been seen with respect to increased blood folate levels and reduced homocysteine levels [56–58]. As described above, homocysteine levels are associated with severity of cerebrovascular disease, including stroke and cerebral atherosclerosis. Furthermore, low folate and high homocysteine are known risk factors for vascular disease, and population-wide reduction of these risk factors may be proposed to reduce incidence of vascular disease, stroke, and subsequent vascular dementia. Supporting this proposal, there has been a reduction in cardiovascular and stroke mortality in the USA and Canada since the introduction of folic acid fortification [59]. It remains to be seen whether folic acid fortification is associated with a change in the incidence of vascular dementia and cognitive decline.

Intervention Trials with B Vitamins

High-dose supplementation with B vitamins, particularly in combination, can decrease homocysteine levels, but the effects on cognition are not clear-cut and the results of intervention trials vary [60]. A study of high-dose supplementation with a combination of vitamins B12, B6, and folic acid in mild to moderate Alzheimer's disease found no effect on cognitive decline over 18 months [61]. A few studies have shown worsening of cognitive functioning following B vitamin intervention [62–64]. Others, however, have shown benefit. In a study of community dwelling elders, homocysteine lowering with 800 μg daily oral folic acid for 3 years had a beneficial effect on memory, information processing speed, and sensorimotor speed [65]. A recent study has also shown that homocysteine lowering with vitamins B6, B12, and folic acid was efficacious in slowing the rate of brain atrophy and cognitive decline in those individuals with amnestic or non-amnestic MCI [66, 67].

There is some evidence that the effect of B vitamin supplementation on cognitive function may depend on nutritional status at baseline. For example, Bryan and colleagues [68] provided women with 750 μg folate for 35 days and conducted cognitive and mood tests pre- and post-supplementation. They found that folate significantly improved performance in a speed-of-processing task for women with initially lower folate levels. In younger women, they found that folate significantly enhanced recall for those with initially lower folate levels. However, the effects of folate supplementation were not consistent across the study or across different age groups. In a trial of B vitamin supplementation in women with or at risk for cardiovascular disease, the results showed no B vitamin supplementation on cognition in women with sufficient dietary intake of B vitamins at baseline, but found that supplementation appeared to preserve cognition among women with low baseline dietary intake [69].

In older adults with cognitive decline, baseline nutrient status or plasma total homocysteine concentrations may also modify the effects of B vitamin supplementation. In a 1997 study by Fioravanti et al. [70], 30 older adults with "abnormal cognitive decline" and folate levels below 3 ng/mL were supplemented with folic acid for 60 days. Their results showed significant improvement in memory and attention efficiency when compared with a placebo group, and found that the degree of improvement in memory was positively correlated with baseline severity of folate deficiency. Nilsson and colleagues [71] studied vitamin B12 and folic acid supplementation in older patients with mild-to-moderate dementia. Performance on cognitive tasks was measured before and after 2 months supplementation

with 5 mg/day folic acid and 1 mg/day cyanocobalamin. They found that in patients with elevated homocysteine, vitamin supplementation decreased homocysteine levels and patients improved on measures of attention, memory, and orientation. Similarly, de Jager and her colleagues [67] evaluated the efficacy of homocysteine lowering with B vitamins on cognitive function in individuals with MCI. While there was an overall treatment effect for executive function, there was an interaction with baseline plasma total homocysteine where those participants with homocysteine levels above 11.3 μmol/L and in the active treatment group had significant benefit in global cognition, episodic memory, and semantic memory. The same study showed a similar interaction between treatment and baseline homocysteine status in the MRI measurement of loss of gray matter over 2 years [72].

There are several considerations in interpreting the findings on vitamin supplementation and cognition. First, as outlined above, the relationship between baseline nutrient status and homocysteine status should be considered. Second, there is no standard dose of vitamin or duration of treatments that is recommended for this type of trial. Third, many trials may have had too few participants to sufficiently determine an effect on cognitive performance. Finally, in people with dementia or other cognitive impairment, the severity of impairment alone is of importance. For example, Nilsson and colleagues [71] excluded severely demented patients from their study because the patients were unable to complete testing either before or after supplementation. As a correlative, the ability of vitamin supplementation to offset existing cognitive decline is somewhat dependent upon the duration and consequent irreversibility of cognitive decline.

Conclusions

The evidence for an association between elevated plasma homocysteine and cognitive dysfunction is compelling. Elevated plasma total homocysteine has been reproducibly linked to diseases of the aging brain including subtle age-related cognitive decline, cerebrovascular disease and stroke, vascular dementia, and Alzheimer's disease. Nevertheless, it is unclear whether elevated homocysteine mediates or is otherwise associated with vascular brain aging. Epidemiological data provide the basis for a hypothesis implicating homocysteine as a mediator of vascular and neuronal pathology. If this is true, then homocysteine-lowering therapies could reduce the incidence of cognitive decline, stroke, and dementia. The evidence for this is not consistent. Highlighting the importance of prevention, some research shows that supplementation with B vitamins, while successful at lowering homocysteine, is not beneficial in mediating cognitive function in individuals with irreversible compromised brain function. However, the role of homocysteine lowering in the long-term prevention of cognitive decline, whether via promotion of vascular health or via direct neural effects, has yet to be adequately established.

The research described in this chapter represents current understanding on the relationships of folate and vitamin B12 nutritional status with cognitive function and dementia in adults and elderly. There is evidence that insufficient B vitamin intake is associated with lower cognitive scores in comparison to adequate intake. Low B vitamin status is associated with increased homocysteine levels. However, higher rates of cognitive decline have been reported with high levels of folate and folic acid intake in adults, and memory performance may be impaired with high folate intake in individuals with low vitamin B12 status. Overall, studies reported lower folate blood levels and a higher prevalence of deficiency among subjects with dementia. In general, vitamin B12 serum levels were lower in patients with dementia relative to nondemented individuals; however, this relationship was not as consistent as that for folate. Stroke mortality has decreased at a greater rate in the USA and Canada subsequent to mandatory folic acid fortification, suggesting a positive effect of fortification on cerebrovascular health. Interventions with folic acid and with combinations of B vitamins were able to improve cognitive function or prevent decline, albeit inconsistently, especially in subjects with low nutrient status.

As with the data for blood nutrient levels, evidence that vitamin B12 treatment improves cognitive function is conflicting and less positive.

Overall, there is support for the concept that diets rich in B vitamins, especially folate and vitamin B12, are beneficial in maintaining brain function in aging. Some smaller experimental trials show that supplementation with vitamin B12 and folic acid may prevent cognitive decline or improve cognitive function to some degree, particularly in individuals with low baseline levels of these nutrients. To date, intervention trials indicate that supplementation with B vitamins for a period of 1.5–3 years, even while adequate to reduce circulating levels of homocysteine, may not be sufficient to prevent cognitive decline. Furthermore, some research indicates that high levels of supplementation with folic acid may have negative consequences on cognitive performance in vitamin B12-deficient individuals.

Recommendations

Adhering to a diet rich in whole grains, leafy greens, as well as lean meats and low-fat dairy may confer the greatest benefit in prevention of decline and maintaining cognitive function. Moreover, following such a diet should be considered a life-long goal rather than an immediate action to treat current decline. Folic acid supplementation is beneficial in those with folate-deficient diets, but may be harmful at high doses in the presence of low vitamin B12 status. Low B12 status is more prevalent in elders than previously known and steps for prevention need to be considered. Given the high prevalence of populations in the low and marginal folate and vitamin B12 status, the results presented here support the concern that cognitive function and risk of age-related decline represent a nutritionally modifiable public health problem.

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Part IV
Diabetes and Obesity

Chapter 18
Childhood Obesity: New Paradigms on Susceptibility, Co-morbidities, and Interventions

Esther Granot

Key Points

- The increased prevalence of obesity is worldwide and is evident in both advantaged societies and developing countries.
- Childhood obesity predicts adult obesity and entails serious health consequences including diabetes, hyperlipidemia, cardiovascular diseases, liver steatosis, and orthopedic complications.
- Factors that predispose children to obesity are both genetic and environmental.
- Currently, common variants in the fat mass and obesity-associated (FTO) gene constitute the strongest known genetic susceptibility loci for obesity.
- Environmental risk factors relate mainly to dietary habits and lack of adequate physical activity.
- Breast-feeding of infants for longer than 6 months is suggested to be inversely associated with development of childhood obesity.
- The Developmental Origins of Health and Disease hypothesis has highlighted the link between prenatal and perinatal conditions, specifically maternal obesity and excessive gestational weight gain, and subsequent development of obesity and metabolic disturbances in the offspring.
- The gut microbiota is presumed to be responsible for weight gain and altered energy metabolism and thus likely plays a pivotal role in the development of the obesity state and its metabolic complications.
- A major challenge facing the pediatric medical community is defining optimal intervention strategies and policies for prevention and early treatment of childhood obesity.

Keywords Obesity • Childhood • Risk factors • Prevention • Intervention

Introduction

We are currently amid a worldwide epidemic of obesity. Obesity among children, adolescents, and adults has emerged as one of the most serious public health concerns in the twenty-first century. The growing prevalence of childhood obesity has serious consequences, leading to appearance of obesity-related comorbid disease entities at an early age and significantly contributing to increasing health care expenditure. Although the major causes for the increasing prevalence of obesity are excessive consumption of energy-dense foods and a sedentary lifestyle, it is now recognized that various genetic,

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_18

physiological, and environmental predispositions constitute risk factors for the development of obesity and its related complications. This chapter will focus on current thoughts relating to susceptibility for development of obesity and on ongoing and possible future interventions for prevention and treatment of obesity in the pediatric age group.

Worldwide Prevalence and Associated Morbidity

According to recent surveys approximately 17 % of children and adolescents (ages 2–19) in the USA are obese (body mass index >95th percentile for age and sex) and about 32 % are overweight (body mass index >85th percentile for age and sex) and it is estimated that the prevalence of obesity is approximately three times greater than it was in 1980 [1]. A survey conducted in 1996 in the UK identified the prevalence of overweight children as 22 % at age 6 and 31 % at age 15 [2]. A later survey, similarly conducted in the UK, found that among 3–4 year old children there was a 60 % increase in prevalence of being overweight and a 70 % increase in the prevalence of obesity during the period between 1989 and 1998 [3]. Internationally, childhood obesity prevalence rates appear to be increasing among economically advantaged countries as well as in developing countries. An analysis of 160 nationally representative cross-sectional surveys from 94 countries showed that the highest prevalence of overweight was located mainly in the Middle East, North Africa, and Latin America [4].

Childhood obesity predicts adult obesity with its magnitude of serious health consequences including type II diabetes, hyperlipidemia, cardiovascular disease, and liver steatosis but it also has emotional consequences including low self-esteem, low confidence, and consequent lower rates of academic achievement (Table 18.1). Furthermore, as a result of this epidemic of childhood obesity some chronic illnesses and risk factors for adult disease are now starting in childhood rather than in adulthood. Childhood obesity is associated with gallbladder disease, steatohepatitis, polycystic ovary disease, hypertension, type II diabetes, and orthopedic complications. Approximately 20–30 % of obese children between 5 and 11 years of age have elevated systolic or diastolic blood pressure and obese adolescents account for up to 50 % of cases of hypertension in this age group [5]. Adolescent obesity, particularly in boys, correlates with increased levels of total cholesterol and LDL-cholesterol with a twofold increase in mortality from cardiovascular disease in adulthood [6]. Childhood obesity is associated with insulin resistance and has even been noted in obese children younger than 10 years. The number of children with type II diabetes increased tenfold between 1982 and 1992 [7] and the

Table 18.1 Morbidity associated with obesity

– Diabetes
– Hypertension
– Cardiovascular disease
– Dyslipidemia
– Metabolic syndrome
– Nonalcoholic fatty liver disease
– Gallbladder disease
– Gastroesophageal reflux
– Orthopedic complications (slipped femoral epiphysis, arthritic pain)
– Polycystic ovarian syndrome
– Pseudotumor cerebri
– Obstructive sleep apnea, asthma, and other respiratory diseases
– Low self-esteem, social isolation, and psychological abnormalities

prevalence of the metabolic syndrome has been shown to increase with severity of obesity, reaching 50 % in severely obese youngsters [8]. In a large cohort of American Indian children, obesity, glucose intolerance, and hypertension in childhood were strongly associated with increased rates of premature death (death occurring before 55 years of age) from endogenous causes [9].

Obesity is associated with increased biliary excretion of cholesterol relative to bile acids and phospholipids secretion, resulting in increased gallstone formation [10]. Liver steatosis, an important metabolic consequence of obesity, is evident in 10–20 % of obese children and 40–50 % of severely obese children. The duration and severity of steatohepatitis are believed to contribute to development of liver fibrosis and cirrhosis [11]. Orthopedic abnormalities are also more common in obese children; slipped femoral epiphysis occurs in obese children at a significantly younger age than in non-obese children, and between 50 and 70 % of children who suffer from bilateral slipped femoral epiphysis are obese [12]. Obesity may cause neurologic manifestations; idiopathic increased intracranial pressure (pseudotumor cerebri) is a less commonly encountered complication of obesity [13].

Few problems in childhood have as significant an impact on emotional development as obesity. Obese children demonstrate disturbance in body image perception, are less likely to enjoy social popularity and are likely to have lower high school grades [14].

Demographic Variables and Obesity

Summary statistics relating to the magnitude of overweight and obesity among children mask important differences in obesity across demographic categories including racial/ethnic group, socioeconomic status, and urban living. In the USA there is a disproportionately high rate of obesity among Hispanics and Native Americans and the risk of obesity is 35 % and 49 % higher, respectively, compared to non-Hispanic whites [15]. Among New York City public school children prevalence of severe obesity (BMI $\geq$ 120 % of 95th percentile) was highest among minority and less economically privileged children [16] and in New Zealand and Australia prevalence of overweight and obesity was higher in Pacific and Maori children and those living in more socioeconomically deprived areas [17, 18]. A recent study from China showed prevalence of overweight and obesity among children and adolescence to be associated with urbanization [19]. These variations within population subgroups are important as they should form the basis for appropriate and more focused interventions for prevention and treatment of childhood obesity.

Overweight and obese children are likely to remain obese into adulthood and are likely to develop serious health problems and confront psychological and social challenges. The obesity epidemic has proven difficult to reverse; it is incredibly difficult to reduce excessive weight gain once it has become established. Thus, children should be considered the priority population for intervention strategies and prevention of this global problem.

Genetic, Environmental, and Nutritional Risk Factors for Development of Obesity

What are the main factors that predispose children to obesity?

Children with an overweight or obese parent are twice as likely to be obese [20, 21]. But these within-family associations are complex; in a study encompassing 10,240 American households, in those with two children, having an obese younger sibling was more strongly associated with elder-child obesity than parent's obesity status and within-family sibling obesity was more strongly patterned between siblings of the same gender than between different genders [21].

Common variants in the fat mass and obesity-associated gene have been shown to be associated with body mass index and the risk of obesity, so that currently FTO is the strongest known genetic susceptibility locus for obesity. In animals, experimental studies suggest the potential roles of FTO in regulating food intake. The interactive relation between FTO variants, dietary intake, and body mass index is complex and highly inconsistent. Results from a recent large scale analysis on data from 177,330 adults from 40 studies showed that the minor allele (A-allele) of the FTO-rs9939609 variant was associated with a higher BMI and with a significantly higher dietary intake of protein. There was only a weak association between the FTO variant and dietary carbohydrate intake. These findings suggest a potential link between the FTO gene, dietary protein intake and adiposity [22]. The FTO-rs9939609 single nucleotide polymorphism (SNP) has also been noted to modify the effect of obesity on high blood pressure in Chinese children, with obese children carrying the homozygous genotype of the FTO-rs9939609 having the highest risk for developing higher blood pressure [23]. The effect of the FTO rs9939609 SNP on obesity risk when adhering to a Mediterranean diet was investigated in a large cohort of over 11,000 participants from five European countries followed for a median of 6.8 years. Interestingly, changes in weight, waist circumference, and waist circumference adjusted for BMI were not associated with the FTO gene [24].

As an inverse association between socioeconomic status and childhood obesity has been shown, in a multitude of studies, it was of interest to determine the interrelation, if any, between parental socioeconomic status and FTO single nucleotide polymorphism rs9939609. Other environmental factors studied were dietary and fitness habits. An interaction between FTO and these parameters was, indeed, observed indicating that children who are not heterozygote or homozygote for the FTO rs9939609 are more protected by a favorable social environment regarding the development of obesity relative to those carrying the FTO rs9939609 genotype [25].

Although genetic factors can predispose to obesity, as the gene pool has obviously not changed, substantially, during the last 2–3 decades, the current obesity epidemic must reflect environmental changes. Two major environmental factors are the accessibility to high caloric, high-fat foods and other dietary habits which promote an increase in energy intake, coupled with a reduction in amount of physical activity that children engage in, thereby decreasing energy expenditure. The observations regarding identifiable genotypes that in association with some environmental factors predispose to obesity while other genotypes have a protective effect may allow, in the future, institution of early intervention programs, in genetically high-risk individuals.

Environmental risk factors for development of obesity relate mainly to dietary habits and to lack of adequate physical activity.

Parents play a crucial role in delineating their children's lifestyle and health behavior. In 1615 German children, aged 7.1 ± 0.6 years, children who had at least one physically active parent spent significantly more time participating in organized sports and children whose parents were both physically active were less likely to be overweight or obese [26]. A study that matched the weight status of kindergarten children aged 3–6 with their kindergarten teachers' weight and physical activity found an association between these parameters, highlighting the importance of psychosocial, non-genetic influences, on children's weight [27]. Children who are less physically active are usually those who watch more television and spend less hours playing outdoors. In a cohort of ~13,000 British children followed since birth, weekdays and weekend TV watching at age 5 years predicted higher BMI z-scores at age 30 years, after adjustment for socioeconomic status, parental BMIs and birth weight. Each additional hour of weekend TV watching at age 5 years increased the risk of adult obesity (BMI $\geq$ 30 kg/m^2) by 7 % [28]. Another study of a cohort of ~8000 American children, enrolled at entry into kindergarten, found that children who watched more television and lived in neighborhoods perceived to be less safe for outdoor play, were more likely to be overweight [29]. A "sedentary" life style, with no or little outdoor playing, no walking to school, spending long hours watching TV or seating opposite computer screens, playing video games and engaging in other electronic near-motionless activities, all contribute to an "obesogenic" energy balance.

Limiting of energy intake is of crucial importance. Type of food and its relative content of carbohydrates, protein, and fats, salt intake, amounts consumed, feeding habits and practices, are all determinants of excess caloric intake and development of overweight and obesity.

Evidence linking breast-feeding for longer than 6 months with prevention of obesity is modest but consistent with breast-feeding suggested to be inversely associated with childhood obesity. Interestingly, overweight or obese mothers tend to breast feed for a shorter duration compared with normal-weight mothers [30, 31].

During the last decades the focus has been on reduction of fat intake. Despite this a dramatic increase in overweight children and adults has occurred. An Italian study prospectively recorded the dietary energy and macronutrient intake of a group of children from birth through their first 10 years of life (at ages 1, 5, 8, and 10 years), together with anthropometric measurements. The authors found a high protein intake at all time points studied, a high lipid intake at age 5 onward, and a high energy intake at age 5. At age 10 almost 25 % of the children were overweight [32]. The relevance of a high intake of carbohydrates with high glycemic index (the glycemic index represents the relative rate of entry of glucose into the bloodstream compared to a reference carbohydrate) for the development of obesity in otherwise healthy children is uncertain. Carbohydrates with high glycemic index are rapidly digested and cause a shorter term of satiety, thereby encouraging higher amounts of food intake, at more frequent intervals. The association between dietary glycemic index, glycemic load (which represents the quality of the carbohydrate containing food and the quantity consumed), and body weight is weak and inconsistent [32, 33].

Nevertheless, fruit juice and sweetened fruit drinks have received considerable attention as potential sources of high-energy beverages that could be related to prevalence of obesity among young children. In a study encompassing 1160 children, types and amounts of beverages consumed was related with weight status. There was no association between weight status and total amount of beverages consumed, nor type of beverages consumed; e.g., 100 % fruit juice, fruit drinks, milk, or soda. Increased beverage consumption was associated with an increase in the children's total energy intake but not with their BMI [34]. A more recent study of 1189 children examined the intake of sugar-sweetened beverage intake in infancy as a predictor of obesity at the age of 6. The prevalence of obesity at 6 years among children who consumed sugar-sweetened beverages during infancy was twice as high as that among children who had not consumed sweetened beverages in infancy (17.0 % vs. 8.6 %). However, among children who consumed sugar-sweetened beverages in infancy, the odds of obesity at 6 years did not differ in accordance with either mean weekly beverage intake or age at introduction of these sweetened beverages, during infancy [35].

Fats have a weak satiating capacity and one can readily overeat when presented with high-fat foods. Proteins have a stronger satiating capacity so from this aspect they hold an advantage over fats. Yet, excess protein intake in early life has been suggested to increase the risk of obesity in later life [36]. Excess high protein intake enhances the secretion of insulin and insulin growth factor 1, which may increase adipogenic activity as well as decrease lipolysis and secretion of growth hormone. This early adiposity rebound may thus be a risk factor for childhood obesity. In a group of Danish children no association was observed between protein intake in infancy and body fat measurements at the age of 10 [37]. An ongoing study, of 1150 non-breast-fed infants randomized to a lower or higher protein formula, and followed from infancy to school age, has been initiated with the aim of providing answers to the many questions regarding protein intake in infancy and risk for obesity later in life [36].

A major source of protein in infancy and early childhood are milk and other dairy products. Despite its importance regarding micronutrient intake and high-quality protein, consumption of milk and dairy products, by children, has waned in recent decades. A review evaluating intake of milk or dairy products and health outcomes in children and adolescents found a neutral or inverse association between consumption of milk and dairy products and indicators of obesity [38] and called for further research regarding intake of milk and health-related outcomes.

The possible effect of salt intake on childhood obesity has also been questioned after one study found that obese children prefer salty snacks. The potential effect of salty snacks on weight gain may be explained, in part, by the observation that these children also consumed more soft drinks. The high salt intake may likely produce a progressive increase in thirst and a parallel increase in intake of beverages with a net increase in energy intake [39].

These observations regarding salt intake have to be further substantiated.

The Effect of Maternal Obesity and Gestational Weight Gain on Obesity and Metabolic Disturbances in the Offspring

The obesity epidemic is not simply a consequence of poor diet and sedentary lifestyles. Obesity is a multifactorial condition in which environmental, biological, and genetic factors all play essential roles. The Developmental Origins of Health and Disease hypothesis [40] has highlighted the link between prenatal, perinatal, and early postnatal exposure to certain environmental factors and subsequent development of obesity and noncommunicable diseases. Maternal obesity and excessive gestational weight gain are major contributors to obesity and metabolic disturbances in the offspring.

Maternal obesity is a stronger determinant of offspring BMI than paternal obesity and its influence is not limited to BMI at birth but is evident also in later infancy with infants born to obese mothers having, at age 1.5–3.5 years, higher BMIs than those of infants born to overweight and normal weight mothers [41].

Infants of obese mothers are not only more likely to be obese but are also at an increased risk of developing diabetes mellitus and cardiovascular diseases in their lifetime.

Gestational diabetes mellitus is one of the most common complications observed in obese pregnant mothers and appears to have a direct impact on the future health of the infant. Fetal programming of metabolic function, induced by obesity and gestational diabetes, may have intergenerational effects that may lead to perpetuation of adverse cardio-metabolic conditions. Pre-pregnancy maternal overweight and obesity, per se, even in the absence of gestational diabetes, accounts for a high proportion of large for gestational age children, as does increased gestational weight gain. In a study of 9835 women of whom 5851 were overweight and obese, obesity without gestational diabetes accounted for 21.6 % of large for gestational age infants and the combination of being overweight or obese and having gestational diabetes accounted for 23.3 % of large for gestational age infants. Increased gestational weight gain was associated with a higher prevalence of large for gestational age infants, independent of maternal weight [42]. Large for gestational age infants have been shown to be at significant risk for having a higher BMI at the age of 2 years [43]. Furthermore, large for gestational age status and maternal obesity were noted to be associated with an approximately twofold increase in risk of developing two or more of the four metabolic syndrome components (obesity, hypertension, glucose intolerance, and dyslipidemia), as early as age 11 years [44].

Interventions before or during pregnancy offer an opportunity to modify the intrauterine environment and maternal lifestyle changes prior to and during gestation may confer health benefits to their children.

The safety of weight loss when obese pregnant women intentionally attempt to lose weight is not substantiated; some observational studies suggest that risks associated with increased weight such as preeclampsia are improved but others indicate that the incidence of small for gestational age infants is increased. A 2013 Cochrane Review concluded that until the safety of weight loss in obese pregnant women can be established there can be no practice recommendations for these women to intentionally lose weight during the pregnancy period [45].

Control of maternal obesity prior to pregnancy and lifestyle intervention during pregnancy, designed to optimize gestational weight gain and modify the metabolic intrauterine environment,

holds promise for the breaking of the vicious cycle that perpetuates the transmission of obesity and its associated cardio-metabolic conditions to the next generation. Unfortunately, results, to date, have been rather disappointing.

In a group of obese women who had taken part in a lifestyle intervention program with a resultant lower gestational weight gain during pregnancy, the percentage of overweight or obese children, and children's body composition (total fat mass, total lean mass and fat percentage) as estimated by dual energy X-ray, did not differ, at mean age of 2.8 years, as compared to the control groups [46]. Despite these results the next few years will undoubtedly see numerous studies focusing on maternal interventions prior to and during pregnancy, in an attempt to modify and prevent the global obesity epidemic.

The Association Between Gut Microbiota and Obesity

Recent research has implicated a "new," previously unrecognized, environmental factor—the gut microbiota. The gut microbiota are currently regarded as responsible for the weight gain and the altered energy metabolism that accompanies the obesity state. It appears that the microbiota function much like a metabolic "organ" influencing nutrient acquisition, energy homeostasis, and ultimately the control of body weight.

Intestinal microbes outnumber our own cells 10 to 1. An early hint that gut microbes might play a role in obesity came from studies comparing intestinal bacteria in obese and lean individuals. The ratio of the two major bacterial phyla, Bacteroidetes and Firmicutes, differs between obese and lean subjects with obese subjects having a higher ratio of Firmicutes to Bacteroidetes. The importance of this ratio is as yet controversial due to conflicting findings in various studies. Researchers do agree that the microbiota of lean individuals is much more diverse than that of obese individuals [47].

Documenting such differences in gut microbiota composition does not mean that these discrepancies are responsible for obesity. But a series of experiments, in mice, has indeed succeeded in proving a "cause and effect"; baby rodents raised in a germ-free environment were populated with intestinal microbes of obese women and their lean twin sisters while receiving a similar diet. Animals that received bacteria from the obese twin grew heavier and had more body fat than the animals that received the microbes from the lean twin. Transfer of bacteria from lean mice to those mice destined to become obese resulted in the obesity-destined mice remaining at a normal, healthy weight. Interestingly, transfer of a markedly large number of varieties of Bacteroidetes bacteria was necessary in order to achieve this change and prevent the development of obesity [48].

Alterations in gut bacteria likely also cause changes in intestinal permeability, with a resultant low grade inflammatory state that contributes to chronic metabolic conditions and especially to development of nonalcoholic fatty liver disease. Indeed, germ-free mice are protected from both obesity and fatty liver injury [49].

Diet is a paramount factor in shaping the gut ecosystem. People consuming a diet of highly processed foods have been shown to have a less diverse gut flora, and mice fed with diets rich in fat and low in fiber grew obese despite cohabiting in cages with mice which harbored bacteria from "lean" donors.

Infants born via vaginal delivery and infants delivered by cesarean section differ in their intestinal flora, as do infants who are solely breast-fed as compared to infants who are formula-fed. It has been suggested that formula-fed infants and infants delivered by cesarean section are at a higher risk of being obese and developing diabetes and this increased risk may be due to differences in gut microflora [50].

In a study of 727 mother–child dyads followed up to age 7, children who had been exposed to maternal use of antibiotics during the second or third trimester of pregnancy were found to be at

higher risk of obesity. Second or third trimester antibiotic exposure was positively associated with BMI z-scores, waist circumference, and % body fat [51]. Antibiotic use during pregnancy may alter maternal–offspring microbiota exchange and thereby contribute to aberrant microbial colonization of the infant gut and increased susceptibility to obesity later in life. Similarly, antibiotics administered to infants during the first year of life are also expected to alter infants' gut microflora. Whether antibiotic use in infancy is related to later development of obesity is intriguing and deserves further studies.

Some investigators are currently conducting studies to determine whether transfer of feces from lean to overweight people will lead to weight loss. These studies are not without risk. A safer approach is to administer probiotics and prebiotics, but, to date, these have not shown any advantage in decreasing obesity or in treating nonalcoholic fatty liver disease. Precise strains of bacteria that promote leanness will have to be identified before a "probiotic pill" can be developed for prevention or treatment of obesity.

Interventions Targeted at Promotion of a Healthy Lifestyle and Prevention of Obesity

Childhood obesity is the harbinger of adult obesity; persistence of obesity is apparent for both the preschool and elementary school period. Children who were overweight at any time point during the preschool period (at ages 24–54 months) are five times more likely to be overweight at age 12 years than those who were below the 85th percentile for BMI at preschool age. During the elementary school period (ages 7–11) the more time points a child is documented to be overweight the greater his odds of being overweight at age 12 years [52].

Pediatricians can be confident in counseling parents to begin to address the at-risk child's eating and activity patterns at an early age rather than delaying counseling in hopes that overweight and the behavior patterns that support it will resolve themselves in due course.

A major challenge facing the pediatric medical community is to find the optimal intervention that will turn the tide, with prevention of obesity and its complications as the best approach. Programs currently emphasize either targeting of a single problem, e.g., reducing intake of sugar-containing beverages in school vending machines, or attempting to improve multiple parameters at once—e.g., modifying physical activity with reduced indoor play and more outdoor sports, reducing snacks and increasing the eating of fruit and vegetables. In order to treat obesity once it sets in, intervention is probably best provided by a team approach encompassing the skills and knowledge of medical, nutritionists, and psychosocial professionals. The team's primary target should be weight maintenance or reduction depending on age and severity. However, it should also define, monitor, and attempt to treat the potential complications of obesity described above.

A Cochrane Database Review published in 2011 analyzed the data from studies which included 27,946 school-aged children (age 6–12 years). Overall, children in the intervention group had a standardized mean difference in adiposity (measured as BMI or zBMI) of −0.15 kg/m^2 with a high level of observed heterogeneity between studies. A broad range of program components were used in these studies so it is not possible to distinguish which of these contributed most to the beneficial effect observed. The following strategies, among others, were assumed to be of promise: a school curriculum that includes healthy eating and importance of physical activity, increased school sessions devoted to sports, improvement in nutritional quality of the food supply in schools, environments and cultural practices that support children eating healthier foods and being active throughout each day, support for teachers and other staff to implement health promotion strategies and activities, parental counseling and home-based interventions that encourage children to be more active, eat more nutritious foods and spend less time in screen-based activities [53]. Assessment of interventions designed with an emphasis on increasing physical activity in school children showed that the results were also modest,

at best. A review of the data based on 36,593 study participants aged 6–18 years, with duration of interventions ranging from 12 weeks to 6 years, demonstrated positive effects on duration of time spent engaging in moderate to vigorous physical activity and on television watching, but with no effect on BMI, blood cholesterol levels or systolic and diastolic blood pressure [54]. A meta-analysis of 11 randomized control trials which included 10,748 children, aged 6–12 years, assessed the effect of physical activity interventions, lasting longer than 6 months, on BMI, blood pressure, and blood lipid levels. Physical activity interventions did not lead to any reductions of BMI. There was a minor reduction in systolic and diastolic blood pressure and triglyceride levels [55].

Although evidence suggests that lifestyle interventions can benefit cognitive function and school achievements in children of normal weight a review of studies in overweight and obese children failed to reach consistent conclusions. Multicomponent lifestyle interventions and interventions comprising only physical activity in which 674 overweight and obese children (3–18 year old) were enrolled, failed to show an effect on reading, vocabulary, and language achievements. Single component physical activity interventions did produce small improvements in mathematics achievement, executive function, and working memory [56]. Future obesity treatment trials will have to address not only physical outcomes but also the effect, if any, on academic and cognitive achievements.

Results of intervention programs initiated in infancy and encompassing preschool-age children (up to age 5 years) may be more promising. A recent review of relevant studies found that mean differences between intervention and control groups ranged from −0.29 to −0.54 kg/m^2 for BMI and −2.9 to −25.6 % for the prevalence of overweight/obesity. Interventions initiated in infancy (under the age of 2) had a positive impact on obesity-related behavior, e.g., diet quality and feeding practices. The more successful interventions were those which entailed high levels of parental engagement and focused on skill building and on links to community resources [57].

Given the overall, generally small magnitude of the positive effects observed, results of these interventions may be viewed by some as rather disappointing. Others are more optimistic and believe that longer periods of time are needed in order to see a stronger impact of these interventions. Furthermore it is urged that research should focus on identifying the more effective intervention components so that they can be embedded within health and care systems and achieve long term sustainable results.

Indeed, the CDC has just reported that in the 2–5 year old group there has been a decrease of 43 % in the prevalence of obesity, during the last decade, from 13.9 % in 2003–2004 to 8.4 % in 2011–2012. Yet, overall childhood obesity prevalence has not changed among the 2–19 year old, age group and remains at ~17 % [58].

New tools that have now been introduced in order to promote nutrition education and improve dietary behavior include games like the Italian board game by the name of Kaledo, intended for the 9–19 age group [59], online web-based educational programs like the EMPOWER intervention, targeting mothers with children aged 4–6 years [60] and educational iPad applications like the one as part of the Body Quest: Food of the Warrior program designed to encourage third grade students to consume more fruits and vegetables [61].

The optimal settings for the interventions targeted at treatment of obese children are as yet debatable. Although pediatric health providers have traditionally assessed and treated childhood obesity and associated health-related conditions in the clinic setting there is a recognized need to expand into the community and integrate primary health care strategies with treatment resources available in the community. In a group of obese children aged 2–5 years, a 1-year program of counseling by a pediatrician in the clinic coupled with home visitations was significantly more successful in decreasing BMI z-score compared with pediatric counseling alone [62]. Other multi-setting programs include components that are provided in the primary-care clinic, community-based services and home-based family interventions. One such multi-setting, multicomponent program is the 3-year, currently undergoing, Stanford Goals trial which enrolled 7–11 year old overweight and obese children, and includes a community-based after school team sports program, a home-based family intervention to reduce

screen time, alter home food/eating environment and promote self-regulatory eating skills and a primary care behavioral counseling intervention linked to the community and home interventions [63].

These programs are expensive and require intensive and complex organization. Lack of funding and shortage of trained health professionals may preclude implementation of such intervention programs in many areas of the world.

Furthermore, multidisciplinary obesity clinics and multi-setting, multicomponent intervention programs may not be acceptable in certain populations. For example, in an ultra-orthodox religious community in Jerusalem children spend long hours in school classes without engaging in recreational sports and without the benefit of school-sponsored physical activities. Obesity in the children of this community is on the rise but parents are reluctant to have their children attend clinics which will expose the children to a large number of health professionals. The feared result of such exposure is that it will "blemish" the child and hinder chances for an arranged marriage in the future. For similar reasons they are unlikely to comply with psychological evaluation and assistance. We therefore devised a simple, inexpensive intervention, based on guidance and reinforcement regarding nutrition and exercise modification. Recruited obese children (7–16 years) were given a basic discussion on what constitutes a healthy diet and on the importance of physical activity (mainly walking) but children in the intervention group were asked to record a daily "diet diary" and the amount of exercise performed and received a weekly telephone call during which diaries were reviewed and children were encouraged to improve their adherence. Despite small numbers, at 6 months there was a clear trend in the direction of improvement in the intervention group; a decrease in BMI and LDL-cholesterol levels (with an increase in the control group) and a positive change in lifestyle parameters studied (drinking of sweetened beverages, eating fruits/vegetables at least once a day, and amount of snacks) [64].

Several minimal intervention studies have been reported; Knoppke et al. performed an uncontrolled study of 141 children aged 8–12 years whose weight at entry was over 120 % of the ideal. The intervention included a 30 min talk with a health professional after which families were given educational material including video films, a drawing booklet, and card games. Children filled out questionnaires every 3 weeks. At 3 months 69.5 % of the children remained in the study and 33 % of them showed some decrease in BMI [65].

It is rather remarkable that simple interventions, of minimal magnitude and expense, can achieve clear trends in the direction of success. Targeting cheaper effective interventions is imperative in order to curb the ongoing obesity epidemic which is now encompassing less socioeconomically privileged populations worldwide. The minimal intervention approach holds promise and in view of its low cost and low requirement for skilled health workers deserves further study in a larger number of children and in different communities [66].

Another approach to tackling the imbalance between the amount of calories children consume and children's energy expenditure is to target environmental factors that urge and induce children to consume high-calorie, low-nutrient "junk" foods. One such potential factor is marketing of food directly to children. Numerous firms conduct marketing research to elucidate the psychological underpinning of children's food choices, exploiting the suggestibility of young children. In marketing to children food companies use intense and aggressive methods of persuasion through television commercials, product placement in toys, games, songs, and movies. Adolescents are reached via cellular-phone text messages and the internet. Realizing that the food industry is thereby contributing to the obesity epidemic, there is now pressure from local and national legislatures to curb and ban food marketing in school and to restrict child-directed fast food TV advertising [67, 68]. A recent report from the Agency for Healthcare Research and Quality on interventions implemented to prevent childhood obesity urged that future studies address the effectiveness of policy changes; imposing regulations on fast food availability, imposing an excise tax, calculated per ounce of sugar, on sugar-sweetened beverages, implementation of regulations regarding retailing and distribution of fruits and vegetables and promotion of health information labeling on food products [69, 70].

Conclusions

The obesity epidemic has proven difficult to reverse. Although some promising news is emerging regarding efforts to curb the obesity crisis with prevalence rates falling, in the USA, among 2–5 year olds [71] substantial disparities in obesity rates exist among population groups and the prevalence of severe obesity is continuing to rise. Diseases associated with obesity continue to affect younger aged individuals and are a major contributor to increasing health care expenditures. There is growing recognition that we will not be able to sustain healthy lifestyles without addressing the environment and culture that currently support an energy balance that provides a surplus of calories and inadvertently promotes sedentary behavior.

Overweight and obesity are largely preventable and therefore children should be considered a priority population for intervention strategies and prevention. A life-course approach to health should commence prior to and during pregnancy with further research directed at mechanisms and processes by which environmental factors affect epigenetic processes in the offspring. Promoting a healthy, sustainable lifestyle requires comprehensive, multilevel, programs that emphasize appropriate nutrition, physical exercise, and behavior modification. Primary care physicians and multidisciplinary health workers play a vital role in these interventions, implemented at home, day-care centers, schools, health care institutions, and the community. Supportive governmental health policies, regulation and control of food marketing and further investment in obesity-related research are also of importance. Obesity is one of the most serious public health challenges of the twenty-first century and all efforts should be directed at its prevention and early treatment.

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Chapter 19
The Women's Health Initiative: Lessons for Preventive Nutrition

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Key Points

- Postmenopausal women represent a subpopulation with unique health issues and variable diet and nutrition influences on health status.
- The Women's Health Initiative (WHI) is the largest cohort of aging women in the USA. Follow-up for assessment for assessment of selected health outcomes will continue through 2020. This effort affords unprecedented opportunity to evaluate diet and health associations in aging women.
- Excess body weight (and adult weight gain) is a significant health risk for cardiovascular disease (CVD), breast cancer, colorectal cancer, and type 2 diabetes in postmenopausal women.
- The WHI Dietary Modification Trial intervention aimed at reducing daily total fat to 20 % of kcal, increasing fruits and vegetables to at least 5 servings/day, and increasing grains to 6 or more servings/day was not associated with reduced risk for breast or colorectal cancer during the trial period or on longer-term follow-up. Likewise, neither CVD nor diabetes was significantly reduced in the WHI Diet Modification study population. In women who reported the highest dietary fat intake at baseline and reported a mean reduction in dietary fat of 12 %, breast cancer risk was significantly reduced during the trial period. Longer-term follow-up after the intervention showed no protective effect for the low fat diet assignment on cancer outcomes.
- Supplementation with calcium and vitamin D did not reduce risk for colorectal cancer nor was it associated with a significant reduction in fracture risk, although women with lower baseline serum vitamin D levels did have some protection against fractures with combined calcium and vitamin D supplementation.

Keywords Diet • Clinical trial • Cardiovascular disease • Cancer • Dietary adherence • Postmenopausal women

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_19

Introduction

Towards the end of the 1980s, considerable evidence was accumulating from observational studies and some short-term trials with non-disease endpoints that identified factors that might benefit women in their postmenopausal years. The balance of randomized trials prior to that point had been focused on men and men's health. The confluence of scientific discovery and political will led to the design and ultimate funding of what was to be known as the WHI. Two main factors in this category were postmenopausal hormone replacement therapy (hormone therapy), suggested to prevent CVD, the number one cause of death among women, and total dietary fat reduction for purposes of reducing risk for breast and colorectal cancers, also major causes of death and disability among women. These became the two, overlapping, main trials that were the core of WHI.

The rationale, sample size estimates, and intervention methodology for each of the main trials were developed separately, and different eligibility criteria were applied to each. Figure 19.1 illustrates how the two trials actually overlapped once the recruitment to all of the studies within WHI was complete.

The WHI is the largest combined clinical and observational trial ever undertaken among postmenopausal women in the USA [1]. The study was originally funded in 1992 out of the Office of the Director of NIH, then Dr. Bernadine Healy; subsequently the funding was moved to the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health, U.S. Department of Health and Human Services [2]. From its inception, the WHI sought to answer significant research questions to inform clinical practice for postmenopausal women over the age of 50 years. Specifically the hypotheses to be tested focused on critical causes of morbidity and mortality in this segment of the population: (1) the role of hormone therapy to prevent CVD and osteoporotic fractures with the potential increase in risk for breast cancer; (2) dietary fat reduction to prevent breast and colorectal cancer and secondarily CVD; and (3) supplemental vitamin D and calcium to prevent both colorectal cancer and hip and other fractures. The study design for this complex clinical trial is illustrated in Fig. 19.1. Briefly, at study onset women were screened for eligibility to either the low fat dietary modification

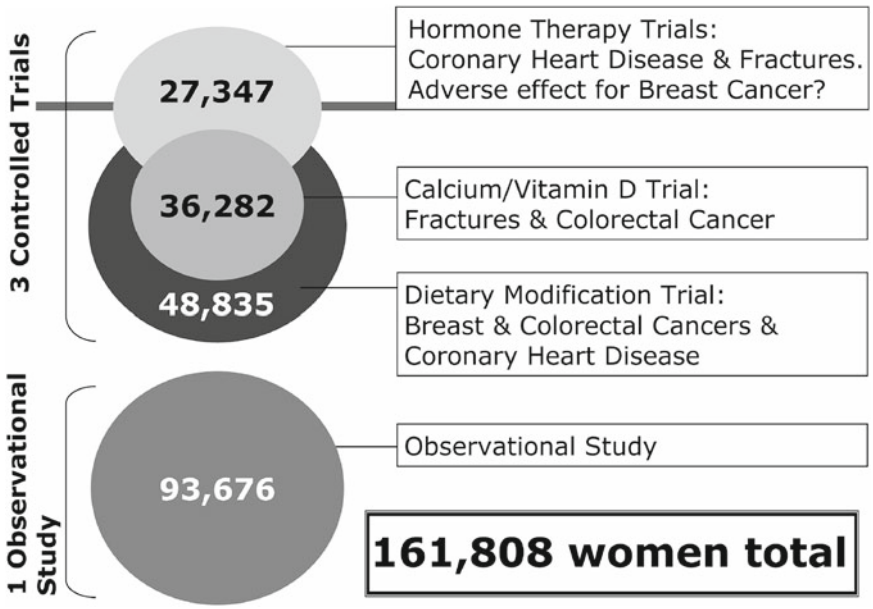

Fig. 19.1 Women's Health Initiative (WHI) study design

trial or the hormone therapy trial, although subjects could participate in both. At the end of the first year, the third clinical trial, studying supplemental calcium and vitamin D at doses of 400 IU vitamin D3 and 1000 mg elemental calcium, was added and again participants were screened for eligibility and randomized to supplementation or placebo; participation in the alternate trials was permitted if the subject met eligibility requirements for the specific trial(s) of interest.

The primary focus of this chapter is the Diet Modification trial because of its relevance to preventive nutrition. The calcium/vitamin D trial will be briefly described; for detailed results from the hormone trial the reader is referred to the primary outcome papers [3–6] (Table 19.2) and more recent results from extended follow-up [7]. Main study outcome papers as well as more recent papers evaluating outcomes during extended follow-up are also presented. [8–14]. In addition to the clinical trials (CT), women not interested or ineligible for these trials could elect to enroll in the WHI Observational Study (OS) where demographic, clinical, dietary, and other lifestyle data were collected periodically and prospectively for use in epidemiological analyses of multiple research questions of relevance in this population of postmenopausal women [1].

Scientific Rationale for the DM Trial

Ever since the international rates of dietary fat consumption and mortality from breast cancer were found to be strongly associated [15], scientists have worked to test this hypothesis in epidemiological studies, with conflicting results [16–18]. Some of the discrepancy in findings from different studies may be partially explained by lack of range of intake in individual level studies compared to the international comparative studies, and also by imperfect adjustment for measurement error and cross-cultural differences in nutrient data bases associated with dietary assessment. By 1993, the time was therefore right to evaluate the hypothesis with a carefully conducted randomized controlled trial. The choice of age groups for this trial was based on statistical power considerations, taking into account the logistics of recruiting very large numbers of women, and the incidence of breast cancer by age group [1].

The intervention was developed based on behavioral research that had demonstrated group sessions resulted in greater behavioral change as compared to lower intensity methods of changing dietary behavior. The feasibility of implementing such a program was evaluated in a series of behavior change trials, including the Women's Health Trial [19], and the WHI Feasibility Study in Minority Populations [20]. These studies showed that not only could women change their dietary fat intake by nearly 50 %, but that the changes they made had some impact on the dietary behavior of their husbands as well [21].

The primary endpoints, breast and colorectal cancers, and the secondary CVD endpoints of the DM Trial of the WHI were selected based on the literature available prior to 1992, scientific evidence which is summarized below.

Breast Cancer

The literature has been summarized in Prentice et al. 2006 [10] and 1988 [22]. Briefly, animal studies in rats and mice showed that higher tumor rates were associated with the high fat feeds [23]. Migrant studies supported the international dietary comparisons by showing that women born in Japan but moving to the USA during their adult life had rates of breast cancer closer to those of the USA than to those of Japan [24]. Reviews of observational studies, using meta-analytic methodology, provided inconsistent conclusions [25] (reference the meta-analyses). Those restricted to case–control studies of breast cancer estimated a significant excess risk associated with higher fat consumption [26]. A meta-analysis that combined case–control and cohort studies also estimated a significantly increased breast cancer risk [27]. On the other hand, a pooled analysis restricted to prospective observational

studies did not find a significant association between dietary fat and risk of breast cancer [28]. One explanation that had been suggested for the inconsistency among the cohort studies was variance in the dietary assessment used to capture diet exposure [29].

Colorectal Cancer

The literature evaluating the association between dietary fat and colorectal cancer has been briefly summarized in Beresford et al. [8]. Like the international studies of breast cancer mortality, Carroll et al. [15] and Prentice and Sheppard [18] demonstrated that colorectal cancer mortality was about one-third lower in countries in which fat intake was estimated to be 50 % lower than that of the US population. Further, migration studies found that women relocating from countries with low fat consumption to countries with high fat consumption experienced the higher colorectal cancer rates of their new country [30, 31]. The preponderance of evidence from within-country observational studies [17, 32–35] suggested that dietary fat was a risk factor for, and fruits, vegetables, and grains were protective against, risk of colorectal cancer.

Cardiovascular Disease

Howard et al. [9] summarized the evidence relating dietary fat to CVD risk where in the relationship is modified by serum cholesterol. Both observational studies and randomized trials in the 1990s identified strong associations between serum cholesterol (especially low-density lipoprotein cholesterol (LDL-C) level) and other CVD risk factors. Elevated serum cholesterol and LDL-C have consistently been associated with increased intake of saturated and *trans* fatty acids and potentially dietary cholesterol while unsaturated fatty acids, plant proteins, grains, and fiber were associated with reduced blood cholesterol levels. A direct relationship between dietary intake of saturated fatty acids and rates of CVD had been found in several observational studies at the time [36–41]. Early dietary intervention trials investigated not only total fat reduction, but more specifically the substitution of one kind of fat for another, or emphasizing one diet type over another. These trials demonstrated cardiovascular risk reduction associated with poly- and monounsaturated as a replacement for saturated fat [42–44], and with Mediterranean-type or very low fat pattern rather than the typical US diet [45–47]. Because this outcome (CVD) was a secondary outcome in the DM trial, the intervention developed for the WHI focused on cancer risk reduction through reduced total fat, and increased fruits, vegetables, and grains, assuming this dietary pattern would likewise reduce cardiovascular risk.

Eligibility Criteria: Clinical Trials and Observational Study

As detailed in the design paper, all women enrolled in the WHI were between the ages of 50 and 79 years at the time of randomization and were required to meet specific eligibility criteria, which varied somewhat across the CT and the OS, prior to enrollment [1]. Overall eligibility required study participants to be postmenopausal, provide written informed consent, and to plan to remain in the general geographical location for a minimum of 3 years. In addition, to be considered eligible for the OS women had to have a predicted survival of 3 years or more, report no dependency on alcohol or drugs, and no dementia or mental illness, all factors that might preclude obtaining reliable study data. Eligibility for the all parts of the CT included no history of breast cancer (including recent clear mammography), no other cancer in previous 10 years, no myocardial infarct, stroke, or transient ischemic attack (TIA) in the previous 6 months, and no severe or chronic hepatitis. Women also had to demonstrate a body mass index (BMI) greater than 18 kg/m^2, hematocrit over 32 %, platelet count above

75,000, and blood pressure of less than 200/105 mmHg; in addition, women were ineligible if currently taking corticosteroids.

Additional exclusion criteria for the hormone trial included endometrial hyperplasia or cancer, malignant melanoma, history of pulmonary emboli or deep vein thrombosis, bleeding disorders, elevated triglycerides, abnormal gynecological exam, previous osteoporotic fracture treated with hormones or use of anticoagulants or Tamoxifen®. Women had to be willing to discontinue hormone therapy in order to be randomized into the treatment or placebo arms of the HT trial; women who had not had a hysterectomy had to be willing to have endometrial aspirations regularly.

The calcium/vitamin D trial further excluded women with a history of renal calculi or hypercalcemia, currently using corticosteroids or taking vitamin D supplementation above 600 IUs/day. Supplementation below this level, as is common in multivitamin supplements, was not an exclusion criterion nor was the use of supplemental calcium. All hormone or CaVitD clinical trial participants had to complete a baseline eligibility visit and successfully complete run-in evaluation on placebo (HT and CaVitD) prior to randomization into the CT arms.

The dietary eligibility criteria for the DM trial were based on an initial food frequency questionnaire (FFQ) screening for dietary fat intake; women were ineligible if they reported an intake of <32 % total daily energy as dietary fat or total daily caloric intake <600 or >5000 kcals/day. Other exclusion criteria included therapeutic diet restriction in conflict with dietary assignments including diabetic or low salt diets, reported intolerance of high fiber intake, history of Type 1 diabetes mellitus, colorectal or breast cancer, reported intake of >10 meals outside home weekly, and inability to complete a 4 day food record.

Recruitment

Over 160,000 women were recruited overall between the fall 1993 and summer 1999. Forty clinical centers from 24 states across the USA participated in recruitment. Ten centers were targeted to enhance minority recruitment including Atlanta, GA, Arizona (Tucson, Phoenix), Birmingham, AL, Chicago-Rush, IL, Detroit, MI, Honolulu, HI, La Jolla, CA, Medlantic, DC, Miami, FL, and San Antonio, TX. In addition, three sites were responsible for collecting more detailed data and clinical assessment of bone health and body composition using Dual-X-ray absorptiometry (DEXA) (Arizona, Pittsburgh, and Birmingham).

The vast majority of study participants were ultimately recruited through direct mail campaigns. However, women were more likely to respond when they had already learned of the study through other channels, such as newspaper and television advertisements, local media reports, public service announcements, health fairs, brochures placed in local health clinics, pharmacies, beauty salons, libraries, churches and clinics, mailings to local healthcare providers, and "name a friend" where additional women were recruited by participants currently active in the study.

Baseline Characteristics of Study Sample

Final enrollment numbers included 93,676 in OS; 27,347 in HT trial, 48,836 in DM, and 36,282 in CaVitD indicating that the DM and HT trials achieved recruitment goals while the CaVitD attained 81 % of goal and the OS 93 %. The majority of women recruited into WHI, both CT and OS, were between the ages of 60 and 69 years, white, achieved an education beyond high school, were married, and overweight. On average, between 15 and 20 % of women screened for study participation entered the CT, while approximately 24 % entered the OS. Recruitment rates were lower for women over age 70 years as well as Native Americans and Asians [48]. An estimated 10 % of women reported a

current smoking habit, the majority consumed 1–7 alcoholic drinks/week and reported low physical activity levels, and greater than 60 % of women took at least one dietary supplement including almost 25 % who reported regular use of calcium supplementation. Clinical tests of lipids, glucose/insulin, and select nutrients were completed for a subsample of WHI participants across all treatment groups. Results of the clinical tests suggested that enrolled women, on average, had borderline hypercholesterolemia (approximately 220 mg/dL across all study groups), triglycerides ranging from 131 to 144 mg/dL; and fasting glucose of 94–102 mg/dL [48]. These indicators along with the elevated BMI and waist circumference of the average study participant suggest that mild to moderate metabolic abnormalities were common in the study population.

Data Collection and Time Points

The amount of data collected for the WHI study is massive and the infrastructure to successfully complete the trial is extremely complex [49]. In addition to numerous questionnaires focused on demographic, lifestyle, and clinical characteristics, biosamples were also collected on the entire study sample with analysis of lipids, nutrients, and select cardiovascular risk factors completed on a 8 % subsample at baseline [6].

A comprehensive list of measures collected and the procedures for collecting clinical measurement data are described in Table 19.1. In addition, interested researchers can visit the National Heart, Lung, Blood Institute (NHLBI) WHI website for more detailed information including PDF copies of all study forms, procedures, and protocols visit https://www.whi.org [www.whi.org - reference #6].

Table 19.1 WHI clinical measurement procedures

Clinical measurement	Procedures	Data entry form
Resting pulse	Measured after 5-min rest at radial artery. Recorded as number of beats in 30 s and then multiplied by 2	Form 80: Physical measurements
Blood pressure	Measured over brachial artery using stethoscope bell and mercury manometer. Cuff size determined by standardized arm circumference measurement. After a 5-min rest, maximal inflation level determined and two blood pressure measurements taken with 30-s rests in between. Systolic value (Phase I) recorded at the first of two or more Korotkoff sounds. Diastolic (Phase V) recorded when the last rhythmic sound heard. Recorded in mmHg to nearest even digit, rounded up	Form 80: Physical measurements
Height	Wall-mounted stadiometer used. Measured at end-inspiration with shoes removed. Recorded to nearest one-tenth centimeter, rounded up	Form 80: Physical measurements
Weight	Calibrated balance beam or digital scale used. Measured with shoes, heavy clothing, and pocket contents removed. Recorded to nearest one-tenth kilogram, rounded up	Form 80: Physical measurements
Waist and hip circumferences	Measured with extra layers of clothes removed (nonbinding undergarments only) at horizontal plane: waist at level of natural waist (narrowest part of torso) at end-expiration; hips at site of maximum extension of buttocks. Recorded to nearest half-centimeter, rounded up	Form 80: Physical measurements
Grip strength	Calibrated Jamar hand-grip dynamometer used. Staff demonstration and participant sub-maximal trial followed by two measurements in dominant arm with staff coaching for maximal performance. Recorded to nearest kilogram, rounded up	Form 90: Functional status

(continued)

Table 19.1 (continued)

Clinical measurement	Procedures	Data entry form
Chair stand	Stop watch and straight-backed, non-padded, flat, armless chair used. If participant able to complete single chair stand without using arms, then two 15-s trials of repeated chair stands performed with arms folded across chest with a 1- to 2-min rest in between trials. Chair stands counted as participant arose; counts for both trials recorded	Form 90: Functional status
Timed walk	Gait course marked with end lines 6 m apart and "X's" marked just beyond each end line. Stop watch used. Participant used ambulatory aids as needed and walked course at usual speed until "X" was reached. Second trial performed to "X" at other end. Recorded as number of seconds (to nearest one-tenth second) from start to when foot completely crossed the end line	Form 90: Functional status
Clinical breast examination	Performed by licensed clinician. Inspection of both breasts with participant in sitting position. Systematic palpation of all four quadrants of both breasts and axillae with participant in supine position. Findings recorded, including follow-up of abnormalities	Form 84: Clinical breast exam
Pelvic examination	Performed by licensed clinician. Inspected external genitalia, pelvic structures (with valsalva), vaginal mucosa, and cervix. Bimanual exam performed with and without valsalva, followed by rectovaginal exam. Findings recorded, including follow-up of abnormalities	Form 81: Pelvic exam
Pap smear	Performed by licensed clinician. Exocervical scraping obtained using wooden spatula and endocervical scraping obtained using cytobrush. Two smears fixed on slide and sent to local lab. Findings and lab report recorded	Form 92: Pap smear
Endometrial aspiration	Performed by licensed clinician. Flexible aspirator used to obtain endometrial tissue from all uterine surfaces. Tip of aspirator cut off and allowed to fall into formalin bottle with specimen. Sent to local lab. Findings and lab report recorded	Form 82: Endometrial aspiration
Transvaginal uterine ultrasound	Performed at local ultrasound center if WHI clinician unable to do endometrial aspiration (e.g., due to cervical stenosis). Recorded thickness of endometrial stripe and any significant pathology	Form 83: Transvaginal uterine ultrasound
Cognitive assessment	Modified Mini Mental Status Examination (3MSE) used. Items asked in order, using standardized strategies and probes. Recorded participant responses to items and computer-scored after data entry	Form 39: Cognitive assessment
Electrocardiogram	MAC-PC electrocardiograph used. Twelve-lead ECG recorded for 5 s with participant in supine position after standard skin preparation, limb lead placement, and chest lead placement using a Heart Square. Digitized ECG recording transmitted to ECG Reading Center	Form 86: ECG
Mammogram	Performed at local mammography center by standard low-dose radiation technique. Radiologist report recorded as negative, benign, probably benign, suspicious, or highly suggestive of malignancy, using ACR classification system. Follow-up of abnormal findings also recorded	Form 85: Mammography
Bone density scan	Dual-energy X-ray absorptiometry (DXA) bone mineral density using Hologic QDR-2000 and standard scanning and analysis procedures for measuring hip, AP lumbar spine and whole body bone density	Form 87: Bone density scan

Progress to Date

The DM trial ended in 2004 and subsequently the women were asked to consent to a longer-term observational follow-up. Over 85 % of the women enrolled in WHI consented to ongoing follow-up; WHI will enter the third extended follow-up period in September 2015, extending outcome collection through 2020. Among women in the DM trial, the control group women were more likely to consent to follow-up than women assigned to the low fat diet. Women consenting for extended

Table 19.2 Publication of main WHI trial findings

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follow-up are slightly younger, white, and more educated. To date, over 1000 manuscripts have been published in the peer-reviewed literature using data collected in the context of the WHI CT and OS. The published manuscripts describing the primary hypotheses tested within the HT, DM, and CaVitD trials are summarized in Table 19.2 and were published in the Journal of the American Medical Association and the New England Journal of Medicine between 2002 and 2006. A comprehensive listing of all study publications and manuscripts in process as well as guidelines for paper proposal submission are available on the WHI Scientific Resources website at: [https://www.whi.org [www.whi.org Ref #6].

In addition to publications, over 150 ancillary studies have been funded which utilize WHI data, biosamples, and/or related resources. Further, WHI scientists participate in over 50 consortiums most designed to pool data across multiple large cohorts to test hypotheses related to rarer events, several involving dietary factors. The ancillary study topics of relevance to preventive nutrition include such areas as diet and age-related eye disease, body composition and breast and bone density, vitamin supplements and cognitive change, gene–environmental interactions in colorectal cancer prevention, serum fatty acids and ischemic stroke, biochemical and anthropometric heterogeneity and obesity, selenium and colorectal cancer risk, and choline/betaine intake and chronic disease. In addition, two dietary and/or physical activity measurement error studies have been completed to develop calibration equations for energy and protein misreporting in the

WHI population: the Nutritional Biomarkers Study and the Nutrition and Physical Activity Assessment Study ([50–52]). These ancillary studies frequently include assessment of additional research biomarkers beyond the core analytes funded under the parent WHI study. Investigators interested in proposing an ancillary study are encouraged to partner with WHI investigative team members. Details are available on the WHI operations website at: http://www.nhlbi.nih.gov/whi and https://www.whi.org [6].

WHI Diet Modification Study

Study Hypotheses

The Women's Health Initiative (WHI) Diet Modification (DM) trial was initiated to test two primary hypotheses:

1. The intervention diet (described below) as compared to usual diet, adhered to over a period of 9 years, would significantly reduce the risk for breast cancer among postmenopausal women.
2. The intervention diet (described below) as compared to usual diet, adhered to over a period of 9 years, would significantly reduce the risk for colorectal cancer among postmenopausal women.

The secondary hypothesis was:

1. The intervention diet, as compared to usual diet, would result in a significantly lower incidence of CVD.

Study Population

The WHI dietary modification trial recruited 48,835 healthy postmenopausal women across 40 clinical sites nationally into a randomized, controlled trial between 1993 and 1999. The details of the DM trial design have been previously published [53]. The inclusion criteria for the diet trial required enrolled women to be between the ages of 50 and 79 years of age; exclusion criteria included cancer diagnosis in previous 10 years; any previous history of breast or colorectal cancer, predicted lifespan of less than 3 years. In addition women completed a FFQ (designed specifically for WHI) [54] at baseline to determine if their dietary fat intake accounted for less than 32 % of total daily energy intake; if so, the women were excluded from the DM trial. The demographic and clinical characteristics of the DM study population have been previously described [48]. Generally the study participants were white (approximately 20 % minority), well-educated, married, retired or unemployed, and were overweight with a mean BMI of just over 27 kg/m^2 and with significant abdominal obesity with a mean waist circumference of 89 cm. Only 7 % were current tobacco smokers. The average physical activity levels were estimated on self-report at 10 METS/day [48].

Dietary Counseling

Study women were randomly assigned in a 40:60 distribution to either a low fat diet or their usual/control diet ($n = 19{,}541$ and 29,294, respectively) (Fig. 19.2). The low fat diet consisted of 20 % of energy as fat. As time went on the intervention further focused on reducing saturated fat intake to <7 % kcal and increasing intake of vegetables or fruits daily to 5+ servings/day as well as increasing whole grains to >6 servings/day [55]. No weight loss component was included.

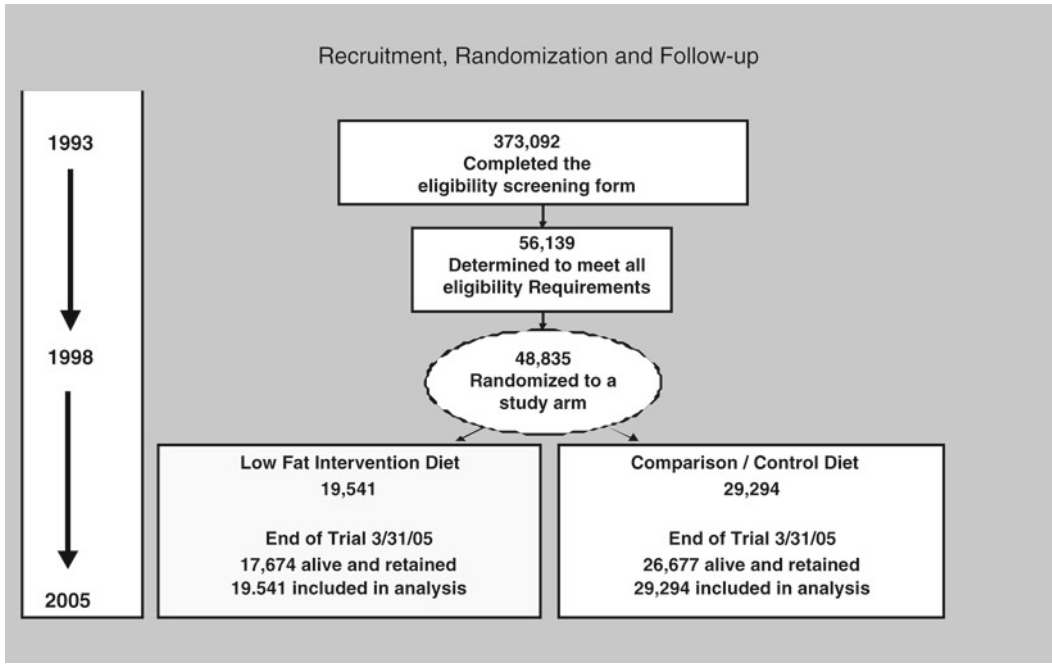

Fig. 19.2 WHI DM trial: recruitment, randomization, and follow-up

Dietary Counseling

In order to achieve and sustain the dietary change goals of the WHI DM low fat diet intervention, study subjects participated in a standardized counseling program that provided women with one individual counseling session with a registered dietitian/nutritionist, in which participants received a fat gram target based on 20 % of reported calories at baseline, followed by 18 small group (8–15 women) during the first 12 months on study and 4 sessions/year during the following years, ending in summer 2004 [53]. The education and counseling regarding fat intake focused on limiting grams of fat. A number of behavioral change theoretical models and tools were applied during the counseling process to promote dietary adherence including self-monitoring, motivational interviewing, goal-setting (daily fat gram goal), targeted messaging, and tailored feedback (Fig. 19.3) [55]. Further, given the emphasis of the study on minority ethnic/racial group recruitment/representation, all written materials were evaluated for acceptance and translation among special populations.

Trial Duration, Data Collection and Time Points

The WHI DM trial continued for 12 years with a mean follow-up period of 8.1 years. Dietary intake was measured at baseline and year 1 for all study participants using the WHI food frequency questionnaire; follow-up measurements using the FFQ continued with a rotating one-third of the population completing the FFQ annually (100 % study sample over 3 years), through year 9. In addition, all subjects provided a 4-day food record at baseline and a 4.6 % random subsample of the population completed a single 24 h recall of dietary intake at year 3, 6, and 9. A subsample of 1311 DM participants enrolled in the follow-up observational period for longer-term evaluation of health outcomes also provided a single 24 h dietary recall an average 2 years after the trial period to assess stability of

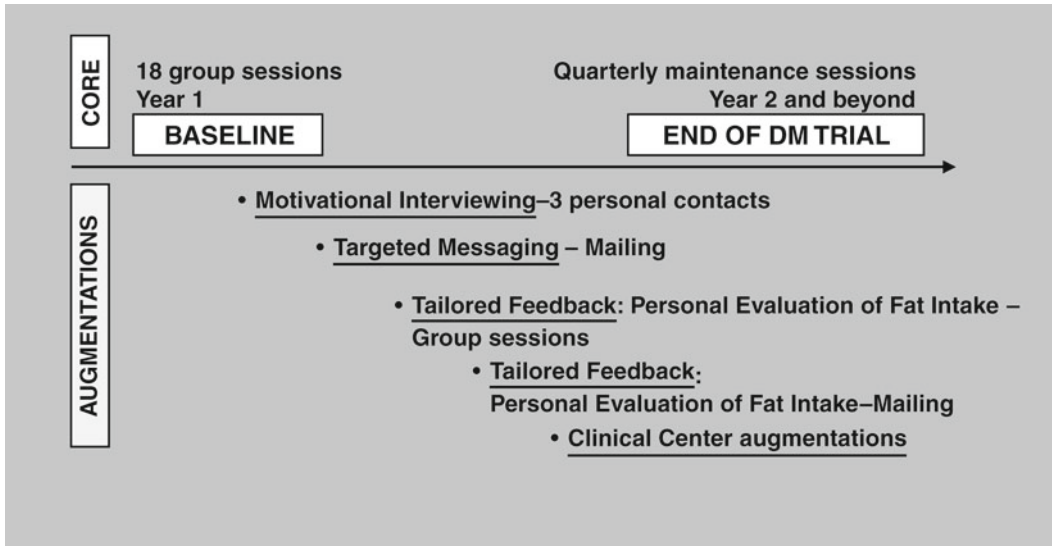

Fig. 19.3 WHI dietary intervention strategies

dietary adherence post-intervention. The FFQ was the primary dietary assessment method for the DM trial [54], but the repeat 24 h recalls and/or diet records are being used to describe consistencies or differences in diet-outcome associations derived from the different diet data collection methods.

In addition to repeated measures of dietary intake, blood samples for biospecimen acquisition were collected on all DM study subjects at baseline and years 1, 3, and 6. Biosamples for a random subsample of DM trial subjects have been evaluated for key nutrients including plasma carotenoids, serum 25(OH)Vitamin D, as well as health indicators such as glucose, insulin, and lipids as part of the core analytes subsample.

Outcomes including breast and colorectal cancer, CVD, and stroke were collected on study participants through bi-annual telephone calls from trained study outcome assessors at the 40 clinical sites across the USA [56]. Once a self-report of one of the key health outcomes was collected, corresponding medical records were collected locally, adjudicated locally, and were sent to the study clinical coordinating center in Seattle to verify the self-report and local adjudication. The medical records were then reviewed by centrally trained medical doctor adjudicators as has been previously described [56]. Self-reports of hospitalizations and major health events were also followed by collection of medical records; for these, adjudication was limited to the local site.

Was Dietary Change Achieved?

At the time of study enrollment, the two randomized groups demonstrated the same dietary intake pattern. Early results from the WHI DM trial demonstrated significant reductions in dietary fat intake and increases in fiber and fruit/vegetable intake in women randomized to the low fat diet arm of the trial [57, 58]. Figure 19.4 illustrates the mean differences in dietary intake between the intervention and control groups at year 3 of the WHI DM trial for select nutrients and food groups which were a target for behavioral modification. At the 3 year time-point the total energy as fat was 25.4 % lower in the intervention compared to the control group. Differences were not only apparent for total fat intake, but also for intake of saturated fat, vitamin E (high in vegetable oils, nuts/seeds), and red meat. Of note, post-intervention analysis of dietary intake longer-term (approximately 2 years post trial end)

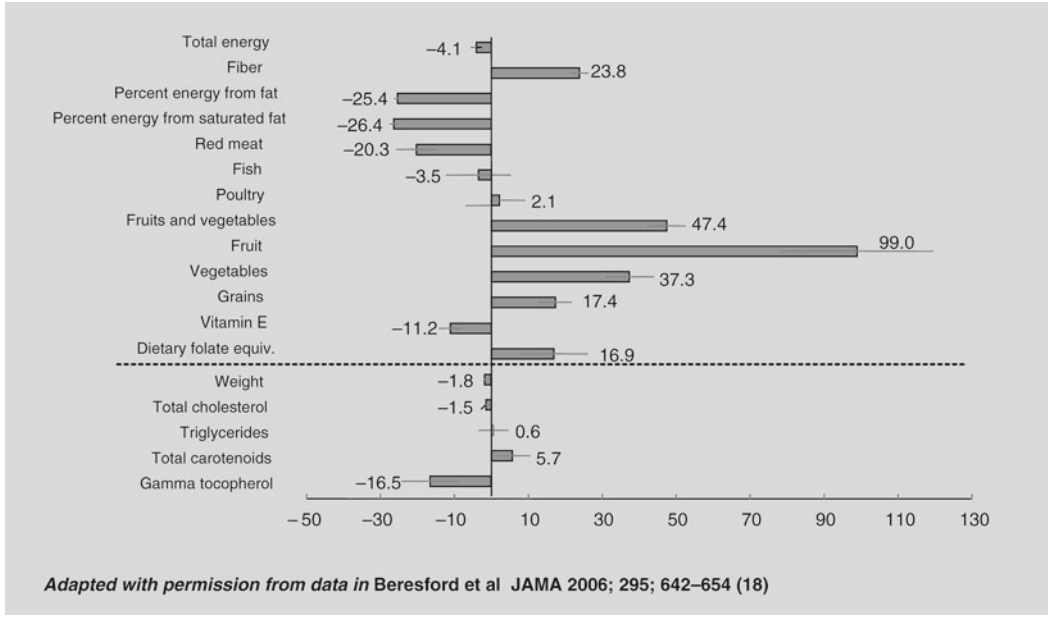

Fig. 19.4 Mean percentage change in intake and clinical measures among women in the WHI-DM low fat diet group

suggested that women randomized to the low fat intervention arm while showing an increase in fat intake over time, maintained an overall fat intake below that of women randomized to the control group [14].

In addition to changes in self-reported dietary intake, changes in select health-related biomarkers were evaluated among a randomly selected 6 % subsample of the DM intervention and control women and supported the self-reported dietary change measures. These analyses showed that the net difference in serum cholesterol favored the intervention by −3.3 %, LDL by −3.5 % and as is common with diets low in saturated fat, HDL decreased slightly while total triglycerides showed an increase in the DM intervention diet group [9]. Gamma tocopherol (vitamin E) decreased by 0.21 μg/dL. In relation to fruit and vegetable intake, total plasma carotenoids increased only slightly by an average 0.04 μg/dL in the intervention diet group. Serum estradiol levels were also significantly reduced while sex hormone binding globulin increased in the study population randomized to the low fat diet group [10].

Several publications have evaluated factors associated with adherence to the low fat diet. Factors reported to be associated with lower adherence to the DM diet included advanced age, minority ethnicity, lower SES, and obesity [59]. Among older WHI participants at eastern state sites of WHI, adherence was highest among assertive women, those who had valued a low fat diet for many years of adult life, and those who felt they had acquired the requisite knowledge and skills for dietary change; in contrast, non-adherent women were unable to effectively resist emotional eating and were more concerned with negative response from others regarding their participation in a low fat diet plan [59]. Of note, similar factors explained non-adherence to the National Cholesterol Education Program dietary guidelines for elevated cholesterol in WHI OS study women [60] with the addition of being married, current smoking and lower physical activity. When a subsample ($n = 100$) of DM trial participants were queried regarding the definition of healthy eating, these women defined healthy eating in terms of greater intake of fruits and vegetables, followed by higher whole grain intake, lower fat and lower meat/protein. Of interest, in WHI DM women 39 % reported “healthy” equivalent to “balanced” [61]. In this sample of women, those who demonstrated a capacity to maintain the WHI dietary goals valued the behavior, “don’t overeat” while non-maintainers described healthy eating as, “consistent/

Women's Health Initiative -- Keeping Track of Goals

Name: _____ Fat Gram Goal: _____ Session # _____ Group # _____

Fat Gram Score: _____ **F/V Score:** _____ **Grain Score:** _____

One Fruit/Vegetable Serving = 1/2 cup cut up fruit or vegetable • 1/2 cup potatoes • 1 medium piece of fruit
1 cup raw leafy vegetable • 3/4 cup juice • 1/4 cup dried fruit

One Grain Serving = 1 slice bread • 1/2 bagel • 1/2 cup cooked cereal or grits • 1 cup cold cereal
8 crackers • 3 cups popcorn • 1/2 cup pasta, rice, or noodles • 1/2 cup cooked dried peas or beans

Date:_____	Date:_____	**Goals/Notes.....**
F/V Serv: □ □ □ □ □ □ □ □ □ =____	F/V Serv: □ □ □ □ □ □ □ □ □ =____	
Grain Serv: □ □ □ □ □ □ □ □ □ =____	Grain Serv: □ □ □ □ □ □ □ □ □ =____	
Fat Grams:	Fat Grams:	
Breakfast = _____	Breakfast = _____	
Lunch = _____	Lunch = _____	
Dinner = _____	Dinner = _____	
Snacks = _____	Snacks = _____	
Total Fat Grams = _____	**Total Fat Grams** = _____	
Date:_____	Date:_____	**Goals/Notes.....**
F/V Serv: □ □ □ □ □ □ □ □ □ =____	F/V Serv: □ □ □ □ □ □ □ □ □ =____	
Grain Serv: □ □ □ □ □ □ □ □ □ =____	Grain Serv: □ □ □ □ □ □ □ □ □ =____	
Fat Grams:	Fat Grams:	
Breakfast = _____	Breakfast = _____	
Lunch = _____	Lunch = _____	
Dinner = _____	Dinner = _____	
Snacks = _____	Snacks = _____	
Total Fat Grams = _____	**Total Fat Grams** = _____	
Date:_____	Date:_____	**Goals/Notes.....**
F/V Serv: □ □ □ □ □ □ □ □ □ =____	F/V Serv: □ □ □ □ □ □ □ □ □ =____	
Grain Serv: □ □ □ □ □ □ □ □ □ =____	Grain Serv: □ □ □ □ □ □ □ □ □ =____	
Fat Grams:	Fat Grams:	
Breakfast = _____	Breakfast = _____	
Lunch = _____	Lunch = _____	
Dinner = _____	Dinner = _____	
Snacks = _____	Snacks = _____	
Total Fat Grams = _____	**Total Fat Grams** = _____	

Fig. 19.5 DM monitoring tools: keeping track of goals

patterned" eating. These results suggest that restricted total intake that is not perceived as inflexible day-to-day may be a significant factor in achieving and maintaining the WHI eating pattern. The use of self-monitoring tools including food diaries, Fat Scan, keeping track goals, Quick Scan, Picture Tracker and eating pattern changes, varied among women assigned to the low fat diet with approximately half of the women using the self-monitoring tools provided. Overall, the number of total days of self-monitoring annually was inversely associated with total percentage of energy intake as fat [62]. Sample self-monitoring tools are shown here as their adoption for other patient counseling programs should be considered as a method to promote dietary adherence to specific dietary goals (Figs. 19.5 and 19.6).

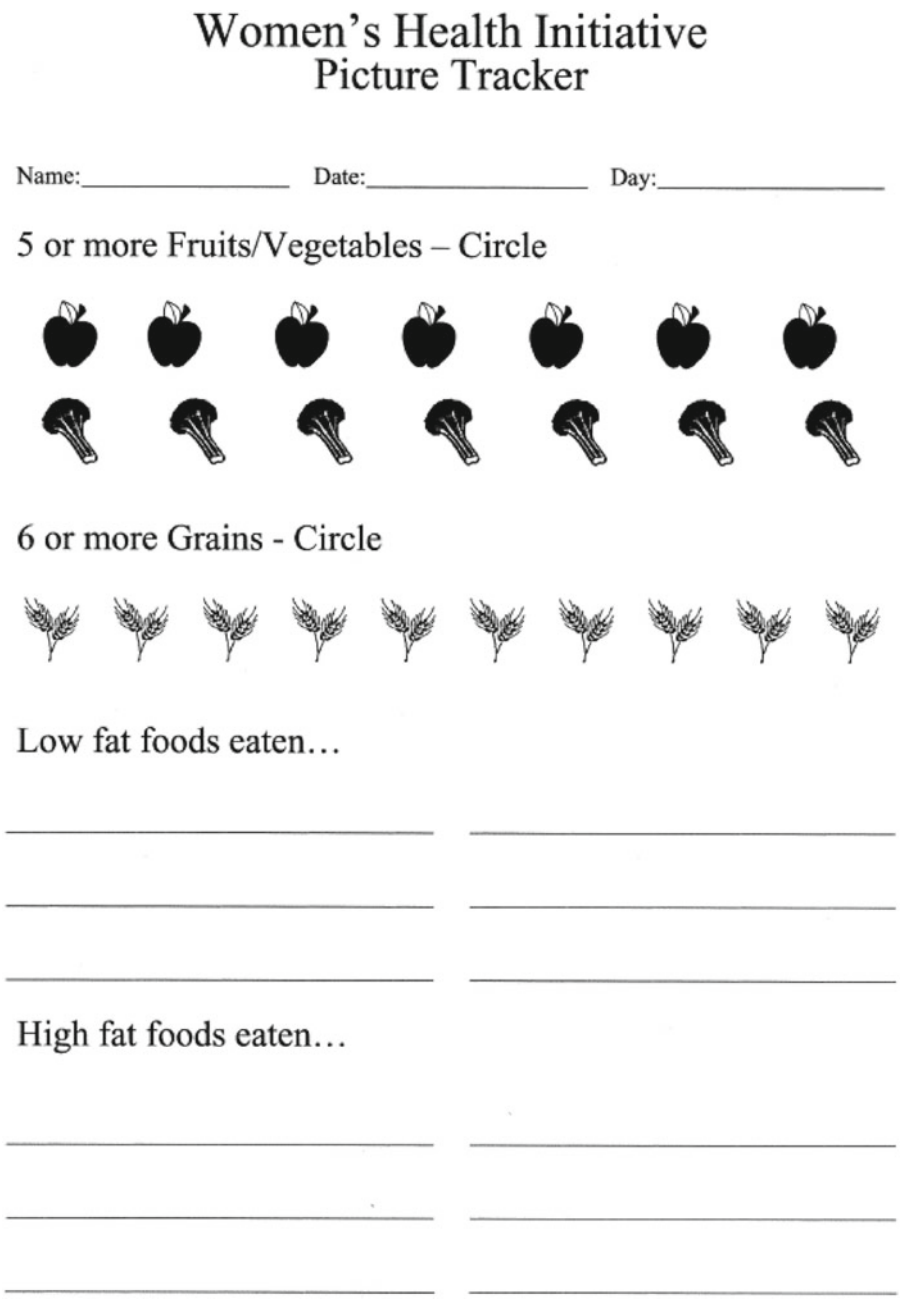

Fig. 19.6 WHI DM monitoring tools: picture tracker

Functional or mental health statuses are also thought to potentially affect success with dietary modification. In an analysis among 13,277 of the 19,542 women in the DM trial, baseline administration of the SF-36 Health Survey, a standardized, validated instrument which includes eight subscale assessments of functional status and well-being, suggested that adherence to the low fat intervention plan was associated with select measures of physical and functional health. For example, greater physical functioning was associated with attendance at more group counseling sessions as well as

self-monitoring of fat intake [63]. Self-monitoring was also more likely in women who scored higher on the mental health, social functioning, and vitality measures. Since fat intake self-monitoring also predicted adherence to the WHI diet, these data are suggestive in that patients with higher SF-36 may be more successful with adopting and complying with medically prescribed changes in dietary behaviors. In support of this assumption, higher optimism was shown to be associated with higher diet quality at baseline as well as improvements in diet quality over time in DM and OS women [64].

Early results from WHI suggest that most women assigned to the low fat arm were able to achieve the daily fat gram goal by making appropriate lower fat or nonfat substitutions in food intake and/or by eliminating added fats such as gravies, sauces, and salad dressings generally [58]. Fat intake also was reduced by restricting dessert, meat, and dairy intake. Added fats and meat contributed the greatest percentage of fat calories in the diet at the time of study enrollment, and were reduced the most in the DM low fat diet arm during year. Of interest, these foods were returned to the diet during year 2 more than other fat sources, suggesting that these specific reductions in intake were challenging for the study participants to sustain long term. Additional predictors of dietary change included higher level of education, younger age, attending more of the dietary counseling sessions as well as reporting a more optimistic approach to life [65].

The WHI DM Trial, Energy Intake, and Body Weight

The WHI study had no dietary goal related to total energy intake nor did the counseling include efforts to promote weight loss among the women on study. Yet, caloric intake was a slight 4.1 % lower, on average, among women randomized to the low fat diet as compared to the usual diet groups [57]. This early reduction in energy intake resulted in the small (approximately 2 kg average) loss of body weight shown in the low fat diet group during the first year on study. Of interest, the initial weight loss during year 1 was followed by a pattern of slow, steady weight regain that paralleled the pattern seen in control group women throughout the study so that, on average, the intervention group women had returned to a mean body weight just above the initial values for body weight at the 8.1 year time point [66]. This pattern of steady body weight gain over several years in both diet groups after year 1 was shown for women under age 60 years. In older women, control group assignment was associated with an initial stable body weight followed by a steady decline over time while intervention provided an initial weight loss during year 1, a slight regain during the next several years, and then a steady decline such that age over 70 years resulted in a net loss of body weight at year 8 of the WHI trial. These differential patterns of weight change over time in relation to diet group assignment as well as age are likely to have clinical relevance in terms of health outcomes in this population as outcomes continue to be collected longitudinally. Analysis of the relationship between the low fat diet and changes in body composition generally supported earlier findings related to weight. Briefly, an analysis of 4311 DM women with DXA measurements showed that assignment to a low fat diet was associated with significant reductions in percentage body fat, fat mass and lean mass as compared to control group assignment [67].

Body weight changes also were evaluated in relation to assignment to the intervention versus placebo arm of the calcium/vitamin D clinical trial within WHI. Caan et al. reported randomization to calcium (1000 mg)/cholecalciferol (440 IU) resulted in a small (–0.13 kg), but statistically significant difference in weight gain at year 3 [68] of the CaVitD trial. Weight control was only significant among women who reported a total calcium intake from diet and supplement of <1200 mg at study initiation, suggesting that the beneficial effects are dependent on baseline exposure, and that supplementation of women with higher baseline calcium intake does not provide additional protection against weight gain in later life. The analysis evaluated the combined exposure to both dietary and supplemental

calcium; based on the outcomes, no specific recommendations can be made regarding diet versus supplemental calcium.

Energy intake is commonly misreported using self-report instruments, particularly FFQs. To address this limitation the WHI conducted two ancillary studies to objectively assess energy intake using doubly weighted water in a sample of over 500 women. These objective measures were used to develop "calibration" equations to "correct" energy exposure from self-report. These calibrations suggested that total energy intake in WHI women is associated with 49 % higher risk for CVD, a 43 % greater risk for invasive breast cancer, and a 4.2-fold greater risk for diabetes [69].

Key Outcomes from the DM trial

The hypotheses of the DM trial have been presented above. The design assumption was that women assigned to the low fat diet would reduce their fat intake from 40 to 20 % of energy as fat, and that after a 9 year period breast cancer risk would be reduced by 50 % and colorectal cancer risk by 30 % [53]. Recruitment efforts at that time, with heightened awareness in the population of the potential relationship between eating excess fat and CVD risk resulted in a sample population that reported baseline dietary fat intake that was much lower than desired. Indeed, an exclusion criterion was implemented such that women consuming diets with <32 % energy from fat were ineligible for study participation. As anticipated, some regression to the mean occurred over time such that the difference across diet groups was maximal at year 1 (10.7 %), and reduced through each subsequent measurement to 9.5 and 8.1 % at years 3 and 6 [70]. Further, a reduction in participating clinics from 44 to 40 resulted in heightened recruitment during the final years; this resulted in a mean average years of follow-up of 8.1 years rather than the 9 years established for initial power estimates. In the end, the difference achieved was only 70 % of the design assumptions and the power to test diet-associated hypotheses was significantly reduced.

Low Fat Diet and Breast Cancer

The primary DM trial results were published in 2006 and suggested that assignment to the low fat diet resulted in a nonsignificant protective association with invasive breast cancer risk (RR: 0.91, 95 % Confidence Interval (CI): 0.83–1.01) [10, 71]. This number was what would be predicted from the initial study assumptions when the decreased fat intake difference and decreased follow-up time were considered. In total 1727 invasive breast cancers were diagnosed and adjudicated during the study period, representing 3.5 % of the DM population [71]. When 4 day food records were used to estimate change in fat intake as a percentage of total energy, a subgroup analysis showed that among women who reported fat intake at the uppermost quartile (>36.8 % fat kcals) and reduced dietary fat by an average of 12.2 % over the course of the study, invasive breast cancer risk was reduced by 22 % (RR: 0.78, 95 % CI: 0.64–0.095) ($p = 0.04$). A separate subgroup analysis indicated that the low fat diet may have proven beneficial in reducing estrogen receptor positive tumors that co-expressed progesterone receptor negativity. In this group the RR was 0.64 (95 % CI: 0.49–0.84); no association was found for any other hormone receptor subgroup. Of interest, when dietary fat and breast cancer outcomes were assessed using the two different dietary measurement instruments, the data showed a significant protection against breast cancer when Food Record data are applied, but not when FFQ diet data are applied, suggesting measurement imprecision known for FFQ data may have accounted for the inability to detect a significant risk association within WHI [72]. In addition, a subsequent analysis of the low fat dietary associations with all cancer sites suggested the low fat diet, after a mean 4 years on this assignment, was potentially protective against ovarian cancer [71].

In 2014, an analysis of longer-term, posttrial intervention cancer outcomes was performed to determine if the DM trial arm assignment was associated with breast (and other cancers) risk. The results suggested that while women in the intervention arm demonstrated an increase in fat intake post-intervention, overall fat intake remained below that of women randomized to the control arm. However, there was no indication of intervention group assignment being associated with longer-term breast cancer risk [14]. Similar to the trial period, there was some evidence of a protective effect of the low fat diet group assignment on risk of estrogen receptor positive/progesterone receptor negative breast cancer longer term.

Low Fat Diet and Colorectal Cancer Risk

Over the course of the DM trial (8.1 years) 480 women were diagnosed with invasive colorectal cancer. Despite a significant reduction in dietary fat intake, which averaged 10.7 % at year 1 and 8.1 % at year 6, there was no significant difference in colorectal cancer rates for women randomized to the low fat or comparison diets (HR 1.08, 95 % CI: 0.90–1.29) [8]. While not statistically significant, the data was suggestive of elevated risk for proximal colon cancers (HR: 1.25, 95 % CI: 0.96–1.61), associations between the low fat intervention and protection from rectal cancer were also nonsignificant, but the number of total cases was small ($n = 117$). Analysis of dietary factors previously reported to be associated with colorectal cancer in the published literature showed that none were associated with increased (energy, fat, red meat, alcohol) or decreased (fruits/vegetables, whole grains, carotenoids, folate, or dietary calcium) risk of colorectal cancer [8]. Longer-term follow-up showed results consistent with the active trial period suggesting no protective effect of the low fat diet assignment on colorectal cancer risk [14].

Low Fat Diet and Cardiovascular Disease Risk

A number of cardiovascular-related diagnoses were evaluated in the context of the WHI DM trial. In relation to hypertension, the low fat diet was associated with a mean decrease in systolic and diastolic blood pressure of −0.2 mmHg and −0.3 mmHg, respectively. Stroke, a common consequence of poorly controlled blood pressure, occurred in a total of 1076 women or 2 % of the DM population. Stroke risk was not associated with low fat dietary assignment [9]. Blood pressure was not modified in relation to treatment assignment for the CaVitD trial subjects [73].

Overall there were 2549 coronary heart disease diagnoses/events (myocardial infarction, bypass, stent, angioplasty) [9]. In relation to heart disease risk, factor VIIC was reduced, on average, by 4.9 % more from baseline to year 3 in the intervention diet versus control group subjects but total CVD rates were not significantly reduced with the low fat diet (RR: 0.98; 85 % CI: 0.92–1.05) nor were CHD or CHD death [9]. With the mildly reduced RR for a number of cardiovascular-related measures, assessment of cardiovascular risk using the Framingham Risk score suggested the WHI low fat diet resulted in a net 3–4 % risk reduction [9].

An analysis of DM trial arm assignment and longer-term effects of CVD outcomes is underway.

Low Fat Diet and Total Mortality and Global Health Index

When comparing global index for the intervention subjects ($n = 2051$ annualized cases) and comparison group subjects ($n = 3207$ annualized cases), no significant difference was demonstrated. Similarly, mortality rates were also comparable across diet groups suggesting the low fat diet also did not modify these more global indicators of health status [70]. A follow-up evaluating the mortality risk in DM

women also showed no protective effect of randomization to the low fat diet group in terms of change in mortality risk [14].

Role of Low Fat Diet in Modifying Other Health Risks

The DM cohort (along with the OS cohort) with the repeated measures of diet and lifestyle factors as well as adjudicated and self-reported health outcomes have provided an unprecedented opportunity to evaluate the role of diet in modifying the risk of a number of common health outcomes. Of particular interest is the role of the diet in influencing risk for diabetes given the known association between diabetes and our primary and secondary outcomes. In 2008, Tinker et al. reported that there was no difference in diabetes incidence across dietary assignments [74]. However, analysis using calibrated versus uncalibrated energy and protein intake in 74,155 WHI participants did suggest that a 30 % increased risk for diabetes in women within the highest quintile of energy intake; similarly calibrated protein intake was also associated with higher diabetes risk [75]. Additionally the calcium and vitamin D trial provides similar opportunities. The results of numerous analyses are summarized in Table 19.3, where association being evaluated, analytical cohort, sample size, and risk ratios are described.

Clinical Applications

The outcomes of the DM study within WHI did not provide statistically significant support for a low fat diet in reducing risk of breast cancer or colorectal cancer. Nor was the diet intervention associated with reduced risk of CVD, obesity, or diabetes. However, there are a number of lessons learned along the way that will help to advance diet–disease association research for years to come [70]. The lessons learned include:

- Self-report of dietary intake using a FFQ may not be a good estimate of actual intake. Having a more detailed dietary record or multiple recalls could provide stronger results. Also having additional biomarker analyses to measure adherence and guide individual change in diet as well as to estimate measurement error within the total population would strengthen future study designs. While the cost associated with such an undertaking was prohibitive during the development of WHI, new and emerging technologies may enable population level dietary assessments at a lower cost. The completed nutritional biomarker studies should also help to advance our understanding of measurement error in diet research [50].
- Adopting a low fat diet late in life may be insufficient "exposure" to reduce or reverse detrimental effects of lifelong eating. Studies targeting women of younger age should be considered. However, there is no indication from subgroup analysis that women in the 50–60 year age group had any different results in terms of low fat diet and health outcomes than the women recruited between the ages of 70 and 79 years.
- The absolute change in dietary fat (and possibly fruits and vegetables) and duration of dietary fat restriction may have been insufficient to modify disease risk. For example, despite a concerted effort to screen out women with lower fat diets, many women, at the time of study entry, had dietary fat intakes below the average for women of the same age in the general population, thus the actual reduction in exposure over time was not as great as was initially predicted. Further, the study was terminated short of the original design estimates and adherence was lessened over time (as has been demonstrated in numerous dietary intervention trials).

Table 19.3 Summary of WHI diet, nutrition research findings from the DM and calcium/vitamin D clinical trials and WHI observational study (as of fall 2014 [84])

First author, journal, Year	Sample population	Dietary/anthropo-metric measure	Outcome measure	Key findings	Comment
Overall health					
Brunner et al., J Am Diet Assoc, 2008 [76]	33, 067 Females (50–79 years) supplement = 1000 mg Ca and 400 IU Vitamin D	Calcium intake	Physical functioning	No significant difference between supplement vs. placebo group	Study findings did not support Vit. D/Ca protecting against physical function decline
Ma et al., Nutrition 2008 [77]	1958 postmenopausal women, observation grp	Dietary fiber intake	Inflammation : Hs-CRP. IL-6, TNF-αR2	Dietary fiber inverse assoc. w/IL-6 and TNF-αR2 (p-value = 0.01 and 0.02, respectively)	No association between hs-CRP and dietary fiber in postmenopausal women
George et al., Am J Epidemiol, 2014 [78]	63,805 WHOI OS women	Dietary quality score	Chronic disease mortality risk	Higher diet quality score(s) were associated with an 18–26 % lower all-cause and CVD mortality; higher HEI, a Mediterranean and DASH score were associated with a 20–23 % lower cancer mortality	Dietary quality appears to protect against mortality in postmenopausal women diagnosed with cancer or CVD
Cancer					
Thomson et al., Cancer Prev Res, 2014 [79]	WHI OS participants	Adherence to cancer prevention guidelines from ACS	Incident cancer and cancer mortality	65,838 women, 8632 incident cancers, 2356 cancer deaths. Adherence to healthy diet, activity and weight control was associated with a 22 % lower risk for breast cancer, 52 % lower risk for CRC, and 20 % lower cancer-specific mortality. Risk was even lower in Hispanic women	Adherence to ACS cancer prevention guidelines is associated with lower cancer risk and higher cancer survival in postmenopausal women
Breast					
Chlebowski, J Natl Ca Inst, 2008 [80]	36,282 WHI women reenrolled in CaVitD trial	Trial arm assignment to CaVitD or placebo	Invasive breast cancer	1074 cases. No difference in risk demonstrated. Baseline 25OH Vitamin D also showed no association with risk	In this sample of relatively well-nourished postmenopausal women, CaVitD supplementation and baseline vitamin D status was not associated with breast cancer risk

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Table 19.3 (continued)

First author, journal, Year	Sample population	Dietary/anthropo-metric measure	Outcome measure	Key findings	Comment
Cui et al., Am J Clin Nutr, 2008 [81]	84,805 WHI OS women	Dietary α and β-carotene, lycopene, Vit. E and Vit. C	Invasive breast cancer	2509 cases. Dietary α and β-carotene, lycopene inversely assoc. w/ER + PR+ breast cancer	Vit. E (regardless of source) and dietary Vit. C not associated w/decrease risk of any type of breast cancer. Supplemental Vit. C showed weak assoc. w/overall breast cancer risk
Duffy et al., Breast Cancer Res Treat, 2009 [82]	93,676 WHI OS women	Alcohol and folate	Incident invasive Breast cancer	1783 cases; linear increase in risk with higher alcohol intake. Relationship not modified by folate intake	Consistent with other studies alcohol was associated with higher breast cancer risk, dietary folate did not modify this risk although this analysis is in the post-fortification era and supplemental folic acid was not evaluated
Gunter et al., J Natl Cancer Inst, 2009 [83]	93,676 WHI OS women-nested case–control subsample	Circulating insulin and insulin-like growth factor-1	Incident invasive breast cancer	835 cases/816 matched controls. Higher fasting insulin was associated with a 46 % higher risk for breast cancer, risk limited to nonusers of hormone therapy	Higher circulating insulin is associated with breast cancer in postmenopausal women not using hormone therapy
Kabat et al., Am J Clin Nutr, 2009 [84]	5450 WHI CT women with baseline measures of serum carotenoids	Baseline and repeat measures of serum carotenoids, tocopherol and retinol	Incident invasive breast cancer	190 incident cases. Baseline α carotene was associated with a 45 % lower risk. Repeat measures showed a trend toward lower risk with higher serum β carotene concentrations (p =0.02)	Higher serum α and β-carotene concentrations, indicators of higher vegetable and fruit intake, may be associated with lower risk for postmenopausal breast cancer
Morimoto et al., Cancer Causes Control, 2002 [85]	85,917 postmenopausal women in the observational group	Body weight, lifetime weight history	Incident invasive breast cancer	1030 women were diagnosed with invasive breast cancer. Among HRT nonusers, heavier women (baseline BMI >31.1) had an elevated risk of postmenopausal breast cancer RR: 2.52; CI: 1.62, 3.93, compared to slimmer women (BMI < 22.6)	Increasing BMI appears to be most pronounced among younger postmenopausal women. Change in BMI since age 18, maximum BMI, and weight were also associated with breast cancer in HRT nonusers. Lifetime weight gain is also a strong predictor of breast cancer. Waist to hip ratio was not related to postmenopausal breast cancer risk
Prentice et al., JAMA, 2006 [10]	48,835 postmenopausal DM group (40 % intervention/60 % obs.)	Dietary fat, fruit, vegetable, and grain intake	Invasive breast cancer	655 breast cancer cases observed in the intervention grp vs. 1072 in comparison grp (HR 0.91, 0.83–1.01)	Low-fat diet did not show a statistically significant reduction in breast cancer; however non-sig. trend suggests decreased risk w/longer follow-up

Cancer Epidemiol, 2013 [86]	OS+CT with DXA measurements		cancer	associated with a 53 % higher risk; trunkal fat was associated with a twofold higher risk. Similar elevated risk was shown for BMI and WHR	adiposity or body fat is consistently associated with higher risk for postmenopausal breast cancer
Shikany et al., Nutr Cancer, 2011 [87]	148,767 DM+OS WHI women	Glycemic index, load and carbohydrate intake	Invasive breast cancer cases	6115 breast cancer cases, HR 1.08, 1.01 and 0.95 for GL, GI and carbohydrate intake, respectively	No association demonstrated
Wassertheil-Smoller et al., Breast Cancer Res Treat, 2013 [88]	WHI OS+CT women with prior diagnosis of invasive breast cancer	Multiple vitamin-mineral use	Mortality after invasive breast cancer cases	7728 cases; 518 deaths. Breast cancer mortality was 30 % lower in MVM users	MVM use may improve survival after breast cancer in postmenopausal women
Colorectal					
Bae et al., Cancer Res, 2014 [89]	Nested case–control sample within WHI OS	Plasma methyl donors (choline, betaine, dimethylglycine, trimethylamine N-oxide)	Incident colorectal cancer	835 matched case–control women. Plasma choline was associated with 2.44> risk for rectal cancer ($p=0.08$); plasma betaine was associated with a 32 % lower risk for colorectal cancer; the ratio was associated with a 44 % lower risk for CRC. Plasma TMAO was associated with a 3.38-fold > risk for rectal cancer	Early evidence suggests plasma choline and associated metabolites are associated with rectal and CRC risk, suggesting a role for the microbiome in setting risk estimates in postmenopausal women
Kabat et al., Cancer Causes Control, 2008 [90]	1476 colorectal cancer cases within WHI (158,800)	Glycemic Index/ Glycemic Load (GI/GL)	Incident colorectal cancer	Total carbohydrate intake, GI, GL, intake of sugars and fiber showed no assoc. w/colorectal cancer	Analyses by cancer subsite yielded null results w/exception of rectal cancer GL(HR between highest and lowest quartiles 1.84, 0.95–3.56)
Kabat et al., Cancer Causes Control, 2013 [91]	11,124 WHI OS+CT women with DXA measurement	Body fat	Incident colorectal cancer	169 new CRC cases; body fat as assessed by DXA or BMI, WHR was not associated with risk	Obesity does not appear to be associated with a higher risk for CRC in postmenopausal women
Wactawski-Wende J et al., N Eng J Med, 2006 [11]	36, 282 Females (50–79 years) supplement= 1000 mg Ca and 400 IU Vit. D	Calcium and Vitamin D intake	Incident colorectal Cancer	168 cancer cases in the supplement grp vs. 154 in comparison grp (HR 1.08, 0.86–1.34, $p=0.51$)	Calcium/Vitamin D supplementation for 7 years had no effect on incidence of colorectal cancer in postmenopausal women
Beresford et al., JAMA, 2006 [8]	48,835 postmenopausal DM group (40 % intervention/60 % obs.)	Dietary fat, fruit, vegetable, and grain intake	Incident colorectal cancer	201 cancer cases in the intervention grp vs. 279 in comparison grp (HR 1.08, 0.90–1.29)	Low-fat dietary pattern did not reduce risk of colorectal cancer. However potential interaction observed between baseline aspirin use and estrogen-progestin use ($p=0.01$ for each)

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Table 19.3 (continued)

First author, journal, Year	Sample population	Dietary/anthropometric measure	Outcome measure	Key findings	Comment
Zschabitz et al., Am J Clin Nutr, 2013 [92]	88,045 women in WHI OS	B vitamin intake	Incident colorectal cancer	1003 incident cases; dietary B-6 was associated with a 20 % lower risk; total riboflavin (diet + supplement) was associated with a 19 % lower risk. Risk for regionally spread disease was also lower	B-6 and riboflavin appeared to be associated with lower risk for CRC in postmenopausal women
Ovarian					
Prentice et al., J Natl Cancer Inst, 2007 [71]	48,835 postmenopausal DM group (40 % intervention/60 % obs.)	Dietary fat, fruit, vegetable, and grain intake	Ovarian Cancer	Ovarian cancer rates statistically lower in the intervention grp vs. comparison grp ($p=0.03$)	For the first 4 years intervention and comparison grp ovarian cancer risk were similar, however over the next 4.1 years intervention grp showed decreased risk of ovarian cancer
Thomson et al., Nutr Cancer, 2008 [93]	133,614 WHI OS + CT women	Dietary antioxidant (and carotenoid) intake	Incident ovarian cancer	No significant associations demonstrated	Antioxidant intake may not modulate ovarian cancer risk in postmenopausal women
Thomson et al., JNCI, 2014 [94]	WHI OS + CT women	Dietary quality as assessed by HEI	Ovarian cancer survival	Higher diet quality was associated a 27 % lower mortality after ovarian cancer	Diet quality may reduce risk of death after ovarian cancer, a cancer with lower overall survival rates
Other cancers					
Cheng, Am J Clin Nutr, 2013 [95]	WHI OS + CT women	Diet plus supplemental vitamin D	Incident lung cancer	1771 cases. No overall association. Among never smokers risk was 63 % lower with vitamin D intake ≥400 IU	Vitamin D (diet and supplemental) may protect against lung cancer in never smokers
Luo et al., Am J Epidemiol, 2007 [96]	140,057 postmenopausal women	Prospective study looking at weight change and waist to hip ratio	Diagnosis of renal cell carcinoma	269 renal cell carcinoma cases. Waist to hip ratio was assoc. with and 80 % higher risk for renal cell carcinoma. Women who experienced weight cycling more than ten times were at 2.6 times greater risk for developing renal cancer vs. women whose weight remained stable	Obesity, specifically central adiposity, is assoc. with increased risk of renal cell carcinoma among postmenopausal women. This research may also indicate that weight cycling is independently assoc. with an increased risk of renal cell carcinoma in this population
Lou et al., Br J Cancer, 2008 [97]	138,503 postmenopausal women	Prospective study looking at weight and waist to hip ratio	Diagnosis of Pancreatic cancer	251 women were diagnosed with pancreatic cancer. Women in the highest quintile of waist to hip ratio had a 70 % (95 % CI: 10–160 %) excess risk of pancreatic cancer compared to women in the lowest quintile	Obesity, especially central adiposity, increases risk of pancreatic cancer in postmenopausal women

Epidemiol, 2008 [98]	women; smokers vs. nonsmokers	looking at weight and waist to hip ratio	cancer	cancer. Baseline BMI was inversely assoc. with lung cancer risk in current and nonsmokers; waist circumference was positively assoc. with lung cancer risk (HR, 1.56, CI: 0.91, 2.69) in current smokers and (HR, 1.50, CI: 0.98, 2.31) in nonsmokers	BMI is inversely associated with lung cancer risk and that waist circumference is positively associated with risk
Reeves et al., Gynecol Oncol, 2011 [99]	86,937 in WHI OS+CT	BMI and waist:hip ratio	Incident endometrial cancer	806 incident cases of endometrial cancer; Higher BMI increase risk by 76 and; WHR increase risk by 33 %. No association with disease stage or grade	As with other studies, obesity is significantly associated with endometrial cancer risk
Simon et al., Cancer Causes and Control [100]	161,809 in WHI OS+CT	Glycemic index and glycemic load	Incident pancreatic cancer	No association demonstrated	Glycemic index, load, fructose and sucrose as assessed by FFQ were not associated with pancreatic cancer risk in postmenopausal women
Tang, J Clin Oncol, 2011 [101]	36,282 WHI women enrolled in the CaVitD trial	Calcium+Vit. D supplementation vs. placebo	Incident Nonmelanoma skin cancer and melanoma	3338 cases. No difference in NMSC demonstrated. In women with prior history of NMSC showed a 57 % lower risk of melanoma if assigned to CaVit. D	Ca+Vit. D may protect against melanoma in high risk subgroup on postmenopausal women; however, numbers were small and results need to be replicated in other cohorts
Cardiovascular disease					
Allison et al., Stroke, 2014 [102]	48,835 WHI DM participants	Low fat vs. control group assignment	Incident carotid artery disease	Low fat assignment was not associated with incident CAD	Low fat diet may not modify risk for CAD in postmenopausal women
Belin et al., Am J Clin Nutr, 2011 [103]	93,676, WHI OS women	Diet quality as assessed using AHEI and dietary modification index (DMI)	Incident CVD of heart failure	Higher diet quality score as assessed using AHEI and DMI was associated with 9–30 % lower risk for CVD and HF	Diet quality is an indicator of lower CVD and HF risk in postmenopausal women
Berry et al., Am J Cardiol, 2010 [104]	44,720 WHI CT women not enrolled in the DM trial	Fish and dietary omega three fatty acid intake	Atrial fibrillation	378 incident cases of AF; No association demonstrated	Dietary fish intake (unknown preparation, fish type) and dietary (not supplemental) *n*-3 fatty acids did not protect against AF in older women

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Table 19.3 (continued)

First author, journal, Year	Sample population	Dietary/anthropometric measure	Outcome measure	Key findings	Comment
Bertoia et al., Am J Clin Nutr, 2014 [105]	93,122 women in WHI OS	Mediterranean diet score and DASH diet score	Sudden cardiac death	Mediterranean diet score was associated with a 36 % lower risk for SCD; no association with DASH diet score	Higher Mediterranean diet score may protect against SCD in older women
Howard et al., JAMA, 2006 [9]	48,835 WHI DM participants	Dietary fat, fruit, vegetable, and grain intake	Fatal and Nonfatal CHD, stroke, and CVD	The numbers who developed CHD, stroke, and CVD (annualized incidence rates) were 1000, 434, and 1357 in the intervention and 1,549,642, and 2088 in the control group. The diet had no significant effects on incidence of CHD, stroke, or CVD	Trends toward greater reductions in CHD risk were observed in those with lower intakes of saturated fat or trans-fat or higher intakes of vegetables/fruits
Hsia et al., Circulation, 2007 [106]	36, 282 Females (50–79 years) supplement = 1000 mg Ca and 400 IU Vit. D	Calcium and vitamin D intake	Myocardial infarction (MI) or coronary heart disease death, stroke	MI or coronary heart disease death confirmed in 499 in supplement grp vs. 475 in comparison grp (HR 1.04, 0.92–1.18). Stroke confirmed in 362 supplement grp vs. 377 comparison grp (HR 1.04, 0.82–1.10)	Women w/higher calcium intake at baseline were at no higher risk for coronary event or stroke ($p = 0.91$ and $p = 0.14$, respectively) if assigned to supplement group
Levitan et al., Circ Heart Fail, 2013 [107]	Women in WHI with heart failure	Mediterranean and DSAH diet scores	Death ($n = 1385$) in women with previous HF	Higher DASH diet score was associated with a 16 % lower mortality in women with HF; Mediterranean diet score showed a nonsignificant 15% lower risk	Healthier diets as qualified using diet pattern scores may reduce mortality in postmenopausal women with HF
Neuhouser et al., Metabolism, 2012 [108]	48,836 WHI-DM women	Assignment to low fat vs control diet	Metabolic syndrome and new cardiac medication use	At year 3, low fat diet assignment was associated with at 17 % lower risk for MetS; new cholesterol and BP medication use was lower at year 1 in low fat group	A low fat diet may protect against MetS and short term when diet adherence is highest, the diet may lower requirements for BP and cholesterol-lowering medications
Rajpathak et al., Eur J Nutr, 2010 [109]	3198 WHI OS women with Type 2 DM and without history of CVD	Alcohol intake	Incident coronary heart disease	336 cases. Women consuming alcohol >2 times/week demonstrated a 35 % lower risk for CHD	Moderate alcohol intake may be associated with lower CHD risk in postmenopausal women with T2DM, similar to reports of protective effects shown in women without T2DM

2012 [110]	sample of women with and without CHD	defined by cluster analysis of FFQ data		vegetable protein, fiber, vitamin K, folate, carotenoids and with > *n*-3:*n*-6 ratio were associated with a 21 % lower risk for CHD; dietary pattern high in energy, total fat, trans fat were assoc. with a 28 % higher risk for CHD	dietary guidance for CV risk reduction may protect against CHD in postmenopausal women; lifestyle factors such as smoking, low physical activity and higher BMI attenuate these protective associations
Bone health					
Jackson et al., N Eng J Med, 2006 [12]	36, 282 Females (50–79 years) supplement = 1000 mg Ca and 400 IU VitD	Calcium and Vitamin D intake	Hip fractures, bone density and kidney stones	Hip bone density was 1.06 % higher in the calcium plus vitamin D group than in the placebo group ($p < 0.01$)	Among healthy postmenopausal women, calcium/vitamin D supplementation resulted in a small but significant improvement in hip bone density, did not significantly reduce hip fracture, and increased the risk of kidney stones
McTiernan et al., Am J Clin Nutr, 2009 [111]	48,835 DM women	DM low fat vs control diet	Hip fracture	Hip BMD was slightly lower in the DM low fat diet group	No significant difference in hip fracture rates between groups
Orchard et al., J Bone Miner Res, 2013 [112]	Nested case–control of hip fracture	Tertiles of RBC fatty acid status	Hip fracture	Higher RBC α-linolenic acid was associated with a 56 % lower risk for hip fracture; EPA and total *n*-3 fatty acids showed similar protection. High *n*-6:*n*-3 ratio associated with doubling of risk	Biomarkers of higher omega three fatty acid intake suggest a reduced risk for hip fracture in postmenopausal women
Diabetes mellitus					
de Boer et al., Diabetes Care, 2008 [113]	33,951 Females (50–79 years) self-reported, nondiabetic supplement = 1000 mg Ca and 400 IU Vit. D vs. placebo (total grp = 36,282)	Calcium and Vitamin D intake	Diagnosis of oral or injection controlled diabetes mellitus	2291 women were newly diagnosed with diabetes. The hazard ratio for incident diabetes associated with calcium/vitamin D treatment was 1.01 (CI 0.94–1.10) based on intention to treat	Calcium plus vitamin D supplementation did not reduce the risk of developing diabetes over 7 years of follow-up in this randomized placebo-controlled trial
Margolis et al., J Nutr, 2011 [114]	82,076 postmenopausal women enrolled in WHI OS	Dairy product intake	Incident treated DM	3946 cases of new DM; RR 40–50 % lower risk for low fat dairy and yogurt	Low fat dairy is associated with lower diabetes risk, particularly in obese women
Parker et al., Ann Epidemiol, 2013 [115]	72,215 WHI OS women free of diabetes at enrollment	Whole grains	Incident DM	3465 cases. Higher whole grain intake was associated with a 25 % lower risk for DM; 43 % reduction in risk in never smokers; no protective association shown in smokers	Consuming more whole grains may reduce DM risk in postmenopausal women who are never smokers

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Table 19.3 (continued)

First author, journal, Year	Sample population	Dietary/anthropometric measure	Outcome measure	Key findings	Comment
Qiao et al., Ethnic Health, 2014 [116]	161,808 WHO CT+OS women	Alternate healthy eating index score	Incident DM	10,307 incident cases of DM; higher AHEI is associated with a 24 % lower risk for DM. Risk reduction was lowest in Hispanic women (32 %)	Higher diet quality score may protect against diabetes in postmenopausal women, particularly Hispanic women
Robinson et al., Diabetes Care, 2011 [117]	Post-hoc analysis of 5140 in three separate nested case–control studies wherein serum 25(OH)D levels	Serum 25(OH) vitamin D levels	Incident DM	317 cases over 7.3 y. No association demonstrated	Serum VitD levels are not associated with DM risk in relatively nourished postmenopausal women
Tinker et al., Arch Intern Med, 2008 [74]	48,835 postmenopausal DM group (40 % intervention/60 % obs.)	Dietary fat, fruit, vegetable, and grain intake	Diagnosis of oral or injection controlled Diabetes Mellitus	Incident treated diabetes in 1303 intervention participants and 2039 comparison participants (HR, 0.96; 0.90–1.03; $p=0.25$). Weight loss occurred in the intervention group, with a difference between intervention and comparison groups of 1.9 kg after 7.5 years ($p<0.001$)	A low-fat dietary pattern among postmenopausal women showed no evidence of reducing diabetes risk. Trends toward reduced incidence were greater with greater decreases in total fat intake and weight loss
Tinker et al., Am J Clin Nutr, 2011 [75]	74,155 WHI OS+CT women	Energy and protein intake—calibrated with objective measures or uncalibrated (self-reported intake)	Incident DM	Uncalibrated energy intake was not associated with DM risk; higher calibrated energy intake was associated with a 2.41-fold greater risk of DM. Uncalibrated protein intake was associated with a 13 % higher risk for DM while calibrated protein intake was assoc. with a 19 % higher risk for DM	Both greater energy and protein intake in postmenopausal women may increase DM risk
Obesity					
Caan et al., Arch Intern Med, 2007 [68]	36, 282 Females (50–79 years) supplement=1000 mg Ca and 400 IU Vit. D	Calcium intake	Change in body weight	Supplement grp=mean wt. loss of 0.13 kg, 95 % CI 0.21–0.05, p-value=0.001	Vit. D/Ca supplement showed small effect on prevention of wt. gain, primarily observed in women with inadequate Ca intake
Carty et al., Am J Clin Nutr, 2011 [67]	48,835 in WHI DM; 4311 with body composition assessments	DM trial arm assignment	Change in body composition by DXA	Low fat assignment associated with significant reduction in % body fat, lean mass and fat mass over 8.1 years	Low fat diet appears to reduce body fat, but may also have undesirable association with loss of lean mass

Obes Relat Metab Disord, 2004 [118]	obtained from 3389 (60 % White, 20 % Black, 12 % Hispanic, and 8 % Asian/Pacific Islander) postmenopausal women from the entire observational study (161,809)	concentration and resistance		were independent predictors of increases in weight in White women and in the combined group. Those in the highest quartile of insulin resistance gained 0.4 kg in 3 year and those in the lowest quartile lost 0.06 kg. A significant interaction between obesity and insulin resistance was observed	predictor of weight gain in postmenopausal women, except among the most obese women
Howard et al., JAMA, 2006 [66]	48,835 postmenopausal DM group (40 % intervention/60 % obs.)	Dietary fat, fruit, vegetable, and grain intake	Change in body weight	Intervention group = mean wt. loss of 2.2 kg, p-value < 0.001 after 1 year; 0.4 kg, p-value 0.01 at 7.5 years	Wt. loss was greatest for women who decreased overall energy from fat. Similar, but lesser trend seen for increasing fruits, vegetables, and fiber
McTiernan et al., Obesity, 2006 [119]	267 postmenopausal women randomly selected from the Diet Modification Group	BMI	Sex hormones	BMI was positively associated with estrone (beta = 0.031, p < 0.001), estradiol (beta = 0.048, p < 0.001), free estradiol (beta = 0.062, p < 0.001), free testosterone (beta = 0.017, p = 0.02), and prolactin (beta = 0.012, p = 0.02) and negatively associated with SHBG (beta = –0.02, p = 0.001). Women with high BMI/low physical activity had the highest mean estrone concentration	The associations between overweight/obesity, sex hormones and sedentary lifestyle on breast cancer risk may in part be explained by this research
McTigue et al., JAMA, 2006 [120]	90,185 postmenopausal women from observation group	BMI	Mortality, coronary heart disease, HTN, DM	Extreme obesity prevalence differed with race/ethnicity, from 1 % among Asian and Pacific Islanders to 10 % among black women. All-cause mortality rates per 10,000 person-years were 68.39 (CI: 65.26–71.68) for normal body mass index, and increased with each BMI category to high of 116.85 (CI: 103.36–132.11) for extreme obesity	All-cause mortality increases with each increase in BMI category. Obesity set as a BMI of 30 or greater may lead to misinterpretation of individuals and populations at risk
Rillamas-Sun et al., JAMA Intern Med, 2014 [121]	36,611 WHO women in OS + CT who reached 85 years of age	Enrollment BMI and waist circumference	Survival (1) "healthy," (2) prevalent disease, (3) incident diseased, (4) disabled, (5) died	Mean baseline age 72.4 year Underweight and obese more likely to die; Overweight and obese higher rates of incident disease and mobility disability; mobility disability increased linearly with advancing obesity classification such that risk was 6.7-fold greater in women with Class III obesity	Obesity is a significant contributor to mobility disability in women reaching age 85 year suggesting weight control is an important clinical goal even in advanced age

- Weight control was not included in the original study design. Weight control is difficult to achieve on an individual basis let alone in the large group counseling setting; the absolute size of the study sample made more individualized diet counseling impossible; had a weight loss component been proposed, it is unlikely that significant weight loss would have occurred or been maintained. The fact that the low fat diet resulted in a lessened weight gain over the study period among women randomized to the low fat diet is promising, but it was insufficient to significantly modify disease risk for obesity-related diagnoses.
- The target study outcomes—breast and colorectal cancer as well as CVD risk—are modifiable through physical activity, and yet no physical activity component was included in the trial design. Again, costs and ability to attain and sustain significant increases in physical activity in older women influenced the decision to not focus on activity as a behavioral goal. Further, physical activity levels did not change significantly during the study. Certainly if physical activity had increased and been maintained at a higher level as adjuvant to the low fat diet, there would have been a greater likelihood of risk reduction.
- In the calcium and vitamin D supplementation trial, study participants were permitted to continue their own independent use of calcium and up to 600 IU of vitamin D daily. This protocol design resulted in some overlap in exposure to these nutrients in the supplemented versus placebo group and may have contributed to the null findings found. Of note, a 2013 analysis of women who were not taking calcium or vitamin D at baseline suggested a 35 % reduction in hip fracture risk in women randomized to calcium plus vitamin D as compared to placebo, suggesting a benefit against hip fracture risk with longer-term supplementation ([122] Osteoporosis).
- Ideally adjudication of additional health outcomes beyond those involved in testing of the primary and secondary hypotheses would allow for more accurate assessment of diet–disease associations (i.e., additional cancer sites, arthritis, and autoimmune diseases) particularly for rare diseases where a large sample size, such as the WHI study provides, would be needed in order to achieve adequate statistical power.
- Translation of research findings can be challenging especially given the multiple interventions, study arms and the inclusion of an observational cohort and CT within the overall study design. On the other hand, having both study types within the larger study population is unprecedented and allows for a thorough comparison of diet–disease hypothesis testing in the context of the two parallel approaches employed in diet research.

Recommendations

The WHI is the largest trial ever undertaken to determine the role of a low fat diet in reducing risk for common chronic diseases of postmenopausal women. In addition, it was the first study to prospectively test in a randomized, placebo-controlled study design the association between supplemental calcium and vitamin D and osteoporotic fracture and/or colorectal cancer risk in a large sample of postmenopausal women. While the paramount finding of the WHI was the results of the HT trial, indicating an increased risk for CVD [9] and breast cancer among postmenopausal women taking hormones [2, 4], the dietary modification trial has and will continue to inform clinical care for women in this age group for years to come.

Despite evidence that the diet intervention designed to achieve a low fat diet did not modulate breast cancer risk in the overall WHI DM trial population, there were some promising results that the diet was efficacious among women with the highest baseline fat intake who adhered to the diet. Further, the low fat diet was associated with a significant reduction in ovarian cancer risk [94]. There was also evidence that the low fat diet may modify risk for a subgroup of breast tumors (estrogen

receptor positive/progesterone receptor negative), effects that should be further evaluated in pooled analyses.

Further, the diet was associated with a significant reduction in ovarian cancer risk after 4 years on trial [71], with the point estimate remaining protective, but not reaching statistical significance for the longer-term, post-intervention follow-up [14].

These findings suggest that postmenopausal women should be provided with appropriate dietary assessment and proper counseling to adopt an eating plan that is low in fat and high in fruits, vegetables, and grains in an effort to reduce these risks. Further, while the reduction in total fat was not protective against CVD, it has subsequently been reported that such an eating plan, specifically reduced in saturated fat and in addition to weight loss in overweight women should be advised per the 2013 American College of Cardiology/American Heart Association Report [124].

Null results of the CaVitD supplementation trial should not lead to a lack of assessment of dietary intake of these nutrients to promote adequacy for optimal bone health. In fact, numerous studies support a protective role for both calcium and vitamin D in reducing osteoporosis and related fracture risk [125–127]. Of note, the 2011 Institute of Medicine report on calcium and vitamin D confirmed the essential role of these nutrients in bone health and in fact increased the RDA for older persons [128]. The subgroup of women with lower serum vitamin D levels did demonstrate reduced fracture risk with calcium/vitamin D supplementation. The "healthy" volunteer effect which was associated with higher self-prescription of calcium supplementation likely was a significant effect modifier for this CT within WHI.

Importantly, the WHI is only just beginning to inform clinical care for aging women. As the population of older women is increasing steadily in the USA so will our need to better understand the role of lifestyle factors, including diet, in reducing disease risk and promoting improved health. The wealth of clinical, lifestyle, demographic, and genetic data generated from this large, longitudinal study will continue to impact clinical knowledge well beyond the initial proposed hypotheses.

Conclusions

The WHI is the largest combined CT and OS of postmenopausal women ever conducted in the USA. The tremendous knowledge gained and evidence developed from this research has had a significant impact on diet and disease prevention. The DM trial alone has supported the development of several public health reports including the Dietary Guidelines for Americans, Healthy People 2010 and 2020 and disease specific guidance published by leading organizations including the American College of Cardiology, The American Cancer Society and The National Osteoporosis Foundation. The majority of this robustly described sample of women is continuing to engage in these research efforts. Their ongoing contributions support an even greater impact of WHI on diet and health in American women for years to come.

Acknowledgments The following dietitians and nutritionists provided support to the implementation of the dietary modification study of WHI: Brooke Adachi; Tanya Agurs-Collins; Gayle Alleman; Joli Allen; Linda Antinoro; Christine Armes; Grace Arriola; Frani Averbach; Heather Backer; Kelly Bailey; Denise Barratt; Mary Paula Baumann; Michelle Bean; Marlene Beddome; Margaret Beesley; Lorna Belden; Randi Belhumeur; Connie Belk; Pat Bergen; Eleanor Betz; Kathy Bierl; Cindy Bjerk; Kerri Bland; Mary Pat Bolton; Charlotte Bragg; Anne Brettschneider; Amy A. Brewer; Mireille Bright-Gbebry; Marilyn Brill; Julie Burgess-Bottom; Deborah Burman; Jill Burns; Elizabeth Burrows; Kathy Burton; Darcy Bushnell; Bette Caan; Arlene Caggiula; Louise Calderera; Virginia Cantrell; Sara Carrion; Michelle Berry Casavale; Aimee Cassulo; Dana Chamberlain; Teresa Clark; Carolyn Clifford; Rosemary Clifford; Dorsey Cokkinias; Amy Coleman; Patricia Collette; Lisa Cooper; Betsy Costello; Janice Cox; Christi Coy; Martha Sue Dale; Dorothy Delessio; Stephanie Dellner; Jan Depper; Janet DiIulio; Nora DiLaura; Pia DiMarzio; Panayiota Doering; Linda Doroshenko; Eva Dugger; Carolyn Ehret; Louise Engar; Jackie Enlund; Margaret Farrell; Jennifer Fine; Darlene

Fontana; Amy Ford; Gail Frank; Trish Freed; Ann Gallagher; Karen Gantt; Angela Gardner; Linda Gay; Judy Gerber; Niki Gernhofer; Vicki Gobel; Sue Goldman; Nancy Goyings; Barbara Graf; Theresa Greco; Lynne Gregor; Peggy Gregson; Adriann Grosz; Andrea Guastamacchio; Valerie Haack; Pamela Haines; Beth Hall; Susan Hall; Mary Hankey; Judith Hartman; Kathy Hattaway; Holly Henry; Judith Hinderlitter; Mary Lynne Hixson; Elizabeth Hoelscher; Donna Hollinger; Leah Hopkins; Gerrilyn Hopper; Heather Horowitz; Kathy Hrovat; Anne Hubbell; Sharon Innes; Sharon Jackson; Carolyn Jennings; Lynn Johnson; Marian Johnson; Bobbette Jones; Donna Toms Jones; Kirsten Jordahl-Nielsen; Sarah Josef; Cheryl Kaplan; Stephanie Keck; Karen Kedrowski; Mark Kestin; Laura Kinzel; Lee-Ann Klein; Heidi Koempel; Sandy Kowal; Jen Kring; Alan Kristal; Brenda Krpata; Danielle Lacina; Phil Lacher; Mary Lamon-Smith; Patti Laqua; Mary Larez; Lynne Larson; Vera I. Lasser; Claire LeBrun; Elisa Lee; Sandy Lemanski; Judy Levin; Barbara Lewin; Ilona Lichty; Marlene Llano; Denise Londergan; Leticia Lopez; MaryAnn Lueck; Pat Lyman; Sally Mackey; Katherine Malville-Shipan; Elizabeth Marchese; Nicole Marrari; Shannon Martin; Barbara Mascitti; Alice McCarley; Gayle McCartney; Carol McDermott; Ann McDonald; Allison McKenny; Kathy McManus; Joan McPherson; Becky Meehan; Renee Melton; Mary Messier; Carole Milas; Audrey Miller; Connie Mobley; Debra Mohns; Carol Molfetta; Deborah Monsegue; Margaret Moonan; Ann Gowan Morrissey; Yasmin Mossavar-Rahmani; Dru Mueller; Mary Mueller; Ann Mylod; Sue Nelson; Marian Neuhouser; Alice Nicolai; Doris Nicolas-Mir; Patricia Ochoa; Deborah Ocken; Annette Okuma; Monica Olsen; Pat Ormond; Christina Padilla; Ruth Patterson; Julia Paulk; Margaret F. Pedersen; Elaine Percival; Lois Peterson; Marie Phipps; Jacqui Pirofalo; Joan Pluess; Rosa Polanco; Megan Porter; Carolyn Prosak; Suzanne Proulx; Diane Quigley; Janet Raines; Susan Rauth; Suzanne Rebro; Cyndi Reeser; Cheryl Ritenbaugh; Connie Roberts; Ramona Robinson-O'Brien; Angela Rodriguez; Nancy Saal; Angelique Salonga; Ethel Sanders; Monica Schoenberger; Lisa Sewell; Julie Shapero; Angie Sharp-Jacobson; Audrey Shweky; Karen Silva; Nancy Simpson; Elise Sinagra; Bali Singh; Donna Sivertsen; Beth Smith; Karen Smith; Nancy Smith; Tracy Smith; Linda Snetselaar; Jennifer Soboslai; Oehme Soule; Karen Southern; Jackie St. Cyr; Sachiko St. Jeor; Lorraine G. Staats; Jennifer Summers; Susie Swenson; Joyce Sydell; Houra Taheri; Marisa Tanko; Kimberly Thedford; Nancy Tiger; Robin Thomas; Alice Thomson; Cyndi Thomson; Lesley Tinker; Maribel Tobar; Cynthia Tucker; Nicki Turner; Alice Valoski; Linda Van Horn; Lucy Villarreal; Eileen Vincent; Shirley Vosburg; Carol Walsh; Christie Wayment; Donna J. Weiss; Maria Welch; Andrea Wenger; Julie West; Catherine Willeford; Lois Wodarski; Dolores Wolongevicz; Judy Wylie-Rosett; Alexandra Wynne; and Heidi Zahrt.

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Chapter 20
Role of Fiber in the Prevention of Type 2 Diabetes

Mark L. Dreher

Key Points

- Dietary fiber (fiber) is an important macronutrient food component for the prevention of diabetes.
- Only about 5 % of the US population meets the recommended fiber adequate intake level and similar low-fiber intake is found in many other western countries. Prospective studies show that healthy, higher fiber diets are associated with a 15–83 % lower risk of developing diabetes compared to low-fiber, western diets.
- A dose response meta-analysis of prospective studies shows a nonlinear relationship between fiber intake and diabetes risk with a linear reduction starting at about 25 g fiber/day.
- A number of randomized trials support healthy, fiber-rich dietary patterns with about 30 g fiber or more/day for lowering diabetes risk and improving fasting insulin levels and insulin resistance scores.
- For reduced diabetes risk, the typical western diet has about a daily 15 g fiber intake deficit. This deficit can be closed by substituting a lower fiber food with a fiber-rich food at each meal and one snack each day.
- Potential mechanisms for fiber's lowering of diabetes risk include (1) delaying postprandial glycemic and insulinemic response rates, (2) promoting lower food energy density and greater macronutrient fecal excretion, (3) improving appetite control to decrease energy intake by affecting signals associated with the stomach, small intestine, and brain, and (4) stimulating the colonic fermentation of short chain fatty acids (SCFA) to affect energy metabolism, appetite control, inflammatory response, and prebiotic microbiota activity.

Keywords Prediabetes • Lifestyle • Insulin resistance • Insulin sensitivity • Visceral fat • Systemic inflammation • Short chain fatty acids • Metabolizable energy • Dietary patterns • Fiber-rich foods • Whole-grains • Fruits • Vegetables • Legumes • Nuts • Seeds • Mediterranean diet • DASH diet

Introduction

Diabetes Overview

Etiology. Type 2 diabetes (diabetes) is a disease that is preventable through the practice of a healthy lifestyle, including the consumption of a healthy, fiber-rich dietary pattern, weight loss/management, and physical activity [1–6]. Only about 5 % of the US and many other westernized populations routinely follow a diabetes preventive lifestyle or consume adequate levels of dietary fiber (fiber) [7–14].

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_20

Prevalence. The prevalence of prediabetes and diabetes has increased globally in parallel with the rising levels of obesity in adults and children, a phenomenon sometimes called diabesity [1, 4, 5]. If this global trend continues, by 2030 about one billion people are expected to be prediabetic and diabetic.

Complications. People with diabetes are at increased risk for other chronic illnesses such as cardiovascular and renal disease, and serious health complications such as retinopathy, neuropathy, shorter life expectancy, and higher medical costs [1, 4, 5]. Consequently, there is an urgent public health need to reexamine the role of fiber in diabetes prevention.

Fiber and Diabetes Risk

Background

Fiber diabetes hypothesis. In the 1970s, the fiber diabetes hypothesis, which associated the change from high-fiber, low-glycemic diets (traditional diets) to low-fiber, high-glycemic diets (western diets) as a primary controllable cause for diabetes was postulated by the early fiber and chronic disease pioneers Drs. Burkitt and Trowell [15, 16]. Their hypothesis was based on a number of convergent lines of evidence. During their time as physicians at different African hospitals during the 1950s and 1960s, Drs. Burkitt, Trowell, Cleave, and Walker observed spikes in diabetes rates as rural Africans moved to the large cities and replaced their traditional (high fiber) diets with western (low fiber) diets. They uncovered health statistics from England and Wales showing a 50 % reduction in diabetes death rates when high-fiber whole-grain flour was mandated during World War II to replace refined flour. A third important piece of the evidence was clinical research by Drs. Jenkins and Anderson demonstrating the direct effects of fiber on postprandial blood glycemic and insulinemic responses. In subsequent years, the fiber diabetes hypothesis has been essentially confirmed by authoritative bodies [8–10].

Fiber recommendations. In the USA, the adequate intake (AI) level for fiber is set at 14 g fiber/1000 kcal, which is roughly 25 g/day for women and 38 g/day for men based on energy intake, from a scientific assessment of fiber's effects on coronary heart disease and diabetes risk [8]. In the EU, the AI is >25 g/day for reduced coronary heart disease, diabetes, and weight gain [9].

Fiber intake. In the USA, there is a significant fiber consumption gap with the mean fiber intake being about 16 g fiber/day for individuals 2 years or older with intakes for males and females at 18 g/day and 15 g/day, respectively [8, 10, 14]. About 95 % of the US population falls short of meeting the AI for fiber and there are major shortfalls in the daily consumption of whole-grains, fruits, and vegetables [7, 8, 10]. In the EU, the average fiber intake is between half and 80 % of the AI, depending on the country [9].

Dietary patterns. Diets associated with a lower risk of developing diabetes consist of (1) fiber-rich diets primarily from whole-grains, fruits and vegetables, pulses, nuts and seeds to achieve a daily fiber intake of about 30 g or more/day, (2) limited intake of red meat, sugar sweetened beverages, high-fat dairy, and refined grains, and (3) controlled energy intake (plus physical activity most days of the week) to limit the risk of gaining body weight [3–17]. Although fiber-rich foods typically contain other potential non-fiber diabetes preventive nutrients and phytochemicals such as magnesium, unsaturated fats, carotenoids, tocopherols, and phenolic acids, fiber has a uniquely important dietary role in reducing the risk of developing diabetes when consumed at high enough levels [12–18, 20, 21].

Prospective Cohort Studies

Nonlinear dose response. Prospective studies find a negative association between the level of fiber intake and risk of developing diabetes. A dose–response meta-analysis of 17 prospective studies found a nonlinear association between fiber intake and diabetes risk (Fig. 20.1) [18]. The effect of fiber intake on diabetes risk is flat until a threshold at about 25 g fiber/day is reached and at ≥25 g fiber/day there is a linear reduction in risk. To put this in perspective, the consumption of (1) low-fiber, high-glycemic, western-type diets (≤15 g fiber/day) can increase diabetes risk by ≥40 %, (2) diets between 20 and <25 g fiber/day have an insignificant single digit lowering diabetes risk, and (3) fiber-rich diets with >25 g fiber/day are associated with progressively significant lowering of diabetes risk [4, 18, 19]. These findings are consistent with several other fiber and diabetes risk dose–response prospective studies [20, 21].

Randomized Trials

Diabetes risk. Several long-term randomized trials are suggestive of the importance of consuming fiber-rich diets containing about 30 g fiber or more/day for reduced risk of developing diabetes. The nut intervention arm of the PREvención con Dieta MEDiterránea [PREDIMED] Diabetes Prevention Trial, which provided about 27 g fiber/day, showed an insignificant reduction in mean diabetes risk by 18 % after 4.1 years compared to the low-fat diet control (24 g fiber/day) [22]. The less than 30 g fiber/day in the diets along with the small difference of fiber intake between the Mediterranean and low-fat control diets appears to account for the insignificant reduction in diabetes risk. The Chinese Da Qing Diabetes Study found after 6 years that healthy diets rich in whole-grain cereal and vegetable fiber significantly lowered diabetes risk by 31 % compared to the lower fiber diets, without exercise guidance and change in BMI [23]. Further, the 3 year Finnish Diabetes Prevention trial, including a

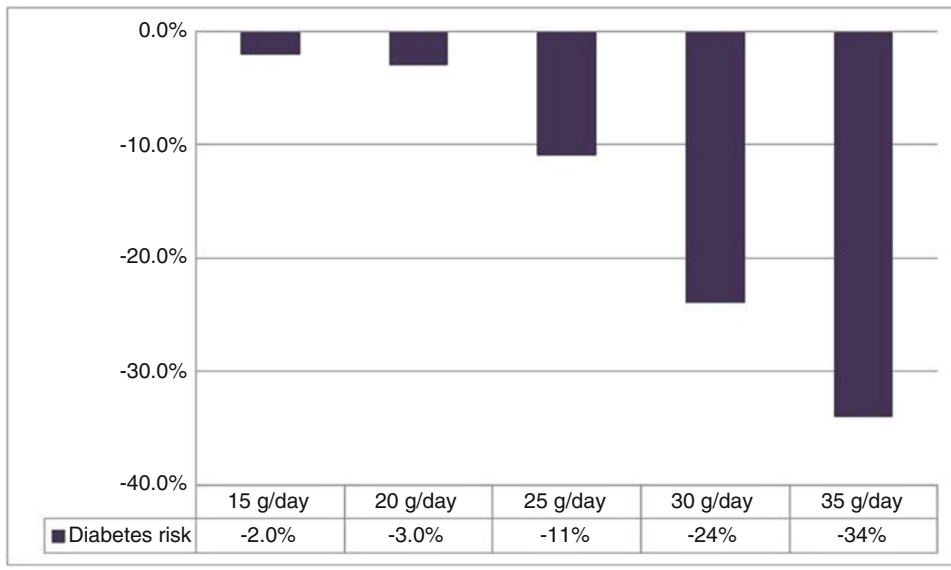


Fig. 20.1 An analysis of total fiber intake and risk of type 2 diabetes from a dose–response analysis of prospective studies found a nonlinear relationship (p for nonlinearity <0.01) [18]

comprehensive lifestyle program with 15 g fiber/1000 kcal, exercise and 5 % weight loss, reported a 58 % lower diabetes risk [24].

Diabetes biomarkers. Key randomized trials support that the daily consumption of about 30 g fiber or more/day from a variety of fiber-rich foods and dietary patterns can improve diabetes biomarkers compared to lower fiber diets. First, a 2 year randomized trial of 180 middle-aged adults with metabolic syndrome consuming a Mediterranean-style diet (32 g fiber/day including 487 g/day of whole-grains, vegetables, fruit, legumes, and nuts) had significantly lower insulin resistance (HOMA IR scores), serum insulin, and plasma glucose compared to those on a control lower fiber diet (15 g fiber/day including 201 g/day of whole-grains, vegetables, fruit, legumes, and nuts) (Fig. 20.2) [25]. This study provides significant evidence in favor of consuming fiber-rich diets to improve diabetes biomarkers compared to low-fiber diets. Second, a multi-phased trial found that high-fiber diets with and without psyllium significantly lowered fasting insulin levels compared to low-fiber diets with and without psyllium (Fig. 20.3) [26]. This study suggests that substituting fiber-rich foods for low-fiber foods is more effective in improving fasting insulin levels than adding a fiber supplement to low-fiber diets. Third, in 240 adults with metabolic syndrome randomized to high-fiber diet (goal to consume 30 g fiber/day) or the multi-component American Heart Association (AHA) hypocaloric dietary guidelines, there were no significant differences in fasting plasma insulin levels, HOMA-IR scores, or fasting glucose levels after 12 months. This study suggests that consuming a healthy diet with about 30 g fiber/day can be as effective as a multi-component, hypocaloric diet in controlling diabetes risk factors [27]. Finally, in the context of an overall DASH-type diet and probably other healthy diets with a fiber intake of ≥30 g fiber/day, using the glycemic index to select specific foods may not improve insulin resistance, if the total carbohydrate levels are 60 % of the energy intake [28]. The primary studies support the 30 g fiber or more/day level to control diabetes risk and biomarkers are listed in Table 20.1.

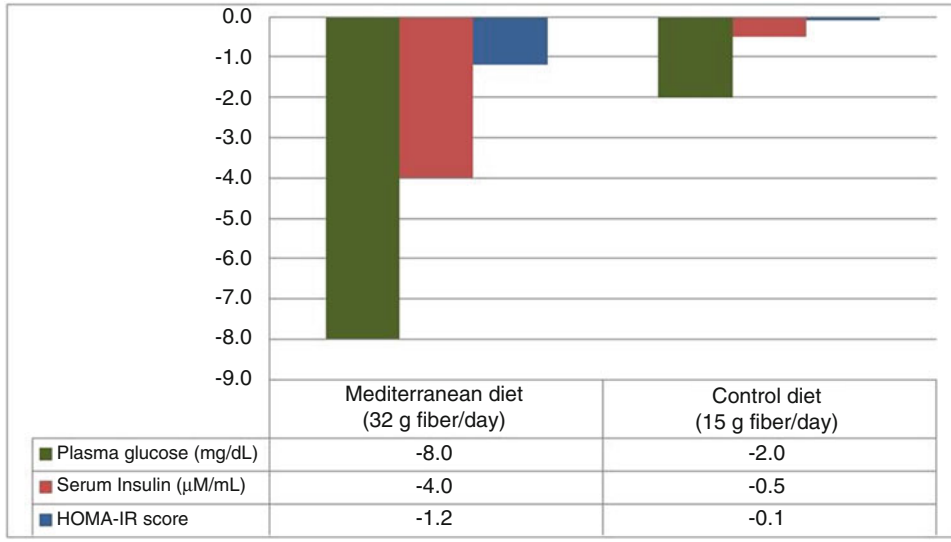


Fig. 20.2 In overweight Italian adults with metabolic syndrome, higher fiber Mediterranean diet was more effective in improving glycemic and insulinemia control from baseline than the lower fiber control after 2 years ($n=180$) [25]. There was significantly improved treatment effect for the high-fiber diet for plasma glucose and HOMA-IR score ($p<0.001$), and serum insulin ($p=0.01$)

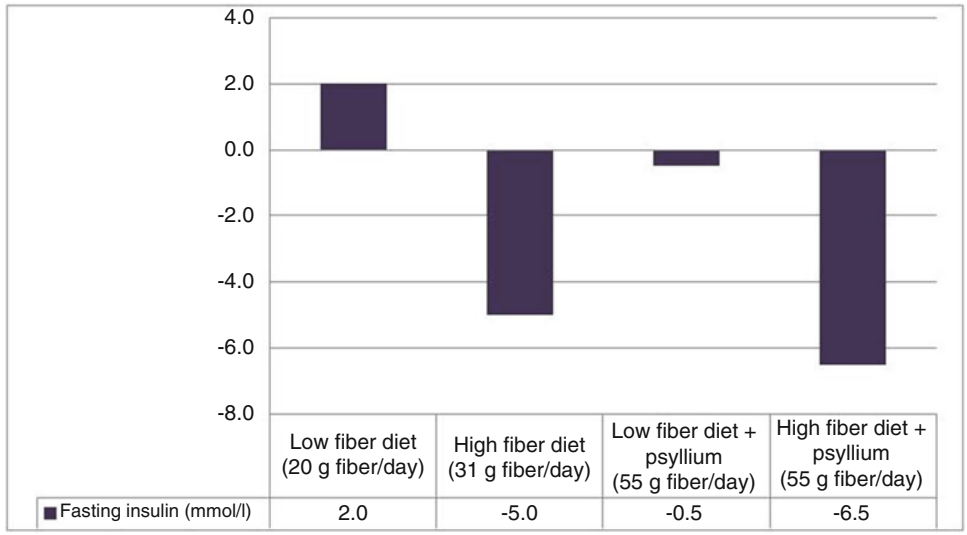


Fig. 20.3 In obese Australian adults, higher fiber diets with and without psyllium significantly lowered mean fasting insulin levels compared to low-fiber diets with and without psyllium (treatment effect $p < 0.05$) after 12 weeks ($n = 72$) [26]

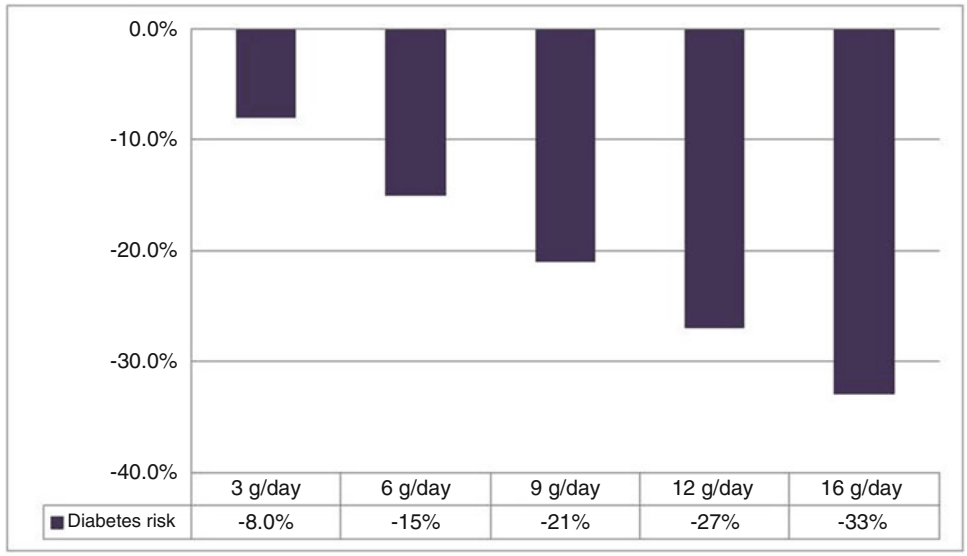


Fig. 20.4 An analysis of cereal fiber intake and risk of type 2 diabetes from a dose–response analysis of prospective studies found a linear relationship (p for nonlinearity = 0.721) [18]

Fiber-Rich Foods/Dietary Patterns and Diabetes Risk

Cereal Fiber and Whole-Grains

Background. Whole-grains have between 3.5 and 18 g cereal fiber per 100 g and provide up to 50 % of the total fiber intake in the western diet [29–31]. The criteria for labeling whole-grain food levels is imprecise, which challenges consumers' ability to optimally select whole-grain foods for diabetes risk

Table 20.1 Primary studies supporting the about 30 g fiber or more/day level to control type 2 diabetes (T2D) risk and biomarkers

Reference	Study details	Results
Prospective studies		
Yao et al. [18]	Dose response meta-analysis with 17 prospective cohort studies of total and cereal fiber intake on risk of T2D	Figures 20.1 and 20.5
Weng et al. [21]	Healthy Taiwanese adults aged ≥30 years ($n=1604$); 4.6 years follow-up	Highest quintile total fiber vs. the lowest quintile (Q) reduced T2D risk by approximately half
	Total fiber: 24.1 g (Q1) vs. ≥38.6 g (Q5)	
Randomized trials		
Salas-Salvado et al. [22]	Spanish men and women aged 55–80 years with high cardiovascular risk ($n=3541$); 4.1 years median follow-up	Mixed nuts had strong trend but insignificant 18 % reduction in T2D risk
	Mediterranean diet with mixed nut group (about 27 g fiber/day) vs. low-fat control diet (about 24 g fiber/day)	
Pan et al. [23]	Chinese men and women with prediabetes ($n=110{,}660$); 6 years follow-up	Higher fiber diets significantly reduced T2D incidence by 31 % ($p<0.03$)
	High cereal and vegetable fiber diets but total fiber level was not disclosed	
Tuomilehto et al. [24]	Finnish middle-aged, pre-diabetic obese subjects (172 men and 350 women); 3.2 years follow-up	Higher fiber lifestyle intervention reduced T2D risk by 58 % ($p<0.001$)
	Lifestyle change with goal to consume 15 g fiber/1000 kcal)	
Esposito et al. [25]	Overweight Italian adults with metabolic syndrome ($n=180$); 2 years duration	See Fig. 20.2
	Mediterranean-style diet (32 g fiber/day) vs. control diet (15 g fiber/day)	
Pal et al. [26]	Obese Australian adults; single-blind, placebo controlled study ($n=72$); 12-week duration	See Fig. 20.3
	Low-fiber diet with and without added psyllium (20–55 g fiber/day) vs. Healthy high-fiber diet with and without psyllium (31–59 g fiber/day)	
Ma et al. [27]	Obese US subjects with the metabolic syndrome ($n=240$); 12 month duration	Higher fiber diets were as effective as an AHA diet in managing diabetes risk factors (treatment effects $p>0.05$).
	High-fiber diet (≥30 g/day goal) vs. hypocaloric diet (about 20 g fiber/day)	

reduction. Thus, future labeling and nutrition tables should use specific grams and type of grains rather than servings of whole-grain (e.g., 16 g whole-grain wheat rather than 28 g whole-grain wheat bread).

Prospective studies. Whole-grains with ≥2 g cereal fiber/serving are the best for diabetes risk reduction as shown in a dose response meta-analysis, which found a 6 % lower risk for each daily 2 g increment in cereal fiber added to the diet (Fig. 20.4) [18]. Several systematic reviews, including 16 prospective studies, concluded that the consumption of 3–5 whole-grain servings (40–80 g)/day, especially from breakfast cereals, breads, or brown rice, lowered diabetes risk by 18–40 % compared to whole-grain free diets [32–34]. A Swedish prospective study found that consuming 4 servings of whole-grains/day, mainly from fiber-rich rye crisp bread, was associated with a 34 % lower risk of developing prediabetes or diabetes compared to consuming only 2 servings of whole-grains/day over an 8 year follow-up [35]. The Physicians' Health Study reported that men consuming whole-grain breakfast cereals had a significant 33 % lower diabetes risk compared to those consuming refined breakfast cereals [36]. The substitution of brown rice for white rice was found to lower the risk of diabetes by 16 % [37].

Randomized trials. The evidence for high cereal fiber or whole-grain diets (>60 % cereal fiber) on insulin sensitivity, HOMA-IR scores, and other related markers are inconsistent with about half the randomized trials showing significant effects [38–47]. Five randomized trials show that diets rich in cereal fiber and whole-grains (approximately 28–40 g cereal fiber or >6 whole-grain servings/day) support a significant 10–25 % improvement in insulin sensitivity and/or HOMA-IR scores compared to diets with ≤18 g fiber/day when subjects maintained their baseline body weight for 3 days to 12 weeks [38–42]. Five randomized trials report that diets rich in cereal fiber and whole-grains (approximately 23–32 g cereal fiber or ≥6 whole-grain servings/day) showed insignificant effects on insulin sensitivity [43–47]. A separate meta-analysis and a systematic review of oat and β-glucan randomized trials with levels in the range of 3 g β-glucan/day found insignificant effects on insulin sensitivity [48, 49]. However, one study suggests that 6 g β-glucan/day may be required to improve insulin sensitivity [50].

Fruit and Vegetables

Background. The fiber content of fruit and vegetables (F&V) ranges from 1 to 10 % for fruit and 1 to 7 % for vegetables [30, 31]. Also, there is a high degree of variability in physical properties (such as whole or processed) and levels of nutrients and phytochemicals.

Combined F&V prospective studies. F&Vs with higher fiber and/or lower glycemic and calorie content are most effective for diabetes risk reduction [51, 52, 55, 56]. A Finnish prospective study found that individuals in the highest quartile for intake of fruit, berries, and vegetables (with the exclusion of potatoes and fruit juices) had a significant 24 % reduction in diabetes risk compared to those in the lowest quartile [52]. The diabetes risk effects of F&V was shown to be related to their fiber content [21].

Fruit prospective studies. For fruit, both the amount and variety or form of fruit consumed determine the effect on diabetes risk [51–56]. The Nurses' Health Studies and the Health Professional Follow-up Study observed a high degree of heterogeneity in the effect of whole fruits on diabetes risk (Fig. 20.5)

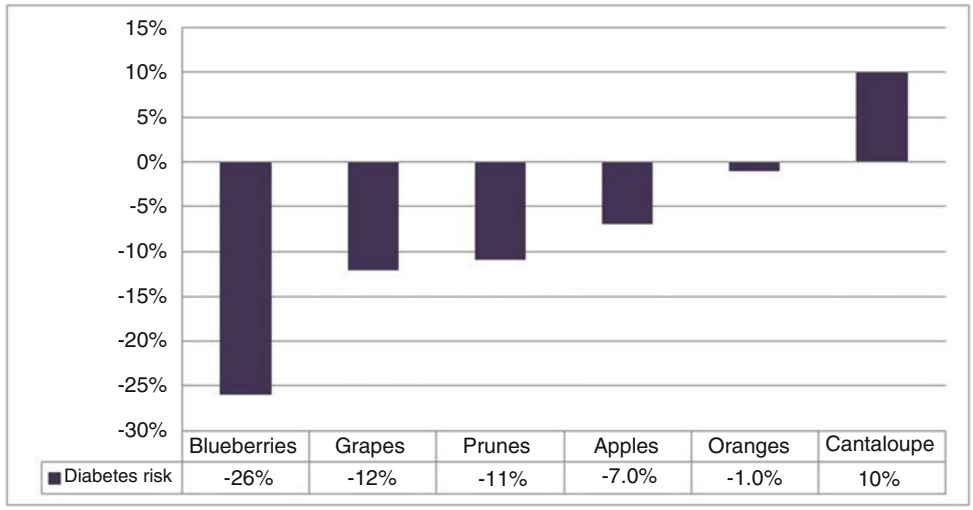


Fig. 20.5 The diabetes risk for every 3 servings/week of total whole fruit consumption differed significantly ($p < 0.001$) based on the pooled Nurses' Health Study, Nurses' Health Study II, and the Health Professionals Follow-up Study cohorts after multivariate adjustment for personal, lifestyle, and dietary risk factors of diabetes [54]

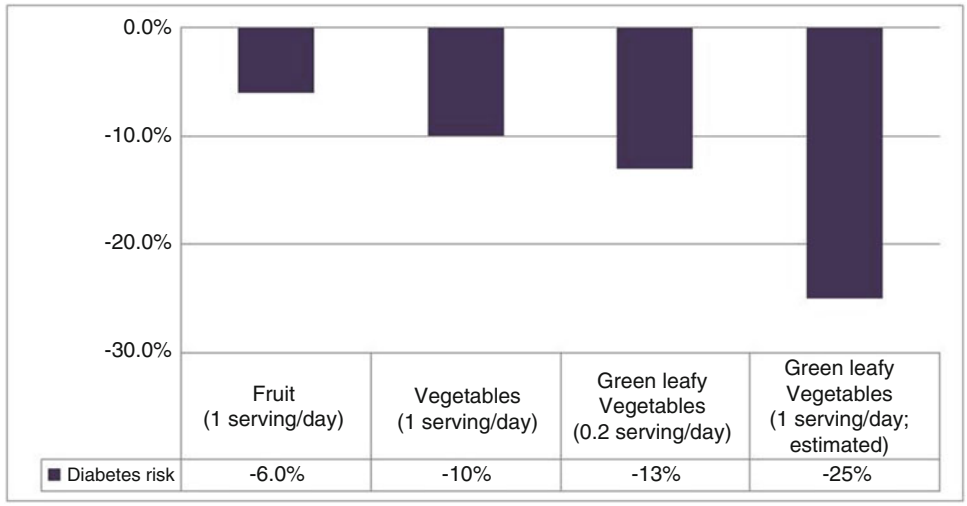


Fig. 20.6 Green leafy vegetable intake have a strong association with lower diabetes risk ($p=0.036$ for nonlinearity; curve linear) based on a meta-analysis of prospective cohort studies. Green leafy vegetables include spinach, kale, Brussels sprouts, romaine lettuce, collard greens, chard, turnip greens, and broccoli [55]

[54]. A dose response meta-analysis of ten prospective studies found a 6 % lower risk of diabetes per 1 serving/day of fruit [55]. However, other studies find a nonlinear relationship in which 3 servings or at least 200 g of whole fruits must be consumed before a significant reduction in diabetes risk is realized [21, 51, 53]. In another prospective study, berries were reported to lower diabetes risk by 35 % [52]. An increase of 3 servings/day in total fruit and vegetable consumption was not associated with development of diabetes, whereas the same increase in whole fruit consumption was associated with an 18 % lower risk of diabetes [56]. For juices, 100 % fruit and vegetable juices have a neutral effect on diabetes risk whereas higher consumption of sugar sweetened fruit juice was associated with a significant 18–28 % increase in diabetes risk [56–58].

Vegetable prospective studies. For vegetables, a greater quantity (3-plus servings vs. 1 serving or less/day) or a greater variety (11 vs. 5 varieties/week) was found to reduce diabetes risk by about 24 % [51]. Although a dose response meta-analysis of vegetables found a 10 % reduction in diabetes risk for each serving consumed/day [55], green leafy vegetables have been most consistently associated with reduced diabetes risk [55, 56, 59]. In a dose response meta-analysis, green leafy vegetables were found to be most effective in lowering diabetes risk, which is likely related to the high fiber to calorie ratio and carotenoid content (Fig. 20.6) [55]. The Nurses' Health Study found a daily serving of potatoes and 2 weekly servings of French fries to be associated with an increased risk of diabetes by 18 % and 16 %, respectively [60]. However, a Brazilian study reported that the consumption of another starchy root staple food, cassava flour, appears to have diabetes protective effects [61].

Randomized trials. For whole F&Vs, several randomized trials indicate that the consumption of 5–7 portions/day has inconsistent effects on insulin sensitivity unless there is specific guidance to consume higher fiber and lower glycemic F&V varieties [62–64]. Snacking on raisins significantly improved glycemic and insulinemic control, and lowered glycated hemoglobin (HbA1c) compared to common high-glycemic snacks [65–67]. In people with metabolic syndrome, the consumption of dehydrated F&V extracts and concentrates had no effect on fasting glucose or insulin after 8 weeks [68].

Legumes

Background. Legumes, including cooked non-oil seed pulses (e.g., chickpeas, beans, peas, lentils), and soybeans contain between 5.5 and 10 g of fiber per 100 g and peanuts contain 2.4 g fiber/oz (28.4 g; 28 peanuts) [31]. Also, soybeans, an oil seed legume, contains a combination of fiber and non-fiber phytochemicals, such as genistein and daidzein [31].

Prospective studies. A Chinese prospective study found that adults consuming the highest level of non-oil seed pulses had a 24 % lower diabetes risk than those with the lowest level of intake [69]. In India's third National Family Health Survey, non-oil seed pulses, such as lentils, were associated with a significant 30 % reduced prevalence of diabetes among women but not men [70]. The Nurses' Health Study found that peanut butter was inversely associated with risk of diabetes. The consumption of ≥140 g (5 oz) peanut butter/week significantly decreased diabetes risk by 21 % [71]. In a study of over 43,000 Chinese Singaporeans, the consumption of plain tofu ≥2/week significantly reduced risk of diabetes after adjusting for BMI [72]. However, two other studies in Hawaii and Japan find no diabetes risk protective benefits for either soy products or genistein in either men or women [73, 74].

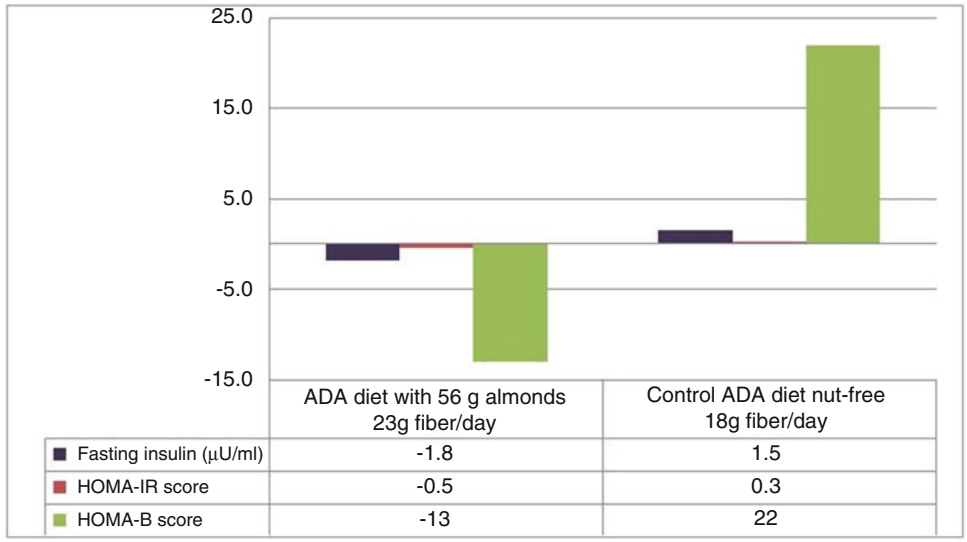
Randomized trials. In a systematic review and meta-analysis of 41 randomized trials, non-oil-seed pulses were shown to modestly improve medium to longer term glycemic control through possible insulin-sparing mechanisms [75]. Two clinical studies with overweight adults have shown that the daily consumption of 100 g cooked chickpeas or 50 g whole pea flour in muffins significantly lowered fasting insulin and insulin resistance within 4–12 weeks [76, 77].

Tree Nuts and Seeds

Background. Nuts such as almonds, pistachios, walnuts, hazel nuts, and pecans contain between 2.0 and 3.5 g fiber/28.4 g (1 oz; about a handful), except for cashews and pine nuts which contain about 1.0 g fiber/28.4 g, and seeds such as flaxseed, pumpkin seeds, and sunflower seeds which contain 3.1–7.9 g fiber/28.4 g [31].

Prospective studies. The Nurses' Health Study observed that the consumption of ≥5 nut servings/week significantly lowered diabetes risk by 27 % compared to never/almost never nut consumption [71]. However, an updated Nurses' Health Study found that ≥2 servings/week of walnuts lowered diabetes risk in women by 23 % after BMI adjustments whereas the consumption of total nuts and other tree nuts was also inversely associated with diabetes risk but these associations were largely explained by BMI [78]. Further, a Nurses' Health Study and Health Professional Follow-up Study analysis of nut intake and cause-specific mortality showed the consumption of ≥5 nuts servings/week lowered diabetes specific mortality by 16 % but this did not reach the level of significance [79]. A systematic review and meta-analysis of five prospective studies and the PREDIMED diabetes randomized trial found that 4 nut servings/week significantly lowered diabetes risk by 12 % [80]. Several other systematic reviews and meta-analyses of prospective studies found significant inverse associations between nuts and ischemic heart disease, overall cardiovascular disease, coronary artery disease, hypertension, and all-cause mortality but not diabetes [81, 82].

Randomized trials. Three randomized clinical studies, in adults with prediabetes or metabolic syndrome, determined that the consumption of 60 g/day of almonds or pistachios or 30 g/day mixed nuts (50 % walnuts, 25 % hazelnuts, and 25 % almonds) significantly improved fasting insulin, insulin sensitivity, insulin resistance (HOMA-IR), and/or β-cell function after 12–16 weeks [83–85]. In an



	ADA diet with 56 g almonds 23g fiber/day	Control ADA diet nut-free 18g fiber/day
Fasting insulin (μU/ml)	-1.8	1.5
HOMA-IR score	-0.5	0.3
HOMA-B score	-13	22

Fig. 20.7 In obese, pre-diabetic middle-aged US adults (74 % women) the American Diabetes Association (ADA) hypocaloric diet with almonds significantly reduced fasting insulin ($p < 0.002$), insulin resistance (HOMA-IR; $p < 0.007$), and beta-cell function (HOMA-B; $p < 0.001$) compared to the nut-free diet after 16 weeks ($n = 65$) [84]

American Diabetes Association (ADA) diet trial, 65 prediabetic adults were randomized to consume either a hypocaloric ADA diet including 56 g whole almonds or a nut-free ADA control diet for 16 weeks [84]. The ADA diet supplemented with almonds significantly improved fasting insulin and HOMA-IR and beta-cell function (HOMA-B) compared to the control ADA diet (Fig. 20.7). This study suggests the healthy hypocaloric diets may reduce the fiber threshold for diabetes risk reduction below 30 g fiber/day. Also, flaxseed (13–40 g/day) has been shown to effectively improve insulin sensitivity in obese and prediabetic adults [86, 87].

Dietary Patterns

Adherence. Systematic reviews of prospective studies estimate that healthy, higher fiber dietary patterns can lower diabetes risk collectively by about 20 % (range 15–83 %) compared to lower fiber, western diets independent of geography, and high adherence to these diets can further lower risk by an additional 15 % [7, 88, 89]. One prospective study found that the combination of low-fiber, western dietary patterns and a sedentary lifestyle increased diabetes risk by 96 % [90]. Moderate to high adherence to healthy, fiber-rich (about 30 g fiber or more/day) diets like the Mediterranean Diet (MedDiet), Dietary Approaches to Stop Hypertension (DASH) diet, alternative healthy eating index diets, and vegetarian diets have been shown to consistently lower diabetes risk [88–101].

Importance of fiber. High adherence to healthy dietary patterns is associated with higher fiber intake. In a Spanish prospective cohort, a higher Mediterranean diet score was associated with higher fiber intake and lower risk for diabetes (Fig. 20.8) [91]. Moderate to high adherence to all forms of vegetarian diets are associated with lower diabetes risk in part because of the about 30 g fiber/day or higher content of these diets [102, 103]. There is progressively lower diabetes risk with increased dietary strictness and fiber content with reductions of 62 % for vegans, 51 % for semi-vegetarians, and 42 % for lacto-ovo vegetarians compared to low-fiber, nonvegetarian western diets.

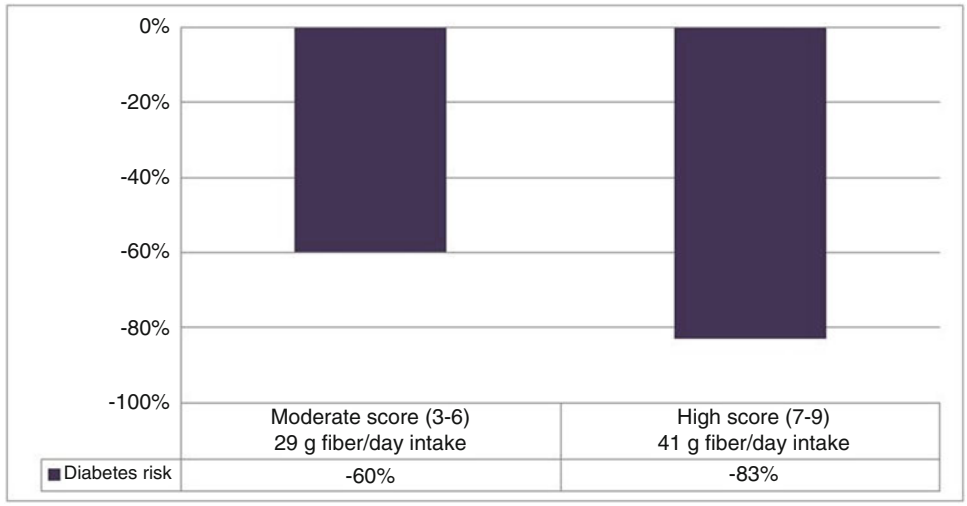


Fig. 20.8 A higher Mediterranean diet adherence score and fiber intake was associated with a significant reduction in diabetes risk compared to the low adherence score (0–2; 18 g fiber/day) after a median of 4.4 years follow-up (*p* for trend = 0.04) [91]

Fiber-Rich Food Guidance

In the usual western diet, there is approximately a 15 g fiber/day gap between current fiber intake and a diet with enough fiber to reduce diabetes risk (about 30 g fiber or more/day) [7–14, 18, 25]. This daily fiber gap can typically be closed by replacing one lower fiber food with a fiber-rich food at each meal and one snack per day. Top fiber-rich foods are listed in Table 20.2. Consuming about 30 g fiber or more/day can be achieved, for example, by daily (1) replacing a low-fiber breakfast cereal with a shredded wheat, raisin bran, or other bran breakfast cereal, (2) eating an apple or pear instead of a cookie at lunch, (3) choosing mixed vegetables or lima beans instead of a tossed salad for dinner or eating raspberries or blueberries instead of ice cream for dessert, and (4) snacking on almonds, pistachios, pumpkin seeds, popcorn, dried fruit, or rye wafer crackers for one snack.

Primary Diabetes Risk Factors

Overview. The primary potential risk factors in the pathogenesis of diabetes are insulin resistance (low insulin sensitivity) and pancreatic β-cell dysfunction, which are adversely effected by elevated visceral fat and chronic inflammation [104–107].

Insulin Sensitivity

Background. Insulin sensitivity is a measure of the body's sensitivity to the effects of insulin with more insulin sensitive people requiring smaller amounts of insulin to lower blood glucose levels than those who have low sensitivity. People with low insulin sensitivity (or insulin resistance) are at increased risk of developing diabetes. Among nutrients and phytochemicals, fiber appears to be one of the most effective on insulin sensitivity [108–120].

Table 20.2 Food sources ranked by amount of fiber per standard food portion plus calories and energy density [29]

Food	Standard portion size	Dietary fiber (g)	Calories (kcal)	Energy Density (calories/g)
High-fiber bran ready-to-eat cereal	1/3 to 3/4 cup (30 g)	9.1–14.3	60–80	2.0–2.6
Navy beans, cooked	1/2 cup cooked (90 g)	9.6	127	1.4
Small white beans, cooked	1/2 cup (90 g)	9.3	127	1.4
Yellow beans, cooked	1/2 cup (90 g)	9.2	127	1.4
Shredded wheat ready-to-eat cereal	1 to 1•1/4 cup (50–60 g)	5.0–9.0	155–220	3.2–3.7
Black bean soup, canned	1/2 cup (130 g)	8.8	117	0.9
Adzuki beans, cooked	1/2 cup (90 g)	8.4	147	1.3
French beans, cooked	1/2 cup (90 g)	8.3	114	1.3
Split peas, cooked	1/2 cup (100 g)	8.2	114	1.2
Chickpeas (Garbanzo) beans, canned	1/2 cup (120 g)	8.1	176	1.4
Lentils, cooked	1/2 cup (100 g)	7.8	115	1.2
Pinto beans, cooked	1/2 cup (90 g)	7.7	122	1.4
Mung beans, cooked	1/2 cup (100 g)	7.7	106	1.1
Black beans, cooked	1/2 cup (90 g)	7.5	114	1.3
Artichoke, global or French, cooked	1/2 cup (84 g)	7.2	45	0.5
Lima beans, cooked	1/2 cup (90 g)	6.6	108	1.2
Great Northern beans, cooked	1/2 cup (90 g)	6.4	149	1.1
White beans, canned	1/2 cup (130 g)	6.3	149	1.1
Kidney beans, all types, cooked	1/2 cup (90 g)	5.7	112	1.3
Wheat bran flakes ready-to-eat cereal	3/4 cup (30 g)	4.9–5.5	90–98	3.1–3.3
Pear with skin	1 medium (180 g)	5.5	100	0.6
Pumpkin seeds, whole, roasted	1 oz (about 28 g)	5.3	126	4.5
Baked beans, canned, plain	1/2 cup (125 g)	5.2	120	0.9
Soybeans, cooked	1/2 cup (90 g)	5.2	150	1.7
Plain rye wafer crackers	2 wafers (22 g)	5.0	73	3.3
Avocado, Hass	1/2 fruit (68 g)	4.6	114	1.7
Broad beans (fava beans), cooked	1/2 cup (85 g)	4.6	94	1.1
Apple, with skin	1 medium (180 g)	4.4	95	0.5
Green peas, cooked (fresh, frozen, canned)	1/2 cup (80 g)	3.5–4.4	59–67	0.7–0.8
Refried beans, canned	1/2 cup (120 g)	4.4	107	0.9
Chia seeds, dried	1 Tbsp (about 12 g)	4.1	58	4.9
Mixed vegetables, cooked from frozen	1/2 cup (45 g)	4.0	59	0.7
Raspberries	1/2 cup (65 g)	3.8	32	0.5
Blackberries	1/2 cup (65 g)	3.8	31	0.4
Collards, cooked	1/2 cup (95 g)	3.8	32	0.3
Soybeans, green, cooked	1/2 cup (75 g)	3.8	127	1.4
Prunes, pitted, stewed	1/2 cup (125 g)	3.8	133	1.1
Sweet potato, baked	1 medium (114 g)	3.8	103	0.9
Multi-grain bread	2 slices regular (52 g)	3.8	140	2.7
Figs, dried	1/4 cup (about 38 g)	3.7	93	2.5
Pumpkin, canned	1/2 cup (125 g)	3.6	42	0.3
Potato baked, with skin	1 medium (173 g)	3.6	163	3.9
Popcorn, air-popped	3 cups (24 g)	3.5	93	3.9
Almonds	1 oz (about 28 g)	3.5	164	5.8
Pears, dried	1/4 cup (45 g)	3.4	118	2.6
Whole wheat spaghetti, cooked	1/2 cup (70 g)	3.2	87	1.2

(continued)

Table 20.2 (continued)

Food	Standard portion size	Dietary fiber (g)	Calories (kcal)	Energy Density (calories/g)
Parsnips, cooked	1/2 cup (78 g)	3.1	55	0.7
Sunflower seed kernels, dry roasted	1 oz (about 28 g)	3.1	165	5.8
Orange	1 medium (130 g)	3.1	69	0.5
Banana	1 medium (118 g)	3.1	105	0.9
Guava	1 fruit (55 g)	3.0	37	0.7
Oat bran muffin	1 small (66 g)	3.0	178	2.7
Vegetable soup	1 cup (245 g)	2.9	91	1.5
Winter squash, cooked	1/2 cup (100 g)	2.9	38	0.4
Dates	1/4 cup (about 38 g)	2.9	104	2.8
Pistachios, dry roasted	1 oz (about 28 g)	2.8	161	5.7
Pecans, oil roasted	1 oz (about 28 g)	2.7	203	7.2
Hazelnuts or filberts	1 oz (about 28 g)	2.7	178	6.3
Peanuts, oil roasted	1 oz (about 28 g)	2.7	170	6.0
Whole wheat paratha bread	1 oz (about 28 g)	2.7	92	3.3
Quinoa, cooked	1/2 cup (90 g)	2.7	92	1.2
Broccoli, cooked	1/2 cup (78 g)	2.6	114	1.5
Potato baked, without skin	1 medium (145 g)	2.3	145	3.9
Baby spinach leaves	3 oz (90 g)	2.1	20	0.2
Blueberries	1/2 cup (74 g)	1.8	178	2.4
Carrot, raw or cooked	1 medium (60 g)	1.7	25	0.4

Observational studies. Increased fiber intake is generally positively associated with improved insulin sensitivity [117–120]. Several cross-sectional studies reported that adequate fiber intake was positively associated with insulin sensitivity and negatively associated with the risk of developing insulin resistance [117–119]. Fiber was shown to be more effective at improving insulin sensitivity than dietary fat [109, 119]. In a study of subjects representing a range of glucose tolerance (2/3 normal and 1/3 impaired) and ethnicity (Hispanic, non-Hispanic white, and African-American), each 10 g/day increased fiber intake lowered fasting insulin by 0.08 pmol/ml and increased insulin sensitivity by 0.123 units [120]. A Swedish prospective study found that fiber-rich whole-grain intake is associated with decreased risk of deteriorating glucose tolerance including progression from normal glucose tolerance to prediabetes by mechanisms related to insulin sensitivity and insulin resistance [35]. Of the whole-grains, dark breads (including whole wheat, rye, pumpernickel, and other high-fiber breads) and breakfast cereals (including high-fiber bran or granola cereals and shredded wheat) were the most positively associated with insulin sensitivity and with lower fasting insulin [117]. In the Multi-Ethnic Study of Atherosclerosis (MESA) cross-sectional study, individuals with a higher MedDiet scores (higher fiber intake) had significantly lower baseline mean insulin levels compared to those with the lowest scores [121].

Randomized trials. Randomized trials on fiber products have reported more variable effects on improving insulin sensitivity than observational studies (e.g., whole-grains/cereal fiber [38–47], F&V [60–66], legumes [72–74], nuts and seeds [83–87], dietary patterns [25, 121], and supplements [26]) depending on the fiber source and level, physical properties of the food, study design, population health status and BMI, and glycemic index. A number of randomized trials support improved fasting insulin or insulin sensitivity when fiber is consumed at about 30 g/day or more with energy from

carbohydrate of about 45–50 % energy from complex carbohydrates [25–27] but excessive consumption of total carbohydrates, especially from retined carbohydrates, such as 58–60 % of energy may overwhelm the effects of fiber [28].

β-Cell Dysfunction and Insulin Secretion

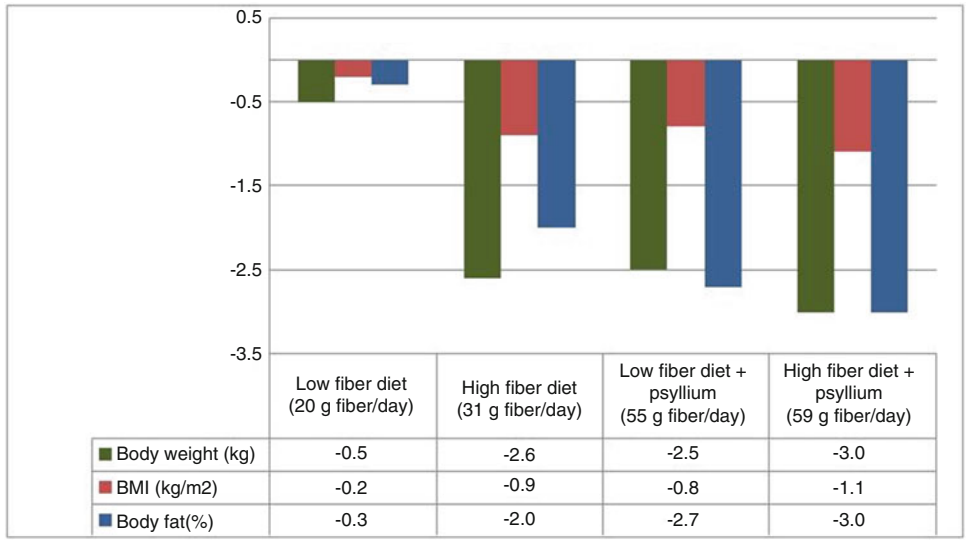
Background. Individuals with metabolic syndrome and women undergoing menopause may especially display a reduction in early-phase insulin secretion, which is an indicator of β-cell dysfunction and susceptibility for developing diabetes [44, 114, 122, 123].

Randomized trial. Three randomized trials indicate that diets rich in cereal grains, such as rye bread or resistant starch diets, can improve early-phase insulin secretion in overweight individuals with insulin resistance, metabolic syndrome or postmenopausal women [44, 122, 123]. The almond-enriched ADA diet group (56 g/day) exhibited greater improvement in beta-cell function compared with the ADA nut-free control group after 16 weeks [84]. Also, a large randomized trial found that total fiber intake was positively associated with improved early-phase insulin secretion in women [114].

Visceral Fat

Background. There is an established association between visceral fat and insulin resistance [105–107]. Related indicators of visceral fat are BMI, waist circumference, waist-to-hip ratio (WHR), and abdominal body fat (%). Increases in visceral fat occur when the subcutaneous fat deposits are full and the extra body fat spills over into visceral fat deposits, which leads to hypertriglyceridemia, ectopic fat deposition (including hepatic steatosis), and insulin resistance in muscle, liver, and pancreatic tissues due to inflammation and inflammatory cytokine production, which causes impairments in insulin receptor function [104–107]. Fiber-rich diets have been shown to help prevent and/or reverse visceral fat accumulation and related central body fat measures [27, 109, 124–143]. A systematic review of prospective and clinical studies concluded that fiber intake was inversely associated with the risk of gaining body weight and waist circumference [124]. The European Food Safety Authority (EFSA) concluded that: “Increased intake of dietary fibre, both naturally fibre-rich foods and added fibre or fibre supplements, has been shown to be related to improved weight maintenance in adults and sustained weight reduction in overweight subjects. Estimated intakes associated with this effect in adults is in the range of >25 g fibre per day (from whole-grain cereals, fruit, and vegetables) and >3.1 g total fibre per MJ” (13 g fiber/1000 kcal) [9].

Observational studies. Observational studies consistently support an inverse relationship between fiber intake and visceral fat measures in both adults and children. Obese individuals tend to have lower fiber intake than those who are normal weight or overweight [125]. In women, high-fiber diets are more effective at preventing weight gain than low-fiber diets and a daily increase of 10 g fiber/1000 kcal was shown to reduce body weight and fat by about 2 kg and 2 % over 20 months, after adjusting for energy intake [126, 127]. A 10-g-higher fiber intake was associated with a 1.9-cm-smaller waist circumference and a 0.80-kg/m^2-lower BMI [119]. Several prospective studies suggest that increasing fiber intake by 10–12 g/day can significantly reduce weight gain, visceral fat accumulation, and waist circumference [128–130]. Two cross-sectional studies in overweight and obese adolescents showed fiber intake to be negatively associated with visceral obesity [131, 132] .



	Low fiber diet (20 g fiber/day)	High fiber diet (31 g fiber/day)	Low fiber diet + psyllium (55 g fiber/day)	High fiber diet + psyllium (59 g fiber/day)
Body weight (kg)	-0.5	-2.6	-2.5	-3.0
BMI (kg/m2)	-0.2	-0.9	-0.8	-1.1
Body fat(%)	-0.3	-2.0	-2.7	-3.0

Fig. 20.9 In Australian obese adults, higher fiber diets with and without psyllium and low-fiber diets with psyllium significantly lowered body weight, BMI, and body fat compared to the lower fiber control diet ($p < 0.05$) after 12 weeks ($n = 72$) [26]

Randomized trials overview. In a number of randomized trials, ad libitum fiber-rich diets containing about 30 g fiber/day or more have consistently been shown to prevent weight gain and provide sustained weight loss compared with fiber diets of <20 g/day [25–27, 133–135]. However, fiber-rich diets with <25 g fiber/day do not appear to significantly reduce body weight or prevent weight regain [136, 137]. The addition of fiber-rich foods to a hypocaloric diet may enhance body weight and/or fat loss depending on the level of fiber or type of food consumed [138–141]. Fiber supplements or fiber-enriched foods can promote improved body weight regulation but their effects are heterogeneous and generally less effective than natural fiber-rich foods [142–147].

Adults. In a placebo controlled trial, 72 obese (mean BMI about 34 kg/m^2) adults were randomized into four diets: (1) control diet plus placebo (20 g fiber/day); (2) control diet plus psyllium supplement (55 g fiber/day); (3) healthy fiber-rich food diet plus placebo (31 g fiber/day); and (4) healthy fiber-rich food diet plus psyllium supplement (59 g fiber/day) for 12 weeks [26]. Compared to the control diet group, all higher fiber diets significantly reduced body weight, BMI, and body fat after 12 weeks (Fig. 20.9). The ab libitum fiber-rich food diet (31 g fiber/day) was as effective as the higher fiber diets with psyllium (>50 g fiber/day). Finally, in obese adults with metabolic syndrome, those on only a high-fiber diet with the goal of consuming ≥30 g fiber/day had insignificant differences in weight, BMI, and waist than those on a more complex, multi-component hypocaloric diet after 12 months [27].

Children/adolescents. Several studies indicate that in overweight children/adolescents, the intake of fiber is inversely related to visceral fat levels. In a longitudinal study, 85 overweight Latino youth ($n = 85$; aged 11–17 years) were evaluated regarding the effect of fiber intake on visceral fat over 2 years [148]. The daily increase or decrease of fiber by 3 g fiber/1000 kcal was a significant determinant of visceral fat area (Fig. 20.10) [148]. A randomized trial of 54 overweight Latino adolescents found that a modest increase of 5 g fiber/day significantly lowered BMI (−4 %) and visceral fat (−10 %) compared to the control diet after 16 weeks [149]. Also, a randomized trial with obese adolescents found that 6 g psyllium/day significantly reduced the android to gynoid fat ratio, which is associated with insulin resistance in obese children and adolescents, by 4 % after only 4 weeks [150].

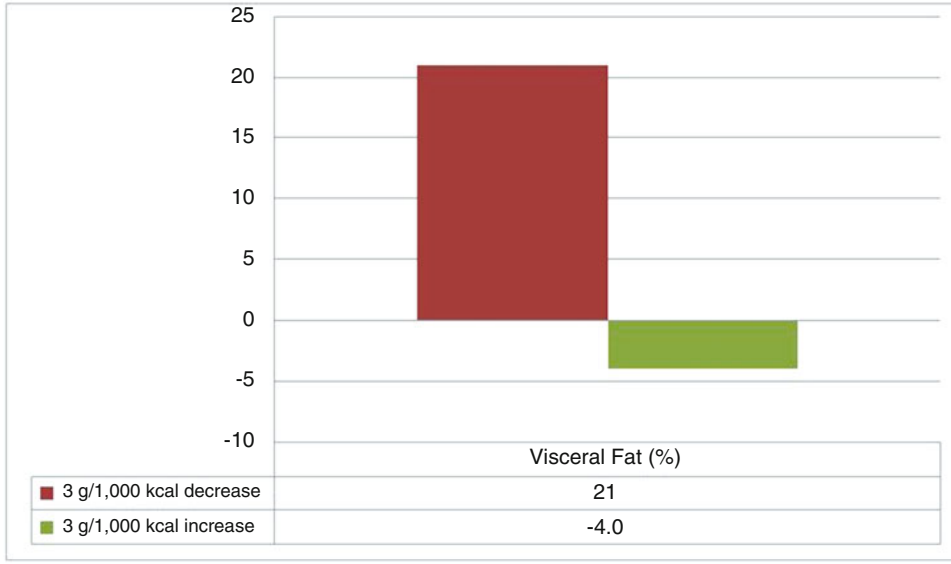


Fig. 20.10 In overweight Latino children and adolescents, a decrease in fiber density significantly increased mean % visceral fat compared with participants who had increased fiber density ($p=0.02$) over a 2 year follow-up ($n=85$) [149]

Systemic Inflammation

Background. Obesity, especially visceral fat, is associated with an increased risk of chronic elevated inflammation and diabetes [151]. The level of systemic inflammation is orchestrated in part by the balance of anti- and pro-inflammatory cytokines that are influenced by both lifestyle and genetics. The anti-inflammatory signals are adiponectin, IL-10, and others, and pro-inflammatory signals are tumor necrosis factor-α (TNF-α), c-Reactive Protein (CRP), interleukin (IL)-6, and others. The inhibition of insulin receptor signaling pathways is a central mechanism through which excess proinflammatory responses increase insulin resistance and diabetes risk [151]. The effects of fiber on helping to control systemic inflammation are through body weight related and unrelated mechanisms such as colonic fermentation of fiber to SCFA [152]. Weight loss, healthy diets, and increased fiber intake are known to increase adiponectin levels [153–155]. Multiple studies have shown a correlation between adiponectin levels and insulin sensitivity, which remains even after adjustment for adiposity [152]. A dietary pattern rich in whole-grain cereals and low-fat dairy products compared with refined cereal grain intake is modestly positively associated with higher plasma adiponectin levels in healthy women [154]. Also, specific fiber types and fiber-rich foods including oligofructose-enriched insulin, β-glucans, and Brazil nuts appear to enhance levels of IL-10 [156–158].

Observational studies. A systematic review of observational studies reported that 13 of 16 fiber studies and 6 of 7 whole-grain studies showed an inverse relationship with fiber intake level and systemic inflammation [159]. Analyses of US NHANES cross-sectional data found that of the macronutrients only fiber was inversely associated with elevated plasma CRP levels [160] and individuals consuming >22.5 fiber g/day had a 34 % lower risk of having elevated CRP compared to those consuming 8 g fiber/day [12]. The Nurses' Health Study found CRP to be inversely associated with healthier, fiber-rich diets and positively associated with low-fiber western diets [161]. A large Swedish longitudinal study of elderly men found that higher fiber intake was associated with both lower inflammation and better kidney function [162]. Also, in US adults 20 years and older, increasing fiber intake was

significantly negatively associated with diabetes risk factors, including elevated CRP (>3.0 mg/L), obesity (BMI > 30 kg/m^2), and metabolic syndrome rate after multi-variate adjustments [12].

Randomized trials. A systematic review found that six of seven randomized trials of high-fiber diets ranging from 14 to 33 g fiber/1000 kcal in the presence of weight loss and healthier dietary fat intakes reported significantly lower CRP concentrations by 21–54 % [163]. Similarly, a systematic review and meta-analysis of 17 clinical trials found that people with high MedDiet adherence (about 30 g fiber or more/day) had significantly increased adiponectin and decreased CRP and IL-6 compared to those on lower fiber diets [164]. The Finnish Diabetes Prevention Study reported that fiber intake was inversely associated with CRP and IL-6 after adjustments for BMIs [165]. A randomized MedDiet trial found that after 2 years subjects on 32 g fiber/day diets had lower CRP (−39 %) and IL-6 (−22 %) than those on 15 g fiber/day diets [25]. For the DASH diet pattern, people consuming 30 g fiber/day from diet alone or from 18 g psyllium fiber supplementation/day added to the usual diet, significantly reduced CRP levels by −14 % and −18 %, respectively, after 3 weeks compared to those on 12 g fiber/day diets [166]. However, in a follow-up study, low-fiber control diets (14 g fiber/day) supplemented with either 7 or 14 g of psyllium fiber/day did not show any effect on CRP or IL-6 levels (these diets were less than the recommended 30 g fiber/day (ranging 21–28 g fiber) [167]. Further, the 14 g psyllium added fiber diet did significantly lower fibrinogen levels compared to the low-fiber control diet. Also, in young smokers, 10 days of consuming 250 g/day of broccoli was shown to significantly decrease CRP by 48 % [168].

Fiber Mechanisms

Overview. Potential mechanisms for fiber's lowering of diabetes risk include (1) delaying postprandial glycemic and insulinemic response rates, (2) promoting lower food energy density and greater macronutrient fecal excretion, (3) improved appetite control to decrease energy intake by affecting signals associated with the stomach, small intestine, and brain, and (4) stimulating the colonic fermentation of SCFA to affect energy metabolism, appetite control, inflammatory response, and prebiotic microbiota activity. Figure 20.11 provides an overview of potential fiber biological mechanisms involved in diabetes prevention.

Delayed Postprandial Glycemic and Insulinemic Response Rates

Mechanism summary. When fiber, either insoluble or soluble fiber, is consumed at about 30 g fiber or more/day, especially with about 10–12 g fiber/meal, there tends to be enough critical mass of fiber to physically increase stomach and/or small intestinal bulk or viscosity to delay the digestion and absorption of available carbohydrates and acutely reduce postprandial glycemic and insulinemic response rates, which can potentially enhance insulin sensitivity if this dietary pattern is consumed as part of the usual diet for most days of the week [169–176].

Reduced Energy Density and Increased Macronutrient Fecal Excretion

Mechanism summary. Fiber-rich foods generally have lower dietary energy density both directly and by displacing higher energy-dense foods, lowering macronutrient bioavailability and leading to increased excretion [177].

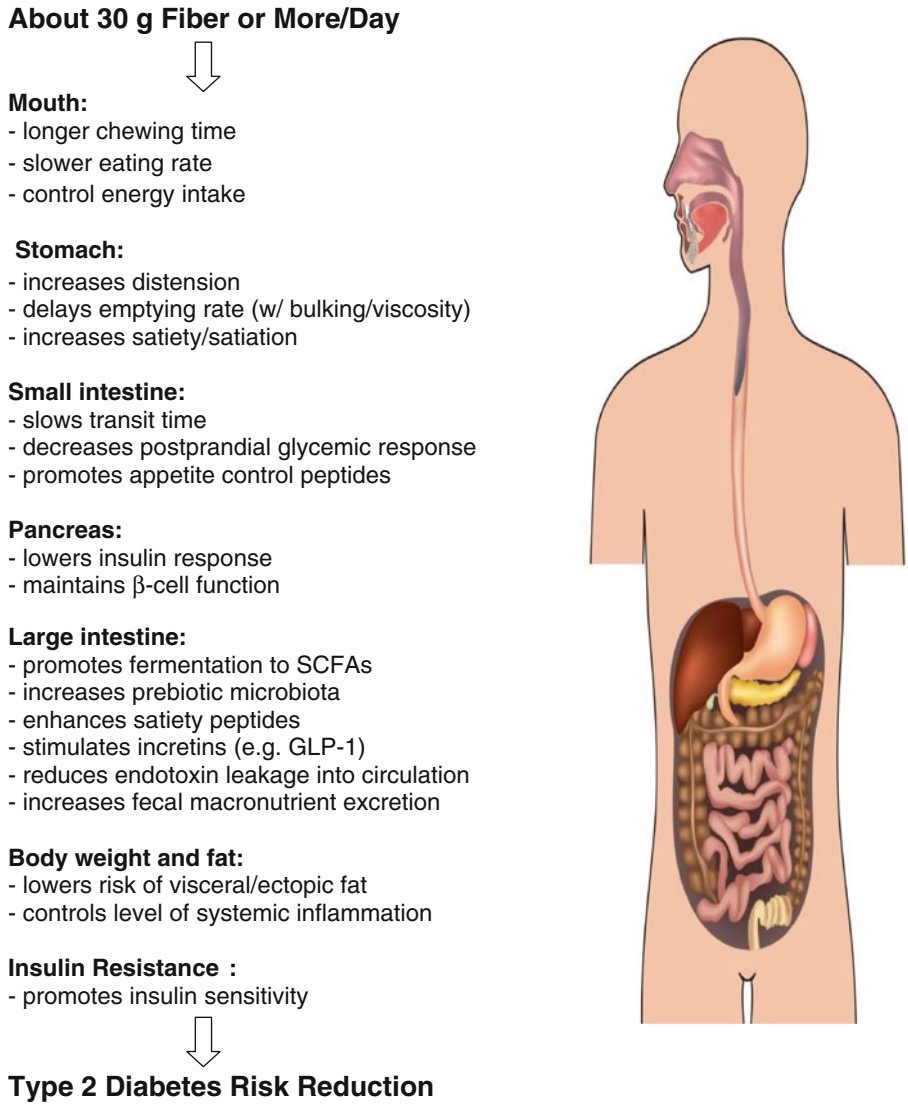


Fig. 20.11 Potential fiber mechanism associated with type 2 diabetes prevention

Energy density. In general, fiber is 2 kcal/g or less compared to 4 kcal/g for sugar and digestible starch, because fiber digestion bypasses the small bowel and is digested by anaerobic fermentation by large bowel microflora to SCFA and the gases carbon dioxide, hydrogen, and methane or remains as indigestible fiber [178, 179]. New studies using breath hydrogen measures indicate that more fibers than previously reported have an energy value of 1 kcal/g [180].

Fecal excretion. The consumption of >25 g fiber/day can reduce macronutrient energy availability by 3–4 %, which is equivalent to about 100 kcal/day [181, 182]. This relationship appears to be dose dependent. One study found that for each additional 5 g/day fiber consumed there was 36 kcal/day increase in fecal energy content [183]. This loss of undigested fecal macronutrient energy is

especially noted for flaxseeds (−6.5 kcal/g flaxseed added to bread vs. control bread) and almonds (32 % lower metabolizable energy (clinically tested) than values calculated from the standard Atwater energy factors used for labeling [184–186].

Improved Appetite Control and Energy Metabolism

Mechanism summary. Fiber may help to delay hunger, increase satiety by slowing the rate of gastric emptying and promote appetite control and fat oxidation by signaling a variety of gastrointestinal hormonal pathways [187, 188]. The hormones include orexigenic ghrelin and anorexigenic and metabolic stimulating peptide YY, cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1) [189–191].

The following is an overview of these hormones:

1. Ghrelin plasma levels are correlated with hunger. In humans, β-glucan and psyllium have been reported to decrease plasma ghrelin levels [191, 192].
2. CCK is both a gastrointestinal hormone and a brain neuropeptide with major biological functionality in decreasing food intake as plasma levels increase [190]. CCK is secreted postprandially from the proximal small intestine to regulate pancreatic activity, gastric emptying, and satiety [190]. The consumption of fiber-rich meals stimulates increased CCK circulation compared to energy matched low-fiber meals [193, 194].
3. Peptide YY is mainly released in the ileum and colon to regulate gastric and pancreatic secretion which is stimulated by fiber [190].
4. GLP-1 is both an important mediator of postprandial insulin secretion and sensitivity, and suppressor of food intake [195–198].

Randomized trials. Two systematic reviews of the effect of fiber-rich food, fiber supplements, or fiber-enriched processed foods on satiation, satiety and daily or next meal energy intake randomized trials are very heterogeneous [199, 200]. Potential variables include fiber type and level, physical characteristics of the fiber source consumed, or timing of intake (before or during a meal), which can promote satiation by increasing gastric distention and alter intestinal satiety hormones to suppress food intake [199, 201]. Whole fiber-rich foods tend to be more effective in appetite, energy intake, and weight control than processed whole foods (e.g., apple sauce or juice), supplements or energy dense foods enriched with added isolated fiber sources [191, 202–212]. However, there are some potential highly functional isolated fiber exceptions such as cactus fiber complex, which appears to help control appetite, reduce energy intake, and promote weight loss when used as a dietary supplement [146].

Increased Colonic SCFA Levels and Prebiotic Microbiota Activity

Mechanism summary. To varying degrees all fibers act as a substrate for the large bowel microbiota production of bioactive SCFAs such as acetate, propionate, and butyrate as approximately 70 % of consumed fiber in mixed meals is fermented [178–180]. SCFA are involved in the crosstalk existing between large bowel microbes and human cells [190]. SCFA appear to be important mediators associated with food intake, insulin sensitivity, and insulin resistance through gut peptides such as GLP-1 and systemic inflammation [116, 195–198, 213–215].

GLP-1 may suppress food intake and promote insulin sensitivity and β-cell mass [190, 195–198]. Colonic epithelial cells have receptors that detect SCFA in luminal contents to stimulate the secretion

of GLP-1. The relationship between fiber fermentation and the modulation of GLP-1 is widely accepted in animal models but the evidence in human study remains a matter of debate. Randomized trials examining the link between fiber microbiota fermentation and GLP-1 secretion have shown mixed outcomes depending on fiber source, subject health profile, and time of consumption [216–224].

Systemic inflammation. Fiber, through its fermentation to SCFA, particularly propionic and butyric acids, has the capacity to reduce systemic inflammation by acting to suppress inflammation activity in the colon and visceral fat [116, 190, 213, 215]. In the colon, butyrate is the preferred energy source for colon cells. Butyrate promotes the assembly of tight endothelial junctions to reduce leakage of intestinal endotoxic bacterial lipopolysaccharides (LPS) into the circulation which helps alleviate systemic inflammation [116]. For visceral fat, propionate has been shown in human adipose tissue obtained from overweight adults to reduce visceral fat inflammation by downregulating the pro-inflammatory cytokine TNF-α [215]. Thus, SCFA produced from fiber fermentation provide another level of defense against system inflammation and risk of developing insulin resistance.

Prebiotic microbiota activity. Many fibers are known to be prebiotics, which can stimulate beneficial bacterial microbiota [213]. Examples of fiber prebiotics include inulin, oligofructose, resistant starch, polydextrose, wheat dextrin, acacia gum, psyllium, and bran. A systematic review of prebiotic clinical studies report significantly increased self-reported feelings of satiety in healthy adults and attenuated postprandial glucose and insulin responses with a number of contradictory findings for energy intake, body weight, PYY and GLP-1 concentrations, insulin sensitivity, and inflammatory response, because of fiber type and level consumed [200]. The consumption of 21 g polydextrose or soluble corn fiber in the form of 3 cereal bars/day for 3 weeks changed the gut microbiota of overweight subjects by shifting the colonic Bacteroidetes to Firmicutes ratio to one that was more typical of lean individuals, independent of caloric restriction [225].

Conclusions

Fiber is an important macronutrient food component for the prevention of diabetes. Only about 5 % of the US population meets the recommended fiber adequate intake level and similar low-fiber intake is found in many other western countries. Prospective studies show that healthy, higher fiber diets are associated with a 15–83 % lower risk of developing diabetes compared to low-fiber, western diets. A dose response meta-analysis of prospective studies found a nonlinear relationship between fiber intake and diabetes risk with a linear reduction starting at about 25 g fiber/day. A number of randomized trials support the consumption of healthy, fiber-rich dietary patterns with about 30 g fiber/day or more for lowering diabetes risk and improving fasting insulin levels and insulin resistance scores. For reduced diabetes risk, the typical western diet has about a daily 15 g fiber intake gap. This gap can be closed by substituting a lower fiber food with a fiber-rich food at each meal and for one snack per day. Fiber has the potential to stimulate a complex and diverse range of intrinsic, hormonal, and colonic protective diabetes mechanisms.

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Chapter 21
Nutrition Issues and Recommendations in the Management of Diabetes and Prediabetes in Older Adults

Barbara Stetson, Stephanie McDonough, and Sri Prakash Mokshagundam

Key Points

- The prevention or delay of long-term complications of high blood glucose and related metabolic abnormalities and improving quality of life are key issues for nutritional considerations in diabetes care.
- Preventive care can substantially add to quality of life in those living with diabetes, including those in their senior years. In the USA, estimates of diabetes prevalence range from 22 to 33 % for adults aged 65 and older. Prevalence is rising as the population ages, there is an increasing proportion of older adults in ethnic groups with particularly high diabetes rates and there is increased longevity in people living with diabetes. Prevention of the development of diabetes in those with risk factors such as "prediabetes" is highly relevant to the care of older adults. The Centers for Disease Control and Prevention (CDC) reports a high prevalence and increasing rates of prediabetes in adults over age 50 with rates from 1999 to 2005 and 2006 to 2010 increasing from 38.5 to 45.9 % in adults aged 50–64 and from 41.3 to 47.9 % in adults aged 65–74 and from 45.1 to 48.9 % in adults aged 75 and older.
- Older adults with diabetes may be functionally limited by the presence of hypoglycemia. Factors that may play a role in the increased risk of hypoglycemia in older adults include poor nutritional status, cognitive dysfunction, polypharmacy, and comorbid illnesses.
- Diabetes prevalence-related comorbidities such as diabetic retinopathy, cardiovascular disease, peripheral vascular disease, and congestive heart failure may result in decreased usual activity and limit activities of daily living, including transportation, shopping for food, and ability to read food labels and restaurant menus.
- Given the high rates of depression in the diabetes population and in the older adults, careful assessment of depressive symptomology and its impact on dietary intake, diabetes self-care, and health outcomes is critical.

Keywords Older adults • Diabetes • Blood glucose • Metabolic syndrome • Hypoglycemia • Quality of life

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Nutrition and Health, DOI 10.1007/978-3-319-22431-2_21,

Introduction

Diabetes mellitus (diabetes) has reached unprecedented proportions worldwide [1]. The WHO estimated that in 2012 there were 347 million individuals with diabetes mellitus worldwide affecting ~9 % of the population >18 years of age. It is projected that between 2005 and 2030 diabetes-related deaths will have doubled. The prevalence of obesity is rising so rapidly in so many countries that the World Health Organization (WHO) has declared that there is now a global epidemic of obesity. Internationally, emergence of new cases of diabetes parallels the increases seen in Western countries with rates increasing particularly rapidly in Asia. The risks of type 2 diabetes in these countries tend to increase at levels of body mass index generally classified as non-obese in Caucasian Westerners. These worldwide changes are due to an accelerated prevalence of obesity, today's predominance of sedentary lifestyle and the rapidly growing population of older adults, including an increased proportion of those belonging to ethnic groups with especially high rates of diabetes [2–4]. In the USA the estimated number of individuals with diabetes was estimated at 25.8 million and 8.3 % of the population. Of these, 18.8 million were diagnosed and 7 million undiagnosed. By 2012, prevalence was 29.1 million and 9.3 % of the population with 21 million diagnosed and 8.1 million undiagnosed [2]. Type 2 diabetes disproportionately affects minority populations, including African Americans (13.2 %), Hispanics (12.8 %), Native Americans (15.9 %), and Asian Americans (9 %) [2]. Risk factors for diabetes that are specific to these populations include genetic, behavioral, and lifestyle factors.

Prevalence of Diabetes in Older Adults

The aging of America is also contributing to the increasing numbers of cases of diabetes, as diabetes prevalence increases with age. In developing countries, the majority of people with diabetes are between 45 and 64 years of age. In developed countries, the majority of people with diabetes are aged 65 or greater. In the USA, the oldest of the large baby boomer cohort are now approaching age 60, and increasing numbers will soon join these ranks. In the USA, estimates of diabetes prevalence range from 22 to 33 % for adults aged 65 and older, depending on the diagnostic criteria that are used. Prevalence is rising as the population ages; there is an increasing proporation of older adults in ethnic groups with high rates of diabetes as well as increased longevity in people living with diabetes [5].

Burden of Diabetes

Diabetes is a chronic disease that leads to a variety of micro- and macrovascular complications that affect almost all systems in the body. While the primary abnormality in diabetes, elevated blood glucose level, remains largely asymptomatic, the consequences of sustained elevation in blood glucose are potentially devastating. Older adults with diabetes have higher rates of functional disability and comorbid conditions relative to those without diabetes. These complications lead to significant morbidity and mortality, and are associated with markedly increased economic burden. The annual economic cost of diabetes mellitus in 2012 in the USA was estimated at $245 billion. The medical expenditures of people with diabetes are 2.3 times that of those without diabetes. Older adults with diabetes have the highest rate of lower extremity amputation [5, 6].

Goals of Diabetes Treatment

The management of diabetes requires a combination of lifestyle interventions and medications. Diabetes is often a progressive disease requiring changing therapeutic strategies. Thus, primary, secondary, and tertiary prevention efforts are all key to public health goals. Clinically, the interaction between lifestyle changes and medications must be carefully considered across the preventive care continuum. This chapter will address secondary and tertiary nutrition prevention, beginning with issues relevant to persons already diagnosed with diabetes and then addressing those with prediabetes.

Dietary intervention to maintain optimal glycemic control is a key component of management of those diagnosed with diabetes. The aims of diabetes treatment are to (1) decrease/prevent the development of long-term complications of high blood glucose and related metabolic abnormalities, (2) improve the quality of life of individuals with diabetes, and (3) treat or prevent the development of symptoms of high or low blood glucose.

Diagnosis and Classification of Diabetes

Diabetes is diagnosed on the basis measurement of Hemoglobin A1c, fasting plasma glucose or the 2-h plasma glucose value after a 75 g glucose load. The American Diabetes Association (ADA) recommendations for the diagnosis of diabetes and prediabetes abnormalities of blood glucose are outlined in Table 21.1. The ADA diagnostic criteria were developed for general use, and apply broadly to all age groups. No specific ADA guidelines exist for older adults.

Typologies of Diabetes in Older Adults

The proper classification of diabetes is important in setting goals for nutritional management. Diabetes is broadly classified into type 1 and type 2 diabetes.

The majority of older adults with diabetes have type 2 diabetes, which is characterized by two defects—insulin resistance and defective insulin secretion. The majority of individuals with type 2 diabetes are obese. However, in the older population, the proportion of who are underweight increases, and could be as high as 20 %. This is particularly true in the nursing home population. Type 2 diabetes results from a combination of insulin resistance, increased hepatic glucose production, and defective insulin secretion. The exact mechanism of insulin resistance in type 2 diabetes is unclear. A variety of genetic and environmental factors lead to decreased insulin sensitivity. Aging is associated with a change in body composition with increase in fat mass and sarcopenia [7]. This could be partly responsible for the increase in insulin resistance with aging. Aging is also associated with a decline in islet function and insulin secretion, particularly a blunting of the first phase insulin secretion [8–10]. Age-related changes in health behaviors, such as increased sedentary lifestyles, may also further compound these changes. Type 2 diabetes is a progressive disorder. The progression of the clinical picture

Table 21.1 Criteria for diagnosis of diabetes and prediabetes

	Prediabetes	Diabetes
Fasting blood glucose (mg/dL)	100–125	>125
Two-hour post 75 g oral glucose challenge blood glucose (mg/dL)	141–199	>200
Hemoglobin A1c (%)	5.7–6.4	≥6.5

with increasing blood glucose levels, requiring increasing doses of medications, is due mainly to a progressive decline in beta-cell function. When beta-cell function is markedly reduced, exogenous insulin will be necessary to regulate blood glucose levels.

Type 1 diabetes is an autoimmune disorder resulting from cell mediated and antibody mediated destruction of beta-cells of the islets [11]. Insulin is required for the management of type 1 diabetes. Failure to treat with insulin results in development of an acute metabolic complication—diabetic ketoacidosis. Although type 1 diabetes most commonly occurs in the first three decades of life, it can develop at any age, even in older adults. With improved life expectancy of individuals with type 1 diabetes, a growing number of them are now in the older age groups [5]. Type 1 diabetes, particularly of long duration, is often very "brittle" with wide fluctuations in blood glucose levels and episodes of recurrent and severe hypoglycemia.

Establishing Medication and Nutritional Management Goals in Older Adults with Diabetes

Once the type of diabetes is established, medication and nutritional management goals should be developed. Current medical nutrition recommendations for both prevention and treatment of diabetes now reflect an evidence-based approach [12, 13]. In addition to tailoring the nutritional recommendation to assist treatment goals associated with glycemic control, consideration of other important risk factors is critical. Obesity, dyslipidemia, hypertension, and insulin resistance are important, and are often overlapping factors, warranting consideration when planning dietary interventions for older adults with type 2 diabetes. Avoidance of hypoglycemia, particularly recurrent and/or severe hypoglycemia, is a major consideration in type 1 diabetes. Lifestyle interventions that have been recommended for the management of diabetes have positive effects on both insulin secretion and insulin resistance. Current nutrition intervention guidelines emphasize the importance of personal preferences including considering cultural and traditional practices, health-related beliefs and economic factors [13].

The major aim of treating diabetes is to decrease the rate of micro- and macrovascular disease progression associated with elevated blood glucose. Much of the previous recommendations for management of diabetes were based on a limited number of clinical trials, which had limited samples of older adults. Furthermore, a number of recent trials designed to obtain "tight" glycemic control in individuals with type 2 diabetes either failed to show any benefit, or had worse outcomes. It has become increasingly clear that several additional factors specific to the older adults, including cognitive impairment, depression, polypharmacy and urinary incontinence need to be considered in setting goals. Studies over the last two decades have also stressed the diversity of responses to aggressive blood pressure and lipid control and have led to revisions in the goals for the management of these comorbidities. Based on the more recent evidence, individualization of treatment is key to effective management of diabetes in older adults. Hence, the current ADA guidelines [14], as well as American Geriatric Society guidelines [15], incorporate a variety of factors in setting goals for the management of diabetes and its comorbidities in older adults (see Table 21.2).

Optimal implementation of the current guidelines and the use of available agents will ultimately depend on expanding the knowledge base of health care providers, and may require far-reaching educational programs that change the way that risk-factor management is viewed by caregivers and patients alike.

Any nutritional approach to the management of diabetes must specifically address the issues related to cardiovascular risk. Cardiovascular risk reduction in diabetes is achieved through a combination of lifestyle changes and pharmacological interventions that address the multiple risk factors. A general outline of lifestyle and pharmacological approaches to diabetes management and this comorbidity is shown in Table 21.3.

Taken together, these lifestyle and pharmacological interventions can play a major role in the management of cardiovascular risk reduction in persons with diabetes. The recommended goals for management of weight, blood pressure, and lipids are noted in Table 21.4.

Table 21.2 Recommend care goals for management of diabetes in older adults[a]

1. Individualize glycemic goals: The goal, in most cases, should usually include the standard A1c target of <7 %. Consider a higher goal, if appropriate, based on the following factors:
– Patient preference
– Diabetes severity
– Life expectancy
– Functional status and social support
2. Keep therapy as simple and inexpensive as possible
3. Encourage diabetes education of the patient and primary caregivers, with the reminder that such education is covered by Medicare
4. Treat hypertension and dyslipidemia to decrease cardiovascular risk
5. Screen for depression and offer therapy promptly if the diagnosis is made
6. Maintain an updated medication list and monitor regularly for adverse drug effects
7. Screen annually for cognitive impairment and other geriatric syndromes (e.g., urinary incontinence, pain, injurious falls)

[a]Adapted from: AGS guidelines for the treatment of diabetes mellitus in geriatric populations [15]

Table 21.3 Considerations for diet and pharmacological approaches to risk factor management in diabetes

Risk factor	Lifestyle intervention	Pharmacological intervention
Hemoglobic A1c	Diet and exercise	Insulin sensitizing agents, insulin secretagogues, insulin
Postprandial glucose/ glycemic excursion	Carbohydrate content of meals (amount, type, timing, personal response to CHO based on post-prandial SMBG feedback)	Repaglinide (prandin), nateglinide (starlix), sitagliptin, exenatide, pramlintide, short acting insulins (insulin lis-pro, insulin aspart, regular insulin)
LDL cholesterol	Low cholesterol diet	Statins
	Exercise	
Triglyceride	Low fat diet	Gemfibrozil/fenofibrate
	Exercise/weight loss	Niacin (long acting)
		Omega 3 fatty acids
Low HDL cholesterol	Exercise	Niacin
	Smoking cessation	Gemfibrozil/fenofibrate
High blood pressure	Low sodium diet	Variety of antihypertensive agents (ace inhibitors preferred)
	Exercise	
	Weight loss	
Procoagulant state	Exercise/weight loss	Aspirin (in some situations)
Proinflammatory state	? Diet (e.g., increased proportion of less-refined CHO, increased vegetable and fruit intake, reduced saturated fat, exercise, weight loss, medication)	Aspirin (?) (? indicates that this is not clearly established)

General Diabetes Dietary Recommendations

The general goals of nutritional recommendations for the management of diabetes are to:

1. Promote and support healthful eating patterns, emphasizing a variety of nutrient dense foods in appropriate portion sizes in order to improve overall health and specifically to:
   (a) Achieve and maintain blood glucose levels as appropriate.

Table 21.4 Framework for considering treatment goals for glycemia, blood pressure, and dyslipidemia in older adults with diabetes[a]

Patient characteristics/ health status	Rationale	Reasonable A1C goal[b]	Fasting or prepran-dial glucose (mg/dL)	Bedtime glucose (mg/dL)	Blood pressure (mmHg)	Lipids
Healthy (few coexisting chronic illnesses, intact cognitive and functional status)	Longer remaining life expectancy	<7.5 %	90–130	90–150	<140/90	Statin unless contraindicated or not tolerated
Complex/intermediate (multiple coexisting chronic illnesses[c] or 21 instrumental ADL impairments or mild-to moderate cognitive impairment)	Intermediate remaining life expectancy, high treatment burden, hypoglycemia vulnerability, fall risk	<8.0 %	90–150	100–180	<140/90	Statin unless contraindicated or not tolerated
Very complex/poor health (long-term care or end stage chronic illnesses[d] or moderate-to-severe cognitive impairment or 2+ ADL dependencies)	Limited remaining life expectancy makes benefit uncertain	<8.5 %[e]	100–180	110–200	<150/90	Consider likelihood of benefit with statin (secondary prevention more so than primary)

This represents a consensus framework for considering treatment goals for glycemia, blood pressure, and dyslipidemia in older adults with diabetes. The patient characteristic categories are general concepts. Not every patient will clearly fall into a particular category. Consideration of patient and caregiver preferences is an important aspect of treatment individualization. Additionally, a patient's health status and preferences may change over time. *ADL* activities of daily living

[a]Adapted From American Diabetes Association Position Statement on Older Adults [96]

[b]A lower Hemoglobin A1c level goal may be set for an individual if achievable without recurrent or severe hypoglycemia or undue treatment burden

[c]Coexisting chronic illnesses are conditions serious enough to require medications or lifestyle management and may include arthritis, cancer, congestive heart failure, depression, emphysema, falls, hypertension, incontinence, stage 3 or worse chronic kidney disease, myocardial infarction, and stroke. By "multiple," we mean at least three, but many patients may have five or more [97]

[d]The presence of a single end-stage chronic illness, such as stage 3–4 congestive heart failure or oxygen-dependent lung disease, chronic kidney disease requiring dialysis, or uncontrolled metastatic cancer, may cause significant symptoms or impairment of functional status and significantly reduce life expectancy

[e]A1C of 8.5 % equates to an estimated average glucose of 200 mg/dL. Looser glycemic targets than this may expose patients to acute risks from glycosuria, dehydration, hyperglycemic hyperosmolar syndrome, and poor wound healing

   (b) Achieve and maintain optimum lipid levels and blood pressure.
   (c) Achieve and maintain reasonable body weight. This would include weight loss, if overweight, and weight gain, if undernourished.
   (d) Delay or prevent chronic complications.
   (e) Prevent acute complications.
   (f) Maintain overall health.

2. Address individual personal needs based on personal and cultural preferences, health literacy and numeracy, access to healthful food choices, willingness and ability to make behavioral changes, as well as barriers to change.
3. Maintain the pleasure of eating by providing positive messages about food choices, and limiting food choices only when indicated by scientific evidence.
4. Provide individual with practical day-to-day meal planning rather than focusing on macronutrients, micronutrients, or special foods.

The above recommendations from the ADA are applicable to the population of diabetics in general. Medical nutrition therapy has been found to be beneficial in older adults with diabetes. However, special considerations may be necessary in older adults. Micronutrient deficiencies might occur due to restrictions in total caloric intake. Also, older adults are at risk of undernutrition due to anorexia, dental/oral health problems, swallowing difficulties, and cognitive impairment. Hence, a rapid, validated nutritional assessment (e.g., with the Mini-Nutritional Assessment Short-Form instrument (http://www.mna-elderly.com/) [16, 17]) is recommended while planning dietary interventions with older adults with diabetes.

While a specific distribution of macronutrients had been previously recommended for individuals with diabetes, recent analyses failed to identify any evidence supporting use of a specific macronutrient composition of diet [18]. Hence, collaborative goals should be developed in discussion with the individual and when appropriate, their caregivers. It is, however, recognized that carbohydrate content of the meal is an important determinant of postprandial blood glucose levels and appropriate diet and medication adjustments need to be made to control postprandial blood glucose. Furthermore, the use of complex carbohydrates from fruits, vegetables, whole grains, and legumes is preferred over use of simple sugars. Similarly, there are no studies that guide the use of specific amount of proteins in subjects with diabetes.

Eating Patterns

A variety of different food groupings and eating patterns have also been evaluated in adults with diabetes. Review of the evidence with these eating pattern plans (Mediterranean [19], vegetarian and vegan [20], low fat, low carbohydrate [21], and DASH—Dietary Approaches to Stop Hypertension [18, 22]) as well as macronutrient distribution data, indicates that several different patterns of eating may beneficially impact glycemic control and cardiovascular risk factors [18]. Description of these patterns and examples of several studies with adults with diabetes may be seen in the ADA position statement on Nutrition therapy [13]. Given these findings, the ADA does not endorse a particular pattern, and recommends that health providers consider personal preferences and needs such as cultural, traditional and religious practices, health beliefs and goals and economics, as well as metabolic goals, when addressing dietary recommendations [13]. Additionally, when considering optimal eating patterns for older adults with diabetes, overall energy intake and portion sizes are important considerations [13].

Special Foods

A number of vitamins, minerals, and dietary supplements have been claimed to be beneficial in individuals with type 2 diabetes. Unfortunately, none of these foods or supplements have been systematically studied to provide enough data supporting their use on a routine basis. It would be reasonable to supplement vitamins when there is a clear demonstration of deficiency, but their benefits on glycemic control and cardiovascular risk have yet to be clearly established. Of particular interest are vitamin D and cinnamon.

The role of vitamin D in the development of diabetes has been explored [23, 24]. Supplementation of vitamin D has been demonstrated to improve inflammatory markers, insulin sensitivity, and beta cell function [23, 25] and improve glycemic control in type 2 diabetes [26]. Recently, the NIH sponsored a clinical trial to assess the role of vitamin D in preventing type 2 diabetes [27]. Until results of these trials are available, no definite recommendations can be made with regard to use of vitamin D. Furthermore, these studies are not specifically designed to address the older adult. A variety of complementary and alternative medicines have been used for the management of

diabetes. Of these, cinnamon, garlic, fenugreek, bitter gourd, and ginger have been widely used. In a recent review of clinical and basic science literature, Medagama and Bandara found that the significant heterogeneity in subjects selection and outcome measures [28], and the fact that these are short-term studies with limited number of subjects made it difficult to make any recommendations on the use of these products.

Gut Microbiota

There is increasing recognition of the role of gut microbiome in the development and progression of diabetes and its complications [29]. Dietary factors play an important role in determining the microbial flora in the gut [30–32]). It is possible to modulate the microbiome through the use of prebiotics and probiotics [33]. Prebiotic is a general term to refer to chemicals that induce the growth and/or activity of commensal microorganisms (e.g., bacteria and fungi) that contribute to the well-being of their host. In diet, prebiotics are typically nondigestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth and/or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them. Probiotics are microorganisms that are believed to provide health benefits when consumed. Small studies have suggested beneficial effects of prebiotics and probiotics in diabetes prevention and treatment [34, 35]). More definitive studies that clearly define the exact nature of these products and the specific populations that might benefit are needed before they can be routinely used in clinical practice.

Balancing Diet and Medication

The interaction of diet and medication is of particular importance in the management of diabetes. A wide range of medications are currently available for the management of diabetes. It is critical to evaluate each individual and evaluate the interactions between medication and diet in managing diabetes. This should be an ongoing process, as medication use and dietary patterns could vary over time. Table 21.5 provides a useful summary of factors to consider in prescribing diet and medications for diabetes. These recommendations are not specific to older adults.

Body Weight and Functional Status in Older Adults with Diabetes

Overweight and Obesity

Overweight is not only an important risk factor for the development of diabetes, but also has a significant impact on diabetes progression and the development of complications [36]. The rate of obesity in older adults continues to increase [37]. Obesity appears to be common in older adults until the eighth decade of life and then declines in the oldest old.

In 2009, over 35 % of adults aged 65 and older were overweight or obese in the USA [38]. Examination of data from the National Health and Nutrition Examination Survey shows ethnic and gender differences in the pattern of changes in obesity prevalence between 1999 and 2008 [2]. Among the young old (aged 65–74), the prevalence of obesity increased from 33 to 40 %; rates decreased in women from 39 to 35 %. Obesity increased among both men and women aged 75 and

Table 21.5 Guidelines for summary coordinating food with type of diabetes medicine[a]

For individuals who take insulin secretagogues:
• Moderate amounts of carbohydrate at each meal and snacks. (To reduce risk of hypoglycemia)
– Eat a source of carbohydrates at meals
– Moderate amounts of carbohydrates at each meal and snacks
– Do not skip meals
– Physical activity may result in low blood glucose depending on when it is performed. Always carry a source of carbohydrates to reduce risk of hypoglycemia*
For individuals who take biguanides (metformin):
• Gradually titrate to minimize gastrointestinal side effects when initiating use:
– Take medication with food or 15 min after a meal if symptoms persist
– If side effects do not resolve over time (a few weeks), follow-up with health care provider
– If taking along with an insulin secretagogue or insulin, may experience hypoglycemia*
For individuals who take a-glucosidase inhibitors:
• Gradually titrate to minimize gastrointestinal side effects when initiating use. Take at start of meal to have maximal effect:
– If taking along with an insulin secretagogue or insulin, may experience hypoglycemia
– If hypoglycemia occurs, eat something containing monosaccharides such as glucose tablets as drug will prevent the digestion of polysaccharides
For individuals who take incretin mimetics (GLP-1):
• Gradually titrate to minimize gastrointestinal side effects when initiating use:
– Injection of daily or twice-daily GLP-1 s should be pre-meal
– If side effects do not resolve over time (a few weeks), follow-up with health care provider
– If taking along with an insulin secretagogue or insulin, may experience hypoglycemia*
– Once-weekly GLP-1 s can be taken at any time during the day regardless of meal times
For individuals with type 1 diabetes and insulin-requiring type 2 diabetes:
• Learn how to count carbohydrates or use another meal planning approach to quantify carbohydrate intake. The objective of using such a meal planning approach is to "match" mealtime insulin to carbohydrates consumed
If on a multiple-daily injection plan or on an insulin pump:
– Take mealtime insulin before eating
– Meals can be consumed at different times
– If physical activity is performed within 1–2 h of mealtime insulin injection, this dose may need to be lowered to reduce risk of hypoglycemia*
If on a premixed insulin plan:
– Insulin doses need to be taken at consistent times every day
– Meals need to be consumed at similar times every day
– Do not skip meals to reduce risk of hypoglycemia
– Physical activity may result in low blood glucose depending on when it is performed. Always carry a source of quick-acting carbohydrates to reduce risk of hypoglycemia*
If on a fixed insulin plan:
– Eat similar amounts of carbohydrates each day to match the set doses of insulin

[a]Adapted from American Diabetes Association Position Statement on Nutrition Therapy Recommendations for the Management of Adults with Diabetes [13]

over across this same time period, with obesity present in just over one-fourth of adults in this age group [2, 39].

Sarcopenic obesity, defined as obesity with decreased muscle mass and function, has been reported in 21 % of older adults [40, 41]. The combination of obesity and sarcopenia has been recognized as a factor increasing risk of disability and mortality than either of these alone.

Unintentional and Intentional Weight Loss

Important implications must be considered for both intentional and unintentional weight loss in older adults. In a 12-year follow-up study examining the effect of intentional weight loss on all-cause mortality in overweight and obese older adults receiving treatment for hypertension, no significant differences were found between intentional weight loss and sodium reduction intervention groups [42]. In a separate study of 3-year mortality in community-dwelling older adults, unintentional weight loss and underweight BMI were associated with elevated mortality. Overweight or obesity and intentional weight loss were not associated with mortality. These results suggest that undernutrition may pose greater mortality risk in older adults than do obesity or intentional weight loss. However, findings are mixed regarding the mortality risks associated with intentional weight loss in older adults overall. Additional research is needed to determine when intentional weight loss may lead to increased health problems. Unintentional weight loss and underweight in older adults are important clinical considerations in both home-based and specialty care settings. One study found that at least 21 % of nursing home patients with type 2 diabetes were underweight [43].

Weight Loss Intervention Issues with Older Adults

Weight loss issues that must be considered for older adults with diabetes include the impact of restrictions on quality of life and potential loss of lean muscle mass from decreased protein intake. In research undertaken with younger adults with diabetes, weight loss programs that combine diet, physical activity, and theoretically guided behavior change techniques have been shown to be the most effective over the short term [44]. A randomized clinical trial examined the effects of diet, exercise, and combined interventions in obese older adults on a number of outcomes including physical function, frailty, and weight loss. Results indicated that greater improvements in physical function, functional status, strength, balance and gait were shown in the diet-exercise combined group (compared to diet and exercise alone conditions). Body weight decreased comparably in the diet group and diet-exercise combined group (10 % and 9 % respectively), but did not decrease in the exercise-only group [45]. Behavioral weight control interventions with persons with type 2 diabetes have found that even reductions of approximately 10 % of weight loss can decrease hypertension and lipid abnormalities and improve glycemic control, with improvements related to the magnitude of weight loss [44].

The Look AHEAD trial was the first large, multisite long-term randomized clinical trial ($N = 5145$; aged 45–74) with type 2 diabetes that compared intensive weight loss intervention to diabetes support and education. Restriction of caloric intake was the primary approach in the weight loss group with a goal to limit total fat calories to 30 %, with a maximum of 10 % saturated fat and minimum of 15 % from protein. Portion controlled diets, structured meal plans, and home-based exercise goals were prescribed. At 1-year follow-up, intervention participants had lost an average of 8.6 % body weight, with greater weight loss, increased cardiovascular fitness, and improved cardiovascular risk factors relative to participants in the control condition. At year 8, participants in the weight loss condition lost an average of 4.7 % of initial weight compared to 2.1 % in the control condition. In addition, 50.3 %

achieved a 5 % or greater weight loss (vs. 30.7 %) and 26.9 % lost 10 % or more (vs. 17.2 %). Further, when compared to the control condition, participants reported greater engagement of key weight-control behaviors. Notably, use of glucose lowering medications decreased in intervention participants and increased in control group participants. Use of antihypertensive medications increased in control group participants but remained unchanged in the intervention group. Use of lipid lowering medications increased in both intervention and control groups, with smaller increases in intervention participants. Findings highlight that substantial weight loss is feasible in persons with type 2 diabetes and results in a wide range of health benefits [46–48].

While weight loss interventions have been established as effective and important for improved health, intentional weight loss could worsen sarcopenia as well as decrease bone mineral density in older adults [5]. Research examining bone mineral density in a subsample of participants ($N = 1274$; M age = 58.4) in the Look AHEAD study at baseline and 1-year follow-up revealed greater bone loss in the weight loss group at the total hip (−1.4 % vs. −0.4 %) and femoral neck (−1.5 % vs. −0.8 %) when compared to the control condition. Bone mineral density for the lumbar spine and whole body did not differ significantly [49]. Other studies have shown that hip bone mineral density decreased less when the intervention emphasized the combination of diet and exercise, compared to a diet-alone intervention [45]. Further research is needed to gain a better understanding of the effects weight loss interventions have on bone mineral density and other important health domains in older adults. The approach to the management of sarcopenic obesity would include both dietary intervention and exercise. Dietary interventions that have been recommended include both an increase in protein intake and reduction in carbohydrate intake [50, 51]. This may be a particularly salient issue for the oldest old, and those who have impaired functional status.

Special Nutrition Intervention Situations in Diabetes Preventive Nutrition

Hospitalization

Barriers that may impact an individual's nutrition status and subsequently affect glycemic control include poor appetite, inability to eat, increased nutrient and calorie needs due to catabolic stress, variation in medications, and the possible need for enteral or parenteral nutrition support. Proper timing of meals and the relation to medications is important. Insulin should be administered immediately before or after a meal. Due to the wide heterogeneity in the hospital population, individualization of nutrition recommendations is key to improving outcomes. The common practice of ordering an "ADA Diet" is strongly discouraged, as the ADA does not endorse any specific diet [13]. The consistent carbohydrate meal planning system is encouraged. For this system to be effective, it is important that nursing and nutrition services coordinate their services.

Management of hyperglycemia in the hospital setting has gained increasing attention over the last few years. Hyperglycemia is common among hospitalized adults with diabetes and has been shown to be associated with higher mortality and morbidity in a variety of studies. This is particularly relevant to the older population, since they are more likely to be admitted to the hospital and have higher rates of diabetes. While early randomized controlled studies in the intensive care unit supported the glycemic goal of <110 mg/dL in the ICU, subsequent studies have raised concerns about worse outcomes with such "tight" control. Currently the ADA recommends blood glucose levels of 140–180 mg/dL for most critically ill patients with hyperglycemia [52]. There are no trials that clearly inform us about a goal blood glucose level in the non-ICU setting. It is the general consensus that in the hospital setting, pre-meal glucose levels of <140 mg/dL and random blood glucose readings of <180 mg/dL would be reasonable [53].

The key areas of focus to improve inpatient glycemic control are:

1. Establishing screening criteria for appropriate referral to a registered dietitian
2. Identifying nutrition-related issues in clinical pathways and patient care plans
3. Implementing and maintaining standardized diet orders such as consistent carbohydrate menus
4. Integrating blood glucose monitoring results with nutrition care plans
5. Using standing orders for diabetes education and diabetes MNT as appropriate
6. Standardizing discharge follow-up orders for MNT and diabetes education post-discharge when necessary [54, 55]

Patients requiring clear or full liquid diets should receive 200 g carbohydrate/day in equally divided amounts at meal and snack times. Liquids should not be sugar free. Patients require carbohydrate and calories, and sugar-free liquids do not meet these nutritional needs. For tube feedings, either a standard enteral formula (50 % carbohydrate) or a lower-carbohydrate content formula (33–40 % carbohydrate) may be used. Calorie needs for most patients are in the range of 25–35 kcal/kg every 24 h. Care must be taken not to overfeed patients because this can exacerbate hyperglycemia. After surgery, food intake should be initiated as quickly as possible. Progression from clear liquids to full liquids to solid foods should be completed as rapidly as tolerated [56].

Long-Term Care

Diabetes is common in Long-Term Care (LTC) affecting ~25 % of the population [57]. Residents of long-term care facilities may face additional or unique problems. They may be underweight, rendering caloric restrictions inappropriate. Low body weight has been associated with higher mortality and morbidity in these settings. In the long-term care setting, restriction of food choices may lead to poor overall nutritional status and has not been shown to improve glycemic control. Hence, the use of "no concentrated sugar," "no sugar added," or "liberal diabetic diet" is discouraged. An additional, and significant, problem in LTCs is frequent staff turnover and lack of familiarity of the staff with the resident, absence of specific glycemia management protocols that result in overall poor glycemic control [58].

Enteral and Parenteral Nutrition

Enteral and parenteral nutrition might also pose challenges in the management of diabetes in older adults. While the glycemic goals for individuals receiving enteral and parenteral nutrition are the same as glycemic goals for the general population of adults with diabetes, achievement of normoglycemia may be more difficult in individuals who are acutely ill. There is evidence that poor glycemic control in individuals on parenteral or enteral nutrition is related to poor outcomes. It is estimated that up to 30 % of patients who receive parenteral nutrition have diabetes. Many of these patients have no previous history of diagnosed diabetes and develop diabetes due to stress-induced increases in counter-regulatory hormones and cytokines.

The relative value of high carbohydrate versus high fat enteral feeds for persons with diabetes has been debated [59]. The most widely used commercial enteral preparations for individuals with diabetes provide 1 cal/mL, 40 % (CHOICEdm TF; Novartis Medical Nutrition) to 34 % (Glucerna; Abbott Laboratories, Inc.) carbohydrate, and 43 % (CHOICEdmTF) to 49 % (Glucerna) fat. They also have high monounsaturated fatty acids (MUFA; 35 % of kcal in Glucerna). MUFA has been shown to be beneficial in improving lipid profile, glycemic control, and lowering insulin level [60]. CHOICEdmTF

has a higher content of medium chain triglycerides and has no fructose. The use of insulin or oral agents in persons receiving enteral nutrition should be tailored to match the timing of feeds. Parenteral nutrition fluids are high in carbohydrate and derive only few calories from fat. In persons with diabetes, particularly in less severely stressed individuals, the proportion of carbohydrate may be decreased but is still very high. The usual rate of glucose infusion is 4–5 g/kg body weight and lipid infusion of 1 to 1/5 g/kg body weight. This requires adequate use of insulin to maintain normoglycemia [61]. Insulin infusion not only maintains glycemic control, but also prevents protein breakdown and promotes protein synthesis.

Preventive Nutrition Issues and Prediabetes

Type 2 diabetes develops over a prolonged period and the earliest abnormalities are now categorized as "prediabetes." The criteria for the diagnosis of prediabetes are outlined in Table 21.1. Analyses of 1999–2010 National Health and Nutrition Examination Surveys (NHANES) data for adults over the age of 50 indicated a high prevalence of prediabetes, with rates reaching nearly 50 % in older adults. Further, data suggest these that rates are continuing to increase [62]. Importantly, individuals with prediabetes progress to develop diabetes at a much higher rate than those with no abnormality of blood glucose. In addition, individuals with prediabetes have other comorbidities which increase their risk of macrovascular disease.

Several large, randomized clinical trials have demonstrated the efficacy of both lifestyle and pharmacological interventions in preventing the progression of prediabetes. The landmark Diabetes Prevention Program (DPP) used a combination of diet and exercise programs in the Intensive Lifestyle Intervention (ILI) arm of their study [63]. This program was designed to achieve a weight loss of ~7 % of initial body weight. The ILI participants were enrolled in a 16 week training program and were counseled on diet and exercise. Exercise recommendations included 150 min of moderate intensity exercise per week. The DPP also demonstrated that older adults were able to actively engage in this program and that they achieved a higher rate of diabetes prevention relative to younger adults. Overall, progression to diabetes was reduced by 65 % in the lifestyle intervention group and by 31 % in the metformin group. In older participants, lifestyle intervention had an even greater impact than metformin. Among participants aged 60 and older, lifestyle intervention reduced the risk of development of diabetes by 71 %. Participants in the 60–85 year old age group were the most likely to achieve weight loss (5–7 % loss in body weight was achieved with dietary change and activity) and physical activity goals. No age differences were noted in reduction of caloric intake. Older participants receiving metformin, the medication intervention arm of the study, did not experience benefits to the degree as younger, heavier participants. Diabetes incidence rates over time fell with increasing age, while in the metformin group, the youngest participants showed the lowest diabetes incidence. A trend was noted that metformin had lower effectiveness relative to lifestyle change with increased participant age, despite comparable to superior medication adherence and greater weight loss in the older metformin group participants. Age differences in response to pharmacologic treatment and physiology, as well as behavior, likely played a role in the findings. Of note, DPP participants were community dwelling, relatively healthy and free of significant physical limitations and frailty; however, the ILI was modified to accommodate participants who developed limitations over the course of the study. The ongoing follow-up of this study, DPP Outcomes Study, has shown long-term benefits of ILI in slowing the progression to diabetes. Furthermore, there was a reduction in cardiovascular risk factors in subjects who had been randomized to lifestyle intervention [63].

The success of this intervention in clinical trial setting has been further replicated in community settings through a number of translational studies, indicating the feasibility of implementing lifestyle intervention in a larger scale [64]. Based on these studies the US Centers for Disease Control launched a National Diabetes Prevention Recognition Program to recognize programs that have shown that they can effectively deliver a proven lifestyle change program (in-person, virtual, or via distance learning) to prevent type 2 diabetes [65].

Clinical Issues Impacting Older Adults with Diabetes

Dietary Habits of Older Adults with Diabetes

Nutrition is associated with functional status and quality of life for older adults with chronic illness, including diabetes [66, 67]. Persons with diabetes must follow a diet that incorporates healthy food choices and spacing of meals to be consistent with exogenous insulin use and physical activity, with the goal of maintaining euglycemia. Older adults, in particular, have unique nutritional requirements and barriers to overcome. Although older adults eat more grains, fruits, and vegetables compared to younger adults, less than 50 % eat the recommended five servings of fruits and vegetables per day. Importantly, starchy vegetables account for a large percentage of daily vegetable intake, with dark green and orange vegetables (nutrient-rich) only accounting for 12–15 %. Often, due to physical health and environmental constraints, they are faced with difficulty accessing, preparing, and consuming important nutrient [66, 67]. As such, this population has an increased risk for undernutrition due to various aging-related barriers, including swallowing difficulties, altered taste and smell, dental problems, and other functional impairments [5].

To determine whether nutrition barriers or undernutrition are present, The Mini-Nutritional Assessment (MNT) may be easily administered to older adults with diabetes. This assessment can assist providers in determining whether nutrition-specific referrals or resources are needed. Concrete recommendations should consider the individual's unique circumstances and may include suggesting smaller more frequent meals, adding liquid nutrition supplements, altering food texture, or fortifying food regularly consumed. Additional community resources might be recommended such as Meals on Wheels, local senior centers, or the U.S. Department of Agriculture's Older Americans Nutrition Program [5].

Hypoglycemia in Older Adults

Hypoglycemia is a major limiting factor in the management of diabetes. A variety of factors may play a role in the increased risk of hypoglycemia in older adults. These include poor nutritional status, cognitive dysfunction, polypharmacy, and comorbid illnesses. Except in the severely malnourished, poor dietary intake by itself does not lead to hypoglycemia. The most common cause of hypoglycemia remains the use of blood glucose lowering agents. Adults over age 75 have been found to have twice the rate of hypoglycemic episodes resulting in more frequent emergency room visits relative to the general population with diabetes [5].

This means that older adults using oral agents or insulin should regularly be assessed for hypoglycemia by asking the individual or caregiver about signs and symptoms and when appropriate, reviewing blood glucose logs. When medication use creates problems of consistent hypoglycemia, patients must learn how to avoid and manage hypoglycemic episodes. Those taking multiple daily insulin injections must understand the importance of balancing eating behavior and insulin use patterns [5]. Older adults taking insulin who have high variability in blood glucose levels, exhibit very low average blood glucose concentrations, have had diabetes for a long duration, have a low body mass index, or who have high levels of vigorous physical activity, may be at particular risk of severe hypoglycemia. Hypoglycemia and cognitive dysfunction have shown bidirectional associations, with severe episodes of hypoglycemia associated with the incidence of dementia and existing cognitive impairment enhancing risk of hypoglycemic episodes [5, 68, 69].

Table 21.6 Dietary management of hypoglycemia

1. Check blood glucose level by glucose monitor
2. If blood glucose less than 60 mg/dL or symptomatic—treat with 15 g of carbohydrate (1/2 cup juice, 1/2 cup regular soft drink, glucose gel)
3. Repeat blood sugar reading in 15 min after treatment and again after 60 min
4. Repeat step 2 until blood glucose is >60 mg/dL
5. If meals are due within 60 min—eat meal now
6. If meals are not due within 60 min follow the glucose treatment with a snack containing carbohydrate and one protein (cheese and crackers, peanut butter and crackers, skim milk and crackers, or a small sandwich)
7. If blood glucose <40 mg/dL and/or subject is stuporous, confused, or unresponsive—give 1 amp of D50W as IV push and start D10W at 60 cm^3/h. Check blood glucose every 5 min and repeat till blood glucose >60 mg/dL or till awake. Give oral carbohydrate once awake

Self-Monitoring and Dietary Treatment of Hypoglycemia

Frequent self-monitoring of blood glucose levels provides specific information that may serve as feedback for guiding decisions about moment-to-moment treatment needs, thus helping individuals to anticipate or prevent severely low glucose levels [70]. However, frequent blood glucose testing may be perceived as expensive, inconvenient, or painful. Unfortunately, rather than performing frequent blood glucose testing, many individuals simply rely on their symptoms or estimates about their blood glucose levels when deciding what to eat or how vigorously to exercise or whether to operate a motor vehicle [71]. By increasing the frequency of blood glucose testing (at least four times per day for persons taking insulin) and making informed decisions about when to eat additional carbohydrate (e.g., eat 15 g of carbohydrate to raise blood glucose levels about 45 mg/dL) or to identify personal sources of vigorous physical activity contributing to low blood glucose levels, patients may learn to prevent severe hypoglycemia. Educating patients about the importance of always carrying glucose tabs or gel or fast-acting carbohydrate snacks or placing them in various locations such as the car, or relative's homes may also aid in the treatment of mild to moderate hypoglycemic episodes. Recommendations for dietary management of hypoglycemia in older adults are presented in Table 21.6.

Individuals with recurrent hypoglycemia may be particularly at risk for hypoglycemia unawareness and for severely low hypoglycemic episodes. Failure to test blood glucose levels regularly can contribute to the problem of hypoglycemia unawareness. This cycle is particularly problematic for older adults who are highly physically active or who skip meals, do not eat sufficient quantities of food to match their insulin doses, or consume a high fat diet which delays carbohydrate absorption and is not accounted for in the timing of insulin administration [71].

Alcohol consumption, while not typically problematic when consumed in moderation, can pose risks for hypoglycemia in older adults taking insulin. In particular, the major risk of alcohol-related hypoglycemia is in persons in a fasting state and those who are alcohol dependent. The disinhibiting effect of alcohol poses the risk of hypoglycemia unawareness, making blood glucose monitoring essential. The potential for a delayed risk of hypoglycemia the morning after evening alcohol intake should also be emphasized. Potential barriers to blood glucose testing or adequate food consumption such as financial constraints, fear of pain, depression, or feelings of being overwhelmed by diabetes should be assessed.

Table 21.7 Provider delivery approaches to optimize care for older adults

Spread self-management education contact over multiple sessions
Provide large print handouts and cues for use at home to facilitate learning and retention
Simplify steps of the self-care regimen
Engage caregivers and family members to enhance self-management education
Factor in impact on day to day quality of life when considering goal of tight glycemic control
Prioritize patients' personal preferences in overall well-being when making care-related decisions

Self-Management Behaviors

Few studies of diabetes self-management education (DSME) have focused on older adults. However, a recent evaluation of a group-based, structured diabetes self-management intervention supports DSME in this population. This secondary analysis of this behavioral trial found that older adults aged 60–75 (mean age 67) achieved similar benefits to younger adults (mean age 47) in frequency of self-care behaviors, glycemic control, depressive symptoms and emotional coping and care-related cognitions such as self-efficacy and frustration with self-care [72]. Efforts to promote behavior change using group or individual approaches must consider individual dietary and psychosocial needs. Some older adults may have limited knowledge and/or understanding of diabetes care. Self-management steps such as home-based blood glucose monitoring, meal planning and adjusting food intake and insulin based on blood glucose levels, and when (and how to adjust) insulin or take oral diabetes agents may be influenced by cognitive functioning, physical status and personal preferences and resources. In instances in which older adults have limited health numeracy skills or low health literacy or have multiple health comorbidities the demands of balancing diet in the context of the other aspects of care may become overwhelming [5]. Physical barriers to optimal diabetes dietary intake may include swallowing difficulties, poor dentition, decreased thirst or appetite, and influence of medications on taste. Psychosocial influences may include limited financial resources, difficulties with transportation, and limited social support. Given the variability in the older adult population, it has been argued that individual self-management intervention may be preferable to group-based intervention. However, studies such as the previously described behavioral trial demonstrated positive outcomes. A systematic review of diabetes education for adults aged 65 and older found modest, long-term benefits [73]. Given the multiple aspects of the self-management demands of diabetes and high rate of comorbidities and barriers in older adults, a simplified self-care regimen is optimal [74, 75] and placing supportive resources in place, such as telemedicine outreach may further enhance efforts to provide meaningful self-management education for older adults [73]. Providing DSME content over multiple contacts and use of memory cues such as large print handouts and cues to use at home can be helpful. A simplified self-care regimen is optimal with goals not only to maintain diabetes control but also to have good quality of life [74, 75].

Provider delivery approaches to DSME to optimize care for older adults may be seen in Table 21.7.

Physical Limitations and Diabetes Self-Management

Diabetes-related comorbidities such diabetic retinopathy, cardiovascular disease, peripheral vascular disease, and congestive heart failure may result in decreased usual activity and limit activities of daily living (ADLs) and instrumental activities in daily living (IADLs), including limited transportation options, which may result in limited ability to shop for food or ability to read restaurant menus.

Self-monitoring may also be influenced by reduced visual acuity. Diminished fine motor skills may also impact ability to functionally conduct a finger stick, conduct the steps necessary for using the glucometer and read the results. Due to such limitations, many older patients may require alternative choices of meters or assistance in blood glucose testing. Comorbid health conditions increase polypharmacy, so pill boxes and mediplanners may also be helpful in simplifying medication management.

Psychosocial and Behavioral Issues Related to Self-Care and Dietary Intake in Older Adults with Diabetes

A recent review highlights the considerable literature on comorbid mental health issues in diabetes [76]. Living with diabetes has been associated with diabetes-related distress due to the disease burden and emotional distress associated with self-care demands, uncertainty and guilt associated with poor self-management or discouraging outcomes. Rates of major depressive disorders in diabetes are at least twice that of the general population. Older adults with diabetes have higher rates of comorbid depression, with many undiagnosed [77]. Anxiety disorders such as generalized anxiety disorder, panic disorder, and post-traumatic stress disorder have also been found to be elevated in diabetes samples. Disordered eating patterns are also elevated in both younger and older samples. These mental health comorbidities pose considerable problems for diabetes care. Depression in those with diabetes is associated with poor self-care management, worse glycemic control, more complications, disability, and reduced quality of life. In the case of anxiety, worries upon diagnosis may escalate to avoidance of care or symptoms of panic or phobias. When the demands of injections or blood draws are present, symptoms of anxiety may mimic hypoglycemia, complicating presentation, and fear of hypoglycemia may lead to intentionally maintaining elevated blood glucose levels. Eating disorders have predominantly been studied in younger adults with type 1 diabetes; women with type 1 diabetes have double the risk of developing subthreshold eating disorders. The presence of binge eating is well known in both type 1 and type 2 diabetes care. Purging through insulin restriction has been well documented [76]. The depression-diabetes link may be particularly salient for older adults. Data from the Epidemiological Catchment Area study of more than 18,000 adults conducted in five sites found depressive symptoms in 15 % of adults over age 65 and lifetime rate of depression in 2 % of women and 3 % of men [78]. In EPESE, death rates were substantially higher when a high level of depressive symptoms was comorbid with diabetes, cardiovascular disease, hypertension, stroke, and cancer. The odds of having died among persons with diabetes with high levels of depressive symptoms were three times that of diabetics without high levels of depressive symptoms. Hence, an interaction between depression and diabetes and the prevalence of other risk factors greatly increased absolute risk of mortality in these large studies of older adults.

In depressed older adults, indirect self-destructive behavior, such as not eating and medication nonadherence, may be more common than overt self-harming gestures such as suicide attempts, and are associated with decreased survival. Many older adults might consider depression to be a normal part of aging and may not report their symptoms to a health care provider. Health providers may also attribute some depressive symptoms to old age or other physical ailments or mood disturbance may be less prominent than multiple somatic complaints. Some older patients with depression may present with “failure to thrive” rather than specific complaints [79]. Given the high rates of depression in this population, careful assessment of depressive symptomology and its impact on dietary intake, related aspects of diabetes self-care and health outcomes are critical. Recidivism of depression in persons with diabetes must also be considered when planning interventions for older adults since these rates appear to be higher than in the general population [80].

Social Isolation

Social isolation is an established risk factor for morbidity and mortality in numerous disease states, including a large body of literature linking it to cardiovascular disease, a common outcome of diabetes. A report on changes in social structure across decades points out the dramatic changes in the USA in recent decades [81]. Social isolation appears to be increasing in midlife and older adults, making this an important consideration when assessing psychosocial function and potential influence on diet and related self-care. In the US rates of adults living alone jumped from 17 % in 1970 to 28 % in 2011. Discussion networks are a third smaller in 2004 than in 1985, and now the modal respondent reports no confidant. A greater decrease in non-family ties has resulted in greater reliance on immediate family and fewer contacts through voluntary associations and neighborhoods [81]. Evaluation of data from 6500 adults aged 52 and older who took part in the English Longitudinal Study of Ageing Found that isolated individuals were more likely to be older, with lower educational and income levels and unmarried and to have more long-standing illnesses and depressive symptoms. Social isolation was associated with all-cause mortality [82].

One avenue by which social support may impact outcomes in chronic diseases such as diabetes is through its impact on self-care behaviors. Recently widowed persons may have limited cooking skills or access to shopping. Such persons may also be depressed and withdraw from usual daily activities, including social and food-related activities such as dining out or even preparing regular meals. In older adults who continue to live with a spouse, husband's food preference, regardless of nutritional content, is often the best predictor of family meals that are eaten [83]. Thus, it is imperative to evaluate the social context of patients' food purchases and dietary intake patterns.

Diabetes education programs that include the older adult's partner may help to promote optimal social support for healthful dietary and lifestyle changes. Substantial improvements were found for diabetes knowledge, psychosocial functioning, and metabolic control in a 6-week diabetes education program for male diabetes patients aged 65–82 years that included their spouses [84].

These studies highlight that to provide optimal care, one must consider the social support system, provide the older adult with encouragement and reinforcement for self-care behaviors, and provide instrumental assistance and technical advice as needed.

Cognitive Dysfunction

Systematic review and meta-analysis indicate that older adults with diabetes have approximately twice the rates of multi-infarct and Alzheimer's type dementia relative to those without diabetes [5, 68]. Cognitive function is an important consideration since impaired function can impact comprehension of health-related information, including instructions regarding self-care, such as procedures for conducting blood glucose testing, making adjustments in dietary intake, and taking multiple medications [85]. Older adults have high rates of cognitive impairment ranging from subtle deficits to memory loss and overt dementia, a phenomenon which was observed in the ACCORD trial. Participants were adults aged ≥55 years with type 2 diabetes and additional cardiovascular risk factors. Referred participants were considered to have the skills to follow a complicated protocol; however, an ancillary trial examining cognitive function in 2956 participants found 20 % to have undiagnosed cognitive dysfunction. The high rate of unidentified impairment warrants screening in advance of implementing complicated dietary plans [5, 86]. Influences likely impacting cognitive functioning in older adults with diabetes include duration of illness, blood glucose control, and age. At present, it remains unclear how and at what threshold cognitive impairment influences acquisition of new diabetes self-care demands.

If frequent self-monitoring and adherence to specific dietary guidelines is within the cognitive abilities of the older adult with diabetes, then attainment of tight blood glucose control may be a reasonable

goal. However, intensive self-management may not always be realistic for many cognitively impaired older adults. Unfortunately, despite the potential physical benefits, intensive management and tight control may require so many day-to-day demands, that this may be difficult to practically achieve. Cognitive dysfunction can make adherence to dietary recommendations particularly difficult. For example, older adults who are cognitively impaired may not remember structured mealtimes that are coordinated with their insulin regimen. Difficulty following a complicated meal plan, such as carbohydrate counting or using a sliding scale to match insulin units to intake, may make such treatment regimens too overwhelming to be practical. For some older individuals, a concrete, structured meal plan can help minimize ambiguity regarding their diabetes diet. Such plans may be made in conjunction with a diabetes educator or dietitian. Helpful strategies include using cues in the environment such as regular meals, setting alarms, providing written information with large print and pictures, training videotapes and assessing comprehension and skill by asking for demonstrations. In addition, provision of home-based caretakers or meal services may also assist the older person with cognitive impairment, significant physical disability or other barriers to obtain access to optimal nutrition that is consistent with diabetes goals.

Cultural Issues and Nutrition in Diabetes

Racial and ethnic minorities are disproportionately affected by diabetes; compared to non-Hispanic Whites, the risk of receiving a diabetes diagnosis is 18 % higher in Asians, 66 % higher in Hispanics, and 77 % higher in non-Hispanic blacks [87]. Studies indicate that minorities face unique barriers in accessing and utilizing critical diabetes-related health care. In one study of 3003 older adults, significant racial and ethnic disparities were noted and examined in both the management of diabetes and use of health care services: (1) Compared to White Americans, African Americans were less likely to both visit a doctor and have a usual source of health care and more likely to utilize the emergency department for foot examinations and regular diabetes care, (2) Asians were less likely than Whites to have foot examinations and regularly self-monitor blood glucose, (3) Hispanics were less likely to take medication in an effort to lower cholesterol but more likely to self-monitor blood glucose, and (4) American Indians/Alaska Natives were less likely than Whites to see a physician and take medication to reduce risk for myocardial infarction, but were more likely to use insulin, oral diabetes medication, or both. Although results from this study may not be generalizable to all ethnic minorities, findings suggest the importance for the development of specific health care interventions targeting these groups [87].

Research also suggests that ethnic minority and older adults may have culturally unique health-related perspectives that are not effectively targeted by traditionally delivered health promotion interventions [88]. While being healthy appears to be viewed as important, and a general awareness of what to do to stay healthy is evident, operational definitions of health in these populations are often different than those typically used in prevention and health promotion efforts. For example, focus group studies with underserved ethnic minorities found that prevailing beliefs were that better health behaviors could build resistance to acute illnesses and maintain health, but that chronic diseases such as diabetes were due to fate and heredity and beyond individual control. In general, participants did not appear to make the cognitive "link" between chronic disease prevention and the importance of diet, physical activity, and weight control. Most participants expressed an interest in "doing better" but were not able to specify *how* such healthful changes might be made.

Qualitative evaluations of cultural influences on diabetes reveal the complexity of psychosocial influences on diabetes lifestyle change and why traditional health provider perspective-based dietary interventions with minority persons often fail. An interview-based study of 20 middle-aged Mexican American women with type 2 diabetes found that personal understanding and interpretation of their diabetes was most heavily based on family's experiences and community influences [89]. From the participants' perspectives, the severity of their diabetes was indicated by being treated with insulin

injections, which indicated provider vigilance, while treatment with oral medications was associated with the perception that the provider approach was lax, which indicated that the diabetes was not severe. Having diabetes was also viewed as a confusing, silent illness, and provider provision of information was often viewed as insufficient. Participant comments revealed the perception that many health provider comments were predominantly focused on negative aspects of behavior, confrontational, and at times, petty or demeaning. Provider focus on positive gains to be made with behavior change, reinforcement for accomplishments and avoiding pejorative terms (e.g., obese) may facilitate patient engagement and enhance a more collaborative relationship.

The strong influence of family and culture on adherence to diabetes diet and physical activity behaviors were evaluated in a study of 70 southern, predominantly rural African American women with type 2 diabetes, of whom 65 % were aged 55 and older [90]. Participants described the psychological impact of diabetes (reported as nervousness, fatigue, worrying and having feelings of dietary deprivation, including craving for sweets) as stronger than the physical impact. Participants reported considerable life stress other than diabetes, particularly having a multi-caregiver role. Family members' resistance of healthy food preparation methods was common. Positive family support for diabetes was evident in the form of instrumental support from adult daughters or other female family members or friends to older, single or widowed women. In addition, spirituality and religiosity emerged as a main theme in all groups. Spirituality was largely viewed as a primary source of emotional support, a positive influence on diabetes and a contributor to quality of life. This study exemplifies the importance of incorporating family and the church in self-care behaviors of many southern African American women.

These qualitative studies demonstrate need for consideration of the social and cultural context of older adult's lives when developing interventions to promote diet and lifestyle change. Collaborative patient provider care and family-centered and church-based approaches may offer more appropriate avenues for promotion of diabetes care and maximizing the effective delivery of dietary interventions for many older adults with diabetes, who are frequently over represented in ethnic minority populations.

Due to sparse medical resources often associated with rural communities, older adults living in rural locations are faced with unique barriers in diabetes management. For example, higher rates of food insecurity (lack of regular access to nutritious food) are seen in rural areas, often due to low-income and lack of variety in food choice. Notably, NHANES data indicate that 60 % of adults with diabetes (M age = 64) met criteria for food insufficiency. In addition, rural communities are often lacking critical diabetes educator and dietician services [91].

In a study examining food insecurity and food choices in rural older adults with diabetes, a nutrition education intervention was delivered utilizing telemedicine technology. Individuals reporting mild food insecurity had significantly higher BMIs and lower total income. Additionally, these individuals were more likely to report food costs as a barrier to nutritious eating. Individuals receiving the intensive nutrition intervention endorsed good nutrition habits and knowledge overall, such as following their dietician's recommendations, limiting fast food intake, and attending to nutritional information. Notably, nearly all participants bought fresh fruits and vegetables when shopping. Findings indicate that telemedicine interventions may be beneficial in delivering critical nutrition education to older adults with diabetes who do not have access to traditional services [91].

Imparting Diabetes Dietary Information to Older Adults

Structured nutrition education may be effective with older adults. A randomized nutrition education intervention with adults 65 and older (who did not have functional limitations) used Social Cognitive Theory and meaningful learning approaches to minimize the presentation of too much information

and maximize learning by breaking information down into small pieces and successively adding upon each concept across the intervention. Strategies included limiting the content introduced at each session, meaningfully organizing the content, integrating preexisting knowledge with new information, and modeling and in-session practice regarding decision-making related to reading food labels, food purchasing, meal planning, and using the information for diabetes self-care. Goal setting, self-monitoring, and feedback were also utilized. The intervention resulted in improved glycemic control [92], greater total knowledge, positive outcome expectancies, decision-making skills and reduced self-management barriers [93].

In order to impart diabetes diet information in a fashion that will lead to actual changes in behavior and maintenance of these changes, it is critical to consider the psychosocial and cultural influences that are present for each individual patient. Simple and concrete statements such as "eat less fat" or "eat less food" or "get more walking in each day" may promote learning and minimize failure. Nutrition information is best presented in sequenced manageable steps that can then be individualized to the patient's setting. Simple tip sheets and problem solving approaches discussed in earlier sections may also be helpful. The National Diabetes Education Program (NDEP), which is a partnership between the National Institutes of Health and the CDC, has prepared materials for adults with type 2 diabetes and prediabetes, adapted from those used in the DPP for use by primary care providers for middle age and older adults. Materials specific to older adults are included. These materials address motivational approaches that consider readiness for change and behavioral relapse. Materials can help to assess a patient's personal readiness for change and help in setting up a walking program. Health provider resources for diabetes include a Consensus Report on Diabetes in Older Adults, an overview of Special Challenges in Elderly Patients With Diabetes, The NDEP toolkit—the NDEP GAMEPLAN (Goals, Accountability, Monitoring, Effectiveness, Prevention through a Lifestyle of Activity and Nutrition) which is copyright free and contains health care provider information and program background information and patient handouts that may be copied (http://ndep.nih.gov/older-adults/) [94].

It is also important to be mindful of the range of functioning in older adults. Older adults of the World War II generation have tended to be characterized as somewhat reverential toward physicians and the health care system. However, baby boomers, who are now entering the realm of older adulthood, tend to differ from previous generations and tend to have high expectations of their health providers and desire additional information. This generation of "new" older adults tends to want a collaborative relationship with their health care provider and desire additional information including resources such as self-help publications, internet, video, and audiotapes. They demand convenience, expect hard evidence of quality and expertise, can be skeptical of advice at face value and are often willing to explore alternative therapies [95]. In order to meet the needs of the range of older adults with diabetes, it is clear that a "one size fits all" approach will not be effective. Rather, issues related to culture and ethnicity and generational cohort must be considered.

Conclusions

Diabetes has reached unprecedented proportions worldwide. Internationally, emergence of new cases of diabetes parallels the increases seen in Western countries. The risks of type 2 diabetes in Asian countries tend to increase at levels of body mass index generally classified as non-obese in Non Hispanic White Westerners. These worldwide changes are due to an accelerated prevalence of obesity, today's predominance of sedentary lifestyle and the rapidly growing population of older adults, including an increased proportion of those belonging to ethnic groups with especially high rates of diabetes. Risk factors for diabetes that are specific to these populations include genetic, behavioral, and lifestyle factors. Medication and nutritional management goals should be developed in older adults with diabetes. Individualization of treatment is key. Treament and dietary goals should consider

health status and comorbidities and functional status. The American Diabetes Association recommends considering personal preferences and needs such as cultural, traditional and religious practices, health beliefs, goals and economics as well as metabolic goals in dietary recommendations. Interactions of diet and medication are of particular importance in management of diabetes. Overweight and obesity significantly impact diabetes progression and the development of complications and should be monitored. Obesity tends to be common on older adults but declines in the oldest old. Unintentional weight loss and underweight and undernutrition may pose greater mortality risk than obesity and are issues for older adults with diabetes. Weight loss issues that must be considered for older adults with diabetes include the impact of dietary restrictions on quality of life and potential loss of muscle mass. Increased protein intake and reduction in carbohydrate intake by be helpful for those with sarcopenic obesity, the oldest old and those with impaired functional status. Special nutrition intervention situations for older adults with diabetes include hospitalization and enteral and parenteral nutrition. The progression of type 2 diabetes has been studied in tracking individuals identified with prediabetes. NHANES data indicates prediabetes rates reaching nearly 50% in older adults. The Diabetes Prevention Program (DPP) found that intensive lifestyle intervention (diet and physical activity) was highly effective in preventing diabetes with at-risk adults, with older adults achieving higher rates of prevention than younger adults. The DPP results have been replicated in community settings. Older adults with diabetes who use oral agents or insulin may be at risk for hypoglycemia and should be regularly assessed for signs and symptoms. Hypoglycemia and cognitive dysfunction show bidirectional associations. Regular self-monitoring of blood glucose self-monitoring (SMBG) may assist in decision making regarding diet or medication adjustments in the context of blood glucose levels. Barriers to SMBG should be considered. Additional considerations related to diet and self-care include depression and anxiety disorders, which are elevated in the diabetes population, social isolation, which is a risk-factor for morbidity and mortality in older adults and in numerous disease states, and cognitive dysfunction, which has relatively higher rates in older adults with diabetes. Racial and ethnic minorities are disproportionately affected by diabetes and may have culturally unique health care behaviors and perspectives. Dietary information may be effectively imparted to older adults with structured, theoretically-based learning approaches.

Recommendations

1. Establish the type of diabetes and medication regimen in order to appropriately integrate dietary goals.
2. Consider the importance of cardiovascular risk—including obesity and lipids—in developing diabetes dietary goals and routine assessment.
3. Work with the patient to set a goal of achieving and maintaining reasonable body weight. For obese older adults, moderate weight loss may achieve dramatic results and exercise may greatly enhance dietary intervention. Maintenance of behavior change and weight loss is critical. For underweight adults, focus on promotion of optimal nutritional intake and functional status.
4. Educate older adults with diabetes about the rationale for diet and lifestyle change and link to health outcomes; promote self-efficacy for change.
5. Consider the risk of hypoglycemia for older adults taking insulin—particularly those with poor nutritional status, cognitive dysfunction, polypharmacy and comorbid illness. Encourage frequent self-monitoring and dietary self-treatment and preventive strategies.
6. Assess older adults' specific dietary patterns such as food choices, quantity eaten, and unplanned snacking and the lifestyle contexts in which they occur.
7. Address psychosocial issues that may influence dietary intake, including depression, social support, cognitive status, attitudes and perceptions, and the impact of the diabetes regimen on quality of life.

8. Address and intervene within individuals' cultural context.
9. Provide a collaborative relationship with each patient, offer resources and provide concrete, behavioral strategies to promote behavior change.
10. When appropriate, provide self-help materials including Internet and NDEP resources (e.g., from the NDEP: http://ndep.nih.gov/older-adults/hcp.aspx, ADA: http://professional.diabetes.org/?loc=bb, The CDC: http://www.cdc.gov/diabetes/, or NIH http://www2.niddk.nih.gov/.

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Chapter 22
The Effects of Diabetes and Obesity on the Skeleton

Jessica Furst, John P. Bilezikian, and Mishaela R. Rubin

Key Points

- Osteoporosis is a disorder of compromised bone strength that increases fracture risk. It is a disease that has reached epidemic proportions affecting 200 million women worldwide.
- Type 2 diabetes (T2D) and obesity have emerged as newly recognized risk factors for osteoporosis.
- Mechanisms for bone loss in T2D include reduced bone formation, abnormal skeletal microstructure, and abnormal bone material properties.
- Many medications for the treatment of osteoporosis have shown efficacy in preserving or improving the bone health of those with T2D.
- Mechanisms for increased fracture risk in obesity include altered distribution of fat depots in the body, some locations of which can be associated with reduced bone quality.

Keywords Osteoporosis • Type 2 diabetes (T2D) • Obesity • Bone mineral density (BMD) • Fracture risk assessment tool (FRAX) • Bone quality • Bisphosphonate • Fracture • Visceral adiposity • Bone marrow fat • White adipose tissue • Brown adipose tissue

Introduction

Nutrition and Skeletal Health

During childhood and early adulthood, the skeleton accrues bone mineral, reaching peak bone mass by the third decade of life, between ages 20 to 30 [1]. During growth and development of the skeleton, calcium looms as a vital nutrient [1]. Recommended intake of calcium for children is 800 mg from ages 3 to 8, 1300 mg from ages 9 to 17 according to the Institute of Medicine (IOM) [1]. Adequate calcium intake is obviously also important to help maintain bone mass after peak bone mass has been achieved. For adults, the recommended daily intake of calcium is between 1000 and 1200 mg per day according to the National Osteoporosis Foundation (NOF) [2]. Unfortunately, only about 25 % of children and 50 % of adults are estimated to comply with these guidelines [1]. Vitamin D intake is also important for both the developing and adult skeleton [1]. The IOM recommends 600–800 international units (IU) of vitamin D daily with slightly more recommended by the NOF, namely 800–1000 IU daily [2, 3]. In addition to adequate intake of calcium and vitamin D, exercise, especially resistance training, is vital for strong bones [1]. Exercise helps in the accrual of bone mass in children and young adults [4] and is believed to help maintain bone mass in adults.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_22,

Osteoporosis

Description, Incidence, and Risk Factors

Osteoporosis is a skeletal disorder characterized by compromised bone strength that increases fracture risk [1]. The skeletal dynamic that is most responsible for osteoporosis is bone mineral loss, due to aging and to other factors. Failure to achieve optimal peak bone mass is also important in the pathogenesis of osteoporosis in later years [1]. Osteoporosis affects both women and men. It is most prevalent among postmenopausal women [1]. Worldwide estimates of hip fractures, the most devastating consequence of this disease, signal an increase from about 1.7 million in 1950 to a projection of about six million in 2050 [5]. Prevalence data suggest that 200 million women worldwide are affected by this disease, 1/3 of whom are between 60 and 70 years old and 2/3 of whom are over the age of 80 [6]. Approximately 30 % of women over the age of 50 have experienced one or more vertebral fractures [7]. In the USA, two million fractures occur annually [8, 9]. A 50-year-old Caucasian woman has a 40–50 % life-time risk of sustaining a low trauma fracture, the hallmark of osteoporosis [8, 9]. The most common sites for osteoporotic fractures in postmenopausal women include the vertebrae, hip, and forearm [8, 9].

Osteoporotic fractures are associated with high morbidity, leading to impaired function and decreased mobility [10–12]. Decreased mobility, in turn, causes more bone loss due to decreased activity [10–12]. Compression fractures of the spine cause conformational deformities that lead to abdominal compression, reducing appetite and leading to weight loss and malnutrition, all of which can further weaken bones [10–12]. Additionally, thoracic compression fractures reduce pulmonary vital capacity and thus impair pulmonary function [10–12]. Osteoporosis is also associated with increased mortality, particularly in association with fractures [10–12]. Hip fracture leads to death in one out of five patients due to associated complications [1]. After the hip fracture, as few as one-third of patients are able to regain their pre-fracture level of function and as many as one-third of patients require placement in assisted care facilities [1]. The costs associated with osteoporosis are well over 10–15 billion dollars annually in the USA [1].

Numerous risk factors for osteoporosis are well established. These include, but are not limited to, estrogen deficiency, low body weight and body mass index, vitamin D deficiency, family history of fracture, disease states such as rheumatoid arthritis and chronic obstructive pulmonary disease, use of medications such as steroids and certain anticonvulsants, smoking, excessive alcohol, and lack of exercise [1]. Notably, most risk factor lists for osteoporosis do not include T2D and obesity. This chapter examines the evidence for the detrimental effects of T2D and obesity on bone [13].

Diagnosis

The diagnosis of osteoporosis is conventionally established through measuring areal bone mineral density (aBMD) by dual-energy X-ray absorptiometry (DXA). DXA has been available as a quantitative tool for measuring bone mass since 1986. It measures the lumbar spine, the hip, and the forearm [1]. DXA is safe, accurate, precise, and correlates with fracture risk. Bone density by DXA predicts fracture risk directly. For every 1 SD decrease in BMD, fracture risk doubles. In 1994, the World Health organization defined osteoporosis in densitometric terms as a bone density 2.5 standard deviations below the mean for young adult women [1]. The FRAX score is an additional clinical tool developed by the World Health Organization (WHO), which calculates an individual's fracture risk based on models that integrate clinical risk factors and BMD at the femoral neck. It is important to appreciate the fact that osteoporosis can also be diagnosed when a patient sustains a low trauma fracture regardless of the actual bone density measurement [14, 15].

Treatment

Treating osteoporosis is multifaceted and includes non-pharmacological interventions such as physical activity and appropriate levels of dietary calcium and vitamin D. Gait and balance training to prevent falls are also helpful [1]. A number of FDA-approved pharmacological therapies exist. While the majority of FDA-approved therapies inhibit bone resorption, the Women's Health Initiative (WHI), a long-term observational study of over 150,000 postmenopausal American women, found that combination estrogen plus progesterone in the form of hormone replacement therapy increased BMD and reduced fracture risk, especially at the hip [16]. While hormone replacement therapy is effective at reducing hip fracture risk, it is no longer considered a first-line treatment because of concerns related to adverse effects such as breast cancer and vascular disease. Currently, bisphosphonates are the most widely used therapy, with efficacy for reduction of both vertebral and non-vertebral fractures established for most agents of this class [1]. Denosumab is a human monoclonal antibody to receptor activator of nuclear factor kappa-B ligand (RANK Ligand), a powerful bone resorbing cytokine. It has been shown also to reduce vertebral and non-vertebral fracture risk. Teriparatide, the only FDA-approved osteoanabolic agent, builds bone and reduces vertebral and non-vertebral fractures.

T2D and Bone Health

Fractures in T2D

Incidence and Contributing Factors

Despite higher bone mineral density (BMD), T2D is associated with an increased risk of fracture, by 40–70 % at the hip [17, 18] and by about 27 % at the spine and extremities [19]. The WHI prospectively studied 93,676 postmenopausal women, comparing those with and without T2D for risk of fracture over a 7-year period [19]. A significantly increased fracture risk at the hip, foot, upper arm, ankle, spine, and forearm was found (Table 22.1) [19]. In two population-based observational studies, T2D was associated with an increased fracture risk [20, 21]. Individuals with inadequately controlled T2D had a 47–62% higher fracture risk than those without diabetes despite having 1.1–5.6% higher BMD by DXA [20]. Similarly, those with glycated hemoglobin (HgA1C) >8.0% had a greater risk of fracture than those with HgA1C <8.0%, suggesting fracture risk is a function of the extent to which hyperglycemia is controlled [21].

The duration of T2D is an important factor in assessing fracture risk [22–24]. Longer duration of T2D is associated with a greater risk of hip fracture [23]. In the Scottish National Registry study of hip fractures in T1D and T2D, men with diabetes had a relative risk of hip fracture of 0.97 (0.92–1.02) and

Table 22.1 Relative risk of fracture in T2D compared to nondiabetic postmenopausal controls (adapted from Bonds JCEM 2006)

Skeletal site	Relative risk for fracture
Hip	1.41
Foot	1.44
Upper arm	1.30
Ankle	1.34
Spine	1.28
Forearm	0.98

women with diabetes had a relative risk of 1.05 (1.01–1.10), suggesting that the risk did not differ significantly from nondiabetics. However, when the data were analyzed including only those with T2D for more than 7 years, both men and women had greatly increased relative risk of hip fracture [men 1.25 (1.08–1.45), women 1.55 (1.38–1.75)], suggesting that T2D takes a progressive toll on skeletal health over time [23]. Of concern, this time-related deterioration of skeletal health may not be reversible as intensive glucose control with resultant lowering of HgA1C may not reduce the incidence of fractures or falls [24]. In the randomized trial known as ACCORD (The Action to Control Cardiovascular Risk in Diabetes), intensive versus standard glycemic control was evaluated with respect to fracture risk and falls [24]. Over a follow-up period of 3.8 years, the average incidence of first non-spine fracture was not statistically different from the intensive control group (13.9 per 1000 person-years) versus the standard control group (13.3 per 1000 person-years) [24]. However, in ACCORD, thiazolidinediones (TZDs) were used frequently. Since TZDs alone are associated with higher fracture risk, they may have obscured a potential beneficial skeletal effect of tighter glycemic control. Further prospective information is needed to determine whether intensive glycemic control can reduce fracture risk.

BMD, FRAX, and Fracture Risk in T2D

For a given BMD, the risk of fracture is higher in those with T2D than in those without T2D [25]. This well-established point does not imply that BMD is a poor predictor of fracture in T2D. DXA is predictive of fracture risk once the underestimation of fracture risk is taken into account [25]. For a given level of fracture risk, the T-score in a woman with T2D is approximately 0.6 units higher [25]. Another predictor of fracture risk is the FRAX score (Fracture Risk Assessment Tool) in which a number of risk factors, independent of BMD, can be used to determine fracture risk. These other risk factors, which are not equally weighted in importance, include age, gender, race, height, BMI, fracture history, parental history of hip fracture, smoking status, alcohol intake, and systemic glucocorticosteroid use. FRAX does not include diabetes as a clinical risk factor [26]. As with DXA, for a given FRAX score, fracture risk is higher than predicted in T2D as compared to fracture risk in those without T2D [25]. Given that DXA and FRAX underestimate fracture risk in T2D, it is likely that in T2D, there are skeletal and perhaps nonskeletal issues, not captured by DXA or FRAX, that are important as risk factors.

Mechanisms for Increased Fracture Risk in T2D

Falls

Falls are important in the assessment of fracture risk because most non-vertebral fractures occur after a fall. A number of factors may exist in a patient with T2D that predisposes to falls. These factors include resultant effects of peripheral neuropathy, retinopathy, cardiovascular disease, and use of insulin. They are manifested clinically as impaired balance and gait, poor vision, osteoarthritis, heart failure, arrhythmias, and hypoglycemia [19]. Despite the increased propensity of type 2 diabetics to fall, when large population studies such as the WHI and the Rotterdam study adjusted for falls, T2D was still associated with increased fracture risk, suggesting that there are other factors, independent of fall risk, that predispose type 2 diabetics to fracture [19, 27].

Diabetes Medications

TZDs have been shown to increase the risk of peripheral fracture in postmenopausal women with T2D [28]. A potential mechanism for this effect is diversion of mesenchymal progenitor stem cells from the osteoblastic to the adipocytic lineage [28]. In an analysis from the ADOPT study (A Diabetes Outcome Progression Trial), Kahn et al. studied fracture risk in women receiving rosiglitazone, a

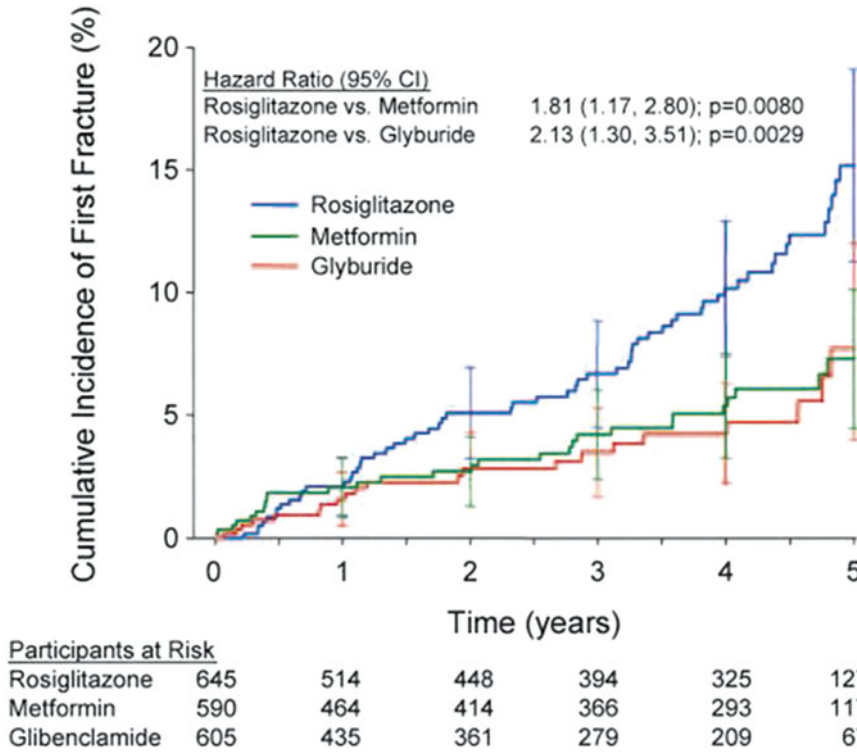

Fig. 22.1 TZD use and fractures. The use of rosiglitazone over 5 years in the ADOPT trial was associated with a higher cumulative incidence of first fracture as compared to metformin or glyburide. Adapted from Kahn, S.E., et al., Rosiglitazone-associated fractures in type 2 diabetes: an Analysis from A Diabetes Outcome Progression Trial (ADOPT). Diabetes Care, 2008. 31(5): p. 845–51

TZD, in comparison to metformin and glyburide [28]. Women taking rosiglitazone had a greater incidence of fracture than women on metformin (HR 1.81, 95 % CI 1.17–2.80; *p* 0.008) or glyburide [HR 2.13 (1.30–3.51) *p* 0.0029] (Fig. 22.1) [28]. Use of TZDs in postmenopausal women with T2D who are at risk for bone loss should therefore be discouraged. However, the data reflecting an increased risk of fracture in T2D cannot be accounted for by TZDs, at least not in a major way because many type 2 diabetics do not take TZDs, especially in more recent years as newer oral diabetic medications are available. The data reflecting an increase in fracture risk in T2D are not well correlated with the use of TZDs. Furthermore, studies that have taken into account TZDs continue to show an increase in fracture risk in T2D. Thus, there are factors that are responsible for increased fracture risk that cannot be accounted for by the use of TZDs.

Reduced Bone Formation

T2D is known to be associated with low bone formation [29–31]. Two bone formation markers, osteocalcin and procollagen type 1 amino-terminal propeptide (P1NP), are reduced in T2D as compared to normal, nondiabetic populations [29–31]. Low bone formation rates in T2D are supported by bone biopsy data showing reduced histomorphometric indices of bone formation, including mineralizing surface and bone formation rate [32, 33]. Postmenopausal type 2 diabetic women were also found to have fewer circulating osteogenic precursor cells, which are thought to be a source of osteoblasts [33]. Other studies have found that circulating sclerostin, an osteocyte product that inhibits bone formation,

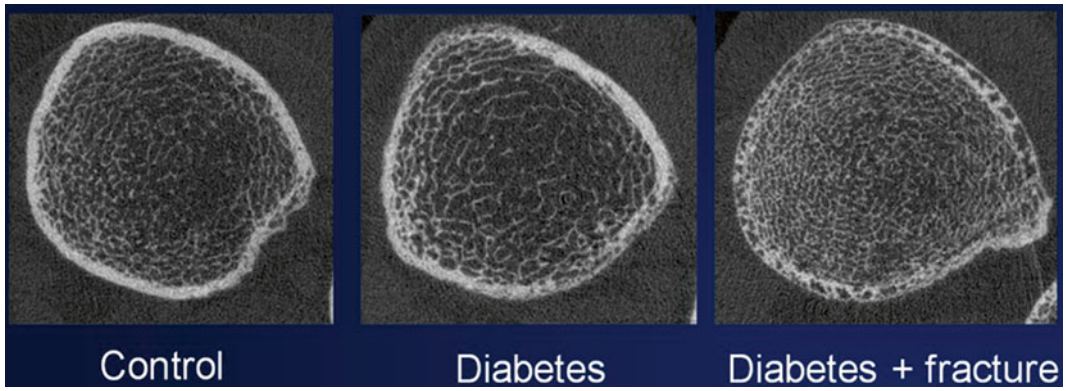

Fig. 22.2 Cortical porosity in T2D. Cortical porosity was increased in postmenopausal women with T2D as compared to nondiabetic controls, particularly in the T2D subjects who had fractured. Adapted from Burghardt, A.J., et al., High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab, 2010. 95(11): p. 5045–55

is increased in T2D [34]. Even after adjusting for age and BMI, sclerostin levels are significantly higher in T2D when compared to normal controls and type 1 diabetics [34]. These data suggest several mechanisms by which bone formation is decreased in T2D.

Abnormal Biomechanics

Biomechanical indices are worse in T2D despite higher areal BMD by DXA [35]. At the femoral neck, for example, where areal BMD by DXA is generally above average, femoral neck strength in T2D is lower relative to the total load when compared to controls [35]. In a multisite, multiethnic study of pre- and peri-menopausal women with and without T2D, diabetic women had worse DXA-derived indices with respect to compression, bending and impact as compared to nondiabetic women [35]. In this report, the greater BMD in T2D was not accompanied by a greater load to strength ratio [35]. In older men, those with T2D had lower total bone area at the radius and tibia by peripheral quantitative computed tomography, resulting in lower bone bending strength at these sites [36]. Thus, despite higher BMD by DXA, when biomechanical indices are assessed, T2D is associated with reduced bone strength and reduced ability to adapt to compression, bending, and impact forces.

Abnormal Bone Quality

High-resolution peripheral quantitative tomography (HRpQCT), a technology that permits high resolution imaging and quantification of non-vertebral microstructure, has yielded new insights about microarchitecture in T2D [37]. In the cortical compartment of bone, cortical porosity is increased by 1.24-fold in type 2 diabetics as compared to nondiabetic controls [38] (Fig. 22.2). Interestingly, the increase in cortical porosity in T2D was only observed in the T2D subjects who had sustained a fracture [39] (Table 22.2). Abnormalities in the trabecular compartment have also been suggested by data using another new imaging technology, Trabecular Bone Score (TBS). TBS is based upon the lumbar spine DXA image, analyzing it as a textural variability analysis [40]. A homogeneous trabecular network will give a higher relative TBS value which has been shown to be associated with greater bone strength [40]. If the textural analysis reveals more heterogeneity, the TBS will be relatively low, a finding

Table 22.2 Cortical porosity in T2D with fractures. Cortical porosity was significantly greater in those with T2D and fracture as compared to those with T2D and no fracture (*$p < 0.05$ between T2D history of fracture and T2D No fracture). Adapted from Patsch, J.M., et al., Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. J Bone Miner Res, 2013. 28(2): p. 313–24

Postmenopausal women				
	No diabetes		T2 diabetes	
	No fracture	History of fracture	No fracture	History of fracture
N	20	20	20	20
Distal radius	1.2%	2.5%	0.8%	**3.9%***
Distal tibia	4.3%	4.8%	3.0%	**5.7%***

associated with reduced bone strength. In a large retrospective study, diabetic women had lower TBS despite higher areal BMD by DXA. TBS was a more powerful predictor of fracture risk than DXA (HR 1.27, 95 % CI 1.10–1.46) [41]. Bear in mind that the fracture prediction by DXA was erroneously low.

Abnormal Bone Matrix

Abnormal bone composition due to the accumulation of Advanced Glycation End Products (AGEs) may provide an explanation for compromised bone strength in T2D. AGEs result from the irreversible addition of a carbohydrate to a protein [42]. Common AGEs include pentosidine and N(6)-carboxymethyllysine (CML) [42]. AGEs alter bone metabolism by leading to nonenzymatic cross-linking, of Type 1 collagen, which normally gives bone its toughness [43]. AGE accumulation occurs with natural aging but AGE accumulation is accelerated in T2D leading to lower bone turnover and a decrease in bone toughness, or the ability of bone to absorb energy before failing [43]. AGEs may decrease bone strength independent of BMD [43]. Urinary pentosidine predicted a higher risk of both vertebral and long bone fractures (HR 1.18, 95 % CI 1.05–1.33, $p < 0.01$) in postmenopausal diabetic women, suggesting that even in the absence of T2D, the accumulation of AGEs increases fracture risk, independent of BMD [44]. In T2D, pentosidine correlates with increased incidence of fracture [43]. Women with T2D and vertebral fracture had higher serum levels of pentosidine than women without vertebral fracture despite having similar BMD [22]. These data suggest that alteration of bone collagen quality may play an important role in increasing skeletal fragility among those with T2D.

In summary, T2D is an important risk factor for fracture. The explanation for increased fracture risk in T2D is multifactorial. While falls and use of certain diabetes medications (TZDs) may play a role, the underlying defects in bone formation, biomechanics, quality, and abnormal bone matrix make T2D a risk factor for fracture despite normal or even increased areal BMD by DXA. Reduced bone formation is associated with decreased levels of osteocalcin and P1NP and with increased levels of sclerostin. Compromised bone strength makes diabetic bone more brittle. Microarchitectural defects in trabecular and cortical bone as well as the accumulation of AGEs in the bone matrix all add to the increased fracture risk in this population as well.

Obesity and Bone Health

Understanding Increased Fracture Risk in Obesity

Until recently, obesity was considered to be protective for skeletal health [45, 46]. Given that higher weight is tightly linked to higher BMD and that low weight is a risk factor for osteoporosis, obesity was conventionally regarded as good for bone [47]. Moreover, higher weight provides a cushioning effect in case of a fall [48]. However, more recent research has demonstrated the opposite, namely that excess adipose tissue is detrimental to skeletal health [49–51]. As for T2D, the mechanisms are multiple. In the bone marrow, where mesenchymal stem cells differentiate into osteoblasts or adipocytes, the fat content of the bone marrow increases with age [52, 53]. Conditions such as glucocorticoid use, anorexia nervosa, menopause, TZDs, and T2D are all associated with a similar increase in marrow fat with a reciprocal decrease in osteoblasts [54, 55]. This diversion of lineage cells is also the case for obesity.

Although increased adiposity is associated with higher BMD, excess fat can also lead to an artifact of the DXA measurement because BMD is influenced by the surrounding soft tissue composition. The BMD therefore can be falsely elevated when obesity is present [49–51]. Other factors further confound an understanding of obesity and fracture risk. Obese individuals often have comorbidities such as T2D and vitamin D deficiency that can have their own adverse skeletal effects [49, 51]. Obese women often experience an early menopause, thus, losing earlier the protective effects of estrogen on bone [50]. Obese men may have lower testosterone levels than non-obese men, which can also lead to weaker bones [50]. Obese individuals may also have increased risk of falls, contributing to their fracture burden [49]. Moreover, when obese patients fall, they have a higher risk of fracture at all ages when compared to non-obese subjects. They are particularly predisposed to higher fracture risk in the upper and lower extremities [51, 56].

Body Composition and Fracture Risk

Body composition is altered in obese individuals with regard to the distribution of lean mass and fat mass. Obese individuals have greater fat mass, which is associated with lower BMD [56, 57]. In older adults, increased adiposity may lead to an increased number of vertebral deformities [58]. These adverse features in body composition begin as early as young adolescence, which is the vital time for skeletal accrual [59]. Overweight children and adolescents have an increased risk of forearm fractures [60]. This may be because obese children have increased cortical porosity, thinner trabeculae, and lower bone mass as compared to their lean counterparts [61].

Visceral Adiposity and BMD

Visceral fat, not subcutaneous fat, has been clearly associated with lower BMD [62]. A cross-sectional study of young adults found that increased visceral fat deposition was associated with detrimental femoral indices such as lower cross-sectional area [62]. Cohen et al. measured body composition by DXA in 40 premenopausal women and found that those with high trunk fat, a measure of visceral adiposity, had lower bone volume fraction as measured by HRpQCT as compared to those with the lowest trunk fat [63]. Those with higher abdominal fat had substantially lower bone volume, trabecular number and thickness, and lower estimated bone stiffness, suggesting that increased visceral adiposity as measured by truncal and abdominal fat is associated with adverse bone quality measures and decreased bone stiffness (Fig. 22.3) [63]. An underlying mechanism for

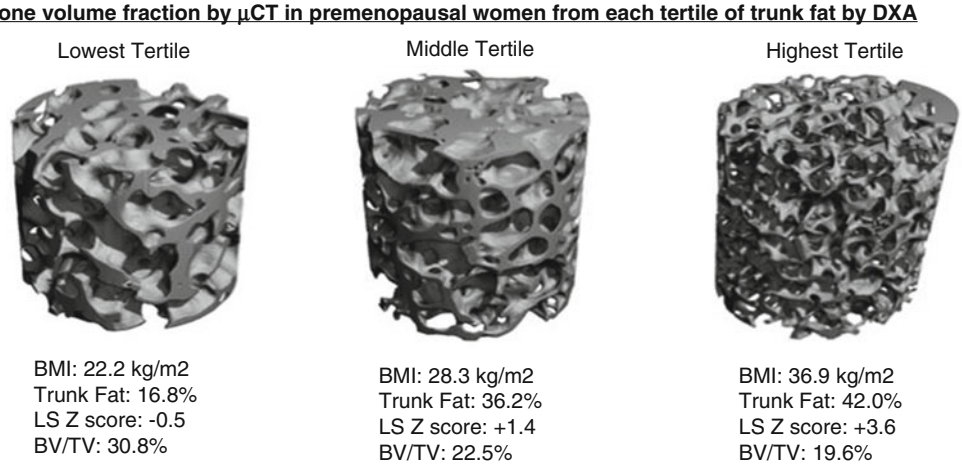

Fig. 22.3 Trunk fat and bone volume fraction. Trabecular bone structure by microCT of transiliac crest bone biopsy samples are shown, with representative subjects from each DXA trunk fat tertile. Subjects with the highest trunk fat had the lowest bone volume fraction. BV/TV = bone volume fraction. Adapted from Cohen, A., et al., Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. J Clin Endocrinol Metab, 2013. 98(6): p. 2562–72

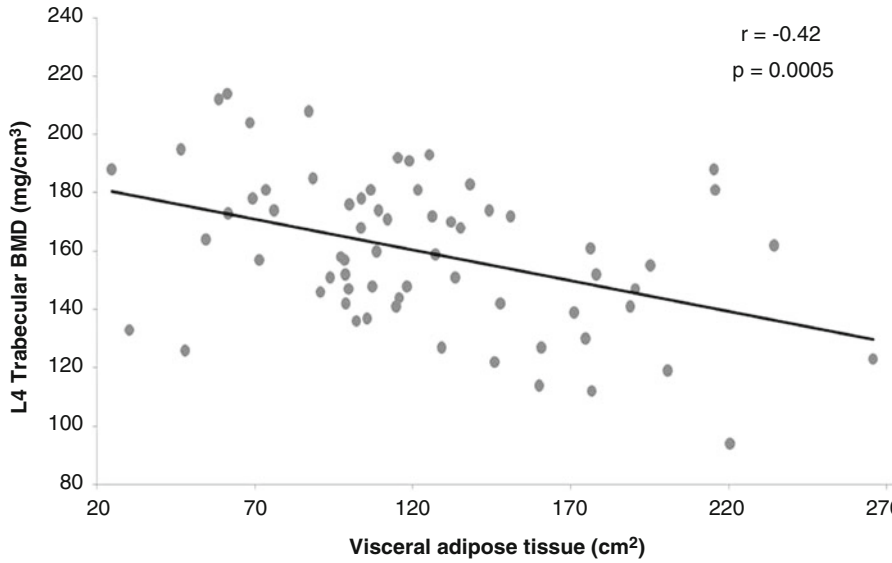

Fig. 22.4 Visceral adiposity and trabecular BMD in obese premenopausal women. As visceral adiposity by quantitative computed tomography (QCT) increases, volumetric trabecular bone mineral density decreases. Adapted from Bredella, M.A., et al., Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. Obesity (Silver Spring), 2011. 19(1): p. 49–53

this increased fracture risk may be that visceral fat alters levels of growth hormone and insulin-like growth factor 1 (IGF-1), both important mediators of skeletal health [57]. In addition to higher visceral fat content, obese women tend to have higher bone marrow fat when compared to non-obese women. Higher bone marrow fat has been associated with lower areal and trabecular BMD and lower levels of IGF-1 [55, 57] (Fig. 22.4).

Fat Composition and BMD

It is interesting to note that while patients with obesity have higher bone marrow fat than non-obese individuals, patients with low BMI also have high bone marrow fat and low BMD [64]. In women with anorexia nervosa, the total marrow fat content of the femur was significantly higher than in normal-weight, age-matched controls [64]. Unsaturated fat depots were found to be inversely associated with total body fat depots, demonstrating that the women with anorexia nervosa had less unsaturated fat depots than the normal-weight women [64]. Anorexia nervosa was therefore associated with higher amount of fatty acid saturation [64]. Higher bone marrow fat was inversely associated with BMD where BMD at the lumbar spine was lower in those with anorexia nervosa as compared to healthy controls suggesting that saturated lipids may negatively affect BMD [64].

Adipose Tissue Subtypes and Bone Quality

In addition to fat distribution, the underlying type of fat may also be a key determinant of bone quality [65]. White adipose tissue (WAT), the primary site of energy storage, is distributed throughout the body [66]. It is comprised of unilocular lipid droplets—85 % lipid composition—virtually all of which is in the form of triglycerides [65]. WAT regulates insulin sensitivity and glucose metabolism in the liver and muscle through multiple mechanisms including IGF-1 [66]. Brown adipose tissue (BAT), on the other hand, exists in discrete lobules in many species, primarily in the intrascapular and peri-renal regions in the newborn [67]. It plays a central role in both basal and inducible energy expenditure by regulating thermogenesis [67]. It is composed of multiple small lipid droplets with abundant mitochondria that oxidize nutrients and generate heat [67]. It is highly vascular and shows increased blood flow when activated [67]. BAT may regulate bone area in developing children and adolescents, while by adulthood it may be present in very small amounts with highly variable detectability [65, 67]. A retrospective, cross-sectional, single-center study examined the relationship between BAT and cross-sectional area of the femur in 40 children and adolescents [65]. PET/CT imaging was used to ascertain the amount of BAT as well as midshaft images of the femur to assess cross-sectional area and cortical bone area [65]. BAT was found to correlate with femoral cross-sectional area and cortical bone area after accounting for height, weight, and gender. These data suggest that BAT may play a key role in determining bone size and potentially bone strength [65].

Therapies for Osteoporosis in T2D and Obesity

Although no prospective randomized trials of osteoporosis agents in T2D and obesity exist, other data are available about the skeletal effects of osteoporosis drugs in these conditions.

Bisphosphonates

The most commonly prescribed medications for osteoporosis are the bisphosphonates, a class of medications that bind to hydroxyapatite in bone and impair osteoclast function, leading to reduced bone resorption [68]. These medications increase BMD and reduce fracture risk at vertebral and non-vertebral sites [69]. A post hoc analysis from the Fracture Intervention Trial (FIT) suggests that postmenopausal women with T2D who are treated with bisphosphonates have similar improvements in

BMD at the spine and hip as those without T2D [70]. Observational data suggest an anti-fracture effect of bisphosphonates in T2D [70, 71]. Data in obese subjects suggest that bisphosphonates are equally effective at increasing bone mass and preventing fracture as in the non-obese [72].

Raloxifene

Raloxifene is a selective estrogen receptor modulator that increases BMD at the spine and hip and reduces the risk of vertebral fractures in postmenopausal women [73]. The Multiple Outcomes of Raloxifene Evaluation (MORE) study assessed 30 risk factors and their impact on raloxifene effectiveness, including T2D and BMI [74]. In those with T2D on raloxifene therapy, the reduction in vertebral fracture was greater than in those without T2D, suggesting that raloxifene has protective skeletal effects in T2D [74]. Despite the reduction in vertebral fracture risk in T2D, the use of raloxifene did not change fracture risk based on BMI [74].

Conclusion

Other Therapies

Newer therapies for osteoporosis, including denosumab, and teriparatide, and older therapies for osteoporosis including estrogen and calcitonin have not been widely evaluated for efficacy in those with T2D or obesity. Estrogen in the form of HRT in postmenopausal women can preserve the natural decline in BMD that occurs at the menopause transition [16]. Calcitonin, which directly inhibits osteoclastic bone resorption has been shown to reduce vertebral fractures, but it has not been shown to reduce hip or non-vertebral fractures and has not been specifically evaluated in diabetes or obesity [75]. Denosumab is a fully humanized monoclonal antibody with affinity for RANK Ligand and binds to RANK receptors on osteoclast surfaces to decrease bone resorption [76]. Denosumab increases BMD at the spine, hip, and distal radius and decreases the incidence of spine, hip and non-vertebral fractures [76]. In postmenopausal women with T2D, denosumab reduced blood glucose levels in those not taking antidiabetic medications suggesting a dual role for denosumab in both decreasing fractures and lessening the toll of high blood sugars on the skeleton [77]. This later observation should be regarded as still speculative. Teriparatide [rhPTH(1-34)] is a recombinant foreshortened form of native parathyroid hormone [PTH(1-84)] that stimulates osteoblast number and function [77, 78]. Teriparatide increases BMD at the spine and hip and decreases risk of spine and non-vertebral fractures in randomized clinical trials both in those with and without T2D [78, 79].

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Chapter 23
Nutritional Concerns for Bariatric Surgery

Sue A. Shapses, Tara Mauro, and Robert E. Brolin

Key Points

- Bariatric surgery is a successful weight loss option for obese individuals and is especially recommended for those with obesity-related comorbidities.
- The surgical procedures are categorized as malabsorptive or restrictive and vary in weight loss, disease remission and complications.
- Alteration of the normal anatomy and physiology of the gastrointestinal tract can lead to nutrient deficiencies, intestinal disorders, and possible malnutrition. There are unique risks and prevention strategies associated with the different surgical procedures.
- Bariatric surgery patients require specialized nutrition care due to decreased absorptive capacity and reduced nutrient intake, and need lifelong micronutrient supplementation. Individualized patient care with the help of the physician and dietitian is needed to prevent long-term metabolic risk.

Keywords Bariatric surgery • Gastrointestinal disorders (bowel obstruction, dumping syndrome, ulcers, microbiome and bacterial overgrowth, gut sensing, bile acids) • Nutritional deficiencies (malabsorption, beriberi, osteopenia) • Obesity • Nutrition therapy • Weight loss

Introduction

Obesity is present in 34 % of the population in the USA, and its presence has increased markedly in other countries at a surprising rate [1, 2]. The obesity rates are expected to rise further in many Asian and Middle Eastern countries, and to a greater extent in former socialist countries and Latin America [3]. Also, the Global Burden of Disease Study 2013 states that while the epidemic might have peaked in developed countries, obesity rates are expected to rise to over 40 % in a number of developing countries [2, 4]. In the past decade more persons have reached the level of stage II and severe obesity making them eligible for bariatric surgery. In addition, for those individuals who reach this level of obesity, usual weight loss diets are often not successful. Bariatric surgery offers

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_23,

these individuals the opportunity to lose weight that is permanent and is often the only way for many to reduce their obesity related comorbidities. Improved techniques in the field have led to a dramatic rise in the number of bariatric procedures performed. Hence, understanding the acute and long-term nutritional outcomes is important as these patients continue to need care from clinicians after their surgical procedures.

Obesity

Obesity, defined by a body mass index (BMI) greater than or equal to 30 kg/m^2, is a worldwide epidemic. Obesity is further classified as Class I (BMI 30.0–34.9 kg/m^2), Class II (BMI 35.0–39.9 kg/m^2), and Class III or extreme obesity (BMI $\geq$ 40 kg/m^2) [5]. 34.9 % of the adult American population is obese, according to the Centers for Disease Control and Prevention, and although the prevalence of obesity has remained stable in the USA from 2003 to 2010, the percentage of the population affected remains high [6]. This issue continues to be a concern because of the chronic comorbidities such as diabetes, hypertension, and sleep apnea that can be attributed to obesity [7, 8].

Weight Loss

Although the etiology of obesity is multifactorial, weight loss is the solution to excess body weight and to its comorbidities [9]. A healthy diet and regular exercise are key factors in weight loss, but may not be effective in some patients with severe obesity [9]. With the high prevalence of obesity in the USA, bariatric surgery is expected to remain a popular weight loss option for severely obese individuals (Class III) and for any obese individual with comorbid conditions [10].

Bariatric Surgery

Surgery as a therapeutic form of treatment for weight loss dates back to the 1950s. The Swedish surgeon, Viktor Henrikson, performed the first procedure, a jejunoileal bypass, which had high rates of complications [11]. During the past six decades weight loss procedures became less invasive (i.e., including laparoscopic techniques), which resulted in fewer complications. Now, bariatric surgeries are categorized as malabsorptive, reducing the amount of nutrients absorbed, restrictive, reducing the functional capacity of the stomach, or a combination of the two [12].

Currently, bariatric surgery is widely accepted as a weight loss option for patients with Class III obesity, and patients categorized with Class II obesity with one or more comorbidities. However, it is also an accepted form of treatment for some patients with Class I obesity who have failed with nonsurgical options of weight loss [13].

The type of bariatric surgery utilized should be individualized to each patient. The American Society for Metabolic and Bariatric Surgery (ASMBS) has approved four types of procedures: the adjustable gastric band (AGB), the sleeve gastrectomy, Roux-en-Y gastric bypass (RYGB), and biliopancreatic diversion with duodenal switch (BPD-DS) (Fig. 23.1) [14]. These operations differ in terms of weight loss outcomes, advantages, and complications.

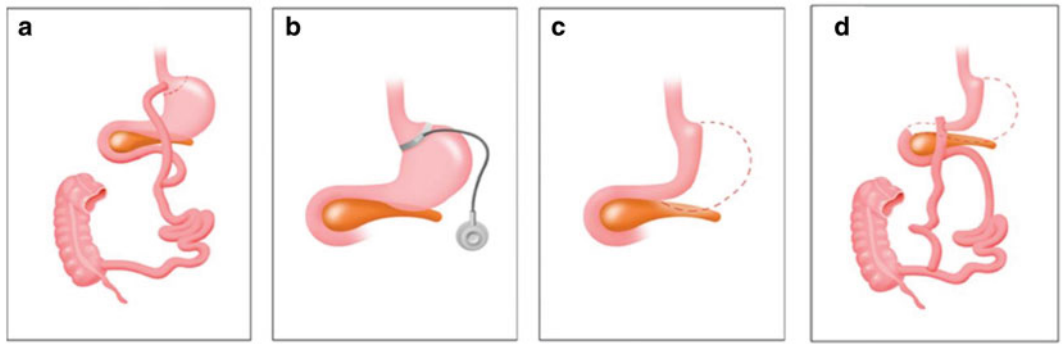

Fig. 23.1 Bariatric surgery procedures: (**a**) Roux en Y gastric bypass (RYGB); (**b**) Adjustable gastric banding (AGB); (**c**) Sleeve gastrectomy (SG); (**d**) Biliopancreatic diversion with duodenal switch (BPD/DS). Modified from Miras AD and leRoux CW [17]

Adjustable Gastric Band

The AGB is a restrictive bariatric operation in which an inflatable plastic band is surgically placed just above the fundus of the stomach. The band creates a small gastric pouch above the band that holds approximately 20 mL [15]. The amount of restriction can be adjusted via saline injected into a port beneath the skin that is connected to the band by silastic tubing. Studies have shown that this bariatric procedure can produce up to 55 % of excess body weight. However, in comparison with other procedures weight loss is much more gradual [16]. The weight loss achieved by the band has been shown to improve or resolve some comorbidities including diabetes, metabolic syndrome, hypertension, and dyslipidemia [17]. This procedure can be reversed by surgically removing the band. It has the lowest risk for vitamin and mineral deficiencies. Conversely, it requires strict adherence to the postoperative diet and the band has the highest rate of reoperation [17].

Sleeve Gastrectomy

The sleeve gastrectomy is a restrictive bariatric procedure, in which up to 85 % of the stomach is removed, leaving less than 60 mL of gastric capacity [18]. Because the fundus is removed, some of the endocrine functioning is lost, which contributes to the successful long-term weight loss of this procedure. Removal of the fundus results in decreased production of the hormone ghrelin, which stimulates appetite [19]. Greater weight loss is achieved by this procedure in comparison with gastric banding. However, there are more serious operative complications including gastric leaks, but these are infrequent [20].

Roux-en-Y Gastric Bypass

This gastric bypass procedure is both restrictive and malabsorptive. In gastric bypass a small gastric pouch is created and connected to a short limb of the jejunum, thereby excluding most of the stomach, all of the duodenum, and a small portion of jejunum [15]. This procedure also affects the endocrine function of the gastrointestinal tract [21]. The gastric hormones peptide YY and glucagon like

peptide 1 have been found to rise after RYGB, which may result in decreased appetite, contributing to the weight loss in these patients [22]. Studies have shown that this procedure can produce approximately 57–67 % loss of excess body weight, and can decrease the incidence of comorbid conditions in many patients [23]. Postoperative complications include dumping syndrome and micronutrient deficiencies [24].

Biliopancreatic Diversion with Duodenal Switch

The BPD-DS is a bariatric procedure that includes a partial gastrectomy with creation of alimentary and biliopancreatic limbs of the small bowel, making it both restrictive and malabsorptive [25]. Approximately three-fourths of the intestine is bypassed, the bypassed segment is connected with the ileum, allowing for food particles and pancreatic enzymes to mix [26]. The weight loss achieved by BPD-DS is slightly better than that of the RYGB. However, BPD-DS is associated with more complications and nutritional deficiencies including calcium and fat-soluble vitamins [27].

Surgical Complications

The surgical complications differ for each type of operation. Early complications include anastomotic leaks and small bowel obstruction, occurring most frequently with RYGB and BPD-DS [28]. Complications also may occur weeks or months postoperatively including internal hernias, stomal stenosis, and staple line complications [28]. All of these complications require readmission and typically involve surgical intervention.

Many patients with AGB need revisional surgery due to band slippage, erosion, or prolapse [29]. Even though this operation is less invasive and requires no re-routing of the gastrointestinal tract, there are still risks. In fact, subsequent bariatric procedures are more common after AGB than RYGB (Fig. 23.2) [30]. In addition, many patients fail the AGB procedure, due to either late surgical complications or inadequate weight loss, and return to the operating room for a malabsorptive procedure [31].

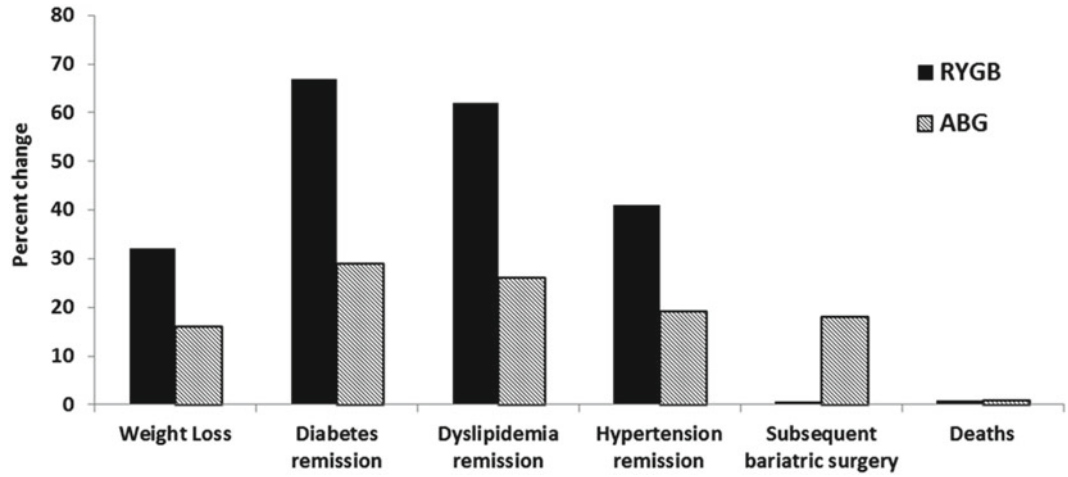

Fig. 23.2 Weight loss, remission of health outcomes, complications resulting in subsequent bariatric surgery and death at 3 years after bariatric surgery with Roux En Y gastric bypass (RYGB) or laparoscopic adjustable gastric banding (ABG) [32]

Weight Loss and the Remission in Comorbidities of Obesity

Surgical Weight Reduction

Studies show that gastric bypass results in greater weight loss, improved body composition, lower fat intake, and greater postprandial satiety hormone responses in comparison with gastric banding [32]. Studies comparing sleeve gastrectomy with RYGB generally show similar excess body weight loss [33]. Gastric banding produces a more gradual weight loss that is generally less than after malabsorptive procedures. A comparative study of weight lose outcomes 1 year postoperatively reported mean excess weight loss of 66.5 %, 56 %, and 39 %, respectively, for RYGB, sleeve gastrectomy, and AGB [34]. At 3 years postoperatively, excess body weight loss dropped, respectively, to 59 %, 46 %, and 37 % for RYGB, sleeve gastrectomy, and AGB. Another study reported 47 % excess body weight loss 10 years after gastric banding [35].

Diabetes and Other Obesity-Related Comorbidities

A systematic Cochrane review showed that bariatric surgery results in greater improvement in weight loss and comorbidities versus medical interventions regardless of the type of procedures used [27]. In a 3-year retrospective cohort study, 1738 participants underwent RYGB, while 610 had laparoscopic AGB [30]. Preoperatively, 33 % had diabetes, 63 % dyslipidemia, and 68 % hypertension. The RYGB patients lost about twice as much weight as the ABG patients, with a similar pattern of improvement observed for remission of diabetes, dyslipidemia, and hypertension (Fig. 23.2). In a systematic review of 29 studies, the percent remission in diabetes, dyslipidemia, and hypertension was similar [36]. Both surgeries resulted in <1 % risk of death [30] (Fig. 23.2).

Weight loss is the most important mechanism of type 2 diabetes mellitus (T2DM) remission. With more weight loss after RYGB (vs. AGB), there is evidence of higher remission of diabetes [27]. The greatest metabolic response to meal ingestion includes increased early postprandial plasma GLP-1 and insulin concentrations. It is not clear if these postprandial changes are also responsible for improving glycemic control and ameliorating T2DM, and whether they act independent of the weight loss is not known. Another study has shown similar changes in insulin sensitivity, β-cell function, and oral glucose tolerance in nondiabetic obese adults after RYGB and laparoscopic ABG [37]. In contrast, BPD is associated with marked improvement in β-cell function and insulin sensitivity after minimal weight loss. This is distinct from other bariatric procedures [38]. The ability of bariatric surgery to ameliorate diabetes and for specific types (i.e., BPD) to have a weight loss independent effect, a reevaluation of the indications for bariatric surgery in patients with class II obesity and T2DM, based on a risk-benefit assessment, is needed. More recently, it was shown that initial BMI did not predict T2DM remission, in a study where patients were grouped according to BMI < or > than 35 kg/m^2 [39]. A study that included gastric bypass patients with T2DM preoperatively had a greater than 40 % relapse of diabetes at 8 years postoperatively [40]. This suggests that the metabolic and anatomical changes due to the surgery are not permanent, yet it remains unclear why only some individuals redevelop metabolic dysfunction or diabetes. Weight regain can occur following any type of bariatric procedure and occasionally leads to the redevelopment of metabolic dysfunction. Therefore strict adherence to healthy eating and lifestyle is required for long-term success. Bariatric surgery should continue to provide new insights into the mechanisms responsible for the regulation of body weight and glucose homeostasis.

Nutritional Consequences and Gastrointestinal Physiology

Micronutrient Deficiencies

Because the malabsorptive procedures, RYGB and BPD-DS, alter the anatomy of the normal gastrointestinal tract and thus alter the ability to absorb nutrients, patients who have these procedures are at risk for micronutrient deficiencies. It is therefore imperative that patients comply with the nutritional supplementation that has been individualized for them by their weight loss team. Vitamin and mineral deficiencies reported in postoperative bariatric surgery patients include iron, vitamin B_{12}, folate, vitamin D, calcium, and thiamin [41–43]. Because the BPD-DS procedure decreases the absorption of biliary salts due to the rerouting of the intestine, this increases the risk for lipid malabsorption [44]. This can lead to fat-soluble vitamin deficiencies. In contrast to BPD-DS, RYGB results in less fat malabsorption and no carbohydrate malabsorption [45, 46].

Surprisingly, studies have found similar micronutrient deficiencies present after both the RYGB and sleeve gastrectomy procedures, despite the fact that sleeve gastrectomy is not a malabsorptive procedure [33, 47, 48]. These studies found that the most common deficiencies in this patient population include vitamin D, vitamin B_6, and vitamin B_1 (thiamin). Specific diseases associated with micronutrient deficiencies after bariatric surgery are discussed below and shown in Table 23.1.

Beriberi

Thiamin deficiency (vitamin B_1) has been found to be one of the most common water-soluble vitamin deficiencies reported after bariatric surgery. Thiamin is necessary for energy metabolism within the body, and thus a deficiency has significant effects. The most common setting for thiamin deficiency after bariatric surgery occurs in patients who develop hyperemesis, who can neither eat nor swallow vitamin supplements. This occurs in both restrictive and malabsorptive procedures. If left untreated, this can result in wet, dry, or cerebral beriberi. Wet beriberi symptoms are rare, and include tachycardia and hypertension. Dry and cerebral beriberi have neurological symptoms including peripheral paresthesias, loss of balance, nystagmus (that can limit vision), mental confusion, and psychosis [49]. A literature review of beriberi in the bariatric surgery population found that females tend to be more

Table 23.1 Nutritional and gastrointestinal conditions post-bariatric surgery

Disease/condition	Symptoms	Management
Beriberi [wet, dry, cerebral][a–d]	Wet beriberi: tachycardia, hypertension, shortness of breath	Thiamin supplementation
	Dry and cerebral beriberi: weakness, lethargy, confusion, loss of sensation, pain, or tingling in limbs	
Bone loss[a]	Increased fractures	Adequate vitamin D and Ca supplementation
Bowel obstruction[a,b]	Nausea, vomiting	Surgical intervention
	Abdominal pain	
Dumping syndrome[a,c]	Nausea, diarrhea	Limit simple carbohydrate intake
	Postprandial hypoglycemia	Acarbose
	Loss of consciousness	
Gastric ulceration[a]	Abdominal pain	Proton pump inhibitors
	Bleeding	Sucralfate therapy
		Surgical intervention

[a–d]Most evidence available for [a]RYGB, [b]AGB, [c]SG, [d]BPD/DS

affected than males. This deficiency typically develops 1–3 months postoperatively and can be life threatening [49]. Early recognition of the neurologic symptoms is key in successful treatment. Suspicion should prompt immediate administration of 50 mg of thiamin with subsequent daily doses of 50–100 mg until improvement is noted.

Bone Loss

It is well established that bone loss occurs with moderate weight loss [50], and evidence suggests that bariatric surgery procedures also have negative skeletal effects, and that vary by surgical type [51]. The presence of osteopenia or extent of bone loss may be related to lower calcium absorption or other factors. An early investigation of RYGB patients who were examined 9 months after surgery found that compared to obese controls, RYGB patients had a significantly higher bone resorption markers (urinary N-telopeptide cross-linked collagen type 1 and osteocalcin) and lower bone mineral density (BMD) and bone mineral content (BMC) at the total hip, total body, and trochanter [52]. The amount of bone loss is influenced by the extent of weight loss, but there is no evidence that serum 25OHD levels, that normally rise after moderate weight loss or after bariatric surgery attenuates bone loss [53–55]. In addition, dietary calcium or vitamin D intake does not seem to modify the extent of bone loss [56]. Another study found no change in serum PTH or 25(OH)D despite doubling of dietary calcium and vitamin D intake to ~2350 mg/day and 1700 IU/day, respectively [53]. They also found increases in bone remodeling markers (urinary N-telopeptide and osteocalcin) and decreased BMD at the femoral neck and total hip [53]. A study in our lab showed elevated markers of bone resorption and secondary hyperparathyroidism 4 years after the surgery despite a modest increase in calcium and vitamin D intake [56]. In another study, we showed that there was a dramatic decrease in calcium absorption by 33 % (absolute fractional decrease from 0.36 to 0.24) 6 months after RYGB surgery in women consuming 1.5 g Ca/day and 400 IU vitamin D [57]. However, it should be noted that the 24 % absorption after RYGB surgery is still at a level that is adequate to maintain calcium balance, suggesting that other mechanisms are increasing bone resorption and loss.

Overall, bariatric patients have been found to have increased indices of bone resorption markers, elevated serum PTH, lower calcium absorption. A meta-analysis of 12 studies examining BMD 1 year after bariatric procedures found a decline in BMD at the hip as well as a total overall reduction in BMD compared to baseline [58], and thus far it appears that the bone loss cannot be attenuated with higher Ca and vitamin D intake after RYGB surgery.

Gastrointestinal Complications and Changes

Bowel Obstruction and Internal Hernias

Bowel obstructions can occur after any type of abdominal surgery. Obstructions are also a complication with bariatric procedures, with a reported incidence of 2–10 % after laparoscopic RYGB [59, 60]. They have etiologies ranging from blood clots to intestinal adhesions [61, 62]. Patients with acute obstructions typically have abdominal pain. A retrospective analysis of 99 cases of small bowel obstruction after RYGB found that 50 % of individuals also had elevated pancreatic enzyme levels and all patients required surgical interventions [63]. In our practice [59], it was shown that the rate of small bowel obstructions and internal mesenteric hernias could be reduced when there was complete (vs. incomplete) closure of mesenteric defects with laparoscopic RYGB. Other surgical methods have been proposed to reduce internal hernias leading to bowel obstructions [60]. A small study of patients who underwent the gastric banding procedures revealed that all patients had some degree of gastric

obstruction and that this commonly resulted in vomiting [64]. Patients often do not see their surgeons when they exhibit symptoms years after their procedures and these symptoms can go unrecognized even though most require surgical intervention.

Dumping Syndrome

Dumping syndrome is typically seen following RYGB, but can also occur after restrictive procedures like the sleeve gastrectomy [41]. Dumping syndrome results from the rapid passage of gastric contents into the small intestine, most commonly after the consumption of simple carbohydrates. Postprandial hypoglycemia can cause weakness, lethargy, or loss of consciousness, and is associated with late dumping syndrome. Treatment of late dumping includes acarbose, which will help to improve glycemic control [65]. Some speculate that the symptoms of dumping syndrome may cause patients to avoid foods, and thus lose even more weight. However, a study of 50 RYGB patients found that while 46 % suffered from dumping syndrome, there was no relationship between having dumping syndrome and restrained eating [66]. RYGB patients report that dumping episodes occur less than once a week [67], however 5–10 % of patients have more frequent symptoms. After surgery, patients (especially RYGB) should be monitored for symptoms of dumping syndrome to prevent hypoglycemia and minimize gastrointestinal disturbances.

Marginal Ulcers

Marginal or anastomotic ulcers constitute the majority of complications after RYGB surgery. They develop on the jejunal side of the gastrojejunostomy after RYGB, and typically present with gastrointestinal bleeding and severe upper abdominal pain. Marginal ulcers occur in 2–15 % of gastric bypass patients, occurring within the first 18 months postoperatively. Most ulcers will heal after 3 months of proton pump inhibitors (PPI) and sucralfate therapy [68]. While bariatric surgery patients are typically prescribed PPI, one study found that 2 % of their patients with ulcers needed surgical or other pharmacological interventions [69]. Ulceration is more common in patients who use nonsteroidal anti-inflammatory drugs and in those who smoke [70]. Cessation of these habits is essential for healing of the ulcer. Postoperative ulcers, while common, can be treated proactively with pharmacology and healthy habits. Only a small population of patients will require additional surgical intervention.

Microbiome and Bacterial Overgrowth

Bacterial transplantation studies have indicated that the gut microbiome may actively play a role in obesity and conditions such as diabetes and inflammation. For example, transfer of gut microbiota from obese (but not lean) rodents to germ-free control rodents leads to an increase in food intake and body weight [71]. Zhang et al. [72] was the first to show a decrease in *Clostridium* bacteria (phylum Firmicutes) after RYGB compared with controls. RYGB surgery reverses the gut microbiota from an obese to a lean phenotype (Table 23.2; [72–82]). While RYGB induced changes in the gut microbiota show the standard decrease in the Firmicutes to Bacteroidetes ratio that accompanies weight loss with dieting alone, microbial sequencing analyses after RYGB consistently indicates an overabundance of the phylum Proteobacteria in the distal gut microbiome [73, 75, 82, 83].

Furet et al. [75] showed that the low levels of *Bacteroid*es/*Prevotella* and *F. prausnitzii* populations in obese subjects before surgery increased after RYGB. In contrast, both the bifidobacterium and lactobacillus/Leuconostoc/Pediococcus groups decreased after RYGB surgery [75]. Importantly, while some gut bacteria groups correlate with energy intake, high body weight, and metabolic changes, *F. prausnitzii* is associated with changes in the inflammatory state and insulin resistance. Hence, a change in the gut

Table 23.2 Microbiome: effects of obesity and effect of weight loss (medical vs. bariatric surgery[a])

Condition	Firmicutes:bacteroides	Other
Obesity	Increased [73–76]	
Medical weight loss	Decreased [73, 77, 78]	Dieting increases diversity and dramatically changes microbiome [79]. Proteobacteria increased [73, 80]. Lactobacillus administration increases weight loss [81]
Roux en Y gastric bypass	Decreased [72, 73, 75, 82]	Bacteroides/*Prevotella* and *F. prausnitzii* populations increased and both the Bifidobacterium and lactobacillus/Leuconostoc/Pediococcus groups decreased [75]
Other procedures	No data	

[a]Adjustable gastric band, sleeve gastrectomy, biliary-pancreatic diversion, duodenal-jejunal bypass

microbiota may affect the amelioration of obesity-related symptoms and diseases after RYGB or other bariatric procedures. One interesting study indicates that the RYGB modifications of gut microbiota are associated with changes in white adipose tissue gene expression [84]. Studies also show that fecal microbiota does not reflect what is happening within other microbial areas of the gut [84, 85]. For example, small intestinal bacterial overgrowth has been reported in nearly 50 % of cases during RYGB reconstruction cases [86]. Hence, a stagnant loop created during the surgery can result in a novel ecological niche. Both colonic and small bowel changes in the microbiota may influence vitamin deficiencies after bariatric surgery. The hypochlorhydria and pH-induced increases in the gut after RYGB can be expected to affect the gut microorganisms. In addition, probiotic supplementation can alter the microbiota. Woodard et al. [81] showed that supplementation with lactobacillus probiotics (phylum Firmicutes) compared to placebo led to greater weight loss and less bacterial overgrowth in post-RYGB patients. A greater understanding of the gut microbiome may explain health outcomes after bariatric surgery and may help define new treatment modalities in obese patients with and without bariatric surgery.

Gut Sensing

Gut nutrient sensing has been studied in animal models using a modified bypass procedure and has shown increased intestinal glucose production after surgery with lower hepatic glucose output [87]. Greater hepatic glucose control has also been shown in an animal model of duodenal-jejunal bypass in a similar manner [88]. This is possibly due to the presence of undigested nutrients, but it is still not entirely clear. Soon after surgery, RYGB produces changes in postprandial glucose, C-peptide, glucagon, and pancreatic polypeptide responses, and appears to enhance insulin clearance after a meal tolerance test [89]. The alteration in gastrointestinal anatomy also causes changes in hormone levels, thus altering the gut nutrient sensing ability, and therefore would occur to a greater extent in the malabsorptive procedures. Research in this area is ongoing and should help to better define disease remission after bariatric surgery.

Bile Acids

Circulating bile acids are blunted in obesity, and increase after malabsorptive surgical procedures such as RYGB and BPD-DS. Increased bile acids may also play a role in the mechanisms regulating weight loss and glycemic improvements after bariatric surgery. Bile acids have been shown to have numerous metabolic effects, such as increasing gut hormone production and energy expenditure, and reducing food intake, gluconeogenesis, and insulin resistance [14, 90]. Others have also shown an increase in both bile salts and FGF21 after RYGB surgery (unlike weight loss) [32, 91]. Overall, the rise in bile acids may help explain why fat malabsorption is not as compromised after RYGB surgery and glycemic control is improved compared to purely restrictive types of surgeries.

Protein Calorie Malnutrition

Most individuals who undergo gastric bypass surgery are highly motivated to improve their health and many will increase physical activity during the period of greatest weight loss in the first year. Because gastric bypass is malabsorptive and leads to greater weight loss than banded gastroplasty, these patients are also at greater risk for malnutrition [32]. One carefully designed study examined regional body composition, muscle proteolysis, and energy expenditure before, 6 and 12 months after RYGB surgery. After 1 year and 44.3 ± 10.2 kg weight loss, lean body mass (LBM) loss constituted 28 ± 10 % of the total weight loss after 12 months, and this primarily occurred in the first 6 months postoperatively [93]. In another study, 114 patients lost 34 kg of body weight at about 6 months after RYGB, with a 37 % reduction in fat tissue mass and 20 % reduction in LBM [94]. These studies show that there is an undesirable large and rapid decrease in LBM after surgery. Also, because LBM reduces energy expenditure, these individuals are more susceptible to weight regain. Therefore, adequate protein intake coupled with increased physical activity, should be encouraged to minimize this rapid loss of LBM. Importantly, both RYGB and BPD-DS bariatric surgery can lead to potentially fatal protein calorie malnutrition [95]. Patients should be monitored regularly for nutritional deficiencies, and treated aggressively to prevent malnutrition and the possibility of life-threatening complications.

Disease Classification

Classification or scoring patient health outcomes due to surgical weight loss, rather than using loss of weight or adiposity as a marker is considered one approach to specifically examine medical, psychological, and functional improvements in clinical trials. No scoring system is currently used in clinical practice, nor is it recommended. The Edmonton Obesity Staging System has been developed [96, 97] and ranks severity of health problems associated with obesity, mental health, and quality of life. Another classification system has been proposed by a group in Ireland called the King's Obesity Staging System and was developed to provide a more objective assessment. This system uses an alphabetic mnemonic that addresses Airways, Body mass index, Cardiovascular, Diabetes, Economic, Functional, Gonadal, Health status (perceived), and (body) Image. For each category, health is scored as follows: 0 ("normal health"), 1 ("at risk"), 2 ("established disease") or 3 ("advanced disease"). These methods are relatively recent and therefore, their use is limited in the bariatric population at this point in time [97, 98]. These classification systems should be considered in future studies to provide more evidenced based support to improve patient health and clinical guidance across different centers performing the surgery. A classification system that specifically addresses nutritional concerns might be a good approach in the future to score nutritional status with a consistent approach between studies.

Nutritional Therapy

Preoperative Preparation

In order for a patient's bariatric surgery to be covered by Medicare, they must not only fulfill BMI and comorbidity requirements, but they must also have had unsuccessful medical treatment of their obesity [99]. Many insurance providers require weight loss attempts prior to surgery approval for coverage [100]. It is the position of the ASBMS that preoperative weight loss is not always beneficial and can be counterproductive, but should be analyzed on a patient individual basis with the input from the entire weight loss team [101].

The preoperative education and counseling is crucial to the successful weight loss of the patient [41]. Bariatric surgery patients are best managed using a team approach including the help of a registered dietitian. Prior to bariatric surgery, nutrition consultation is essential in order to ensure the success of the patient. A nutrition assessment is performed to evaluate the patient's current eating behavior and to screen for nutritional deficiencies. Typically, an individualized nutrition action plan is created for the patient [102]. The most common nutrient deficiencies found in bariatric surgery patients preoperatively include vitamin D and vitamin B_{12} as well as iron and zinc [103, 104]. Nutritional supplementation prior to surgery to correct for nutritional deficiency may be necessary [105]. Proper nutrition education includes implementation of a diet plan before bariatric surgery that is specific to that patient.

Postoperative Follow-Up

Immediately after surgery, the postoperative concerns are similar to other types of gastrointestinal surgical procedures, as well as some that are unique to bariatric surgery. Surgical healing and the return of gastrointestinal function expected due to the surgery are the goals during this period. The postoperative follow-up of the bariatric surgery patient is just as important as the preoperative care. Follow-up appointments with the surgeon and dietitian generally occur 1–2 weeks later, and again postoperatively 3, 6, and 9 months later. They may also be individualized. Yearly appointments are recommended subsequently [18].

Postoperative diet advancement follows the progression from clear liquids, to pureed foods, to soft foods and then to regular foods. This diet advancement takes place at the discretion of the surgeon, dietitian, and the patient's tolerance of foods and should be monitored and individualized [106]. The patient generally remains on a clear liquid diet up to 1 week postoperatively, and this progression can be adapted to fit the needs of the patient [107]. Advancement to pureed foods takes place over the next 2–3 weeks. Soft, solid foods are then gradually introduced into the diet, as tolerated. Finally regular foods are reintroduced into the diet. Throughout the diet advancement, patients are repeatedly instructed to eat slowly and to chew foods thoroughly. This means that food intake can take a couple of hours, but this approach should help to prevent nausea or vomiting [107].

During the early postoperative period, adequate protein intake and fluid consumption is a major concern. Protein is necessary for the maintenance of LBM, wound healing, and prevention of malnutrition [108]. Patients are instructed to consume the protein portion of their meals first, primarily as a method to maintain reduced food intake. The DRI for protein for normal, healthy adults is 0.8 g/kg/day, and it is recommended that bariatric surgery patients consume 1.1 g/kg of ideal body weight/day [18, 108]. Patients are encouraged to consume 48–64 fluid oz/day to maintain adequate hydration [109]. Many bariatric surgery patients who have restrictive procedures will need to separate the consumption of solids and liquids to minimize nausea and vomiting. Patients should drink before eating and then are counseled to wait a 30–60 min before drinking again [18, 109].

Supplementation

Most bariatric surgery patients will need lifelong daily micronutrient supplementation; however, this varies with the type of procedure. All patients should consume a daily multivitamin/mineral supplement. In addition to multivitamin/mineral supplement, RYGB and BPD-DS patients will need a calcium supplement to total 1.5 g/day and additional vitamin D is also recommended. In addition to both of these, it is recommended that patients who have undergone RYGB and BPD-DS procedures need to take 150 mg elemental iron and 500 μg of vitamin B_{12} daily. It has been recommended that BPD-DS patients additionally take a supplementation of the fat soluble vitamins, A, D, E, and K due to fat malabsorption following this procedure [109]. Patient compliance with the prescribed supplementation regimen is important for the prevention of nutritional deficiencies [47].

Special Population: Adolescents

Obesity has become increasingly common in children and adolescents in the USA and worldwide, and is often associated with the same comorbidities as in the adult population. Some severely obese adolescents may require bariatric surgery for successful weight loss [110]. The most commonly performed surgeries within this population include RYGB, sleeve gastrectomy, and AGB [111]. DuCoin et al. [112] reported that adolescents with a mean age of 18 years who underwent RYGB had a mean excess weight loss of 55 % at 2 years postoperatively, with a reduction of comorbidities and no postoperative complications. These findings support the effectiveness and safety of weight loss surgery in this population. Another study investigated 135 adolescents, with a median age of 19 years, who had the sleeve procedure and reported mean excess weight loss of 77 % and 86 % for females and males, respectively, complete resolution of diabetes, and a postoperative complication rate of only 4 % [113]. These authors concluded that there is a high rate of safety and efficacy for the sleeve gastrectomy procedure in the adolescent population. Postoperative nutritional recommendations are similar for adolescents as recommended for adults [110]. It is imperative that these adolescents comply with the nutrition supplement regimen to ensure normal growth and maturation.

Long-Term Nutritional Care

Due to the large number of bariatric operations performed in the USA, it is becoming increasingly important for clinicians to specifically obtain a history regarding bariatric surgery because of the higher risk of long-term nutritional complications in these patients that would require specialized monitoring. These include low bone mass, gastrointestinal issues, and micronutrient deficiencies. Noncompliance with vitamin/mineral supplementation is a major concern, since some patients forget about their greater micronutrient needs. Primary care physicians and nurses need to be able to recognize and address these problems.

Conclusions

Bariatric surgery has a significant role in the treatment of severe obesity. Because it has significant physiological and nutritional consequences, it brings a unique mix of new medical and nutritional requirements in this population. Future nonsurgical obesity treatments include a focus on taste/food preferences, gut microbiota, bile acid signaling and methods to preserve β-cell function and hepatic glucose output. Nonsurgical interventions that mimic the metabolic benefits of bariatric surgery represent areas of future investigation. Currently, improvements in surgical techniques using minimally invasive methods have made it even more viable for a greater number of patients. Health-care professionals must understand and address the long-term medical comorbidities in these patients that are responsible for increased healthcare costs and premature deaths.

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Chapter 24
Composition, Production, Consumption, and Health Effects of Added Sugars

James M. Rippe and John S. White

Key Points

- Fructose containing sugars are among the most misunderstood nutrients in all of nutrition.
- Sucrose and high fructose corn syrup (HFCS) undergo similar manufacturing processes utilizing many of the same procedures.
- Sucrose and HFCS are absorbed into the body the same way and enter the blood stream as free fructose and free glucose. They have the same sweetness and calories.
- Numerous studies suggest there are no differences between sucrose and HFCS with regards to multiple nutritional metabolic and health related parameters and no adverse effects from either when consumed within the normal range of human consumption.

Keywords Sucrose • High fructose corn syrup • Fructose

Introduction

The relationship between added sugars and potential health consequences has engendered considerable controversy and debate, particularly over the past decade [1–14]. Numerous research studies have been published related to the effects of added sugars in the diet, ranging from animal studies to epidemiologic and cohort studies as well as randomized controlled trials and meta-analyses. In addition, many research studies have compared pure fructose to pure glucose with regard to metabolic and health effects [15–17], although these two monosaccharides are rarely consumed in isolation in the human diet.

Fructose containing sugars—namely fructose itself, HFCS and sucrose—are also the subject of multiple misconceptions and are among the most poorly understood nutrients in all of nutrition. Potential effects of added sugars figure not only in important scientific questions, but are of considerable interest to the media, the public and even regulatory bodies. Added sugars have been blamed for contributing to the obesity epidemic [4, 6]; contributing to or even causing diabetes [18, 19]; increasing the likelihood of coronary heart disease (CHD) [9]; and contributing to the rise of the metabolic

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_24,

syndrome [20], nonalcoholic fatty liver disease (NAFLD) [7], hypertension [20], and various lipid disorders [21]. Some investigators have even maintained that added sugars may be "addictive," leading to overconsumption of calories [7, 8, 22, 23]. Other researchers, however, strongly dispute the assertion that sugars are "addictive" and have even challenged the fundamental concept of whether "sugar addiction"—or more broadly "food addiction"—exists except under narrowly restricted experimental conditions [24–26].

Scientific organizations like the American Heart Association (AHA) [27], World Health Organization (WHO) [28], and the Scientific Advisory Committee on Nutrition (SACN) in England [29] have recommended significant restrictions on the amount of calories consumed from added sugars, with the recommended upper limit of no more than 10 % of calories from sugars. Other organizations like the *Dietary Guidelines for Americans 2010* (DGA 2010) [30] and the Institute of Medicine carbohydrate report (IOM Report) [31]—upon which the DGA 2010 value was based—recommended a higher upper limit of 25 % of calories for added sugars.

The putative link between sugar sweetened beverages (SSBs) and multiple adverse health consequences has even caused some school systems to eliminate chocolate milk from their lunch menus [32]. A number of state legislatures have also looked into potentially banning added sugars from school lunch menus [33]. New York City's former mayor Bloomberg advocated prohibiting certain size SSBs from restaurants regulated by the Department of Health in New York City, based on the belief this would make a meaningful contribution to obesity prevention; this initiative was disputed by many scientists and ultimately struck down by the courts [33]. Several prominent scientists have suggested taxation or other regulatory measures [34] to limit consumption of SSBs, although little evidence exists suggesting for a link between taxation and consumption [33].

Over the past 40 years the amount of fructose containing sugars consumed in the USA and worldwide has increased along with other major sources of calories in the diet including fats, flours, and cereal products [13]. While a few epidemiologic studies have linked consumption of SSBs with risk of heart disease [35, 36], obesity [4, 6, 9], hypertension [37, 38] and a decrease in dietary quality, others have disputed these findings [39–41], leading to an intense scientific debate.

Against this background of controversy, the current chapter will attempt to provide basic facts concerning the composition, production, and consumption of added sugars followed by a description of the modern science related to health effects of these sweeteners. While there are numerous types of added sugars, this chapter will focus on fructose containing sugars including sucrose, HFCS, and fructose itself since the first two are the predominant added sugars in the human diet and all three have been at the center of controversies related to potential adverse health consequences [42]. The reader should keep in mind, however, that additional fructose containing sweeteners in the diet include honey, fruit juice concentrates, and agave nectar.

Historical Perspective

The first nutritive (caloric) sweeteners available to early man were the naturally occurring sugars in fruits, vegetables, nuts and honey. The sugars in these gathered foods consisted largely of fructose, glucose, and sucrose in varying ratios and amounts. Because availability was limited by climate, geography, and season, sugars were accessible only to the immediate gatherers or those with the means to afford them. It was only in the past two centuries that sweeteners became more widely available and affordable, and only during the twentieth century that added sugars became a regular part of the human diet.

The two dominant sweeteners in use today are refined sugar (sucrose) and HFCS. Their development into commodity ingredients closely tracks the history of agricultural expansion, exploration, conquest, colonization, industrialization, and technological innovation [43–52]. Important milestones are detailed below.

Sugar

Although sugar is now refined both from sugarcane and sugar beets, its original source was sugarcane. The earliest sugarcane was domesticated around 8000 BC in New Guinea and spread from there to Southeast Asia, China, and India. The oldest known variety of sugar beet was chard, originally used for food and medication as early as 2000 BC by the Greeks and Romans.

The development of a process to mold cooled sugar syrup enabled regional sugar trade in India around 500 BC. Darius the First and Alexander the Great learned of sugar during conquests in India and Western Asia; exports to Greece and Rome followed, as sugar became valued for its sweetening and medicinal qualities. By 300 AD, selective breeding produced bulbous red and white beetroots—predecessors of the modern sugar beet—however, they were used primarily as a food source in Italy.

An early crystallization process for granulated sugar was developed in India (400 AD), producing a more stable and readily transported product. Sugar become a major trade item by 500 AD, introduced by merchants throughout the Indian Ocean and China; invading Arabs acquired sugarcane from Persia and traded it throughout the Mediterranean. By 1200 AD, Indian sugar production methods were adopted throughout the Middle East and Asia. Crusaders returned home with sugar and Venetian merchants imported it to Europe. Juice yields doubled by 1300 AD due to technical innovations in sugar pressing.

An important goal of global exploration (1400–1700 AD) was the discovery of suitable land and climates for sugarcane cultivation. It was by this means that Dutch, Spanish, and Portuguese explorers expanded sugarcane cultivation to the Caribbean region and Central and South America. At about the same time, a comprehensive refining and distribution center was established in Antwerp in direct competition with the Venetians.

By the 1700s, the Caribbean had become the global low cost sugar producer due to an abundant slave and indentured workforce. And sugar beets were becoming recognized as a source of sucrose in non-temperate climates: mangel-wurzel, the large rooted beets commonly used for animal feed in Germany, Holland, and England, were discovered by Marggraf to contain sucrose. The first sugar beet factory was built in what is now Poland to refine sugar from beets.

The half-century from 1800 to 1850 saw the rise of sugar as a dietary staple, spurred by lower cost, more abundant supply and new uses for sugar in preserves, candies, desserts, beverages, and processed foods. Because of an abundance of arable land and one of the few remaining pro-slavery policies, Cuba became the most affluent Caribbean nation. This period also witnessed a number of technical advances in sugar refining, including the closed kettle vacuum pan (reducing energy costs and heat-induced sucrose losses); multiple-effect evaporation (reducing energy costs); and centrifugation (improving sucrose recovery from molasses).

European sugar beet refining received a boost during the Napoleonic Wars, stimulated by British blockades curbing cane sugar imports. Beet sugar production became more competitive when the Caribbean labor advantage was lost with the abolition of slavery after 1850. After several failed attempts in other regions, the first successful American beet sugar plant was built in Central California. The global beet sugar industry thrives today, mostly in northern climes.

High Fructose Corn Syrup

Against the historical backdrop of sugar, HFCS is a relative newcomer. Although the technology for producing HFCS was developed between 1940 and 1970, its history is closely tied to the development of starch-based agriculture and technical innovations.

The first documented use of starch occurred in 6000 BC, when the Egyptians used it as an adhesive to glue papyrus strips together. One of the first starch refining processes was described by Cato, incorporating some of the same separation and refining techniques used in modern corn wet milling.

Starch can be isolated from a number of botanical sources besides corn. Wheat starch was first produced in Holland in the 1500s AD. Early uses included laundry sizing and white hair powdering. When it proved to be a more economical source, potato starch production began in Germany in 1765. It wasn't until the early nineteenth century that the first American starch plant was built to supply a host of new uses, including textiles, paper, color printing, adhesives, and food thickening.

The first use of corn as a raw material for starch production was by the Colgate Company in the mid-1800s; they eventually became the largest starch producer in the world. This was closely followed by the Union Sugar Company with the development of corn syrup, an aqueous solution of glucose and glucose oligomers (*no* fructose), a concept originally demonstrated by the Russian chemist, Kirchoff. Corn syrup is mildly sweet, has good thickening properties, was cheaper than sugar, and was widely adopted by both consumers and the food industry.

Though corn syrup was an innovative and successful product, its applications were limited because of low sweetness and large functional differences in comparison to sugar. Studies by Cantor and Hobbs in the 1940s demonstrated that alkaline isomerization of glucose to fructose was theoretically possible. The resulting mixture of glucose and fructose was closer to sucrose in sweetness than corn syrup, but was not commercially viable due to the formation of undesirable sugars degradation products. Later studies in the 1950s by Clinton Corn Processing Company (Clinton)—stimulated by erratic sugar supply from Cuba and concomitant price spikes—used microbial enzymes to partially isomerize the glucose in fully hydrolyzed starch. Though this process wasn't economically viable due to the instability of the enzyme, it proved to be a breakthrough in principle for the development of a corn-derived sweetener that could compete with sugar on a sweetness and functionality basis.

A more robust, heat-stable bacterial enzyme (xylose isomerase) was isolated in 1965 by Takasaki of the Japanese Agency of Industrial Science and Technology (AIST). Clinton partnered with AIST to further develop the technology and by 1968 had produced a batch of HFCS with 42 % fructose, dubbed "first generation" HFCS. By 1972, Clinton incorporated immobilized enzyme in the first continuous process to make HFCS-42. Because HFCS-42 has only 92 % of the sweetness of sucrose, there was a need to boost the fructose content higher. Moving bed chromatographic separation was adapted to the HFCS process enabling enrichment of fructose and production of HFCS-55, "second generation" HFCS. This product had sweetness similar to sucrose. Although HFCS was successfully substituted for sucrose in many non-beverage applications, it wasn't until 1984 that sufficient advancements in refining increased the purity of HFCS-55 to the point it was acceptable as a 100 % substitute for sucrose in the high-volume carbonated soft drink market.

It is worth noting that development of both beet sugar and HFCS was driven largely by the periodic fluctuations in sugar availability and price that inconvenienced home users and frustrated food manufacturers. Who knows if these sweeteners would have fully developed without this catalyst?

Composition

The sugars compositions of the most common nutritive sweeteners are listed in Table 24.1. The two most important HFCS products in commerce contain 42 % fructose (HFCS-42) and 55 % fructose (HFCS-55). The remaining carbohydrates in HFCS are free glucose and minor amounts of bound glucose: predominantly maltose (glucose dimer), maltotriose (glucose trimer), and higher saccharides (glucose oligomers) [53]. Although HFCS-42 and HFCS-55 are distinct products, they have fructose and glucose compositions comparable to sucrose, invert sugar, and honey.

HFCS is occasionally confused with common corn syrup, but they are clearly dissimilar products. Corn syrup is actually a family of ingredients made up only of glucose—some free, but most bonded to itself in chains of various lengths up to approximately 10, depending on the specific corn syrup product [56]. HFCS is also frequently confused with pure fructose. The compositional differences

Table 24.1 Sugars composition of common nutritive (caloric) sweeteners, *as consumed*

Component (%)	HFCS-42	HFCS-55	Corn syrup	Fructose	Sucrose	Invert sugar[a]	Honey
Fructose	42	55	0	100	50	45	49
Glucose	53	42	100	0	50	45	43
Others	5[b]	3[b]	0	0	0	10[c]	5[d]
Moisture	29	23	20	5	5	25	18

Adapted from [13, 53–55]
[a]Total invert sugar—nearly completely hydrolyzed (inverted) sucrose using acid or enzyme (invertase)
[b]Readily hydrolysable oligomers of glucose
[c]Unhydrolyzed sucrose
[d]Sucrose and minor amounts of other carbohydrates
HFCS high fructose corn syrup

between HFCS and pure fructose are obvious: the former contains roughly equal levels of glucose and fructose and is a liquid sweetener, while the latter contains no glucose and is a low-moisture crystalline material. It must be recognized that, from a composition standpoint, pure fructose is a poor experimental model for HFCS, as they are completely different products.

The glucose-to-fructose ratio in HFCS is nearly 1:1 on a hydrolyzed, post-digestion basis, similar to the ratio in sucrose, invert sugar, and honey. The same 1:1 ratio is also found in over 50 fruits/fruit juices, vegetables, and nuts [57]. The main distinction in composition between sucrose and other fructose containing sweeteners is the presence of a bond linking fructose and glucose. The glucose and fructose in HFCS, total invert sugar, honey, and fruit juice concentrates is principally monosaccharide (free, unbonded).

Invert sugar is the name given to sucrose in which some (medium invert sugar) or most (total invert sugar) of the bonds linking fructose and glucose have been hydrolyzed (broken). This may be accomplished either with acid or enzyme (invertase). Acid catalyzed inversion of sucrose is accelerated by increased temperature and reduced pH, and takes place within time spans as short as minutes to as long as months [58]. Because carbonated beverages are low in pH (colas are near pH 3.5) and are stored in warehouses at ambient (and sometimes very hot, especially in summer) temperature—sometimes for weeks before they reach supermarket shelves—considerable inversion of sucrose can take place before the product reaches the consumer. Ironically, those seeking to consume sucrose-sweetened sodas often end up drinking a sweetener composition more similar to HFCS than intact sucrose, and have been doing so since the first cola was formulated in the 1880s. Because the chemical bond between fructose and glucose has been intentionally broken during manufacturing of invert sugar (or unintentionally during storage of sucrose-sweetened beverages), the human body does not need to break that bond, to the extent of the inversion, before metabolizing fructose and glucose.

Production

Viewed with perspective, HFCS and sucrose manufacturing processes are more similar than they are different. The manufacturing processes used for HFCS and sugar are compared in Table 24.2. Both exploit sophisticated separations and refining technology to produce valued sweeteners. Food and beverage manufacturers have strict requirements for sweeteners used in mainstream products. They must have low color, flavor, and odor in order not to overshadow desired product characteristics. Because HFCS and sugar are both derived from complex living organisms—corn and cane or beets, respectively—there is a rich milieu of colored, flavored, and odorous biochemical compounds from which the sugars must be purified. Both industries draw from the same pool of manufacturing and refining techniques to isolate and separate their respective sweeteners [60–63].

Table 24.2 Similarities in manufacturing processes for HFCS and sugar

Sources: products	Physical disruption	Sugars extraction	Purification
Corn—high fructose corn syrup (HFCS)	Steep, mill, grind, screen, wash	• Liquefaction	• Filtration
		• Saccharification	• Refining—carbon, ion exchange
		• Isomerization	• Chromatographic separation
		• Enzymes [59]	• Blending
		– Starch hydrolysis	
		– Isomerization	
Cane and beet—sucrose and invert sugar	Chip, shred, slice, mill, wash, diffuse	• Sulfitation	• Affination
		• Clarification	• Carbonatation/phosphatation
		• Enzymes [59]	• Refining—carbon, ion exchange
		– Filtration aid	• Crystallization
		– Invert sugar production	• Refining, chromatographic separation, blending
			• Crystallization, conditioning
			• Inversion

After harvest, transport, and cleaning, the raw materials must undergo *physical disruption* to allow isolation of the carbohydrates within. In corn wet milling, water is used to move successively purer material through the process, while non-carbohydrate fractions are removed and sold as byproducts. Cleaned corn is soaked in water and sulfur dioxide (SO_2) to soften the fibrous hull in preparation for physical disruption. As the hull softens, SO_2 diffuses into the kernel and loosens the tightly bound starch granules. SO_2 also serves to control pH, color formation, and microbial growth. The corn hull and germ are separated from starch through milling, grinding, screening, and washing. Corn oil is recovered from the germ through pressing, while protein (gluten) and other insolubles are removed by mud centrifugation. Spent germ, fiber, protein, and insolubles are commonly sold as animal feed. High purity starch moves on from the wet mill to the refinery. Sugarcane and sugar beets also undergo physical disruption after harvest, transport and cleaning. Cane is chipped, shredded, and milled, while beets are sliced and diffused.

The next steps in the sweetener manufacturing process (Table 24.2) are designed for *sugars extraction*. HFCS processing uses acid and enzymes to sequentially reduce the size of high molecular weight starch molecules (polymers of glucose) in two processes called liquefaction and saccharification. In liquefaction, combinations of acid and the enzyme α-amylase make random cuts in starch molecules, shortening the chain lengths and creating commercial products called maltodextrins and corn syrups. In saccharification, the enzyme glucoamylase is used to hydrolyze the remaining oligosaccharides to monosaccharide glucose. The enzyme xylose isomerase is used by the corn wet milling industry to catalyze the isomerization of glucose to fructose in the production of HFCS.

Disrupted material from cane and beets also undergoes sugars extraction starting with a process called sulfitation—steeping in water containing lime and dissolved SO_2. Clarifiers similar to mud centrifuges in corn wet milling separate insoluble particles created during sulfitation. A series of evaporation, crystallization, and drying steps produce raw sugar, a relatively low grade and impure product that requires further refining for human consumption. Sugar cane and beets sometimes contain starches, raffinose, and dextrans that interfere with filtration, impede crystallization and reduce sugar yields. Use of enzymes (amylase, raffinase, and dextranase) as process aides to remove these carbohydrate polymers is becoming more common in sugar processing [59, 64–66]. The sugar industry also uses the enzyme invertase in the production of invert sugar, in which sucrose is hydrolyzed (inverted) to a mixture of glucose, fructose, and residual sucrose.

Extracted sugars next undergo a series of *purification* steps to remove impurities (Table 24.2). In corn wet milling, glucose (dextrose) is the product of liquefaction and saccharification process steps. It is purified by filtration, carbon treatment and ion exchange chromatography to remove large

particles, unwanted color and flavor, and charged compounds. Isomerization of glucose produces HFCS-42. HFCS-55 is made by enriching the fructose made through isomerization using moving-bed chromatographic separation, and blending the enriched fructose stream with HFCS-42. Analogous to sugar production, crystallization is also used by the corn wet milling industry to make pure crystalline fructose from the enriched fructose stream described above.

Raw sugar received by the refinery is remelted (affination) and then undergoes further purification via carbonatation (steeping in aqueous carbon dioxide and lime) and/or phosphatation (steeping in aqueous phosphoric acid and lime), followed by carbon treatment and ion exchange chromatography. Carbonatation and phosphatation denature protein, absorb color compounds, and destroy monosaccharides (largely glucose and fructose) that cause unwanted color and reduce sucrose yields. Crystallization is the final purification step, producing white sugar. Non-crystallized material can undergo chromatographic separation to recover sucrose that is blended back into the process; the remainder is sold as molasses.

Sucrose is sometimes described as "natural" and HFCS as "processed." Given the similarities described above, however, it makes little sense to differentiate HFCS and sucrose in this manner on the basis of the processes used to manufacture and refine them: they are either both natural or both processed.

Functionality: Uses in Foods

Consumers sometimes express surprise at seeing a sweetener on an unexpected product label. Many don't realize that sugars play additional roles in foods in addition to providing sweetness. Sugars affect the taste, texture, and shelf-life of foods by performing the following roles [57]:

- Providing sweetness
- Serving as preservatives in jams and jellies
- Increasing the boiling point or reduces the freezing point of foods
- Allowing fermentation by yeast
- Reacting with amino acids to produce color and flavor compounds important to the appealing flavor and color of baked goods
- Extending shelf-life by controlling moisture and inhibiting microbial growth
- Improving the palatability of low moisture, high fiber foods

Consumption

There are many misconceptions about the consumption of nutritive sweeteners that have resulted in a misguided focus on HFCS and added sugars as the driving force for obesity and other public health concerns [6]. Figure 24.1 compares trends in consumption of sugar, HFCS, fructose and added sugars. It is derived from USDA-ERS per capita availability data, adjusted for loss—a reasonable estimate of consumption based on production figures after correction for amounts wasted (uneaten) that occur between manufacturing and ingestion [67]. These data are consistent with estimates derived from other distinct data sets, including the NHANES database [68]. The following points are clear after studying Fig. 24.1:

- Sugar and HFCS consumption curves are near mirror images. Shortly after its introduction to the food and beverage industry in 1970, HFCS began replacing sugar in food and beverage products. As HFCS use (and consumption) increased between 1970 and 1985, sugar declined. The one-to-one replacement occurred due to close similarities between the two sweeteners in functionality and sweetness.

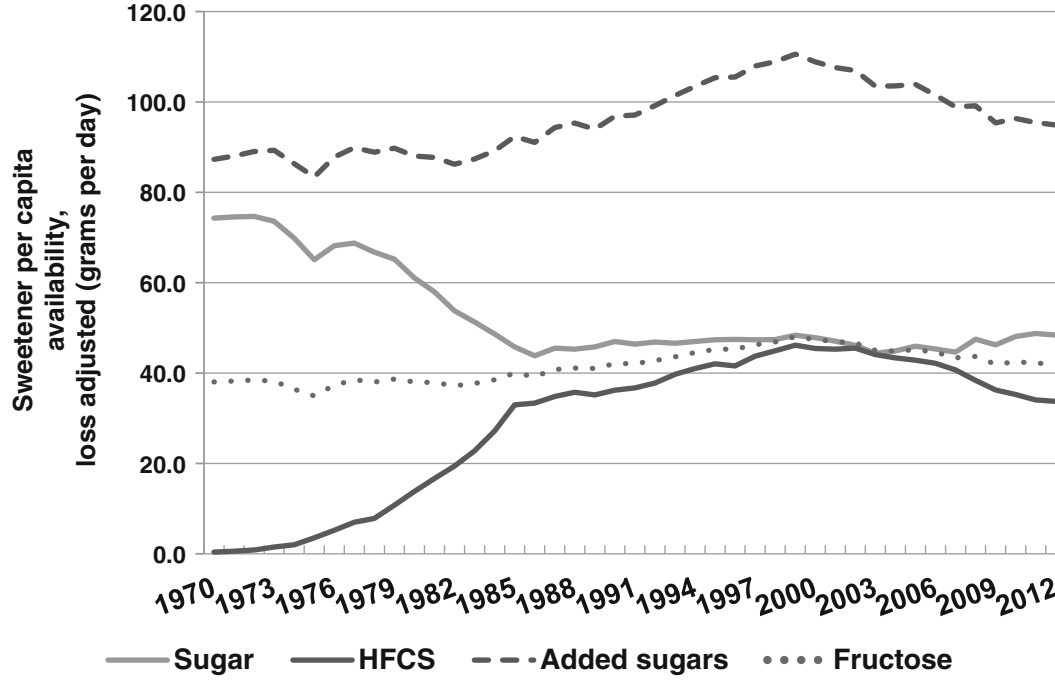

Fig. 24.1 Comparison of trends in per capita availability (loss adjusted) of nutritive sweeteners

- Consumption of HFCS slowed after 1985 and peaked in 1999. Contrary to popular belief, HFCS use has been in continual decline for the past 15 years. Consumption in 2012 was comparable to that 1987.
- Despite the misconception that "HFCS is in everything," Americans today consume nearly 1.5 times as much sugar as HFCS.
- Other than a modest rise between 1985 and its peak in 1999, consumption of fructose—the sugar component in nutritive sweeteners claimed by some to be an important contributor to many significant diseases—has been relatively constant for the past 40 years. In fact, USDA records going back to 1910 show that over the past 90 years, fructose intake has averaged 39 ± 4 g/day/person with a variation of just 16 kcal/day [14].
- Per capita consumption of all added sugars increased over the 30-year period beginning in 1970, however the upward trend peaked in 1999, the same year as HFCS and fructose. And added sugars consumption has been in significant decline ever since.
- Obesity rates rose throughout the decade from 2000 to 2010, however, consumption of HFCS, fructose and added sugars was in decline. Consequently, the reported correlation between HFCS and obesity that focused attention on HFCS as a unique cause [6] has now been invalid for over a decade.
- Sugar (sucrose) consumption has enjoyed a resurgence since 2003 at the expense of HFCS.

The current emphasis on added sugars suggests that sugars consumption is increasing disproportionately in the US diet. However, it was recently calculated [14] that of the 449 kcal/day increase in daily energy intake between 1970 and 2010, the increased energy from caloric sweeteners was minor, accounting for just 34 kcal/day. More than 90 % of the daily calorie increase came from increased consumption of added fats and flour/cereal products. Americans are not overweight and obese because of fructose or HFCS or added sugars; they're overweight because they eat too much of *everything*.

Finally, it is important to understand that HFCS is predominantly a US sweetener for a variety of reasons, including lack of a stable starch or water source, technology barriers and domestic sugar protectionist policies. Globally, sucrose consumption exceeds HFCS by a ratio of more than 10:1 [14].

Metabolism

In reviewing the metabolism and metabolic effects of fructose-containing sugars, it is important to do so from a "real world diet" perspective; that is, in the amounts and dietary patterns in which humans ingest them:

- Humans rarely consume fructose alone. It is found together with equivalent glucose in nearly every dietary source, including added sugars (sucrose, HFCS, honey, fruit juice concentrates) and natural sources (fruits, vegetables, nuts). The lone exception is crystalline fructose; however, this specialty sweetener is used in such limited quantities as to be insignificant compared to other sources.
- A persistent myth about HFCS is that, because of its free fructose, it is metabolized differently than sucrose with its bound fructose (Fig. 24.2). However, any discussion of the metabolic consequences of sucrose and HFCS must consider their *post-digestion*—not *pre-digestion*—composition, since this is what enters the bloodstream and is ultimately processed by the liver and other tissues. Sucrose is hydrolyzed (inverted) in three ways: (1) prior to digestion by the action of food and beverage acids on the labile sucrose bond (as in canned tomato preparations or carbonated soft

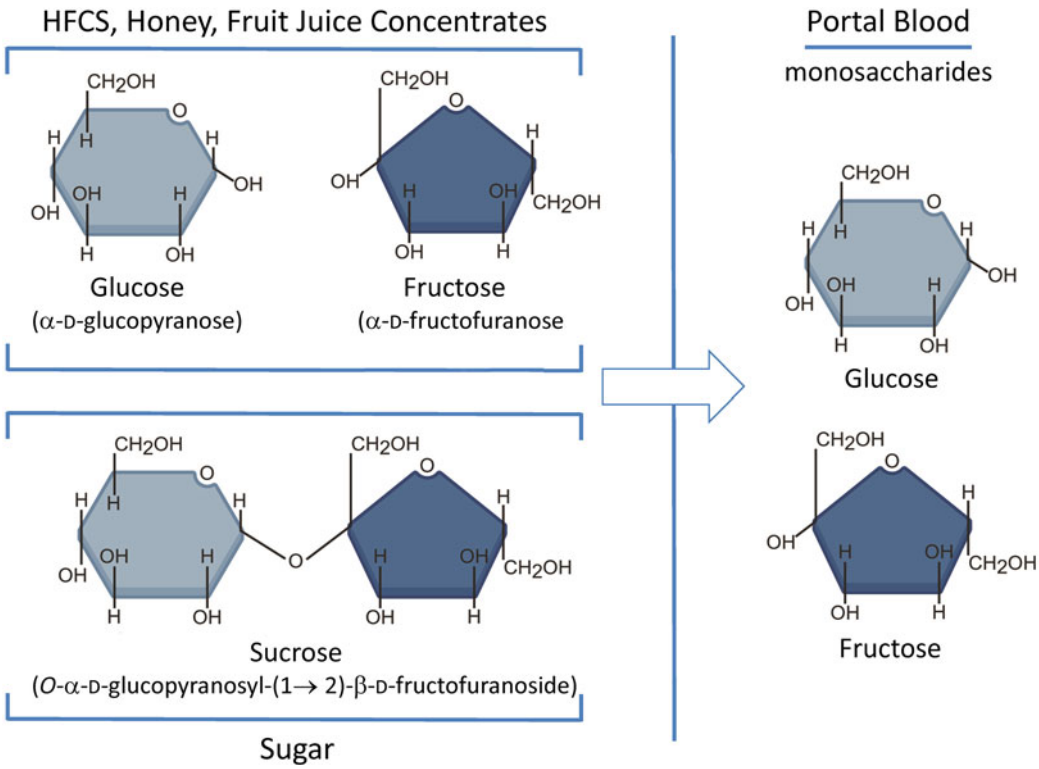

Fig. 24.2 Structures of fructose sweeteners before and after digestion

drinks); (2) prior to digestion by purposeful hydrolysis of the sucrose bond by acid or enzyme to make invert sugar; and (3) during digestion by the plentiful and fast-acting enzyme sucrase in the lumen of the small intestine. Thus, fructose and glucose enter the bloodstream as monosaccharides after digestion of *all* fructose sweeteners, no matter the mode of sucrose hydrolysis (Fig. 24.2). Any distinctions about monosaccharide origin are lost once they reach the bloodstream. If the ratios of fructose:glucose are similar—as they are in sucrose, HFCS, honey, and grape juice concentrates—one would hypothesize *equivalent* rather than *distinct* metabolism of fructose sweeteners. Clinical data that confirm this hypothesis are presented later in this chapter.

- The consequences of sugars consumption should be viewed in the context of the whole diet, not as isolated nutrients. In a diet rich in fat, complex carbohydrates, and protein, sugars are only one nutrient group contributing to the overall net body metabolism. And it has been estimated that dietary glucose from all sources exceeds fructose by a ratio between 3:1 and 5:1—due in large part to the increased intake of starchy foods over the past 40 years—so it may be expected that fructose effects are tempered by the overwhelming amounts of glucose in the diet [14, 77].
- It is important to test the effects of fructose sugars within the range of normal human consumption. Marriott used NHANES data to estimate fructose exposures in the US population and reported that mean and 95th percentile fructose intakes as a percentage of energy were 9.1 % and 14.5 %, respectively. The highest fructose consumers were men and women aged 19–30 years with intakes of 15.5 % and 18 %, respectively [69]. White calculated that contemporary animal and human research protocols frequently test fructose levels as high as three times the 95th percentile [14].

Intestinal Absorption

Sucrose is hydrolyzed prior to absorption into the portal vein by the enzyme sucrase in the lumen of the small intestine, with release of free fructose and glucose. Fructose is transported into enterocytes via facilitated diffusion by the transporter GLUT5. Glucose and sodium ions are co-transported into enterocytes via sodium-glucose transporter 1 (SGLT1). Some researchers have suggested that hydrolysis of the disaccharide bond in sucrose is a differentiating event, resulting in different rates of uptake—and hence, metabolism—of fructose from sucrose and HFCS [78–82]. However, there is no reliable human evidence to support such a distinction; on the contrary, recent human clinical trials by Rippe et al. demonstrate *no* metabolic distinction between the two sweeteners [1].

Liver Metabolism

Absorbed fructose and glucose pass to the liver via the portal blood. It is estimated the liver metabolizes up to 90 % of absorbed fructose, but only 20 % of absorbed glucose—a major distinction. But considering there is 3–5× more glucose than fructose in the diet and in circulation, the liver may well process comparable levels of each.

Metabolic pathways for fructose and glucose are distinct, yet interactive, as illustrated in merged form in Fig. 24.3. Both sugars diffuse from circulation into the liver via the same facilitated GLUT2 transporter. Once in the liver both are phosphorylated: glucose-6-phosphate is formed by glucokinase and fructose-1-phosphate is formed by fructokinase.

Glucose uptake and phosphorylation are regulated by the glucose concentration in the portal blood and subsequent disposition of glucose-6-phosphate is regulated by current energy needs. If energy is required, glucose-6-phosphate is catabolized via glycolysis to pyruvate, decarboxylated to acetyl-CoA and transported into the mitochondria, where energy is produced. Glycolysis is regulated by insulin, cytosolic ATP, and citrate; the latter two provide feedback inhibition of phosphofructokinase,

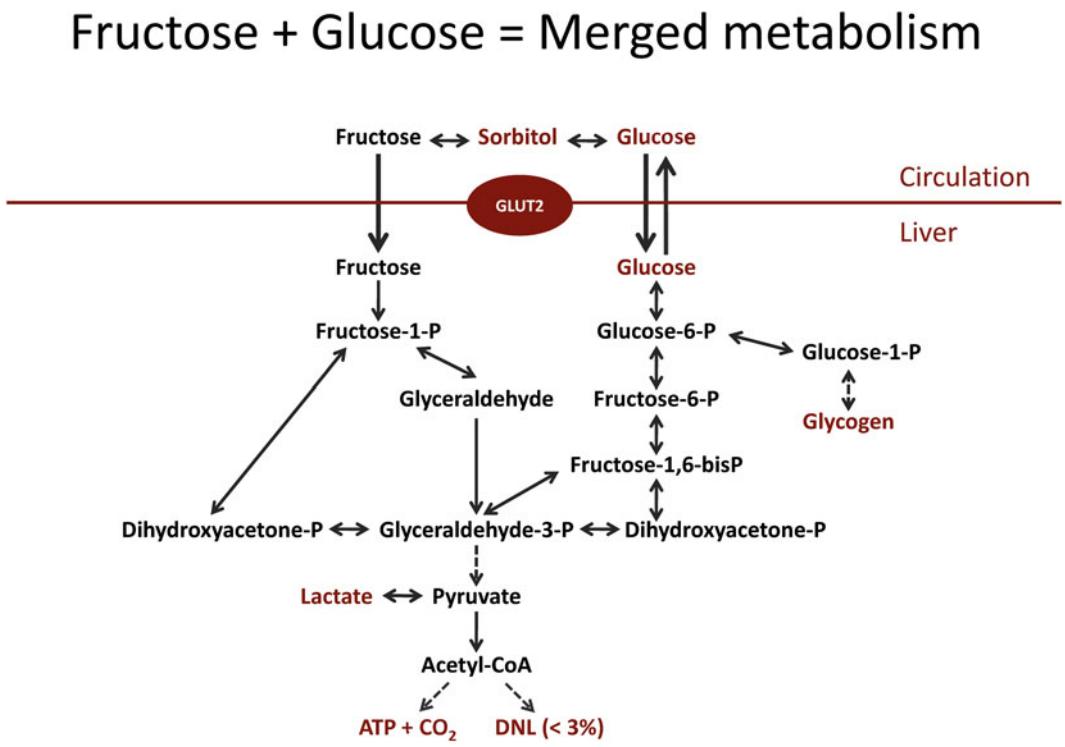

Fig. 24.3 Merged metabolic pathways of fructose and glucose

responsible for production of fructose-6-phosphate in the second step of glycolysis. In times of energy surplus, glucose-6-phosphate is diverted to restore liver glycogen stores.

Because of the high affinity of fructokinase for fructose, essentially all that enters the liver is rapidly phosphorylated to fructose-1-phosphate. This serves to reduce the concentration of free fructose in the liver and ensure its efficient removal from the portal blood. Unlike glucose metabolism, the catabolism of fructose-1-phosphate to dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate is not regulated by insulin, ATP or citrate. Since fructose catabolism tends to produce more triose-phosphates than are needed for energy, resources are diverted to production of other intermediates. Radioisotope labeling reveals that in times of energy surplus, fructose is metabolized preferentially to lactate (25 %), glucose (50 %), and glycogen (>15 %) [71]. Claims that fructose is responsible through fructokinase-ATP depletion for elevated uric acid and from there plays a role in gout, hypertension and NAFLD are based largely on animal or human studies at very high levels of pure fructose; these studies are not applicable to typical human experience [72, 73]. Other claims use one of its ultimate fates—de novo lipogenesis—to promote fructose as a unique and important cause of fatty liver disease. However, radioisotope labeling indicates that de novo lipogenesis normally constitutes less than 3 % of fructose disposition in the liver [70, 75].

Sugars Intolerance and Malabsorption

Genetic sucrase-isomaltase deficiency is a disorder in which the body lacks the enzymes (sucrase, amylases) necessary to digest sucrose and glucose-based polymers and oligomers like starch, maltodextrins, and corn syrup. These undigested carbohydrates pass into the large intestines, drawing with

them considerable water, where they are welcome substrates for gut bacteria. Symptoms include chronic watery diarrhea, gas, bloating, and abdominal pain.

Similar symptoms appear in subjects with fructose malabsorption, a disorder in which fructose transporters are insufficient to handle normal dietary levels. It is detected by measuring appearance in the breath of hydrogen or methane, byproducts of gut bacterial fermentation of incompletely absorbed fructose [83]. It has been reported that up to 50 % and 80 % of subjects are unable to absorb 25 g and 50 g, respectively, of a pure fructose test load [84, 85]. Estimates of fructose malabsorption in the general population are exaggerated in many cases by the amount of fructose administered in the test, which often exceeds the normal absorption capacity for fructose [74]. Simultaneous administration of glucose—as it appears in added and naturally occurring sources—efficiently attenuates fructose malabsorption.

Hereditary fructose intolerance results from a genetic deficiency of the liver enzyme aldolase B, responsible for catalyzing the conversion of fructose-1-phosphate to glyceraldehyde + dihydroxyacetone-phosphate [85]. The normal rapid action of fructokinase combined with aldolase B deficiency causes a buildup of fructose-1-phosphate, effectively trapping inorganic phosphate with the following consequences: inhibition of gluconeogenesis and glucose production via aldolase A; a rapid drop in blood glucose via inhibition of glycogenolysis; and substantial ATP hydrolysis coupled with impaired regeneration, ultimately resulting in increased uric acid and impaired protein synthesis. The population incidence in central Europe has been estimated at 1:26,000. Those with true fructose intolerance are advised to eliminate all sources of fructose—naturally occurring as well as added—from the diet.

Effect of Sweeteners on Energy Regulating Hormones

Early studies in both animals and humans often utilized a model comparing pure fructose to pure glucose, often at very high dosages, to compare their effects on energy regulating hormones [15, 16]. The rationale for this approach came from the well-known differences in hepatic metabolism between these two monosaccharides [71]. It should be noted, however, that this type of experimentation creates a very artificial environment since neither of these monosaccharides is consumed to any appreciable degree in the human diet.

Teff et al. compared fructose-sweetened to glucose-sweetened beverages where both were consumed at 25 % of calories [15]. They demonstrated increased 24 h circulating insulin, glucose and leptin and decreased postprandial suppression of plasma ghrelin when comparing these two monosaccharides. Other investigators, utilizing a similar model comparing fructose to glucose sweetened beverages at similar dosage levels, demonstrated increases in postprandial triglycerides particularly in overweight/obese subjects compared to normal weight subjects and in men versus women [16].

Given that insulin, leptin, and ghrelin interact with each other and play important roles in energy consumption, these data were extrapolated to speculate that prolonged consumption of energy from fructose could contribute to increased caloric intake and ultimately weight gain and obesity. When these experiments were repeated, however, utilizing similar levels of the more commonly consumed sugars—HFCS and sucrose (each of which consists of approximately 50 % fructose and 50 % glucose)—no differences occurred with respect to energy regulating hormones, appetite, or ad libitum energy consumption the day after consuming 30 % of calories from either of these two sweeteners (Fig. 24.4) [86].

While these initial investigations comparing HFCS to sucrose were conducted in normal weight women, subsequent acute experiments also demonstrated a similar sugars equivalence in obese women [87]. The same group also conducted a study utilizing two stays in the metabolic unit to measure energy regulating hormones separated by 10 weeks of a free living environment, where dosages comparing 8 % of calories (25 % percent population consumption level of fructose) to 18 % of calories (the 50th percentile population consumption level of fructose) to 30 % of calories (95th percentile

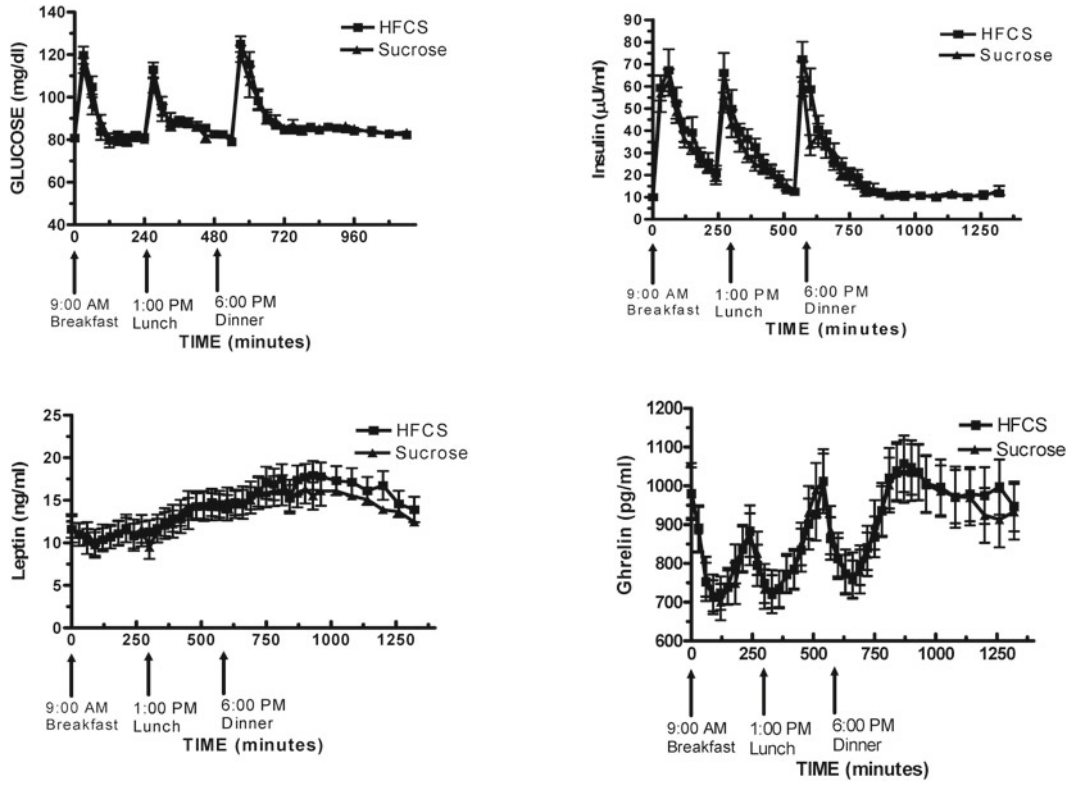

Fig. 24.4 The effects of 30 % of calories consumed as HFCS versus 30 % of calories consumed as sucrose on energy regulating hormones in healthy weight women

population consumption level of fructose) [88]. This study also demonstrated no differences between HFCS and sucrose with regard to energy regulating hormones.

These findings have subsequently been corroborated by the original investigators, who conducted fructose versus glucose investigations [89]. They are also consistent with the meta-analysis published by Dolan et al. comparing multiple studies in normal weight [90], and overweight and obese individuals [91] consuming up to the 95th percentile population consumption level of fructose. These studies did not report any metabolic abnormalities including weight gain. Thus, it appears that there are no meaningful differences in energy regulating hormones when the commonly consumed fructose and glucose are consumed together either in acute experiments or following a 10 week free living protocol. Thus, the findings comparing the monosaccharides fructose versus glucose, which found differences with regard to energy regulating hormones, must be treated with extreme caution since fructose and glucose are invariably consumed together and since studies comparing the most commonly consumed combination of fructose and glucose (HFCS and sucrose) have yielded different results.

Sugars and Obesity

In retrospect, modern concern about a potential role for sugars as a unique cause of obesity can be traced back to a commentary in the *American Journal of Clinical Nutrition* by Bray and Popkin in 2004 [6]. In this commentary, the authors argued there was a temporal association between the rapid

increase in obesity prevalence and the use of HFCS in the US food supply. They argued that the metabolism of fructose compared to glucose differed in such a way that energy consumption could be increased following fructose consumption, thereby increasing the risk of obesity as well as cardiovascular disease, diabetes, and the metabolic syndrome.

In a sense, these arguments were not new since similar ones were raised about sucrose in 1972 by John Yudkin in his book *Pure*, *White and Deadly*: *How Sugar is Killing us and What we can do to Stop it* (updated in 1986 and 2012) [92]. Yudkin's book was the first to suggest nutritional differences between simple sugars and complex carbohydrates, and proposed that sugars had adverse effects when consumed at levels typical in the Western diet. This argument was further buttressed by a series of scientific papers by Sheldon Reiser (USDA, Beltsville, MD) and Gerald Reaven (Stanford University) who focused attention on the fructose component of sucrose and HFCS as a potential cause of obesity, heart disease, and the metabolic syndrome. Many of the arguments from these investigators were addressed in the 1993 Fructose Nutrition monograph edited by Alan Forbes and Barbara Bowman (published as a supplement to the *American Journal of Clinical Nutrition*), which concluded that "on the basis of currently available information there is little basis for recommending increase/decrease use of fructose in the general food supply or in products for special dietary use" [93].

Following the Bray/Nielson/Popkin commentary, numerous research trials failed to support a unique linkage between HFCS and obesity [5, 87, 94]. Multiple studies have now demonstrated, for example, that HFCS and sucrose are virtually identical with regard to calories, sweetness, and absorption [89, 91] and that these two sweeteners as well as fructose itself, when substituted isocalorically for other carbohydrates are not uniquely related to weight gain or obesity [90, 91, 95]. As already indicated, HFCS and sucrose are also virtually identical with regard to glucose, insulin, leptin, ghrelin, and appetite responses in normal weight and obese individuals [86, 87]. The equivalence of HFCS and sucrose has been supported by consensus statements from both the American Medical Association [96] and the Academy of Nutrition and Dietetics [97].

Once the equivalence of HFCS and sucrose was established, focus turned to whether or not fructose containing sugars in general, might be uniquely linked to obesity. Three systematic reviews and meta-analyses of randomized controlled trials explored sweetened beverage consumption and body weight [39–41]. These meta-analyses all demonstrated that replacing sugar with other energy equivalent macronutrients had no effect on body weight. Some evidence from these studies suggested that increased energy consumption by increasing sugar intake on top of the normal diet in adults could lead to modest weight increase. However, that increase did not appear due to sugar per se but the increase in energy consumed in these hypercaloric RCTs, where individuals were told to increase their sugar consumption in addition to their usual caloric intake.

Prospective cohort trials have yielded similar results. Recent summary articles have also drawn the same conclusion—that there is a lack of evidence linking sugars to obesity [3, 39]. Thus, evidence from a variety of sources does not suggest that sugar per se makes a unique contribution to obesity. Furthermore, in a disease as complicated as obesity, it is highly unlikely that any single nutrient is a unique cause. This view is consistent with the recent scientific statement from the American Society for Nutrition, which emphasized that energy regulation and weight are complicated, and cautioned against isolating one component of the diet as a primary cause of weight gain or obesity [98].

Sweeteners and Diabetes

There has been a dramatic worldwide increase in type 2 diabetes in the past 28 years, making it a major global public health concern [99]. It has been estimated that 6.4 % of the world population is currently diabetic. This estimate is expected to rise to 7.7 % worldwide by the year 2030 [99, 100]. The increase in diabetes has paralleled the dramatic worldwide increases in obesity [101] and insulin

resistance [102]. It has also led to an exploration of a variety of dietary factors in the etiology of both diseases.

Considerable attention has been focused on the consumption of fructose containing sugars and their possible role in promoting type 2 diabetes. Several epidemiologic studies have linked consumption of SSBs to increased risk of diabetes [103, 104]. Two recent ecologic studies have linked the rise in fructose availability (either from HFCS or sucrose) to an increase in the prevalence of diabetes in the USA and around the world [18, 19]. These ecological analyses, however, are considered a weak form of evidence. Furthermore, it should also be noted that not all ecological studies have shown a positive correlation between sugar intake and diabetes. In the USA, for example, total sugar consumption has decreased substantially between 1980 and 2003 as it did in both Australia and the UK [105]. In Australia, for example, there was a 10 % decrease in caloric consumption from SSBs, yet significant increases in both obesity and diabetes occurred. This has been called the "Australian Paradox." Similar "paradoxes" have been seen in the USA and the UK [105].

Prospective cohort studies provide mixed evidence concerning sugar consumption and diabetes. Malik et al. reported a meta-analysis of cohort studies related to SSBs and incident diabetes [103]. Eight studies were analyzed—four did not find a significant effect of SSBs on the incidence of diabetes, and five did not adjust findings for energy intake and body weight. A large cohort study (Health Professionals Follow-up Study) reported no association between diabetes risk and SSB consumption once data were adjusted for total energy intake [104]. Other prospective cohort studies have also failed to find significant associations between sugar intake and diabetes [106–108].

Meta-analyses of RCTs also do not provide support for association between sugar intake and diabetes. Cozma et al. reported a systematic review and meta-analysis of randomized and non-randomized controlled trials of fructose and diabetes [109]. Of the 18 feeding studies identified, fructose had no impact on fasting insulin, glucose, or glycated blood proteins (including HbA_{1c}). Most randomized controlled trials in non-diabetic subjects which have substituted fructose containing sugars for other carbohydrates in controlled eucaloric diets have not yielded adverse effects on multiple risk factors for diabetes, including insulin, postprandial glucose, and fasting glucose [110–114]. Taken together there is little direct evidence that sugar consumption increases the risk of diabetes.

Sugars and Nonalcoholic Fatty Liver Disease

Fatty infiltration of the liver leading to NAFLD has increased dramatically throughout the world in the last 20 years [115]. NAFLD now represents the leading cause for worldwide chronic liver failure and the need for liver transplantation. The rise in NAFLD has largely paralleled the rise in obesity [115].

Concern about the interaction between consumption of fructose containing sugars and NAFLD is based on the differential metabolism between fructose and glucose in the liver [71]. As depicted in Fig. 24.2, fructose is metabolized differently in the liver than glucose which can ultimately result in the creation of free fatty acids which could theoretically lead to fatty infiltration of the liver. As illustrated in this figure, however, the pathways between fructose and glucose metabolism in the liver are interactive.

Multiple, randomized controlled trials have not demonstrated an effect of fructose containing sugars leading to increased fat in the liver when consumed at dosage levels within the normal human consumption [116–118]. Moreover, two recent systematic reviews and meta-analyses also failed to find a linkage between fructose consumption and NAFLD. When investigators have given 25 % of energy as glucose or fructose, however, it has been demonstrated that some increase in liver fat occurs [119]. Also, in one study, very large doses of fructose given to descendants of diabetics demonstrated some increase in liver fat [120]. The findings in these investigations, however, should be taken with great caution given that the dosages used are far in excess of the normal levels of human consumption and employed fructose in isolation rather than the normally consumed sucrose or HFCS where it is

combined with glucose. Other investigators have not found normally consumed levels of fructose containing sugars to lead to fatty infiltration of the liver [121, 122]. Two recent systematic reviews and a meta-analyses also did not find a link between fructose consumption and NAFLD [123, 124]. There appears, thus, to be little support for a link between fructose containing sugars and NAFLD at normal levels of human consumption.

Fructose and Cardiovascular Disease

There are no reported randomized controlled trials examining the effect of fructose containing sugars on CHD itself. Several prospective cohort studies have explored an association between SSB consumption and incidence of CHD [36, 125, 126]. In the Male Health Professionals Follow-up Study, a significant association was found between CHD events and the highest quintile of SSB consumption compared to the lowest [125]. The Nurses' Health Study found significantly elevated associated risk between two or more servings of SSBs a day and CHD compared to less than one serving per month [36]. However, Eshak et al., in a large prospective cohort study, found no association between SSBs and myocardial infarction [125]. All of these studies, however, are subject to the limitations of prospective cohort studies and do not establish cause and effect.

Since RCTs are lacking in exploring the link between added sugar and cardiovascular disease, more focus has been devoted to sugars and risk factors for cardiovascular disease. A number of studies have explored the potential linkage between consumption of added sugars and dyslipidemias [21, 127]. The AHA has recommended restricting consumption of fructose containing sugars as one mechanism for controlling triglycerides [127]. The data to support this recommendation are inconclusive as reported in several recent systematic reviews and meta-analyses [128–130]. Chiavaroli et al. found no link between fructose consumption and fasting triglycerides [128]. Wang et al. reported no effect of fructose on postprandial triglycerides when they were substituted isocalorically for other carbohydrates [129]. The meta-analysis of Livesey et al. did not report an overall adverse effect on lipids at normal levels of human consumption [131]. These investigators did, however, suggest a dose threshold for triglyceride-elevating effects of fructose and isocaloric substitution for other carbohydrates at 100 and 50 g/day for postprandial triglycerides.

Sievenpiper et al. proposed a threshold of greater than 50 g/day for fructose containing sugars for fasting triglycerides in individuals with diabetes [132]. A recent RCT demonstrated that individuals who consumed either fructose or HFCS at 10 or 20 % of total calories (25th or 50th percentile population intake levels of fructose) in an isocaloric diet in a free living environment for 10 weeks showed no changes in cholesterol, triglycerides, LDL, or ApoB. Another recent RCT showed that fructose containing sugars (fructose, HFCS, or sucrose) compared to glucose did not result in changes in HDL or LDL, however, a 10 % rise in triglycerides did occur [133]. This result, however, was confounded by the fact that subjects in the sugar consuming groups gained an average of approximately 2½ pounds and consumed an average of more than 300 cal above baseline during the trial. Thus, it would appear that adverse effects of sugars are likely to be small and typically the result of excess energy rather than sugar per se.

Several RCTs have explored the effect of fructose containing sugars on blood pressure. In one study, 21 overweight individuals were supplemented with either sucrose or artificial sweeteners [37]. After 10 weeks the blood pressure in the sucrose group was significantly higher than controls. These data, however, are confounded by the fact that sucrose subjects also gained an average of 2.6 kg more than controls. Other studies have not found increases in blood pressure related to fructose administration [134, 135]. A recent meta-analysis of 13 randomized and non-randomized trials, where fructose was given in an isocaloric exchange for other carbohydrates, did not show any effect on systolic blood pressure [136]. Several recent RCTs have also corroborated no adverse effect on blood pressure from

fructose containing sugars when consumed at up to the 90th percentile population consumption level for fructose [137]. Thus, there is little evidence to support that sugar consumption per se is a significant risk factor for elevating blood pressure.

Sugars and the Brain

Some animal experiments have suggested a differential response to fructose compared to glucose in the brain [138, 139]. Many of the studies were conducted in rodents. These experiments must be treated with great caution, however, since animal brains differ in significant ways from the human brain.

The emergence of noninvasive testing utilizing functional magnetic resonance imaging (fMRI) has created the potential to explore differential neurologic responses to various sugars in human beings. Utilizing this technology, Page et al. compared 75 g of oral bolus fructose to 75 g of oral bolus glucose in 20 young, healthy volunteers in a randomized, blinded study [22]. They reported differences in hypothalamic blood flow, with glucose suppressing hypothalamic blood flow compared to fructose as assessed by arterial spin labeling. Purnell et al. explored neurologic responses to 25 g of either fructose or glucose delivered as an intravenous bolus [23]. They found no changes in hypothalamic blood flow, but did find differences between fructose and glucose with regard to blood flow to the cerebral cortex. Both sets of experiments must be treated with great caution, however, since as already indicated, fructose and glucose are seldom consumed in isolation in the human diet. Moreover, when given through an atypical route such as intravenously, an additional confounder is introduced.

A recent study employing a model of various levels of sugar versus various levels of fat in isocaloric milk shakes reported that sugar activates reward, gustatory and somatosensory pathways more than does fat [140]. Other experiments, however, have shown exactly the opposite [141, 142]. A recent pilot study compared average levels of HFCS (18 % of calories) versus 9 % of calories from either fructose or glucose in the context of mixed nutrient meals and found no differences in hypothalamic blood flow or reward pathways [143]. Thus, in this emerging field, there is a need for a larger randomized controlled trial to sort out whether or not brain pathways differ in response to various sugars.

Are Sugars Addictive?

Several investigators have suggested that the sweetness inherent to fructose containing sugars creates the potential for individuals to become “addicted” to sugar. Much of the research in this area, however, has been based on animal models [7, 76]. Several recent reviews have called into question the fundamental concept of either “food addiction” or “sugar addiction” except in very limited situations, such as binge eating disorder [24–26]. These reviews have suggested that much of the food related pathology that is seen by clinicians can be explained and treated without invoking addiction as the explanation for behavioral eating problems.

Sugar Intake in Children and Adolescents: Effects on Heath

While this chapter is focused largely on research trials in adults, it should also be noted that considerable interest has been generated concerning potential effects of sugar intake on children and adolescents [144]. In particular, the dramatic rise of obesity in children has stimulated investigators to look for potential causes including sugar consumption. Recent reviews of this literature, however, have

suggested that while children prefer sweetened foods and consume larger portions from daily caloric intake from sugars than adults, sugars do not appear to be directly related to obesity or other health issues aside from dental caries [144]. Moreover, focusing on single factor of nutrition is unlikely to yield desired health changes in children, adolescents, or adults. Thus, policies to restrict access to added sugars do not appear to lead to desired outcomes, such as impacting body weight.

Does Dosage Matter?

Disparities between recommended upper limits of added sugar consumption between those proposed by the AHA [27], WHO [28], and SACN [29] compared to DGA 2010 [30] and IOM [31] recommendations have caused some investigators to explore whether or not there is a scientific basis for the more significant restrictions offered by some of these organizations. In several research trials which have compared 8 % of calories from added sugar (roughly the upper limit amount recommended by the AHA, WHO, and SACN) compared to 18 % of calories from added sugars (average consumption of fructose in the USA) to 30 % of calories from added sugars (roughly the 95th percentile population consumption level of the USA and similar to DGA 2010 and IOM recommendations), no differences have been found in risk factors for cardiovascular disease, diabetes, or metabolic syndrome [72] amongst these three levels of sugar consumption. Thus, while it may appear politically prudent to restrict sugar consumption, the scientific basis for this recommendation is far from secure.

Conclusions

Evidence from multiple sources including systematic reviews, meta-analyses, and multiple RCTs suggests there is nothing unique with regard to sugar consumption and health consequences, provided the sugar is substituted isocalorically for other carbohydrates and consumed in the normal range of human consumption. Questions remain about whether or not when sugar is added to the normal diet in hypercaloric trials, adverse health consequences may occur. Future research trials will be necessary to settle this issue.

For now, we believe it is safe to conclude that the current scientific evidence does not support a unique relationship between sugar consumption and changes in energy regulating hormones, obesity, diabetes, NAFLD, or risk factors for cardiovascular disease. Neurologic responses to sugar remain an area of active research. Concepts such as sugar "addiction," however, do not appear to be currently supported by research trials in human beings or by expert opinion. And the evidence supports the conclusion that fructose-containing sweeteners with similar composition—like HFCS and sucrose—are nutritionally and metabolically equivalent.

The focus on sugar, with the suggestion that it somehow uniquely causes multiple health problems, does not appear supported based on current scientific evidence. Further research trials will continue to clarify issues and improve public health while guiding wise public policy and informing individual nutritional decisions in the area of added sugars and health.

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Chapter 25
Low Calorie Sweeteners and Weight Management

Danielle Greenberg, Richard Black, and Catherine Cioffi

Key Points

- Low calorie sweeteners (LCS) can be beneficial for weight loss and long-term weight loss maintenance.
- Randomized control trials (RCTs) demonstrate that substituting LCS for full calorie sweeteners helps reduce calorie intake and thereby promote weight loss in obese and overweight people.
- Epidemiological studies sometimes uncover apparent positive associations between LCS consumption and BMI. However, this is most likely due to reverse causality. That is, the association is not due to LCS having a causal effect on weight gain but rather that overweight or obese people seeking to lose weight switch to LCS in order to reduce their calorie intake.
- Consuming LCS can be an aid to support weight maintenance in successful weight losers.
- Little data are available to determine if consuming LCS help normal weight people prevent weight gain over time. Research on this topic is needed.
- The suggestion that the high taste intensity of LCS may lead to hyperstimulation of sweet sensors is not supported by the evidence. LCS do not stimulate sweet receptors to a greater extent than does sucrose, nor do food manufacturers make products with LCS sweeter than their full calorie products.
- LCS do not appear to increase preferences for sweet foods or beverages.
- Despite repeated efforts, human studies have failed to show any disruption of normal physiological responses to food or any increased weight gain, drawing into question the relevance of such research in animal models.
- An emerging topic is the impact of LCS on the gut microbiome. The body of research is very limited and further research on this topic is warranted.

Keywords Low calorie sweeteners • Artificial sweeteners • High-intensity sweeteners • Nonnutritive sweeteners • Saccharine • Aspartame • Sucralose • Acesulfame Postassium • Energy balance • Calorie balance • Weight management

Abbreviations In order to avoid confusion the abbreviation LCS (low calorie sweeteners) is used throughout this manuscript including in quoting citations where other terms were used. Some of the abbreviations used where the abbreviation LCS was substituted included AS (artificial sweeteners), ASB (artificially sweetened beverages), HIS (high-intensity sweeteners), and NNS (nonnutritive sweeteners).

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_25

History of Low Calorie Sweeteners

Saccharin was the first low calorie sweetener to be used. It was discovered in 1879 in the laboratory of Ira Remsen at Johns Hopkins University by Constantine Fahlberg [1]. Like most low calorie sweeteners (LCS), the discovery of the sweetening characteristics of saccharin was serendipitous. Also like most LCS the discovery of saccharin was mired in controversy from the very beginning. The accident was that a beaker boiled over creating saccharin while looking for other products. The controversy was that Fahlberg claimed and patented the discovery while Remsen believed it to be his idea and believed Fahlberg to be an intellectual thief.

Soon after its discovery saccharin was prescribed by doctors for weight loss and for a number of unrelated conditions including headaches and nausea. Once the Pure Food and Drug Act of 1906 passed, Harvey Wiley, the director of the bureau of chemistry for the USDA, questioned the safety of saccharin because it was a coal tar derivative. Wiley also believed that saccharin should be banned because it could be used to deceive people as it could be substituted for sugar without informing the public and thus deprive them of needed calories without their knowledge. President Theodore Roosevelt, who was a regular user of saccharin as an aid for weight loss, strongly disagreed, fired Wiley, and stated that: "Anybody who says saccharin is injurious to health is an idiot." [1]

Since those early controversies saccharin has been confirmed as safe by numerous regulatory bodies worldwide and is an FDA approved food additive [2]. But the controversy surrounding use of LCS for weight management continues to this day. This chapter will not address the issue of LCS safety as that topic has been reviewed numerous times elsewhere [3–5]. Only those LCS that are permitted for use in the USA will be discussed, with a focus on their use in weight management. In addition to saccharin, LCSs that are currently permitted for use are: Aspartame, which was discovered in 1956 while looking for an ulcer medication; Acesulfame potassium (Ace-K), which was discovered in 1967 again while looking for a different molecule; Sucralose, which was discovered in 1989 while looking for non-sweetener uses of halogenated sucrose molecules; Neotame (a more potent variant on aspartame), which was discovered in 1999 based on LCS research [6]; Advantame (an additional variant of aspartame), which was discovered in 2009 based on LCS research; Steviol glycosides, which are derived from the Stevia plant and have been used as sweeteners in many cultures for centuries, though purified steviol glycosides were first permitted for use in 2008; Luo Han Guo fruit extracts, which have also been used in many cultures for a long period of time but were first permitted for use in 2010; finally there is a group of LCS known as sugar alcohols or polyols which have been used in foods since the 1980s, and have about half the calories of sucrose. They have a variety of sweetening characteristics and are often used in small quantities in food items such as gums and candies or they are used in combination with other LCS as large quantities of some polyols can have a laxative effect.

A critical factor in interpretation of studies on LCS is that these various sweeteners have vastly different chemical structures. This is notable for several reasons, namely, individual sweeteners do not activate the same taste receptors [7], do not release the same gut hormones [8], and do not have the same metabolic fate [3]. Thus research on one particular sweetener cannot necessarily be expected to apply to all LCS. Another critical factor is that many species respond differently to specific sweeteners and, more importantly, differently from humans. This fact is critical when determining the implication of animal studies for humans. For example, old world simians find aspartame sweet while prosimians do not [7, 9, 10]. Importantly, rats do not respond to aspartame as sweet or particularly rewarding [11, 12] and in contrast, rats do taste maltodextrin as sweet and find it rewarding while humans do not [13, 14]. Thus research on one LCS cannot be assumed to apply to all LCS and research in animals cannot be assumed to apply to humans.

Low Calorie Sweeteners and Weight Loss

Clinical Trials

As early as 1914 Gustav Gaertner from Harvard recommended the substitution of saccharin for sugar in the dietetic treatment for obesity [15] but at that time there was no research to support this recommendation. Saccharin consumption had become widespread during World War I as sugar shortages led to saccharin use in foods, especially items like canned fruits. But this widespread use did not relate to any research on weight management.

Early experiments in animal models demonstrated that the sweet taste of saccharin was rewarding to rats. For example, rats preferred the taste of both saccharin and sucrose to water [16]. Further demonstrating the reward value of saccharin, Sheffield and Roby showed that hungry rats would learn a task where saccharin was used as a reward while satiated rats would not learn the task [17]. It was not until the 1950s that the question of whether LCS consumption could aid in weight loss was explored in humans. McCann et al. followed obese subjects for 3 years and compared those who typically consumed LCS to those who did not. They found no difference in weight gain, weight loss, or weight stability between groups [18].

Since then numerous clinical trials in humans substituting LCS for nutritive sweeteners have demonstrated benefits of LCS consumption as a strategy for calorie reduction and weight loss. Early studies in a clinical metabolic unit where all foods consumed were determined and monitored by the experimenters showed that both lean and obese subjects ate approximately 25 % fewer calories when aspartame replaced sugar covertly [19]. An early study of college students found that among those who reported daily saccharin consumption, 24-h recalls showed significantly lower calorie intake than for nonusers [20].

It should be noted that in a series of early experiments Blundell and his colleagues reported that consumption of aspartame led to increases in subjective ratings of appetite [21, 22] but further studies showed that LCS did not increase actual food intake [23]. Black and his colleagues found, in a series of studies, that ingestion of soft drinks containing aspartame did not increase short-term subjective hunger or food intake [24–26]. An early intervention trial examining the effects of LCS on caloric intake and body weight was by Tordoff et al. where participants consumed about 40 oz/day of soda sweetened with high-fructose corn syrup (HFCS) or with aspartame compared to a no beverage control in counterbalanced order. Relative to no beverage consumption, those drinking aspartame-sweetened soda reduced calorie intake and showed a small reduction in body weight. In contrast, those drinking HCFS-sweetened soda had increases in total calorie intake and in body weight. Interestingly consuming both types of sodas reduced intake of calories from the remaining diet to about the same extent. For both groups this was the result of reductions in sugars intake from other foods in the diet. Since the participants were not informed of the sweetener type they were given, the study confirmed the previous finding that when LCS is covertly substituted for sugar there is a decrease in daily calorie intake and tendency toward weight loss [19, 27].

A seminal study in George Blackburn's laboratory [28] tested over 150 obese people who were assigned to either consume or not consume aspartame-sweetened foods and beverages in a 2 year trial. There was a weight loss phase of 16 weeks and a weight maintenance program that continued for 2 years. The aspartame group lost significantly more weight during the weight loss phase and regained significantly less weight during the 2 year follow-up compared to those not consuming aspartame-sweetened foods and beverages. The authors concluded that including aspartame-sweetened foods and beverages in a weight loss program may help in the weight loss and long-term maintenance of reduced body weight.

Raben et al. [29] studied overweight subjects and examined the effects of consuming both foods and drinks sweetened with either LCS or sucrose on total food intake and body weight. The LCS used included aspartame, acesulfame K, cyclamates, and saccharin. After the 10 week intervention, the LCS sweetener group decreased sucrose intake and total energy while the sucrose group increased total energy, sucrose and carbohydrate intakes. The LCS group reduced body weight and fat mass while the sucrose group increased both body weight and fat mass. In addition, systolic and diastolic blood pressure decreased in the LCS group and increased in the sucrose group (possibly attributable to the weight changes). After the intervention, the number of subjects with controlled eating behavior increased in the LCS group and decreased in the sucrose group demonstrating an overall benefit for LCS substitution over a longer time period. Many current weight loss studies also track participants for at least 1 year in order to determine efficacy of any interventions.

Anton et al. [30] examined the short-term effects of preloads containing stevia, aspartame, or sucrose on food intake, satiety, and postprandial glucose and insulin levels in both lean and obese individuals. Participants received preloads containing stevia (290 kcal), aspartame (290 kcal), or sucrose (493 kcal) before lunch and dinner meals were consumed ad libitum. Even though there were fewer calories in the LCS preloads, participants did not overcompensate by eating more calories at lunch or dinner. In fact when preloads of LCS were consumed there was about a 300 kcal decrease in intake over the entire day compared to when a sucrose preload was consumed. There were also no differences in self-reported hunger and fullness levels assessed by visual analog scales though it must be acknowledged that such differences are notoriously difficult to discern. Importantly LCS led to reduced blood glucose and lower insulin levels compared to sucrose. Caloric reduction when LCS-sweetened beverages were consumed was also seen in a similar experiment by DellaValle et al. [31] In this study the subjects were given a lunch accompanied by water, diet cola, regular cola, orange juice, or 1 % milk. When caloric beverages were consumed with the meal, energy intake was on average 104 kcal greater than when the diet soda or water was consumed.

More recently two weight loss studies have compared the effects of consuming LCS-sweetened beverages or water within a weight loss program and found that LCS improved outcomes. Tate et al. [32] evaluated outcomes of the CHOICE (Choose Healthy Options Consciously Everyday) study, and found that those consuming LCS beverages achieved a 5 % reduction of body weight after 6 months, while those in the water group or an attention control did not. In addition, the diet beverage group reduced their caloric beverage intake by 70 kcal more per day at both 3 and 6 months compared with the water group. The most likely explanation of this effect is that it is a result of improved compliance rather than any metabolic or other effect of LCS. Those consuming diet beverages were able to fulfill any desires for sweet beverages with the zero calorie diet beverages while those who were instructed to avoid LCS-sweetened beverages were not able to make this type of substitution. A second experiment with this same study population focused on which specific foods and beverages were reduced during weight loss found that the LCS group significantly reduced their intake of all sweet foods compared to those consuming water (see Fig. 25.1) [33]. This observation is especially enlightening as directly addresses the concerns expressed by some [34] that consuming LCS-sweetened foods would increase cravings for sweet foods. Such speculation is not supported by the evidence to date.

Another recent study examined the effects of a 12-week weight loss program where participants had regular meetings with Registered Dieticians and supervised exercise sessions [35]. The study randomly assigned over 300 overweight or obese subjects to consume LCS or water as a control group. Those in the LCS group were asked to consume at least 24 fluid ounces of beverages sweetened with LCS per day while their water consumption was not restricted. Those in the water group were asked to consume at least 24 fluid ounces of water per day, and not drink any beverages sweetened with LCS. While both groups lost weight and had other metabolic improvements the LCS group lost 44 % more weight than the control (water) group, reported feeling significantly less hungry overall, had significantly greater improvements in serum levels of total cholesterol and low-density lipoprotein (LDL) and saw a significant reduction in serum triglycerides. Again the most likely explanation

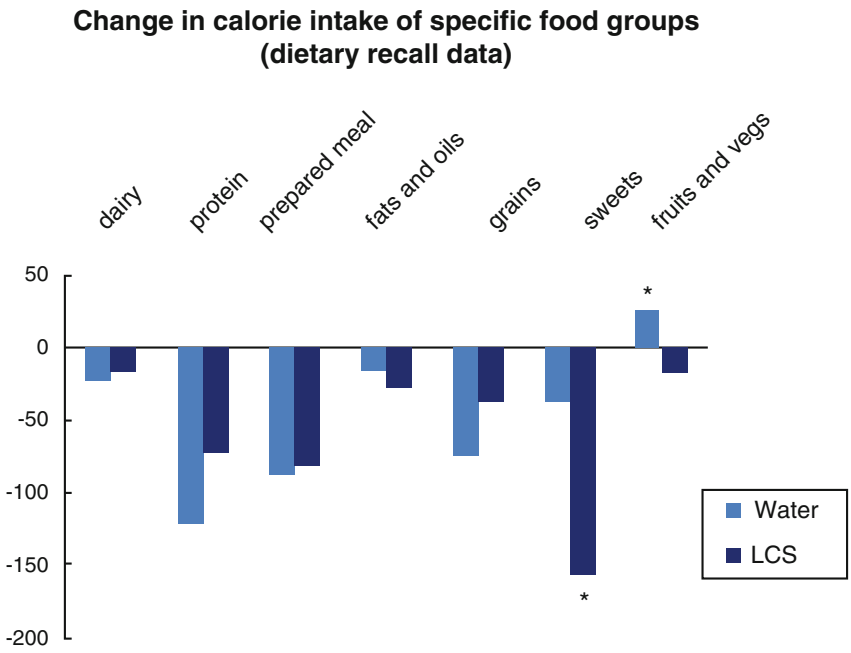

Fig. 25.1 Intake of food groups as assessed by dietary recall at 6 months in the CHOICE trial weight loss study. Intake of all foods except fruits decreased in both groups. Intake of fruits was higher in the water group. Intake of sweets was significantly lower in the LCS group compared to the water group. Subjects were $n=85$ in the water group and $n=84$ in the LCS group. Sweets included those added in processing and preparation and did not include naturally occurring sugars such as fructose in fruit. The LCS included saccharin, aspartame, sucralose, and acesulfame K. From Piernas et al. 2013 by permission

for these findings is improvements in compliance with the weight loss program in those assigned to the LCS group.

Sorensen et al. [36] recently investigated not only changes in intake but also in energy expenditure and ratings of hunger and fullness in overweight subjects assigned to either a sucrose or LCS treatment group for 10 weeks. Body weight increased in the sucrose group and decreased in the LCS group. The sucrose group increased calorie intake and reported greater hunger and less fullness then the LCS group. Basal metabolic rate increased in the sucrose group, while energy expenditure over 24 h increased in both groups to an equivalent level. The authors concluded that the decreases in body weight seen in response to LCS were attributable to changes in food intake rather than in energy expenditure. These findings again support the concept that LCS assist in compliance with weight loss programs rather than having any metabolic effect.

Meta-analyses and reviews of clinical trials examining the effects of LCS in weight loss efforts have concluded that they are beneficial. In an early review Rolls [37] found that "Aspartame has not been found to increase food intake; indeed, both short-term and long-term studies have shown that consumption of aspartame-sweetened foods or drinks is associated with either no change or a reduction in food intake. Preliminary clinical trials suggest that aspartame may be a useful aid in a complete diet-and-exercise program or in weight maintenance. Intense sweeteners have never been found to cause weight gain in humans." In a meta-analysis deLaHunty et al. [38] found "a significant reduction in energy intakes was seen with aspartame compared with all types of control…the most relevant comparisons are the parallel design studies which compare the effects of aspartame with sucrose. These had an overall effect of a mean reduction of about 10 % of energy intake." More recently Miller and Perez [39] reported "Findings from the meta-analysis of 15 RCTs—the gold standard study design

in medical research—indicate that substituting LCS for sugar modestly reduces body weight, BMI, fat mass and waist circumference." The Dietary Guidelines for Americans 2015 Advisory Committee reviewed the evidence on LCS in body weight management and concluded that "Evidence from randomized controlled trials consistently indicates that LCS sweetened foods and beverages (vs. sugar-containing foods and beverages) modestly reduces body weight in adults. When evidence from adults and children are combined, LCSs modestly reduce BMI, fat mass and waist circumference." [40]

While clinical trials have constantly shown benefits of LCS on body weight management, observational studies have not consistently found this effect and some observational studies have found a paradoxical association between LCS intake and weight gain.

Observational Studies

Observational studies have shown all possible results of LCS consumption on body weight; some have demonstrated associations of LCS consumption with weight loss, others with no change and still others with weight gain. The thoughtful interpretation of these studies is critical in understanding the actual effect of LCS consumption in weight management.

Parker et al. [41] in a 4 year prospective cohort study using The Pawtucket Heart Health Program assessed dietary factors including reported sucrose and saccharin use to determine their associations with weight gain. Although total energy intake was associated with weight gain, no specific nutrient including saccharin was significantly associated with weight gain. However, they did find greater weight gain of those in the highest tertile of saccharin use compared to those with less saccharin use. An early study by Stellman and Garfinkel [42] using a large database from the American Cancer Society found that obesity was positively correlated with LCS consumption. They found BMI was higher in those consuming the most LCS compared to those consuming the least. In addition when comparing consumers and nonconsumers a greater percent gained weight and gained a larger amount of weight. However, the authors noted that mean differences in weight gain was less than two pounds and thus was not clinically meaningful. Nonetheless, this small association generated considerable debate on the efficacy of LCS for weight management.

Other studies have had more mixed findings. For example, in a 10 year study on adolescents, using data from the National Heart, Lung, and Blood Institute (NHLBI) Growth and Health Study, Striegel-Moore et al. [43] found that consumption of diet soda was associated with a significant decline in sucrose intake, a significant increase in calcium intake, and a small but nonsignificant decrease in BMI. Those consuming diet beverages also showed smaller increases in total energy intakes, which were increasing as the children in the study grew into adolescence, compared to those consuming any other beverage type including milk, juices, regular sodas, and coffee or tea.

More recently in an article that gained much publicity Fowler et al. [44] found that adjusted BMIs were higher among LCS consumers as compared to nonconsumers in the San Antonio Heart Study. It should be noted that in their own conclusion statements they suggested that this could be due to reverse causality, stating that: "there may be no causal relationship between artificial sweetener consumption and weight gain"...."as individuals seeking to lose weight often switch to diet beverages in order to reduce their caloric intake", and that low calorie sweetener consumption "might therefore simply be a marker for individuals already on weight-gain trajectories…and this is the most obvious possible explanation of our findings."

In the most recent meta-analysis of these results Miller and Perez [39] summarized the findings of observational studies as having statistically nonsignificant associations of LCS consumption with body weight and fat mass. They did however, find a significant, albeit modest, positive association with BMI. Pereira and Odegaard [45] also performed a meta-analysis of the evidence on LCS and body weight and concluded that experimental evidence does not support the concept that LCS could

cause overweight, obesity, or chronic disease. They suggested that "experimental studies in humans suggest LCS may be effective for weight loss when replacing sugar-sweetened beverages." In addition they stated that the concept of "reverse causality" was the most likely explanation for the association that is found in epidemiologic observational studies between overweight and obesity and consumption of LCS. Simply stated, it is not the LCS that are leading to overweight or obesity, but rather it is being overweight or obese that is likely driving the consumption of LCS. Those at higher risk of weight gain choose to consume LCS in an attempt to control their body weight leading to the associations that are consistently seen. There is no evidence that the LCS are a causal factor in weight gain; Pereira and Odegaard along with Rolls and others [37, 45, 46] have noted that this is a particular problem faced in interpreting the results of the epidemiologic studies on this topic. It is impossible to interpret the evidence of an association of overweight or obesity and LCS consumption from epidemiological surveys as having a causal basis.

Theories That Question the Use of LCS in Weight Management

Some have suggested that the consumption of LCS leads to weight gain rather than weight loss or maintenance of weight loss [44, 47, 48]. These hypotheses are based primarily on the observational studies mentioned above, despite contradiction of the totality of the evidence from clinical trials. The hypotheses for how consuming LCS could lead to weight gain can be summarized as follows. First there are theories based on the associations frequently seen in epidemiological studies which are erroneously interpreted as causal but are most likely explained by reverse causality.

A second set of theories suggesting LCS consumption could lead to weight gain are based on the misconception that the "intensity" of LCS leads to overstimulation of sweet receptors and thus results in increasing preferences for sweet foods and beverages. For example, Ludwig [34] has suggested that LCS lead to "overstimulation of sugar receptors" and that "individuals who habitually consume LCS may find more satiating but less intensely sweet foods (e.g., fruit) less appealing" and "unsweet foods (e.g., vegetables, legumes) less palatable, reducing overall diet quality in ways that might contribute to excessive weight gain." This hypothesis reflects a misunderstanding not only of how LCS are used in manufacturing, but it is also not supported by evidence on the sensory psychophysics of responses to LCS. Manufacturers develop reduced calorie products by using LCS in quantities that lead to matching as closely as possible the taste characteristics of the full calorie products. Manufacturers invest in extensive product development and testing, at considerable cost, to meet this goal of matching low- and full-calorie products, and not exceeding sweet taste characteristics [49]. In addition, LCS are not more "intense" than nutritive sweeteners. There is considerable confusion concerning the differentiation between intensity and potency. While LCS do have a greater potency, meaning that on milligram to milligram comparison with sucrose (or any other nutritive sweetener) much less LCS is needed to elicit a similar sweet perception (Table 25.1), that does not mean that they are more intense. Describing LCS as high intensity implies they evoke a sweetness response greater than natural sugars like sucrose but there is no evidence that this is true. A large body of evidence demonstrates that there are no concentrations of LCS that are perceived as more sweet than sucrose [6, 49–52].

For example, the perception of the intensity of sweet tastes in response to LCS was recently tested by Antenucci and Hayes [50]. Subjects rated the perceived sweetness of increasing concentrations of the LCS acesulfame K, rebaudioside A, aspartame, and sucralose and also rated the perceived sweetness of the caloric sweeteners: sucrose, maple syrup, and agave nectar. The most intense sweetness ratings were in response to the caloric sweeteners. In addition, increasing concentrations of all the nutritive sweeteners led to increasing perception of sweet intensity while for the LCS increasing concentrations reached a maximal sweet intensity that declined with further increasing concentration while the perception of bitterness increased. This research confirms that although LCS may have

Table 25.1 Relative sweetness of LCS compared to sugar

Sweetener	Sweetness rating
Acesulfame-K	200×
Advantame	20,000×
Aspartame	200×
Neotame	7000–13,000×
Saccharin	200–700×
Stevia (Rebaudioside A)	200–400×
Sucralose	600×

Relative potency of LCS compared to sucrose. From FDA: http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm397725.htm#Saccharin

greater binding affinity to sweet receptors, this does not imply that LCS overstimulate sweet receptors [50] and in terms of perceived sweetness, LCS cannot be considered "supernormal stimuli." This research also does not support the hypothesis that LCS overstimulate sweet receptors to produce elevated sweet sensations. Thus, in the manner that LCS are used by manufactures and in sweet perception in response to increasing concentrations, the data do not support the concept that LCS would train people to like more intense sweet tastes.

Regarding LCS and the intake of other sweetened foods, a particularly compelling line of supporting evidence derives from the work of Binkley and Golub. They examined the grocery purchases of people who consumed LCS compared to those who did not, and found that those who purchased diet soda made better nutrition choices in the other foods they purchased and specifically purchased fewer desserts or other sweetened foods [53]. Also, as previously mentioned, Piernas et al. [33] found that people consuming diet beverages in a weight loss study had greater decreases in the intake of sweet foods than did subjects consuming water (Fig. 25.1). Perhaps the more telling observation, "sweet foods" was the main category where those in the diet beverage group consumed significantly less than the control (water) group. The authors [33] concluded that their research did not provide evidence that consumption of LCS increased preferences for sweet foods or beverages. The body of evidence that exists does not support the theory that LCS act to train people to prefer other sweet tasting foods.

A third set of hypotheses are based on learning theory studies in rodent models that primarily use saccharin as the sweetener. As noted previously rodents do not have the same taste and physiological responses to LCS as do humans, thus these studies need to be interpreted with caution [11–14]. When consumed in isolation—that is with no other foods—presumably in either a diet beverage or in coffee or tea without any milk or other caloric substance added, LCS do not provide the same post-ingestive consequences such as raising blood glucose or releasing insulin as would caloric sweeteners. This has led to hypotheses that consumption of LCS therefore disrupts the normal physiological responses that lead to the sensation of fullness when consuming sweet tasting foods [47, 54]. For both rats and people, foods elicit not only responses to the taste, aroma, and context of the consumption but also a cascade of postingestive consequences that are the result of the calorie and macronutrient content of the food. LCS do elicit the hedonic responses to sweet stimuli but these theories hold that LCS would not elicit the postingestive response to calories. These theories are also complicated by the fact that there may be some postingestive responses to LCS; for example, aspartame has been shown to lead to the release of GLP-1 which is thought to be a satiety hormone and has some effect in reducing intake [8, 55].

More importantly, the experimental designs used to support this idea of Pavlovian conditioning to sweet tastes are highly controlled and have not, to date, been demonstrated in humans. The theory on dissociation of sweet taste and caloric response leading to weight gain is based on the concept that these postingestive consequences serve as unconditioned stimuli in a classical Pavlovian learning scheme while the sweet taste associated with LCS serve as the conditioned stimulus [54]. In this learning paradigm the unconditioned stimuli would be analogous to meat in the classic Pavlov's dog experiment while the conditioned stimulus for salvation would in that circumstance be the bell. With

consistent pairing and/or signaling the dog learns to salivate to the bell [56]. For Pavlovian conditioning to occur the necessary and sufficient conditions include consistent pairing of, or signaling for, the unconditioned stimulus with the conditioned stimulus. In the case of LCS, for this learning to occur there would need to be consistent pairing or signaling of LCS with no or lessoned caloric consequences. While this is true in the animal studies where the experimenter controls when the rats consume the saccharin or sucrose, the consistent pairing or signaling is not likely to exist in the way people consume LCS as LCS are often consumed at eating occasions where calories are present from other foods [57]. In addition, LCS in the human food supply are sometimes present at the same time caloric sweeteners are present [57].

The research in rats on Pavlovian conditioning that suggests saccharin can lead to weight gain has most frequently used one of two experimental paradigms [58, 59]. In the first design rats are exposed to water flavored with either saccharin or sucrose as a preload for a set period of time and then subsequently the available food is rat chow. Those in the saccharin group gain more weight over time as they consume more of the available food presumably due to learning that taste is dissociated from postingestive consequences [58]. In the second test condition the preload is saccharin-sweetened yogurt, plain yogurt where the only sweet component is from the naturally occurring lactose, or sugar-sweetened yogurt, and again the available food is rat chow. There are variations in these studies but again the rats consuming saccharin gain more weight [59]. In the first test situation rats consuming saccharin would presumably learn that sweet taste does not have any caloric consequences and it is theorized that they then learn to ignore postingestive cues, which results in overeating. In the second test condition rats would learn that sweet tastes are associated with diminished rather than absent postingestive cues but nonetheless it is theorized that they learn to overconsume. These tightly controlled experimental designs have consistency as the sweet stimuli are always the same for an individual experimental animal. Thus a given animal experiences only one type of sweet taste: saccharin with or without lactose, always as lactose alone, or always as sucrose with or without lactose. The eating patterns of people do not reflect this tight control. People are exposed to sweet tastes that at time come from different LCS, at time from nutritive sweeteners, and at times from both. Additionally these pairings are not consistent thus learning would not be likely to occur. In the case of the yogurt some calories are coming from the yogurt but still the sweet tastes always is consistent either from the saccharin mixed with the naturally occurring lactose or from the sugar along with the naturally occurring lactose. It is this consistency that is likely leading to the animals learning that saccharin-sweetened yogurt has reduced postingestive consequences. In addition, there may be an experimental confound in the case of yogurt as rats are known to be lactose intolerant after weaning and develop conditioned taste aversions to lactose [60]. Thus the interpretation of the studies where rats are given saccharin-sweetened yogurt needs to include this possible confounding factor.

In sum, before the concept of Pavlovian conditioning to overeat in response to LCS consumption is applied to the question of obesity in humans, the random manner in which humans actually consume LCS in foods and beverages, with or without other calories present needs to be taken into account. The supporting research to date is strictly derived from animal studies, thus there is a need for research in humans prior to adopting this as an explanation for human behavior.

Another set of hypotheses on possible adverse effects of LCS in weight management are based on changes in the gut microbiome seen in response to consumption of LCS. The gut microbiome is an area of exciting new research where the implications for body weight and disease states are only beginning to be explored. For example, dietary patterns have been shown to influence the makeup of the microbiome, and the makeup of the microbiome may have a role in the etiology of obesity [61, 62]. To date there are two studies that examine the effects of LCS intake on the gut microbiome and they have conflicting findings. Daly et al. [63] gave either lactose or saccharin combined with neohesperidin dihydrochalcone, a citrus-based LCS, in the feed of young pigs. Both lactose and LCS increased the Lactobacillus population in the gut and also led to increases in lactic acid concentrations. Such increases have been reported to have benefits for gut health and may help with

maintaining a normal body weight [63]. Suez et al. [64] in contrast found that saccharin could be harmful to gut microbiota and lead to glucose intolerance. The complex report described a series of experiments in both mice and humans. The researchers first examined the effects of several LCS including saccharin, sucralose, and aspartame in mice and observed an alteration of the gut microbiome and some glucose intolerance was observed in the mice.

Since the mice had the greatest adverse response to saccharin feeding, the remaining studies in mice used only saccharin. While the researchers simply assumed saccharin to be a "prototypical" sweetener, as mentioned previously there is no reason to expect that responses to one sweetener would predict responses to any other. Thus the thesis that saccharin would be "prototypical" is unlikely [64]. However the researchers did find that fecal transplants from the gut of saccharin-fed mice into germ-free mice led to glucose intolerance in the germ-free mice. The authors concluded that alterations in the gut microbiota elicited by saccharin feeding resulted in the glucose intolerance. The authors also used data from an ongoing study that involved the monitoring of human nutrition and found an association of LCS with obesity and its concomitant clinical features, including glucose intolerance. Subsequently, they performed a 1 week long clinical trial with only seven subjects who did not usually consume saccharin. Over this short time the subjects were given the maximal acceptable daily intake of saccharin each day in bolus doses. Four of the seven subjects developed an impaired (reduced) glycemic response while the other three did not. Fecal samples from the subjects were transferred into germ-free mice and the mice that had received samples from subjects with the impaired glycemic response displayed significant glucose intolerance while the mice with transplants from the subjects with normal glucose tolerance exhibited the same normal glucose tolerance. Thus the effect on glycemic response was interpreted as being the result of changes in the gut microbiome. While Suez et al. [64] referred to four subject "responders" and three "non-responders," it is unlikely that a significant treatment effect would have been detected by any robust statistical test, given the small sample size and roughly equal split of subjects with differing responses. Interpreting the results of this study in the broader context must consider the long and robust literature showing that saccharin and the other LCS do not have any effects on blood glucose levels. As early as 1955 the National Academy of Sciences found no deleterious effects of saccharin on blood sugar, kidney function, vitamin utilization, blood coagulation, or enzyme activity in man [65]. Renwick [66] performed a review of the literature and found that "What is very clear from all of these studies is that intense sweeteners do not significantly affect blood glucose levels." In a review published just last year Bryant et al. [67] found no class effect of LCS on glycemic or appetite responses. As acknowledged by Suez et al. [64], the notion that LCS adversely affects the gut microbiome in a way that leads to glucose intolerance should be considered preliminary at best. What is clear, however, is that there could be effects of LCS on the gut microbiome and this area of research should be explored more fully to determine if there are clinically meaningful effects from these possible alterations.

Low Calorie Sweeteners and Weight Maintenance

Another important question for LCS in weight management is whether they can help those who have previously lost weight to maintain that weight loss. Maintaining weight loss over the long term is extremely difficult and rare. Of those who attempt to lose weight only 10 % maintain any reduction in body weight for more than 1 year [68, 69]. In addition, nearly 90 % of those who have attempted weight loss regain the weight within 3–5 years [70]. The early study of Blackburn et al. [28] where those consuming aspartame over a 2 year period were better able to maintain weight loss suggested that LCS consumption may be beneficial for weight loss maintenance. In addition there have been studies of those who have achieved long and meaningful weight loss through dietary and lifestyle changes. These successful weight loss maintainers are part of the National Weight Control Registry.

This registry is not a random sample, but rather people voluntarily join as long as they meet the minimum requirement for entry; that is, having lost at least 30 lb and maintained that weight loss for over 1 year. Many in this registry have obtained weight losses exceeding 75 lb and maintained those losses for over 10 years [71, 72]. Two studies to date have studied the consumption of LCS in this population. Phelan et al. [73] examined a cohort of women ($n=172$) from the registry who had lost an average of over 60 lb, were at normal weight, and had maintained that weight loss for over 10 years. They compared this cohort to women who had always been at normal weight ($n=131$). The successful weight losers consumed three times the amount of diet beverages compared to those who had never lost weight. Based on these findings the authors suggested that consuming beverages sweetened with LCS might be one way to promote weight management for both prevention and treatment of obesity. More recently, a different sample from the Weight Control registry was administered an online survey regarding their consumption of beverages including LCS and sugar-sweetened beverages. The online survey was given to a cohort ($n=434$) who had lost an average of 75 lb and maintained that loss for over 8 years. Fifty-four percent of those surveyed consumed beverages made with LCS once or more per day. By way of comparison, about 28 % of the general population consumes one or more beverage made with LCS per day [74]. It should be noted that in these studies LCS-sweetened beverages include coffee or tea to which tabletop LCS packets have been added. Importantly, nearly 80 % of these successful weight loss maintainers indicated that consuming LCS-sweetened beverages helped them control or reduce their total caloric intake. Taken together these studies suggest that consumption of LCS can be a useful strategy for maintenance of weight loss.

Low Calorie Sweeteners for Prevention of Weight Gain

A critical Public Health question is whether consumption of LCS by those at normal weight will help prevent weight gain. However, there is very little data on this question. Some studies of beverage consumption in adolescents suggest that those who habitually consume diet drinks are less likely to become obese but the data is very limited [75, 76]. What is needed, possibly through a public private partnership, are clinical trials that assign normal weight subjects to either consume LCS or avoid such products for at least a 5 year time period. Such studies could help reveal whether LCS consumption is useful in preventing weight gain. Adults tend to gain weight progressively through middle age. This is particularly important as the average weight gain across the population in the USA is 0.5–1 kg per year and it has been suggested that this relatively modest weight gain can lead to obesity over time [77]. While there is research on a variety of strategies to prevent weight gain a recent review found that evidence is mostly limited concerning which interventions and approaches may prevent weight gain and found that there is not strong evidence to promote any particular weight gain prevention strategy [77]. Thus the study of whether LCS consumption can be a useful weight gain prevention strategy takes on even more importance.

Conclusion

In evaluating the body of evidence for the use of LCS in weight management numerous health organizations, including the Academy of Nutrition and Dietetics, the American Heart Association, the American Diabetes Association, and The Dietary Guidelines for Americans 2015 Advisory committee, have concluded that the consumption of LCS can be beneficial for weight loss and maintenance [40, 78–80]. For example, as mentioned previously, the draft key findings of the Dietary Guidelines for Americans 2015 Advisory Committee found that LCS consumption modestly reduces body weight

in adults. In their position statement on nutritive and nonnutritive sweeteners the Academy of Nutrition and Dietetics rated as "good" the evidence that the "use of aspartame and aspartame-sweetened products as part of a comprehensive weight loss or maintenance program by individuals may be associated with greater weight loss and may assist individuals with weight maintenance over time." [78]

Overall consuming LCS appears to support weight loss and help prevent weight regain in humans. Randomized trials show LCS consumption leads to weight loss, and successful weight loss maintainers regularly consume beverages sweetened with LCS. While there is an association between LCS consumption and higher BMI, causality has not been demonstrated as individuals seeking to lose weight often switch to beverages made with LCS in order to reduce their caloric intake. Important areas for future research are the influence of LCS on the gut microbiome, and subsequent metabolic outcomes, and whether or not consuming LCS helps prevent weight gain over time in those of normal weight.

Conflict of Interest Disclosure D. Greenberg and R. Black are full-time employees of PepsiCo Inc. C. Cioffi is a contract employee of PepsiCo Inc. The views and opinions expressed herein are those of D. Greenberg, C. Cioffi, and R. Black and do not necessarily reflect the position or policies of PepsiCo Inc.

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Part V
Prevention of Major Disabilities: Geriatrics

Chapter 26
Diet, Osteoporosis, and Fracture Prevention: The Totality of the Evidence

Laura A.G. Armas and Robert P. Heaney

Key Points

- Bone health requires total nutrition. Bone tissue depends on the integrity of its cells, which like most other tissues, needs a broad array of macro- and micronutrients. Additionally, calcium and protein play key structural roles, since the bulk of the bony material is made up of these substances.
- Calcium is a threshold nutrient. The minimum daily requirement is the intake at which bony response plateaus. To ensure reaching this threshold, calcium intake should be 1500 mg/day both during growth and once again after age 50. Risk of osteoporotic hip and other non-spine fractures can be reduced by 30–50 % with life-long calcium intakes in this range.
- Vitamin D is produced predominantly in the skin. Currently recommended daily oral intakes are sufficient only to prevent the most extreme bony manifestations of vitamin D deficiency. Optimal vitamin D status is ensured by serum 25(OH)D values ≥80 nmol/L (32 ng/mL). Lower values are associated with impaired regulation of calcium absorption and increased osteoporotic fracture risk. Daily utilization of vitamin D may be as high as 4000 IU (100 μg). For most elderly individuals a daily oral dose of 1000–2000 IU is necessary to sustain adequate serum 25(OH)D concentrations.
- Protein, once thought to be potentially harmful to bone when ingested in large quantities, is now best understood as complementary to calcium. Together the two nutrients provide the bulk constituents of bony material. To achieve the full benefit of either, the intake of the other must be adequate as well. Protein intakes that optimize bony response are uncertain, but appear from available data to be above 1.0 g/kg/day.
- Recovery from hip fracture can be substantially improved with aggressive attention to the nutritional status of hip fracture patients, with special emphasis on repairing the protein malnutrition common in such patients.
- Even though typical magnesium intakes are below the RDA (310 and 400 mg/day for women and men, respectively), there appear to be few skeletal consequences of the shortfall. Supplemental magnesium does not improve calcium absorption in individuals consuming typical diets and has no recognized effect on calcium balance.
- Vitamin K, zinc, manganese, and copper are involved in various aspects of bone matrix formation, but it is not known whether deficiency of any of them contributes to the development or severity of typical osteoporosis.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_26,

Keywords Calcium • Parathyroid hormone (PTH) • Hip fracture • Phosphorus • Protein • Sodium • Vitamin D • Vitamin K • Magnesium • Nutrition

Introduction

Osteoporosis is currently defined as "a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength primarily reflects the integration of bone density and bone quality" [1]. Bone matrix is composed of collagen and other proteins which are mineralized with calcium and phosphorus. Nutritional intake of these proteins, calcium, and phosphorus are obviously needed to provide these important structural components. Nutritional intake of other vitamins and minerals are needed for the cellular processes of bone tissue deposition, maintenance, and repair. In this chapter, we will review the main micronutrients involved in bone growth, remodeling, and repair; but bear in mind that nutrition comprises all nutrients and micronutrients consumed as food, and as such, food is a sum of more than its component parts. Simply supplementing with adequate intakes of the various nutrients does not make a balanced diet, and likely will not have the same benefits. Some micronutrients may be more important to bone than we realize, but separating them out from the diet and focusing on them as a single actor may not be possible. Micronutrients are not drugs and shouldn't be separated from a balanced diet in practical application. As these are nutrients, the advantage in adding them to a diet will be most beneficial for those with deficiency. For example, adding additional calcium to an already calcium replete diet offers no additional benefit. However, much of the population has inadequate intakes of calcium to build and maintain their skeletons.

The skeleton is not only a structure that supports the body, it is also a nutrient reserve of calcium and phosphorus, and ultimately the size of that reserve and strength of the structure is dependent on the daily balance between absorbed intake and excretory loss of these two minerals.

Calcium

The function of the skeleton includes a reserve for calcium and phosphorus. As a reserve, it offsets short term shortages and is a place to safely store dietary surplus. We see this in the skeletons of laboratory animals such as cats, rats, and dogs. When they are placed on a low calcium intake, they maintain extracellular calcium levels at the expense of their bone mass [2]. The calcium level is maintained in a very narrow range by parathyroid hormone (PTH) which releases calcium into the circulation by resorbing bone tissue [3].

This reserve is meant to be used for the short term. When intake is inadequate, the reserve is the first in line for depletion. With most nutrients, the short term depletion of the reserve has no noticeable effect on the function of the organism until the reserve is exhausted. At this point clinical disease begins to be apparent. For some nutrients (i.e., vitamin A, energy) the reserve can be quite large and it may take months to see the effect of inadequate intake. For others (i.e., water soluble vitamins) the reserve can be very small and dysfunction develops quickly with inadequate intake.

Calcium is unique in that it is primarily stored in the skeleton. So not only is the skeleton the calcium reserve, it is also critically important for body structure, function, and mobility. The skeleton stores a very large amount of calcium, especially in comparison to the cellular and extracellular pools. As a result of PTH's ability to respond to dips in extracellular calcium by resorbing bone and releasing needed calcium, dietary deficiency almost never impairs tissue functions that are dependent on calcium. However, it does this at expense to the skeletal mass, and as bone strength is a function of bone mass, over time the skeleton will suffer a decrease in strength. As the calcium reserve is large, this deficiency takes years to become apparent.

Calcium Requirements

Calcium is a nutrient that has a threshold of response similar to iron. This means that below some critical value, the effect is limited by the available supply, whereas above that value or threshold there is no benefit in increasing the supply. This relationship is illustrated in Fig. 26.1, in which the intake–effect relationship is shown (Fig. 26.1a) and then using actual data from Forbes et al. [4] to show the calcium intake effect on bone calcium in a growing animal (Fig. 26.1b). The minimum calcium requirement is the intake at which the curve becomes flat. The threshold of calcium requirement can vary throughout life with higher values required during growth and pregnancy and lower values at skeletal maturity.

There have been several recommendations for ideal calcium intake from expert panels throughout the years with the most recent released by the Institute of Medicine in 2010 (see Table 26.1). The evidence for their recommendations is summarized in their report [5] and in reviews on the calcium recommendations [5]. The overall conclusion from the review of the evidence is that current calcium intakes in the USA, by both men and women, are too low for optimal bone health. This is shown by several randomized controlled trials that showed a reduction in bone loss and fractures by giving additional calcium [6–13]. If the subjects prevailing calcium intake was adequate (above the threshold) additional calcium would have shown no effect.

This is also the reason behind some trials demonstration of a null effect of calcium on bone mass or fracture [14, 15]. Adding calcium to an already calcium replete diet has no effect, as we would expect.

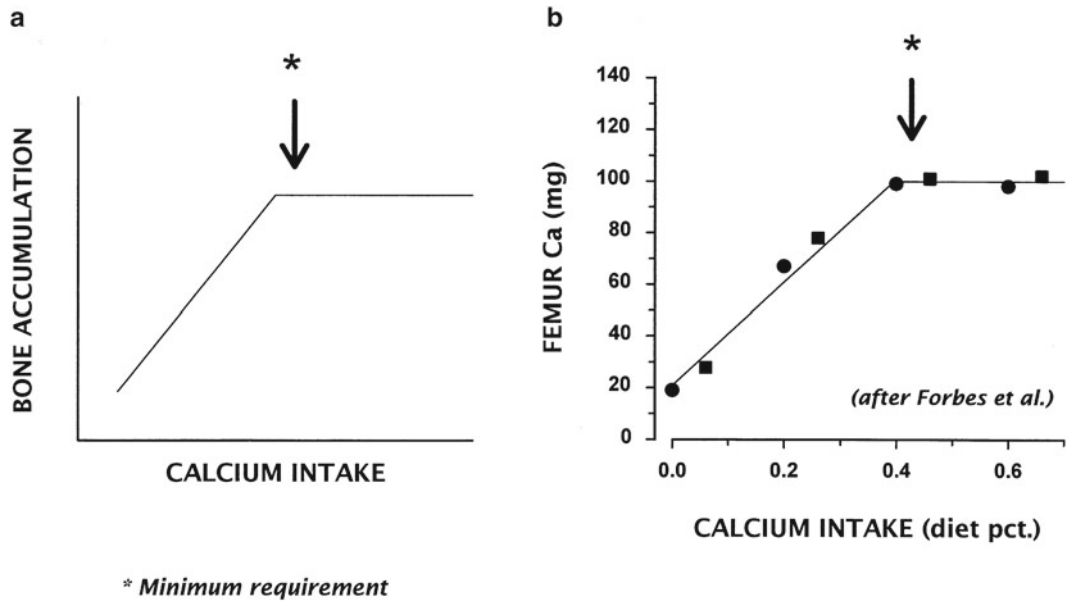

Fig. 26.1 Threshold behavior of calcium intake. (**a**) Theoretical relationship of bone accumulation to intake. Below a certain value—the threshold—bone accumulation is a linear function of intake (the *ascending line*); in other words, the amount of bone that can be accumulated is limited by the amount of calcium ingested. Above the threshold (the *horizontal line*), bone accumulation is limited by other factors and is no longer related to changes in calcium intake. (**b**) Actual data from two experiments in growing rats, showing how bone accumulation does, in fact, exhibit a threshold pattern (Redrawn from data in Forbes RM et al. [4]) (Copyright Robert P. Heaney, 1992. Reproduced with permission)

Table 26.1 Dietary reference intakes for calcium [23]

Life stage group	Estimated average requirement (mg/day)	Recommended dietary allowance (mg/day)	Upper level intake (mg/day)
Infants 0–6 months	[a]	[a]	1000
Infants 6–12 months	[a]	[a]	1500
1–3 years old	500	700	2500
4–8 years old	800	1000	2500
9–13 years old	1100	1300	3000
14–18 years old	1100	1300	3000
19–30 years old	800	1000	2500
31–50 years old	800	1000	2500
51–70 years old males	800	1000	2000
51–70 years old females	1000	1200	2000
>70 years old	1000	1200	2000
14–18 years old, pregnant/lactating	1100	1300	3000
19–50 years old, pregnant/lactating	800	1000	2500

[a]For infants, adequate intake is 200 mg/day for 0–6 months of age and 260 mg/day for 6–12 months of age

Primary Prevention or Reaching Peak Bone Mass

The human skeleton at birth contains about 25–30 g of calcium and in mature women, 1000–1200 g. This difference must be obtained through the diet. This is hampered by the fact that the amount of calcium retained by the body is much less than the amount ingested. This is because calcium absorption efficiency is relatively low (~30 %) and because calcium is lost through shed skin, nails, hair, and sweat, as well as in urine and digestive secretions. In infancy and puberty, calcium retention may be as much as 25–30 % of intake, but during much of the growth period, it is as low as 4–8 % of intake. So a large amount of calcium intake is needed to retain a relatively small proportion. This is because in primitive conditions, there was an abundance of calcium and the body had no need to retain it.

When calcium intake is less than optimal, the balance between bone formation and bone resorption (which should be positive during growth) falls. This occurs because PTH increases bone resorption at the endosteal trabecular surface of growing bones in order to provide enough calcium to mineralize the periosteum and growth plates. So growth in bone size will continue, but without adequate additional calcium from the diet, the body will simply redistribute the existing skeletal calcium over a larger volume.

Net bone accumulation during growth will increase as calcium intake increases, but only to a peak bone mass that is determined by genetics. Above that level, as shown in Fig. 26.1b, further calcium intake will not increase bone mass. The intake required to increase bone mass to its genetically programmed peak is the intake that corresponds to the threshold or beginning of the plateau in Fig. 26.1. This value will be different for different stages of growth [16].

There have been many published studies of calcium balance during growth and when these are examined in aggregate, it is possible to estimate the calcium intake values that correspond to the threshold [16, 17]. Figure 26.2 is an example of the relationship between calcium intake and retention in a study of adolescents [18]. It shows the plateau threshold of approximately 1500–1600 mg calcium per day is needed to optimize calcium balance. Below that value, intake is suboptimal, i.e., calcium balance is limited by intake. The best estimates for the value of this daily calcium threshold from balance studies performed at other stages of growth are 1400 mg/day in children and 1000 mg/day in young

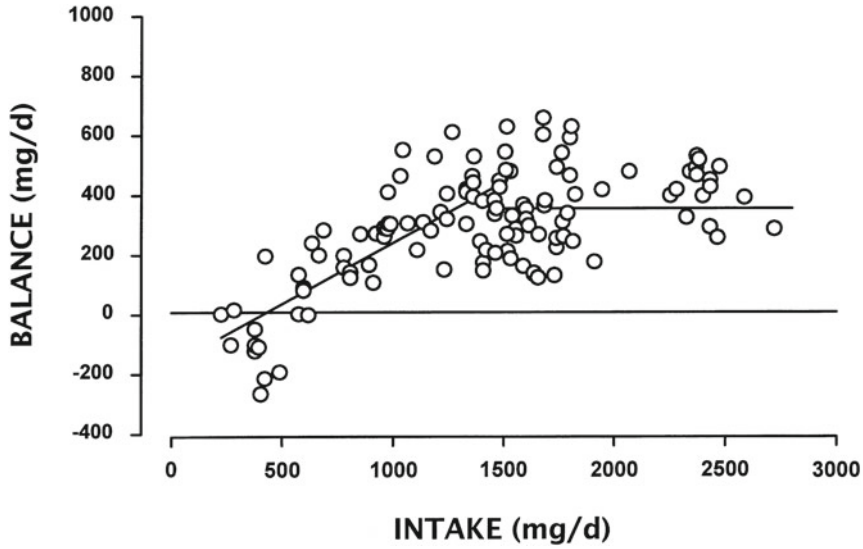

Fig. 26.2 The relationship of calcium intake, on the *horizontal axis*, to calcium retention (balance), on the *vertical axis*, for a subset of the adolescents described by Matkovic and Heaney [18, 19]. Note that, despite the "noisiness" that is inevitable in measurements of balance in humans, there is clear evidence of an intake plateau, as observed in the animal experiments of Fig. 26.1. Note also that, for this age, the threshold of the plateau occurs at about 1500 mg Ca/day (Copyright Robert P. Heaney, 1992. Reproduced with permission)

adults [19]. Jackman et al. found that 1300 mg/day in adolescents was the lowest intake consistent with their model for the retention plateau [17].

These balance studies are supported by several randomized controlled trials of calcium supplementation in children and adolescents [9, 10, 20, 21] and by a longitudinal observational study in young adults [22]. Recker et al. [22] in a longitudinal study of young women showed that bone mass continues to accumulate into the third decade. The gain in total body bone mineral was ~1.25 % per year. This bone accumulation was directly related to calcium intake. The rate of bone mass accumulation was inversely proportion to age, with the estimate that bone continues to accumulate until age 29–30 years.

Secondary Prevention or Conserving Bone Mass

In the latest IOM recommendations [23], the RDA for calcium in 30–50 year olds is 1000 mg/day. This is compatible with the studies that have been done in premenopausal women. Heaney et al. [24] in a balance study of women ingesting their habitual calcium intake found zero calcium balance at a mean intake of slightly under 1000 mg/day. In contrast, Nordin et al. [25] found a figure closer to 600 mg/day and Recker et al. [22] in a 2 year prospective study of bone mass in premenopausal women found no detectable bone loss on a mean calcium intake of 651 mg/day. So, even after peak bone mass is reached, maintenance of the skeletal bone mass relies on adequate calcium intake. It also should be noted that, for women, these years are times of peak reproduction and additional calcium is needed for the growing fetal skeleton during pregnancy and the baby's skeleton during nursing. Again, the physiologic principles are the same, the body will maintain fetal development and breast milk calcium at the expense of the mother's skeleton, so it is imperative that calcium intake be increased during these crucial periods [26, 27].

Menopause

Estrogen deficiency at menopause has two main effects on calcium. The first is a mechanical one. The body's response to estrogen deficiency is to lower the amount of bone needed. So when women lose ovarian function by any means, natural, surgical, or as a result of anorexia nervosa or functional amenorrhea, the remodeling of bone is increased and overall net effect of more resorption and lower bone formation is the loss of bone mass. (The same effect is seen in men with the loss of gonadal hormones.) The amount of bone lost during menopause varies by skeletal site but at the spine it can be about 12–15 % of the bone mass a woman had before menopause [28]. The second effect of estrogen deficiency is lower intestinal calcium absorption and lower renal calcium conservation [28–30]. The synergistic effect of estrogen loss and low calcium intake can be especially detrimental to the skeleton.

The loss of estrogen has a profound effect on bone mass that cannot be overcome by diet or calcium supplementation, although it could be further worsened by poor calcium intake. Almost all randomized controlled trials of calcium supplementation within 5 years after menopause failed to prevent bone loss. Even a very high calcium intake of >3100 mg/day only slowed menopausal bone loss, but it did not prevent it [31]. There are a few reports of a small calcium effect in menopause, which indicates that there are those women who are losing bone mass after menopause because of *both* calcium and estrogen deficiency.

The menopause results in a dramatic drop in bone mass, but if nutrition is adequate the skeleton reaches a new steady state where bone mass is maintained over time. This is where the peak bone mass reached during adolescence and early adulthood becomes of crucial importance. One standard deviation for lumbar spine bone mineral content in women is about 1–15 % of the young adult mean, and for total body, about 10–12 %. So a woman with a bone mass of one standard deviation above the mean can lose 12–15 % over the menopause and still end up with about as much bone as the average woman has before menopause. By contrast, a woman at or under one standard deviation below the young adult mean before menopause, who loses an additional one standard deviation of bone mass at menopause is already osteopenic and close to osteoporotic according to the WHO criterion [32].

As mentioned previously, because of the lower calcium intestinal absorption [29] and renal calcium retention, the estrogen deficient woman needs an even higher dietary calcium intake and unless she raises her dietary intake after menopause, she will continue to lose bone even beyond the bone loss that occurred because of estrogen deficiency. An ongoing calcium deficiency will continue to deplete the skeleton of its calcium reserves for the remainder of her life. Unfortunately, rather than increasing calcium intake, women in the USA take in even less calcium as they age, exacerbating the problem further [33]. In fact, 50 % of older women have an inadequate intake of calcium [23].

Senescence

Both men and women suffer from age related bone loss after age 50. As we will discuss below, calling this age related bone loss is a bit of a misnomer as it can be overcome by additional calcium (and vitamin D) intake as evidenced by many of the trials we will discuss in this section. As we have previously mentioned, in women, the largest effect on bone at menopause is a result of the dramatic effect of estrogen deficiency on bone mass. In comparison to the dramatic change in bone mass related to estrogen deficiency, the rate of bone loss with ageing is about 0.3–1.0 % per year during the sixth and seventh decades and then accelerates with advancing age. For example, in the control subjects (average age 86) of a study by Chapuy et al. [7], the rate of loss from the hip was 3 % per year. The age related loss involves both cortical and trabecular bone and results from several mechanisms or combinations of mechanisms: disuse, remodeling errors, and nutritional deficiency.

Nutritional deficiency in this population is exceedingly common with at least 50 % of women not even reaching the estimated average requirement instituted by the IOM [23] which is a conservative value. As we have mentioned previously, intestinal calcium absorption declines with age [29] at the same time as calcium and other nutrient intake declines [33], resulting in a diet that becomes more and more inadequate. As Chapuy et al. [7] demonstrated in his study of calcium and vitamin D supplementation in a group of elderly institutionalized women, adequate replacement of these nutrients can obliterate the "age" (nutritional inadequacy) related bone loss.

In another example, McKane et al. [34] showed that the high PTH levels in elderly women are due to calcium deficiency and can be completely normalized by calcium intakes of 2400 mg/day and Peacock et al. [35] showed that elevating calcium intake from ~550 mg/day to ~1300 mg/day effectively stopped bone loss at the hip and total body over a 4 year period.

It is in this group of elderly, malnourished people that we see the most dramatic and persuasive evidence for fracture prevention by calcium and vitamin D supplementation [7, 11, 36–39]. This is partly because the incidence of fragility fractures rises with age, so the ability to see a benefit is greater. This is also because the calcium intake at baseline is low on the response curve (see Fig. 26.1) so any increase along the intake axis has a proportional improvement on the outcome. This effect has been demonstrated by Chapuy et al. [7] who showed 43 % reduction in hip fracture and 32 % reduction in other fractures with 18 months of calcium and vitamin D supplementation. Dawson-Hughes et al. [6] produced >50 % reduction in non-vertebral fractures with 36 months of calcium and vitamin D supplementation, and Chevalley et al. [11] compared calcium supplements of 800 mg daily to placebo and found the calcium group had reduced vertebral fracture incidence. Recker et al. [37] showed a decrease in vertebral fractures and a decrease in age related bone loss in a 4 year trial of calcium in elderly women.

As you can see by these trials, vitamin D and calcium are typically given together (appropriately given their physiology). We will discuss vitamin D in greater depth in the next section, but their effects are intertwined and synergistic, so separating out their individual effects in a clinical trial is impossible and reviews that have attempted this have not had great success [40, 41]. Across the population, both calcium and vitamin D deficiency are common, with the elderly having the highest prevalence of deficiency in both nutrients [23].

The calcium intake achieved in these trials can shed light on optimal requirements in this age group. The Chapuy study reached ~1700 mg/day; Chevalley, ~1400 mg/day; Dawson-Hughes, ~1300 mg/day; and Recker, ~1600 mg/day. These are consistent with Heaney's data of healthy estrogen-deprived older women which found calcium requirements of ~1500–1700 mg/day [24].

As we saw in some of the trials, calcium protects from "age" related bone loss, but this is not the only factor in calcium's effect on fracture reduction. In fact the fracture benefit is much larger than the bone mass difference would predict and the fracture reduction is apparent within only a few months after starting calcium (and vitamin D) supplementation [7, 36, 42]—well before much effect on bone mass could have occurred. The best explanation for this would be a reduction in excessively high remodeling activity that is common in postmenopausal women [43] and in the elderly in general [43]. Remodeling activity is an important risk factor for fragility fracture and any agent (pharmaceutical or nutritional) that reduces remodeling, reduces fracture risk, irrespective of its effect on bone mass [42, 44].

An important factor of these controlled trials in elderly individuals is that bone mass was low in both treated and control groups at the start of study and while a significant difference in fracture was seen with calcium supplementation, even the supplemented groups had an unacceptably high fracture rate. What these studies and other short term fracture trials do not establish is what effect a lifetime of optimal calcium intake would have on the fracture rate. This information can only be gleaned from observational studies. The studies of Matkovic et al. [45] and Holbrook et al. [46] found a hip fracture rate ~60 % lower in elderly persons who had habitually high calcium intakes.

Despite the consistency of the body of evidence linking calcium intake to bone mass and fracture risk, the publication and publicity of the calcium-D arm of the Women's Health Initiative (WHI) trial

[14] cast sufficient doubt in the mind of practitioners and the public that calcium supplement sales dropped in the 6 months following publication. The WHI trial was a large trial of 36,282 women over age 50 who were randomized to 1000 mg calcium (as $CaCO_3$) plus 400 IU vitamin D_3 or placebo (other arms of the study included hormone replacement, so more than half the women were on HRT which, as we have discussed, has a separate effect on calcium absorption, calcium retention, and bone resorption). Ignoring that point though, in the intention-to-treat analysis, there was a statistically significant reduction in age related bone mass, a nonsignificant (12 %) reduction in hip fracture and a 30 % (significant) reduction in women not using personal calcium supplements. The per-protocol analysis of those who were treatment-adherent did show a statistically significant, 29 % reduction in hip fractures. However, since treatment adherence may be associated with other factors that could lower the fracture risk, it isn't seen as persuasive as the intention-to-treat analysis.

There are three crucial differences between the WHI trial and the preceding studies. First, the WHI had no low calcium control group to compare to. Mean calcium intake at baseline was between 1100 and 1200 mg/day, i.e., the RDA for women in this age group. As we discussed at the beginning of this chapter, we would expect to see no or very little effect beyond the threshold need for calcium. Second, the baseline vitamin D status was very low, 25(OH)D was 17 ng/mL and vitamin D dose (~200 IU/day after factoring in compliance) would have only raised the 25(OH)D level by 2 ng/mL. At this level of 25(OH)D, calcium absorption efficiency would have been impaired [47]. Finally, hip fracture incidences of the whole cohort was less than expected, perhaps an effect of the estrogen replacement, so the possibility of seeing an effect was limited. So the partial effect observed in the WHI trial supports the model of calcium as a threshold nutrient. Only those individuals below the threshold can be expected to have a response to additional calcium.

In brief, the evidence from all sources supports that adequate calcium intake is essential for bone health and that at least 1200–1500 mg/daily is needed in the elderly. It should also be stressed that osteoporosis is multifactorial and while calcium is essential, other nutrients and risk factors (i.e., muscle weakness, poor vision, environmental conditions, and concomitant medications) also need to be addressed in the elderly population.

Nutrient: Nutrient Interactions or Other Factors That Influence Calcium

There are several nutritional factors which influence or have been proposed to influence calcium absorption or excretion. These are less important if calcium intake is at the recommended levels, but may have an impact at low levels. The principal interacting nutrients are sodium, protein, caffeine, and fiber. Fiber and caffeine have a minor effect on calcium's intestinal absorption [48–51] while sodium and protein influence urinary excretion of calcium [51, 52] and have significant effects on calcium balance when calcium intake is low. The effect of phosphorus and fat are minor or nonexistent.

Figure 26.3 shows the various elements influence on calcium balance. These were taken from Dr. Heaney's work of 560 balance studies in middle-aged women studied at their typical intakes. Surprisingly, only 11 % of variance in calcium balance was explained by differences in intake. Absorption efficiency explained about 15 %, while urinary losses explained over 50 % of the variance.

Intestinal Absorption of Calcium

Fiber

The effect of fiber on calcium absorption is highly variable. Many kinds of fiber such as that found in green leafy vegetables have no effect at all on calcium absorption [53]. The dietary supplement, wheat dextrin, also has no effect on calcium absorption [54]. In contrast, the fiber in wheat bran reduces

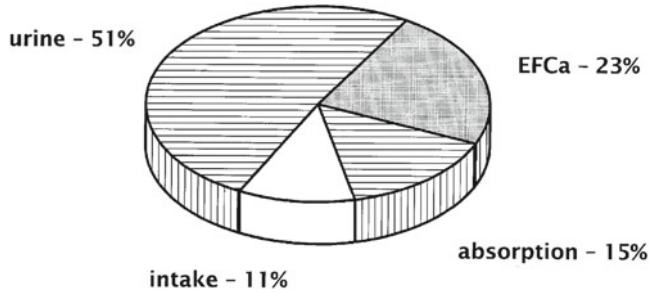

Fig. 26.3 Partition of variance in calcium balance in normal women among the input–output processes involved in calculation of balance (Copyright Robert P. Heaney, 1994. Reproduced with permission)

absorption of coingested calcium, but except for the extremes of fiber intake [55] the overall effect is relatively small. Other plant food constituents such as phytate and oxalate can reduce the availability of calcium contained in the same food, but unlike bran, generally do not affect coingested calcium from other sources. For example, for equal calcium loads, the calcium of beans is only about half available as the calcium of milk [56] while the calcium of spinach and rhubarb is nearly totally unavailable [57]. For spinach and rhubarb, the inhibition is mostly due to oxalate. For beans, phytate and oxalate are each responsible for interference with calcium absorption. Overall, the effects of phytate and oxalate on calcium absorption are highly variable from food to food.

Caffeine

Often considered to have a harmful effect on calcium balance, caffeine actually has the smallest effect of the known interacting nutrients [49]. A single cup of brewed coffee decreases calcium absorption by about 3 mg [50, 51, 58]. This small effect is more than adequately offset by adding a tablespoon or two of milk [50, 58]. While overall, the trend in the USA is towards lower milk consumption; at least the popularity of designer coffee drinks has added a source of calcium to the diet.

Renal Conservation of Calcium

Protein and Sodium

The effects of protein and sodium on urinary calcium loss can be substantial [25, 30, 51]. Both nutrients can increase urinary calcium loss across the full range of their own intakes, from very low to very high—so it's not a question of harmful effects of an *excess* of these nutrients, but rather one of offsetting the urinary calcium loss with adequate calcium intake. The mechanisms for urinary calcium loss differ between the two nutrients. For sodium, calcium and sodium share the same transport system in the proximal tubule, and every 2300 mg of sodium excreted by the kidney also pulls out 20–60 mg of calcium with it. For protein, the excretion of calcium is likely due to the excretion of the sulfate load produced by metabolizing sulfur-containing amino acids. For every gram of typical protein (animal or vegetable) metabolized in adults, about 1 mg of calcium is lost in the urine.

It is important to note that the anion has an effect. Most of the table salt consumed is in the form of sodium chloride, while sodium bicarbonate does not have the same effect on urine calcium excretion [59]. The principal determinant of urinary calcium in young women reported by Matkovic et al. [60, 61] is sodium intake, not calcium intake. At low salt and protein intakes, the minimum calcium requirement for a premenopausal female may be as little as 450 mg/day [52] whereas if both nutrient intakes are high, she may require as much as 2000 mg/day to maintain calcium balance. With our current

higher intake of sodium (3400 mg/day) [62], much more calcium is needed to offset the urinary calcium loss. For diets high in calcium, as would have been typical for hunter-gatherer ancestors, high protein plus high sodium intakes could have been handled by the body perfectly well. The calcium intakes were high enough that intestinal absorption was predominantly passive; there was no need for active calcium absorption. At our population's low calcium intakes, an individual's active calcium absorption is close to maximal. In brief, protein plus sodium intake only cause problems to the calcium balance when calcium intakes are low.

Phosphorus

Phosphorus is commonly believed to reduce calcium absorption but there is little evidence to support that. In fact, metabolic balance studies by Heaney et al. in healthy, middle-aged women on their usual diets, a sixfold variation in phosphorus intake had no detectable effect on calcium absorption [63]. And in a controlled study, Spencer found no effect of even large amounts of phosphorus intake on calcium balance at low, normal, or high calcium intakes [64].

Phosphorus does decrease urinary calcium loss and increase digestive juice calcium output by approximately equal amounts, with little or no net effect on balance [65]. Calcium phosphate salts have similar absorbability to other calcium salts and of course, phosphorus is a major nutrient in food sources of calcium (i.e., dairy products).

Aluminum

Although aluminum is not a nutrient, it is included in this section because when ingested in the form of aluminum-containing antacids, it exerts a significant effect on urinary calcium loss [66]. It does this by binding phosphorus in the gut, which reduces phosphorus absorption, lowering the serum phosphorus levels and elevating urine calcium loss. Therapeutic doses of aluminum-containing antacids can elevate urine calcium by >50 mg/day.

Calcium Sources

The best calcium sources are foods. In a modern, Western diet, foods that provide more than 100 mg of calcium per serving are dairy products, greens in the mustard family (collards, kale, mustard), calcium-set tofu, sardines, and a few nuts (hazelnuts and almonds); but with the exception of shellfish, most meats, poultry, or fish are low in calcium. As previously mentioned, calcium in vegetables with a high amount of oxalate (such as spinach and rhubarb) is almost completely unavailable. Figure 26.4 displays the available calcium in a variety of foods. Available calcium takes into account both the absorbability of calcium in a food and its total calcium content. It is the actual gross amount of calcium a particular food delivers.

In general, most diets devoid of dairy products have a total calcium nutrient density <20 mg Ca^{2+}/100 kcal and even lower *available* calcium densities. If the average total energy intake for adult women is 1400–1800 kCal/day, most diets low in dairy products will be low in calcium; at about 300–400 mg Ca^{2+}/day, far below optimal levels. The lack of dairy food intake at a population level is apparent. Milk intake in particular, the best low calorie source of calcium, has decreased to less than a serving a day per capita in 2012 [67].

In response to this, a variety of foods have been fortified with calcium throughout the last two decades ranging from fruit juice, to bread and breakfast cereals. This level of fortification has varied

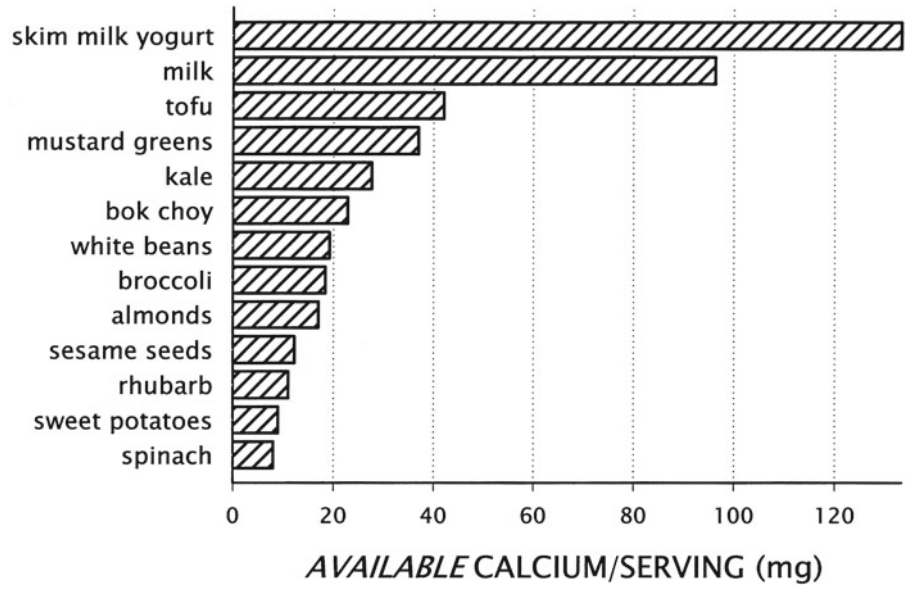

Fig. 26.4 Available calcium per serving for a variety of foods. "Available calcium" is the product of the calcium content of a food and its fractional absorbability (Copyright Robert P. Heaney, 1998. Reproduced with permission)

throughout the years as food trends have come and gone. When the bioavailability of the calcium in these foods has been ensured, these fortified foods help improve calcium intake at a population level. However, simply adding calcium to a food does not ensure its bioavailability. Tests of soy and rice beverages as well as several calcium-fortified orange juices proved disappointing [68]. For example, soy beverages fortified with the same amount of calcium as cow's milk only delivers 75 % of the amount of calcium as cow's milk [63] when the fortificant was optimally suspended in the beverage, and as little as 30 % if it had settled to the bottom of the carton [63]. The IOM estimates median calcium intake from food alone is ~700 mg/day for women over 50 years of age. This means that many women need to either increase calcium rich foods in their diets (the best choice) or take calcium supplements (the most expedient, if not best, choice). The principal calcium supplement in the USA is calcium carbonate, although calcium citrate and calcium citrate malate are also available. When the supplements are competently formulated, so they disintegrate in the stomach, the calcium is quite well absorbed. All of the calcium supplements, regardless of type of salt, have better absorption if taken as part of a meal [19]. Since absorption fraction is an inverse function of calcium load size, absorption is most efficient if the calcium doses are divided. Calcium from all sources interferes with iron absorption when both are ingested at the same meal. This is likely not a large problem in the healthy population, but if an adult is iron deficient and taking an iron supplement, it is probably best not to take the iron at a meal with a large amount of calcium.

Calcium Controversy

While it has been well accepted that calcium is needed for bone health, recent reports of post hoc analyses of cardiovascular events in trials of calcium supplements [69–71] received much media attention, causing many patients with osteoporosis and those at risk for calcium deficiency to discontinue their calcium supplements. The first study was a 5 year study of a calcium supplement vs. placebo in women. In the analysis of the secondary outcomes myocardial infarction (MI), stroke,

or sudden death was statistically significant, but after adjusting for known cardiovascular risk factors at baseline, the effect was lost. The second report was a subgroup analysis of the WHI trial data comparing those who used personal calcium supplements during the trial to those who didn't. Confusingly enough, the results showed those who *didn't* use personal calcium in addition to their trial allocated calcium and vitamin D had a higher risk of MI than those who did [71]. These reports and their methodologies have been widely refuted by members of the scientific community [72–76], but unfortunately have received wide media attention with little concern over the issues of bias and confounding factors which riddle the results. One hopes that physicians are giving thoughtful advice to their patients that isn't based on newspaper headlines, but unfortunately in this information glutted society those with the "loudest" headline get the attention of the public and practitioners.

Vitamin D

Vitamin D is inexorably linked to calcium in its role in skeletal health. The main reason is that vitamin D facilitates active absorption of calcium occurring in the intestinal mucosa. This function is particularly important at low calcium intakes. At high calcium intakes more calcium can absorb passively via paracellular diffusion and is independent of vitamin D status. However, in the majority of people, because of low calcium intake, calcium absorption is directly related to their vitamin D status. Figure 26.5 illustrates the relationship between vitamin D mediated active absorption and calcium

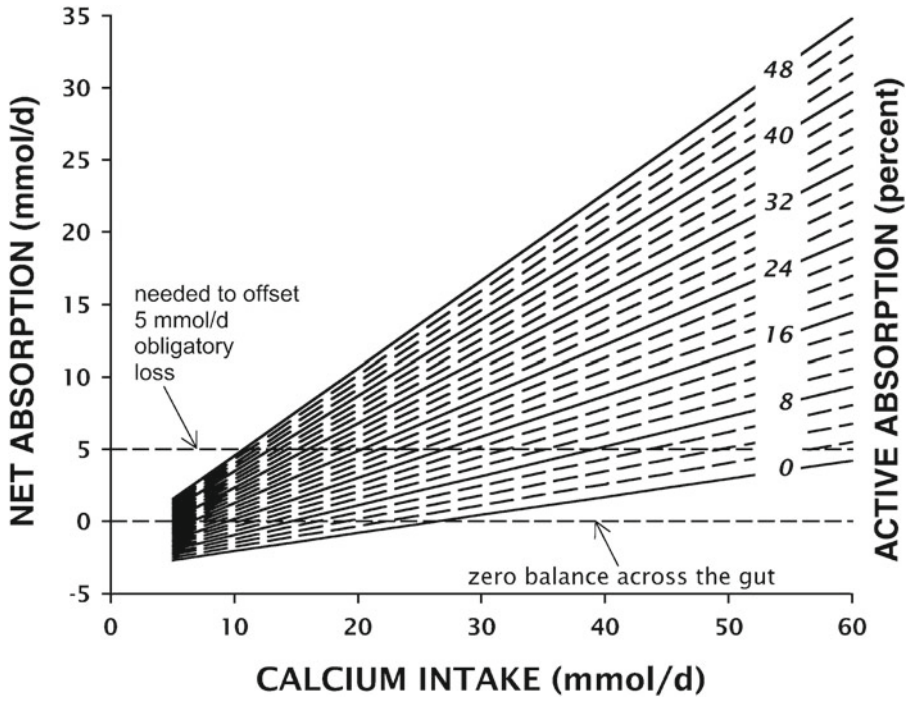

Fig. 26.5 Relationship of vitamin D-mediated, active calcium absorption, calcium intake, and net calcium gain across the gut. Each of the *contours* represents a different level of active absorption above a baseline passive absorption of 12.5 %. (The values along each *contour* represent the sum total of passive and variable active absorption.) The *horizontal dashed lines* indicate 0 and 5 mmol/day net absorption, respectively. The former is the value at which the gut switches from a net excretory to a net absorptive mode, and the latter is the value needed to offset typical urinary and cutaneous losses in mature adults (Copyright Robert P. Heaney, 1999. Reproduced with permission)

intake on the net quantity of calcium absorbed. As you can see, some degree of active absorption is needed to offset excretory losses. Heaney et al. reported active calcium absorption's dependence on vitamin D status with calcium absorption directly increasing with greater 25(OH)D levels up to ~32 ng/mL above which it has no further effect [47].

Vitamin D is well accepted as important in promoting bone health as evidenced by the most recent IOMs [77] review of the current evidence. However, there is much more controversy surrounding the amount needed or the ideal 25(OH)D level needed to achieve optimal bone health [78, 79]. Much of the controversy comes from the difficulty in quantifying the multiple sources of vitamin D, including sun, food, and to a lesser extent, supplements. The sun and food must provide enough vitamin D to achieve 25(OH)D levels of between 20–40 ng/mL in unsupplemented individuals in Europe and North America. Estimates from Heaney et al. suggest that to maintain these 25(OH)D levels, these persons must be getting between 1000 and 4000 IU/day from all sources, much higher than the recent RDA recommended by the IOM (600 IU–800 IU/day depending on age).

Regardless of where the 25(OH)D level of optimal bone health lies (at 20 or 30 ng/mL), or what it takes to get there, it is well known that vitamin D status deteriorates in the elderly [80] due to decreased solar exposure, the presence of less vitamin D precursor in their skin [81], and decreased food intake. In addition, they have other vitamin D abnormalities that further impair their ability to adapt to a reduced calcium intake. They have decreased responsiveness of the renal 1-α-hydroxylase (which activates vitamin D) to parathyroid hormone [82] and possibly a decreased mucosal responsiveness to calcitriol [83].

There is good acceptance that vitamin D deficiency plays a role in the development of osteoporosis and ensuing fracture and many trials have included vitamin D with calcium supplementation [6, 7, 11]. In fact, the trials of antiresorptive medications used for the treatment of osteoporosis have all used concomitant calcium and vitamin D supplementation [84–86].

Again, as with calcium, trials will only be able to find an effect of vitamin D if the baseline levels are sufficiently low and the doses are sufficiently large enough to raise the 25(OH)D levels. Many studies have not shown an effect for just these reasons [87].

The previous discussion has focused solely on vitamin D's role in preventing osteoporosis and fracture simply by its role in improving calcium absorption. In recent years much progress has been made of understanding vitamin D's independent effects in other systems and cells. It appears to function in ways that would impact fracture, such as on balance and muscle, and on other functions, such as blood pressure, insulin resistance, cancer expression, and autoimmune function. Optimal 25(OH)D levels for these functions are likely higher than those needed for bone and several reviews summarize this [88–91].

Protein

While we don't think of protein as a major nutrient involved in skeletal health, protein is the main component of bone. Because of the extensive modification of the collagen molecule, the amino acids released during bone resorption cannot be recycled. So, bone turnover requires a continuous supply of fresh protein to replace what is removed. Another important role of protein is in elevating IGF-1 (insulin-like growth factor) which is trophic for bone [92, 93]. A *minimum* amount of calories (20 kcal/kg/day) and protein (0.6 g/kg of protein) are necessary to maintain normal IGF-1 levels.

As with calcium and vitamin D, there appears to be an interaction or synergism between protein and calcium on its effect on the skeleton. Dawson-Hughes et al. [94] reported a randomized controlled trial of calcium supplementation on bone mass in a group of elderly men and women. They found that the bone gain associated with calcium supplementation was confined to those subjects with the highest protein intake, while in the placebo group there was a slight detrimental effect on bone mass as

protein intake rose. As we discussed in the calcium-nutrient section, we would expect some urinary calcium loss with protein intake and as this trial demonstrated, if this is not replaced with additional calcium, we can expect some bone loss. Another observational study by Heaney et al. found that protein intake in mid-life women positively correlated with calcium balance [95], and this increase in calcium balance was associated with increased calcium intake only in women with protein intakes >62 g/day (~1.0 g/kg/day).

There have been reports over the years that have led to some misunderstandings of the effects of protein foods effects on calcium and bone health. First, it has been shown that protein can increase urinary calcium loss [51, 96, 97]. This has been most clearly shown when purified protein or protein hydrolysates were used, but if the protein was an animal protein (i.e., ground beef) the urinary calcium did not rise [98]. This is likely a result of the phosphorus contained in meat. This just exemplifies the difficulties of singling out specific nutrients out of the diet. Removed from its context in a whole food or even a meal, the solo effect on the body of a single nutrient does not reflect the true physiologic processes. To see the effect of habitual diet intake on the whole organism, we must turn to epidemiological or observational studies. For example, the Framingham osteoporosis cohort found that age related bone loss in postmenopausal women was inversely related to protein intake [99]. Protein was also shown to aid in hip fracture recovery [100, 101].

Again, as with calcium and vitamin D, protein intake also decreases with age as the individuals' tastes change, their ability or desire to cook wanes, and their appetite lessens. This is a crucial time for proper nutrition.

Vitamin K

The chemistry and physiology of vitamin K has been extensively reviewed elsewhere [102–104]. Vitamin K is derived from two sources: as vitamin K1 in green vegetables such as broccoli and spinach and from plant oils and as K2 which is synthesized by gut microflora [105, 106]. Its major role is in coagulation pathways, vitamin K is the coenzyme in the carboxylation of carboxyglutamic acid by gamma-glutamyl carboxylase. The link between vitamin K and bone is the bone specific protein, osteocalcin, which is a protein synthesized by osteoblasts and then posttranslationally modified which requires vitamin K for carboxylation of glutamic acid residues. The gamma-carboxyglutamic acid residues bind calcium. It appears that osteocalcin is key in stimulating bone mineral maturation and in attracting osteoclasts in initiating remodeling activity [107]. Not all osteocalcin is gamma-carboxylated, approximately half is not fully carboxylated [107].

Clinically, serum levels of osteocalcin are used as an indicator of bone turnover. In vitamin K deficiency, such as occurs with anticoagulant treatment which are vitamin K antagonists, the degree of carboxylation of the circulating osteocalcin declines dramatically. While it would seem that vitamin K deficiency would have detectable skeletal effects, they have been difficult to find, for two reasons. First, the measurements of total osteocalcin have included both the carboxylated and uncarboxylated types of osteocalcin. Measuring uncarboxylated osteocalcin is the most sensitive indicator of vitamin K deficiency and when uncarboxylated osteocalcin has been measured in humans, there is a good correlation with high uncarboxylated osteocalcin and low BMD [108, 109]. However, the major food sources of vitamin K are also linked to healthy diets and a healthy lifestyle so it is difficult to separate out the role of vitamin K in an observational study.

Multiple randomized controlled trials have used various forms of vitamin K to prevent bone loss in older adults [110–116]. And while they did decrease the amount of uncarboxylated osteocalcin, only one reported a beneficial effect of vitamin K on bone loss at the hip [112]. This sheds doubt on the role of vitamin K supplementation in addition to a healthy diet. This is not to say that vitamin K is not needed for bone, and that people at risk of vitamin K deficiency wouldn't benefit, but that additional

vitamin K in excess of what is supplied by a healthy diet in a healthy population is not needed for bone health. In addition, these short term trials don't address what effect a lifetime of sufficiency or insufficiency has on bone.

Magnesium

The NHANES survey of 2005–2006 found that the majority of Americans get less than the recommended amount of magnesium from food. As the body's supply of magnesium resides intracellularly or in the bone matrix, it is not easy to accurately assess magnesium status. Severe magnesium deficiency is well described and common in very ill hospitalized patients, but it is unknown whether the general healthy population suffers from marginal magnesium status as it has not been assessed in larger surveys.

Magnesium's role in bone is primarily in the release of parathyroid hormone from PTH cells, PTH's action on bones, and in vitamin D metabolism. Cross-sectional studies have found a direct association between magnesium intake and bone mineral density [117, 118]. As with vitamin K, this finding could be confounded by a healthy diet and lifestyle associated with foods high in magnesium (i.e., fresh leafy vegetables, whole grains, and nuts). While a short term study of magnesium supplementation found that bone turnover was suppressed [119], again this doesn't tell us about long term effects. Therefore, there is no clear evidence that supplemental magnesium helps in prevention or treatment of osteoporosis.

Trace Minerals

Several trace minerals, especially zinc, manganese, and copper, are essential cofactors for enzymes involved in production of various pieces of bone matrix. Vitamin C (with zinc) is needed for collagen cross-linkage. In growing animals, diets deficient in these nutrients produce skeletal abnormalities [120]. Zinc deficiency is well known to produce growth retardation and other abnormalities in humans, but it is not known if zinc deficiency per se contributes to osteoporosis.

Copper deficiency is reported to be associated with osteoporotic lesions in sheep, cattle, and rats [121], and copper deficient animals develop reduced collagen cross-links, a factor known to reduce bone strength [122]. Little is known about copper deficiency's role in osteoporotic humans.

In one four-way, randomized controlled trial, copper, zinc, and manganese with or without calcium was studied in postmenopausal women [123]. The calcium slowed bone loss and there may have been some small additional benefit for the trace minerals. This could mean that there is no role of trace minerals supplementation in osteoporosis or it could mean that some of the women were deficient and were helped with supplements.

Nutrition and Hip Fracture

Up until this point, we have focused on nutrition's role in prevention of fracture, in the building and maintaining of bone throughout life. Now we will turn to nutrition's role after fracture. The most devastating consequences occur after hip fractures, with a 15–20 % mortality and 50 % institutionalization rate. Typically the fractures occur in the elderly with multiple disabilities. The osteoporotic elderly generally have a depleted lean body mass and fat mass and have low circulating values for

several key nutritional variables including serum albumin, ferritin, and vitamins A, D, and K [100]. Overall, nutrition is inversely correlated with outcome. Using serum albumin as a biomarker, survival 2 years after fracture is four times higher in people with serum albumin >3.5 g/dL compared to those below 3.0 g/dL [100].

Rectifying the relative malnutrition these patients suffer has been shown to improve outcomes. Delmi et al. [100] in a randomized trial of a protein-based nutrient supplement given to patients newly hospitalized for hip fracture, found a "good" outcome in 26 % of unsupplemented subjects, vs. 60 % in supplemented individuals. One of the keys to this study was that while the hospital diets supplied to both groups was nutritionally adequate, it was frequently unconsumed while the study investigators ensured compliance with the nutritional supplement. Other studies have also found similar benefits from nutritional supplements in such patients [124] and others have found that nutritional supplements retard bone loss in the other hip [101]. The difficulty now lies in how to implement these types of measures and improve outcomes in this fragile group of patients.

Conclusion

Calcium intake is crucial throughout life. Food sources are best, but for maximum calcium per calorie, low fat milk and yogurt are the best sources. Calcium-fortified foods will also help meet the calcium requirements. Calcium supplements are convenient and often necessary, but they do not replace a good diet. Other nutrients such as vitamin D and protein are also key players in bone health. Prevention of fractures is the ultimate goal, but we must not forget nutrition's important role in fracture repair after fracture.

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Chapter 27
Optimizing Nutrition to Delay Age Related Macular Degeneration

Molly Schleicher, Elizabeth Whitcomb, and Allen Taylor

Key Points

- Age related macular degeneration is the primary cause of blindness in the developed world.
- Rates of the disease are accelerating. Optimizing nutrition appears to offer a means to diminish risk for onset or progress of disease.
- It may even be possible to reverse disease at earlier stages with optimal nutrition. Randomized blinded control clinical studies indicate the value of maintaining dietary supplies of vitamin C (500 mg/day), vitamin E (400 IU/day), Zn (25 mg/day), and apparently, lutein (10 mg/day).
- Epidemiological studies, backed by laboratory tests in mice, indicate that lower glycemia diets and perhaps omega fatty acids can also be beneficial.

Keywords Age related macular degeneration • Eye • Retina • Drusen • Photoreceptor • Randomized blinded control study • Epidemiology • Antioxidants • Vitamin C • Vitamin E • Ascorbate • Zinc • Lutein • Zeaxanthin • Beta-carotene • Vitamin A • Vitamin D • Carbohydrate • Glycemic index • Lutein • Docosahexaenoic acid (DHA) • Eicosapentaenoic acid (EPA) • Lycopene • Dietary pattern

Introduction

Age related macular degeneration (AMD) is the leading cause of visual loss in older adults, affecting 30–50 million individuals worldwide and over two million individuals in the USA [1, 2]. Over 15 % of the elderly are affected. While AMD does not result in complete blindness on its own, the loss of central vision can impair the ability to perform everyday activities, decreasing quality of life [3]. Costs associated with AMD are in excess of $340 billion in the USA, and for the majority of AMD patients in the USA, there is no clinical treatment [1]. Given the aging population, and changing diets toward diets that may enhance risk for AMD (see below), the prevalence of AMD is projected to grow, with over five million individuals being affected by 2050 [2]. Treatment options for AMD are limited to treating only the most advanced stages of AMD, and for only the 15 % of AMD sufferers with "wet" AMD, in which some vision is already lost [4]. Given the limited therapeutic options available

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_27,

for AMD, preventative interventions through dietary modification are attractive strategies. Here we review studies that suggest benefits of certain nutrients with respect to AMD, with few, if any, adverse side effects. For an exhaustive review of the literature available for these nutrients please see review by Weikel et al. [3].

What Is AMD?

Light is focused on the retina at the back of the eye (Fig. 27.1a). The retina comprises multiple layers of cells (Fig. 27.1b). Photoreceptors are the light-sensing cells and are responsible for receiving light signals and converting them to chemical and then electrical impulses that are sent to the brain, where they become the images that we see [2, 3]. The macula is a small spot near the center of retina (Fig. 27.1c). It contains a high density of photoreceptors and is responsible for high resolution central vision. AMD is characterized by damage to the macula of the eye resulting in a blurry, distorted or dark center field of view as well as the loss of central vision [2].

The retinal pigmented epithelium (RPE) is responsible for the flow of nutrients into the retina, and debris out of the retina [3]. Photoreceptors are exposed to damaging energies of light and oxygen each day. Each night the outer 10 % of photoreceptor segments are shed and degraded by the RPE. Aging, inadequate nutrition, and the inability to properly degrade and dispose of cellular debris lead to the formation of basal laminar deposits in the RPE. As the health of the RPE deteriorates further, these deposits may accumulate. Basal laminar deposits are thought to precede the formation of drusen, which are yellow deposits beneath the retina. While small drusen may develop as a normal part of the aging process, medium or large drusen are clinical indicators of AMD [1, 2] (Fig. 27.2b, c).

Although AMD is a progressive disease, it is generally graded in 3–5 stages. In general, one describes three stages of AMD—early, intermediate, and late. Early AMD (categories 1 and 2, Fig. 27.2a, b) is indicated by small (<63 μm) and/or a few medium (<125 μm) sized drusen. Intermediate AMD is indicated by large drusen and/or pigment changes in the RPE (category 3, Fig. 27.2c). Patients with intermediate AMD may have some vision loss. There are two forms of the most severe stage or late AMD: geographic atrophy (also known as dry AMD, Fig. 27.2d) and neovascular (also known as wet AMD, Fig. 27.2e). Dry AMD is indicated by depigmentation in the RPE as well as large and abundant drusen, and death of the RPE and photoreceptors. As a result, there is significant vision loss. There are currently no available therapies to treat dry AMD. Wet AMD occurs when abnormal blood vessels grow under the retina from the choroid, causing damage to the RPE and photoreceptors (see Fig. 27.1b). These vessels are prone to leak, which can cause the macula to bulge, resulting in distorted vision and straight lines may appear curved [2, 3] (Fig. 27.2f). While there are treatment options for those with wet AMD, they are used only after the patients have lost some vision [1]. Approximately 90 % of AMD patients in the USA have early or dry AMD, while only 10 % exhibit the more visually debilitating wet form [2, 3]. The progression from early to late stages of AMD can occur in as few as 5 years, and dry AMD can progress to wet AMD.

Risk Factors for AMD

Non-modifiable risk factors for AMD include age, race, gender, and genetics. Ten percent of those aged 66–75 have early AMD, and this rises to nearly 30 % in those over the age of 75 [5]. AMD is more common among Caucasians than non-Hispanic Blacks and Hispanics at age 80. However, there is a higher rate of dry AMD among 60-year-old Asians than Caucasians [3]. Women appear to have a slightly higher risk for developing AMD [6]. Genetics also plays a role in risk for AMD. Some analyses have shown that having a primary relative with AMD increases one's own risk for AMD.

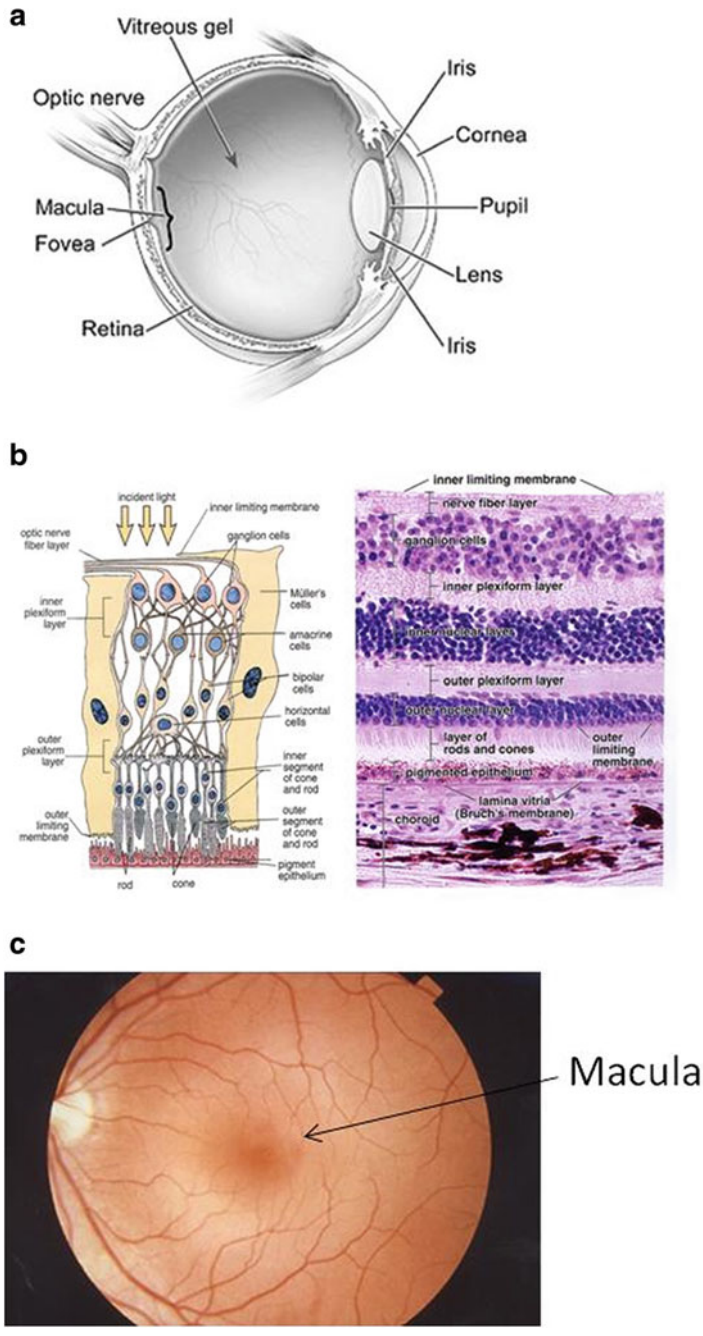

Fig. 27.1 (**a**) Anatomy of the eye. (**b**) Hematoxylin and Eosin stain of a normal retina, cellular layers are defined. (**c**) Fundus photograph of a normal retina, macula is shown by *arrow* [*Acknowledgements*: (**a**, **c**) National Eye Institute, National Institutes of Health (**b**)] http://quizlet.com/8191975/eye-flash-cards/)

In addition, there are several single nucleotide polymorphisms associated with AMD risk [7], with the most widely known being genes involved in the Complement pathway (CFH, CFB, C3). More in-depth details regarding genetic influences on AMD can be found elsewhere [3, 7–14].

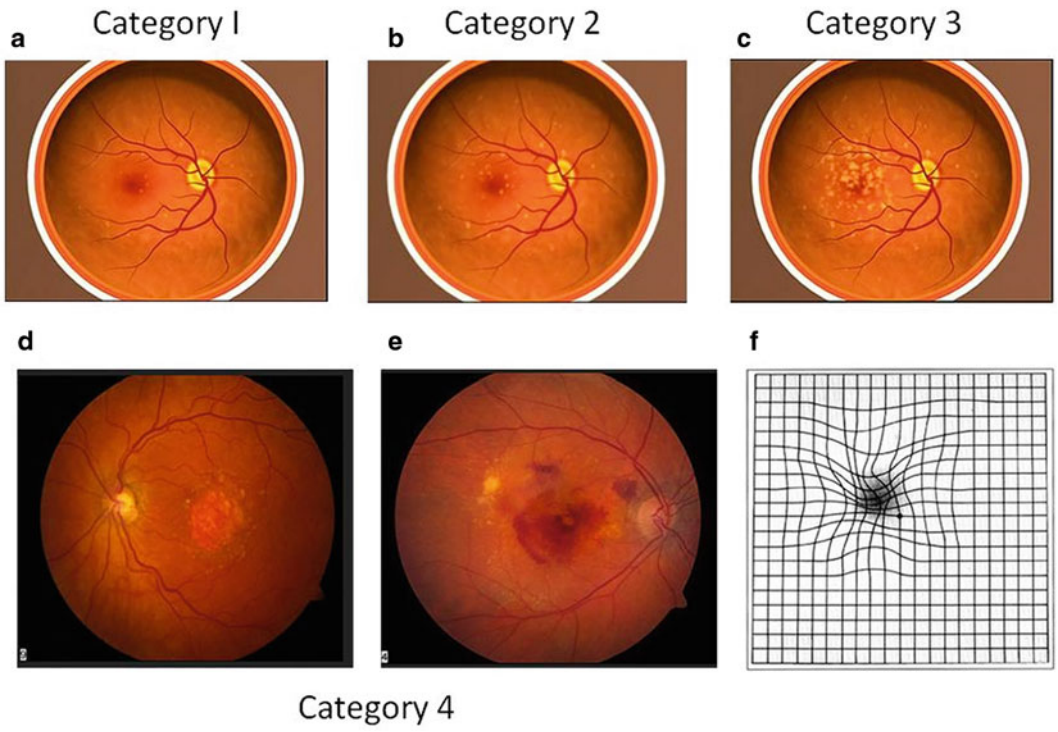

Fig. 27.2 Age-related macular degeneration: (**a**–**c**) Cartoons of retinal fundus photographs of early and intermediate AMD. (**d**) Fundus photograph of geographic atrophy. (**e**) Fundus photograph of choroidal neovascular AMD. (**f**) Hypothetical Amsler grid as observed by AMD patient. All images courtesy of the National Eye Institute, National Institutes of Health

Modifiable risk factors include smoking and obesity. Smoking is the most consistently reported modifiable risk factor for AMD. It has been suggested that smoking increases risk for AMD up to sevenfold [15]. Obesity is also a significant modifiable risk factor for AMD as it has been shown that a 3 % reduction in the waist-to-hip ratio decreases risk for AMD by 20 % [16].

Nutrients and AMD

The sections below highlight the roles of various nutrients in the development and/or progression of AMD. Emphasis is placed on nutrients for which most evidence suggests a beneficial role. For an exhaustive review of the role of various nutrients in AMD, please see the review by Weikel et al. [3].

Dietary Carbohydrate

Several studies have investigated the role of carbohydrates in AMD risk. These studies have focused primarily on the glycemic index of a food. Glycemic index is a measure of the ability of 50 g of a specific food to increase blood glucose levels compared to the ability of 50 g of a standard food (typically glucose or white bread) [17]. Consuming a high glycemic index food will result in higher levels of blood glucose 2 h after consumption than a low glycemic index food. Examples of high

glycemic index foods include Russet potatoes, white bread, and short grain white rice. Examples of low glycemic index foods include most fruits, non-starchy vegetables, and 100 % stone ground whole wheat bread [18].

All epidemiologic data published to date indicates that consuming foods with a higher glycemic index is associated with greater risk for AMD or AMD progression [19, 20]. Chiu et al. reported that participants in the Age-Related Eye Disease Study (AREDS) with the highest glycemic index diets were at increased risk for large drusen compared to those with the lowest glycemic index. There was also a trend for increasing risk for large drusen with increasing glycemic index. In addition, increasing glycemic index increased risk for neovascular AMD. Chiu et al. also found that those with the highest dietary glycemic index had a 39 % greater risk for progressing to more advanced stages of AMD than those with the lowest dietary glycemic index, and predicted that approximately 100,000 cases of AMD could be avoided in 5 years with only slight changes to the diet [21]. Results from studies in mice models support and propose pathophysiologic mechanisms for this association, specifically the deleterious effects of sudden increases in a combination of advanced glycation end products and other oxidants, all of which modify multiple cellular constituents, elicit inflammatory responses, and compromise the usual cellular protein editing capacities [22].

While consuming a low glycemic index diet alone may be beneficial for preventing AMD development and progression, consuming higher levels of DHA and EPA (described below—"Polyunsaturated Fat") along with a low dietary glycemic index diet may be of even more benefit [23]. Similarly, consuming a low dietary glycemic index diet with the AREDS supplement (described below) may also be protective [20].

Lastly, some studies have found that calorie restricted diets based on low glycemic index foods contribute to greater weight loss than those based on high glycemic index foods. This indicates a potential role for glycemic index in reducing risk for obesity, an important risk factor for AMD [24, 25]. While more research is still needed, the data available, and multiple benefits to be gained, encourages the use of low glycemic index diets in lowering risk for AMD.

Dietary Fat

Polyunsaturated fats: Polyunsaturated fats are typically liquid at room temperature and include omega-3 and omega-6 fatty acid. Good food sources include vegetable oils (soybean, safflower) and fatty fish (salmon, trout) [26].

1. Omega-3
   Omega-3 fatty acids include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and have been associated with improvement in a number of chronic diseases, such as coronary heart disease and AMD [27, 28]. Many studies regarding omega-3 fatty acids and AMD suggest that increased consumption may reduce risk for or progression of AMD [3]. For example, analysis of AREDS participants found that increasing EPA+DHA intake was associated with a decreased risk of progression to central geographic atrophy over 12 years, and that progression was lowest in subjects with the highest intakes of DHA, EPA, or EPA+DHA [29, 30]. Furthermore those consuming the highest amounts of DHA, EPA, or EPA+DHA were at reduced risk for progression to neovascular AMD compared to those consuming the lowest amounts [30]. Chiu et al. reported that those who consumed 64 mg/day DHA had a reduced risk for progression to advanced AMD compared to those with intakes below 26 mg/day. In addition, those who consumed at least 42.3 mg EPA per day were at reduced risk for advanced AMD compared to those consuming less than 12.7 mg/day [23]. These upper levels are readily achieved by consuming a serving of most types of fish. Furthermore, a clinical trial of 12 women found that supplementation with 800 mg DHA per day for 4 months significantly increased macular pigment optical density (MPOD), a surrogate marker for retinal health, after 2 and 4 months of supplementation [31].

Despite the studies that have shown a beneficial role of omega-3 fatty acids in eye health, some studies have found no association, or even deleterious effects [32–35]. For example, results from the Carotenoids in Age-Related Eye Disease Study (CAREDS) found that those consuming the highest amounts of omega-3 fatty acids had an increased risk for intermediate AMD compared to those who consumed the least omega-3 fatty acids [36]. In addition, results from the large AREDS2 trial found that addition of DHA+EPA to the original AREDS formulation (see below for more details) did not further reduce risk of progression to advanced AMD [37].

Lastly, fish is one of the most common dietary sources of omega-3 fatty acids, and as a result its role in AMD has been investigated. While the evidence for fish is not as strong as that for EPA and DHA, it appears that 2 servings/week of fish per se may offer some benefit [3].

2. Omega-6
   Omega-6 fatty acids, including linoleic and arachidonic acids, have also been investigated for a potential role in AMD, but there is no evidence to date that suggests a benefit of increased omega-6 fatty acid consumption on AMD [3, 29, 32, 36, 38].

Monounsaturated Fats

Monounsaturated fats are typically liquid at room temperature but turn solid when chilled. Food sources include vegetable oils, peanut butter, and other nuts and seeds [26]. To date the evidence regarding monounsaturated fat does not suggest a beneficial role in AMD prevention or progression [29, 32, 36, 38, 39]. There have also been reports of increased risk of AMD with increasing consumption of monounsaturated fatty acids [29, 32, 34].

Saturated Fats

Saturated fats are typically solid at room temperature and food sources include butter, cheese, and lard [38]. Elevated saturated fat intake has been associated with several adverse health outcomes [40], and no retinal benefits have been seen with increased saturated fat consumption [33, 36, 38, 39, 41]. Indeed, those with a high consumption of saturated fatty acids appear to be at increased risk for AMD [29, 32].

Cholesterol

Cholesterol intake is often monitored due to its relationship with poor heart health. Cholesterol has been related to AMD with mixed findings with most studies finding no association [3, 29, 42]. There have also been some reports of increased risk for early and late AMD with increased cholesterol intake [3, 29, 32–34, 39].

Total Fat

Similar to cholesterol, the findings regarding total fat intake and AMD risk/progression are mixed with most studies finding no association [32, 33, 39]. In addition, several prospective studies have found that a high total fat intake may increase risk for AMD [3, 33, 41, 43].

Carotenoids

Briefly, carotenoids are the yellow, orange, and red pigments that are synthesized by plants and give certain foods color. For example, carrots are a good source of beta-carotene, a bright orange carotenoid. The body can turn some carotenoids into vitamin A, a nutrient that plays a role in signaling in photoreceptors.

1. Lutein and Zeaxanthin
   Lutein and zeaxanthin are the only carotenoids found at appreciable levels in the macula, and therefore may play an important role in eye health [44–48]. Lutein and zeaxanthin function in the eye as antioxidants as well as filters of damaging blue light [3]. Observational studies suggest that lutein and zeaxanthin are associated with delaying progression of AMD. Several studies have reported that increased lutein and zeaxanthin intakes and blood levels may be associated with reduced risk for neovascular AMD [49–51]. Interestingly, the effects of lutein/zeaxanthin on AMD risk may be related to the age of those consuming them, as analysis of national survey data (NHANES III) revealed that among those aged 60–79, those who consumed the highest level of lutein/zeaxanthin had reduced risk for late AMD, but this trend was not seen in the overall population [3, 52].

   Several intervention trials have found that supplementation with lutein/zeaxanthin resulted in increased MPOD, visual acuity, and contrast sensitivity. One study reported a 43 % reduction in risk for advanced AMD in those with the highest intake of dietary carotenoids compared to those with the lowest [49, 53]. Another study, the Lutein Antioxidant Supplement Trial (LAST), suggested that lutein supplementation may be of the most benefit for high risk patients, i.e., those with the lowest baseline levels [54].

   However, despite these encouraging results, some studies have reported no effect or of lutein/zeaxanthin on AMD risk [3, 55–61]. This may be due to competition between the carotenoids in the eye, or different absorptions in different people [3, 62].

   One of the goals of the large scale multicenter placebo controlled randomized clinical trial, AREDS2, was to evaluate the efficacy and safety of lutein and zeaxanthin supplementation in reducing the risk for AMD development. Primary analysis did not reveal any clear benefit from the supplementation of lutein/zeaxanthin on AMD risk. However, secondary exploratory analysis suggested that lutein/zeaxanthin are helpful for reducing AMD risk, reporting a 10 % reduction in progression to advanced AMD in those taking the AREDS formulation with lutein/zeaxanthin compared to those who took the AREDS supplement without beta-carotene [63]. It was suggested that lutein/zeaxanthin may serve as an appropriate substitute for beta-carotene for new AREDS supplement formulations [64].
2. Beta-carotene
   Beta-carotene is a carotenoid commonly found in fruits, vegetables, and whole grains. Beta-carotene is often referred to as pro-vitamin A as it is converted to vitamin A after absorption [3]. One study found that increased intake and blood levels of beta-carotene reduced risk for neovascular AMD and another also found that those with late AMD had significantly lower blood levels of beta-carotene [49, 50]. In contrast, an increased risk for the development of AMD has been reported in those with high dietary intake of beta-carotene [65–67]. Additional studies have not found an association between beta-carotene and AMD risk [3].

   It is important to note that there may also be adverse side effects of beta-carotene supplementation, particularly the development of lung cancer in smokers [68]. Given the potential adverse events, and the lack of evidence supporting a role of beta-carotene in preventing or delaying AMD, increased beta-carotene consumption for the purpose of AMD prevention should be discouraged [67].
3. Alpha-carotene
   Alpha-carotene is an isomer of beta-carotene. One study found that higher blood levels of alpha-carotene were associated with a decreased risk for neovascular AMD [50]. Another reported that

those with higher past intake of dietary alpha-carotene were at reduced risk for large drusen [69]. However, the remainder of studies on alpha-carotene do not report any significant role of alpha-carotene on AMD risk [3]. The current lack of evidence supporting a role of alpha-carotene in AMD prevention suggests that further research should be done before recommendations of alpha-carotene for AMD prevention can be made.

4. Lycopene
   Lycopene is a carotenoid that is commonly found in the American diet in the form of tomato-based products [70]. Lycopene cannot be converted to vitamin A but can serve as an antioxidant in the body. Results of two studies suggested that higher lycopene blood levels were associated with a decreased risk for AMD [3, 57, 60]. However, the results from several observational studies have not found an association of lycopene intake or blood levels with AMD risk [3]. Further research should be done before drawing definitive conclusions regarding lycopene and AMD.
5. Cryptoxanthin
   Cryptoxanthin is another carotenoid that is commonly found in avocados, basil, and mangoes. In the body, cryptoxanthin can be converted to vitamin A and can serve as an antioxidant. Similarly to beta-carotene, it has been reported that increased blood levels of cryptoxanthin were associated with decreased risk for neovascular AMD and those with late AMD have significantly lower levels of beta-cryptoxanthin [50, 56]. The remaining studies regarding cryptoxanthin and AMD risk report no significant effect [3].
6. Total Carotenoids
   Levels of total carotenoids have also been analyzed for their potential role in preventing or delaying AMD. It has been reported that increasing intake and blood levels of total carotenoids reduces risk for neovascular AMD [49]. Interestingly, a small case control study found that total carotenoid plasma levels were lower in patients with late AMD compared to those in earlier stages of the disease, although there was no difference between those with AMD and healthy controls [71]. This may imply that carotenoids play a more important role in progression of AMD as opposed to AMD onset [3]. Additional studies have not found any association of total carotenoids and AMD risk, warranting further research [3].

Vitamins

1. Vitamin A
   Vitamin A is an important nutrient for eye health as it is needed by the retina to form light absorbing molecules. In addition, many carotenoids are by-products of vitamin A. As a result, vitamin A has been investigated for its role in AMD. While two studies have found a reduction in risk for AMD or appearance of drusen with increase vitamin A intake [69, 72], the majority of studies have no found significant associations of vitamin A intake and AMD [3].
2. Vitamin C
   Vitamin C is a potent antioxidant. Given that oxidative stress plays a role in AMD development, one might anticipate that vitamin C would be related to risk for AMD. However, the majority of studies have found little evidence to suggest that vitamin C alone is related to the development and/or progression of AMD [3]. If one subscribes to the hypothesis that an aqueous antioxidant might work best in aqueous environments, then perhaps is it not surprising that vitamin C is without major effect in the lipid rich retina. Nevertheless, elevated the AREDS supplement provides 500 mg/day.
3. Vitamin D
   Vitamin D is being investigated for its role and potential benefits in various chronic diseases. There are a small number of studies investigating the role of vitamin D in AMD. One study reported a reduced risk for soft drusen in those with the highest serum levels of vitamin D compared to those with the lowest levels [73]. Another study reported a significant association between higher levels

of vitamin D and decreased risk for AMD in women over the age of 75 [74]. Studies looking at milk and fish consumption, two of the most common sources of vitamin D in the American diet, have reported a reduced risk for certain types of AMD with regular milk and fish consumption. The data available to date suggests a protective effect of vitamin D on risk of certain types of AMD, and further research is warranted [3].

4. Vitamin E
   Vitamin E is thought to be a lipophylic antioxidant that has been suggested to play a role in AMD as several case control and cross-sectional studies have found that increased vitamin E intake may reduce risk for AMD [56, 71, 75–81]. While the AREDS supplements do contain high levels of vitamin E, the salutary effect of vitamin E has yet to be confirmed by clinical trials. Moreover, two studies have suggested that vitamin E supplementation may increase risk for AMD [65, 82]. Given this, vitamin E may not play a strong preventative role in AMD by itself [3].

Minerals

1. Zinc
   Zinc is an important trace element for the body, with roles in a number of physiological processes such as immunity, reproduction, and neuronal development [67, 83, 84]. The concentration of zinc is very high in the retina, and thus it has been hypothesized that zinc supplementation may be of benefit for retinal health [3]. Data regarding the role of zinc in AMD development and progression is mixed. While some observational studies report that those with the highest intake of zinc have reduced risk for AMD, others have found no effect [61, 85].

   In the AREDS trial, zinc was included in the antioxidant cocktail at 80 mg of zinc oxide. Among participants with later stages of AMD, those who consumed zinc were less likely to progress to more advanced stages of AMD than those who did not consume zinc. However, this was not significant when analysis included those with earlier stages of AMD [3, 86]. One intervention trial found that 100 mg of zinc twice a day for 2 years reduced vision loss without any adverse effects [87], and another trial found that supplementation of 25 mg zinc twice a day for 6 months improved several indicators of retinal function such as visual acuity and contrast sensitivity [88]. In contrast, two intervention trials have found either no association between risk for AMD and zinc intake [89] or an increased risk for AMD with increased zinc [82]. Importantly, the AREDS 2 trial lowered the zinc level in the antioxidant cocktail to 25 mg of zinc, and reported no significant changes in the effectiveness of the formulation [90]. Presently, the evidence available to date suggests that zinc may promote retinal health, but further investigation is warranted [3].

Combinations/Multivitamins

Nutrients often work together to affect metabolism, so groups of nutrients may be more effective in modulating disease progression than single nutrients [3]. The AREDS study, in which participants received either an antioxidant cocktail or placebo, indicated that supplementation with the cocktail of beta-carotene, vitamin E, vitamin C, zinc, and copper reduced progression from intermediate to advanced AMD by 34 % over approximately 6 years of follow up [91, 92]. The AREDS2 trial tested modifications to the original formulation including adding lutein/zeaxanthin, omega-3 fatty acids, removing beta-carotene, and lowering the dose of zinc. Overall, changes in the formulation did not result in significant changes in the effectiveness of the cocktail. For participants with very low levels of lutein/zeaxanthin in their diet, the new formulation did help lower their risk for advanced AMD. In addition, given the concerns regarding beta-carotene and lung cancer in smokers, it was found that removing beta-carotene did

not significantly change the effectiveness of the formulation [90]. Thus, the current standard of care is recommendation of AREDS supplements to patients with incipient AMD.

Another trial showed that supplementation with vitamin C, vitamin E, zinc, copper, lutein, zeaxanthin, and astaxanthin improved function in the central retina [93]. Yet another trial reported that those supplemented with vitamin E, zinc, magnesium, vitamin B6, and folate for 18 months maintained visual acuity compared to the placebo group which experienced a decrease in visual acuity [94]. In addition, another trial found that an antioxidant and omega-3 fatty acid supplement also maintained visual acuity over 56 months compared to a placebo group, which lost visual acuity [95].

There is some evidence that challenges a role for antioxidant combinations in preventing AMD, with several studies reporting no effect of multivitamin/antioxidant supplements on risk for AMD [51, 61, 85, 96, 97]. The use of antioxidant combinations for diminishing the risk of AMD deserves additional study. However, given that multivitamins do not appear to have harmful effects to the eye, their use may be advisable [3].

Dietary Patterns

As shown throughout the above sections, different nutritional factors may influence the development or progression of AMD. The role of single nutrients in AMD risk is often hard to determine given inconsistent findings across studies. In addition, we eat combinations of nutrients, not single nutrients, and it is often difficult to disentangle single nutrients from the diet or lifestyle as a whole. Examining the diet as a whole in relation to AMD may better account for the relationships among different components of the diet [3]. As a result, diet may be a modifiable risk factor for AMD. Chiu et al. recently reported that overall diet is significantly associated with the odds of AMD. It was found that the Oriental dietary pattern (consisting of high intake of vegetables, legumes, fruits, whole grains, tomatoes, and seafood) was associated with reduced odds for AMD, with closer adherents to this pattern gaining larger benefits. In contrast, the Western dietary pattern (consisting of high red meat, processed meat, high fat dairy, French fries, refined grains, and egg intake) was associated with increased odds for AMD [98]. Similarly, analysis of food frequency data from the Melbourne Collaborative Cohort Study found that a dietary pattern that was high in fruit, vegetables, chicken, and nuts, and a pattern low in red meat was associated with a lower prevalence of advanced AMD [99]. Analysis of women who completed a Healthy Eating Index assessment (a measure of diet quality according to federal dietary guidelines) found that women who had the highest modified Healthy Eating Index (HEI) Score were at 46 % lower odds for early AMD than those women who had the lowest mHEI score. The diet of those women with a high mHEI score contained a higher median servings of fish, vegetables, dairy, grains, and meats. Women who had the highest healthy lifestyle score (HLS), which reflected healthy physical activity, smoking and diet scores, had 71 % lower odds for early AMD compared to those with lower scores [100]. It is important to note that the results of studies from dietary pattern analysis do not reveal absolute amounts of certain foods to eat or to avoid. They do however, provide guidelines of what pattern of food intake to consider when trying to reduce AMD risk [99].

Conclusion

AMD is the leading cause of vision loss in the elderly, and the aging population. Therefore, it is crucial to determine effective means to prevent or delay the onset of AMD. Dietary modifications are attractive strategies because they may be more affordable than clinical therapies, do not require specialists for administration and may be of benefit with few, if any, side effects [4]. Several adjustments of diet may

also be achievable with minor dietary modification. It appears that a diet rich in fruits and vegetables, containing adequate fish and low glycemic index may support retinal health. Supplementation can be considered when there is not sufficient nutrient supply from the diet, and a multivitamin may be advisable and safe addition to the diet.

Disclaimer Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Department of Agriculture.

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Chapter 28
Micronutrients and Immunity in Older People

John D. Bogden and Donald B. Louria

Key Points

- Placebo-controlled clinical trials, despite their limitations, are the best approach for studying effects of micronutrients on immunity.
- High doses of some single nutrients may improve immunity in relatively short time periods—weeks to months, but persistence of these effects is not known at this time. High doses of other micronutrients may adversely affect immunity.
- Some micronutrients may interfere with the beneficial effects of other micronutrients on immunity; this effect will depend on relative doses.
- Low to moderate dose multivitamin/mineral supplements may require considerable time (6 months to a year or more) before they enhance immune functions and reduce susceptibility to infectious diseases, and the timing of their effects may differ in men and women.
- High and even low-dose micronutrient supplements may enhance immunity even in the absence of evidence of underlying deficiencies.
- Long-term ingestion of single nutrient supplements, especially at high doses, may have beneficial and/or adverse effects on immunity and other outcomes.
- Micronutrient supplements are not a substitute for a good diet and regular exercise, but rather are a complementary measure.
- Micronutrient status may influence outcomes of pneumonia and other infections in older people.

Keywords Elderly • Immunity • Infection • Micronutrients • Minerals • Vitamins

Introduction

Aging has been described as a group of processes that promote vulnerability to challenges, thereby increasing the likelihood of death. Since there is evidence that depressed immunity can increase the risk of death, it is likely that changes in immunity with age are key factors in the aging process.

Theories of aging include the free radical, programmed senescence, and immunologic theories [1]. Evidence for the immunologic theory of aging is based largely on the well-described changes with age that occur in various species that have been studied, including humans, and on observations from cross-sectional studies that demonstrate an association between maintenance of good immune function and longevity [1, 2].

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_28,

A limitation of this theory is that it lacks the universality of other theories, such as the free radical theory of aging, since it is not applicable to lower organisms that do not have well-developed immune systems. Of course, the complexity of aging may require the use of more than one theory to understand it, and the various theories are not necessarily independent of one another. For example, there is evidence that demonstrates that antioxidant nutrients that reduce free radical damage can improve immunity in older people [3], suggesting that the free radical and immunologic theories may be complementary.

Aging and Immunity

General Changes in Immunity with Aging

It is useful to distinguish between primary changes that develop due to the age-dependent intrinsic decline of immunity and secondary changes that are the result of "environmental" factors such as prescription and nonprescription drug use, physical activity, and diet. In fact, Lesourd and Mazari [4] have suggested that secondary rather than primary changes in immunity with age are more likely to explain the increased incidence and severity of infectious diseases in older people.

Changes in immunity with aging include inhibited T lymphocyte functions, decreased antibody production and responses, increased autoimmune activity with compromised self nonself discrimination, and greater heterogeneity in immunologic responses [5–9]. With regard to the latter, depressed T cell function is the most common and may begin as early as the sixth decade. However, T cell dysfunction is neither inevitable nor predictable. For example, we [8] measured delayed hypersensitivity skin test responses in 100 people aged 60–89. We found that although 41 % were anergic to a panel of seven skin test antigens and an additional 29 % were "relatively anergic," responding to only one of the seven antigens, the remaining 30 % were reactive, responding to two or more of the skin test antigens, often with sizable reactions (Fig. 28.1).

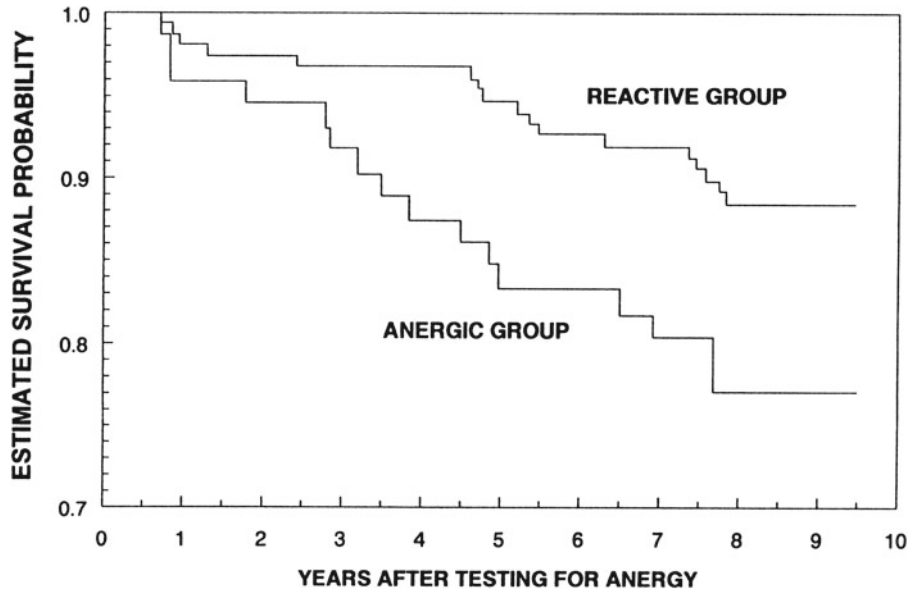

Fig. 28.1 Kaplan–Meier curves of all-cause mortality for initially anergic and reactive older people during 10 year follow-up period. Subjects ($n=273$) were aged 60 or older and apparently healthy at enrollment. Participants were considered anergic if their responses to each of four skin test antigens were less than 5 mm of induration. Most of the excess mortality in the anergic group occurred within 5 years of enrollment. Adapted from Wayne et al. [20]

Table 28.1 Some specific changes in immunity with aging

Involution of the thymus
Decreased thymic hormone concentrations
Decreased delayed hypersensitivity skin test responses
Decreased interleukin-2 secretion
Decreased lymphocyte proliferative responses to mitogens
Lower antibody titers after vaccination
Increased serum autoantibodies
Increased soluble interleukin-2 receptors
Reduced phagocytosis by polymorphonuclear leukocytes
Reduced intracellular killing by polymorphonuclear leukocytes

Specific Changes in Immunity with Aging

Involution of the Thymus

The most striking changes in immunity with increasing age are inhibited T cell functions (Table 28.1). These are likely related to the well-known involution of the thymus [10]. The differentiation process by which stem cells become T lymphocytes occurs in this organ. It is a two-lobed structure in mammals, located in the thorax above the heart. There are several stages in the process by which immature stem cells (pre-T cells) become mature T cells. These include migration to the thymus, where some cells are stimulated to grow and others die; differentiation, in which the mature phenotype of T cells develops in the thymus, including surface expression of accessory molecules; positive selection, in which self-major histocompatibility complex (MHC)-restricted T cells are selected and other cells rejected; and negative selection, which ensures that surviving mature T cells are self-tolerant. The selective survival or death of cells results in a self-MHC-restricted, self-antigen-tolerant, mature T cell population [10].

The thymus is the principal site of T cell maturation. Involution with age occurs soon after puberty. Since some maturation of T cells continues throughout adult life, it is likely that a remnant of the thymus or some other tissue continues to effect T cell maturation [10]. However, since memory T cells have a long life span of 20 years or more [10], the involution of the thymus does not cause compromised immunity in young adults, but is likely to contribute to depressed immunity as the time since thymic involution increases.

The involution of the thymus prior to the peak reproductive years suggests that this process may provide an evolutionary advantage. One hypothesis is that involution provides a net benefit since it reduces the risk of autoimmune reactions [11]. According to this theory, the increased risk of cancer or infectious diseases due to depressed cellular immunity is a detriment that is offset by a reduced risk of autoimmune disease that accompanies thymic involution. Although attractive, this theory of immunologic "trade-offs" as an adaptation to aging requires additional supporting evidence.

An alternative hypothesis has been proposed by Siskind [12], who suggests that adaptation to environmental pathogens occurs early in life and, thereafter, relative constancy of immune function rather than adaptability may be most beneficial. He further speculates that efforts to modify cellular immunity in later life, e.g., by pharmacological or nutritional means, may do more harm than good. Though interesting, this hypothesis is not widely supported and not consistent with the known association between good cellular immunity and reduced morbidity and mortality in older people.

T Lymphocyte Functions

Changes in T lymphocytes with aging include a shift in relative percentages of subpopulations, and qualitative changes in cell surface receptors [13]. In comparison to T cells from younger people, cells

of the elderly are deficient in in vitro production of certain T cell growth factors such as interleukin-2 (IL-2) and have a decreased ability to bind and respond to it [14–17]. McMurray [15] has outlined evidence that implicates nutrient-mediated effects at virtually every step in the development and expression of T cell immunity, from direct effects on the thymus and thymic hormone production through T cell maturation and distribution, antigen reactivity, lymphokine production, and even composition of the T cell membrane.

Delayed hypersensitivity skin test (DTH) responses involve T lymphocyte proliferation, production of IL-2 and other lymphokines and infiltration of the test site with mononuclear cells, resulting 24–72 h later in induration and erythema; it is the T cell parameter that is most consistently and profoundly affected by nutritional status [15]. Reduced DTH is also the immune parameter most consistently associated in older people with increased infectious disease morbidity and mortality from all causes, as found by Meakins et al. [18], and Christou et al. [19] for surgery patients and Wayne et al. [20] and Roberts-Thomson et al. [21] for initially healthy people aged 60 or older.

In their investigation, Christou et al. [19] studied the relationship between presurgery DTH responses and postsurgical sepsis-related death in 245 subjects with a median age of 67 years and a range of 24–98 years. Initially anergic subjects experienced significantly more postsurgical mortality than those who were reactive. Since all the subjects had gastrointestinal cancers that prompted the decision to operate, it could be argued that the initial severity of the disease increased both the incidence of anergy and the risk of dying postoperatively. Thus, initial disease severity could explain the apparent strong relationship between preoperative DTH responses and postsurgical mortality. However, the study of Wayne et al. [20] did not have this confounder since they looked prospectively at healthy adults over a 10-year time period. In this investigation, the authors followed 273 initially healthy subjects aged 60 or older with no history of serious medical problems. DTH responses to four recall antigens were measured at enrollment. Anergy (failure to respond to any skin test antigen) at enrollment in the study was associated with a significantly increased risk of dying in the 10 year follow-up period. At the end of 10 years, 11 % of the initially reactive subjects had died compared to 22 % of the anergic participants. The study demonstrates that anergy to skin test antigens, even when present in healthy older people, is associated with subsequently increased all-cause mortality. The authors also found a 2½-fold increase in cancer mortality in the initially anergic group in comparison to the reactive group. However, this was not statistically significant, probably because of the relatively small number of cancer deaths observed.

Evidence for the decline in T cell function with age includes a considerable number of studies that demonstrate reduced lymphocyte proliferative responses (LPR) to mitogens or antigens, as well as depressed DTH responses to recall antigens [7, 16–23]. Indeed, these two measures of T cell function have been the most widely studied functional tests done in conjunction with assessment of the effects of nutritional intervention on immunity. A problem with lymphocyte proliferative responses to mitogens is the considerable variability of these assays, even in laboratories with rigid quality control procedures.

There is some evidence for changes in T lymphocyte subsets with aging, in particular decreases in CD4+, increases in CD8+ cells, and decreases in the CD4+/CD8+ ratio [9]. There is also evidence that lymphocyte subsets are altered in older people who are ill. For example, Markewitz et al. [24] have found that immunosuppression in cardiopulmonary bypass surgery patients aged 55 or older is associated with decreased CD4+ T cells and increases in CD8+ T cells. Higa et al. [25] have found that increases in CD8+ T cells predict a longer period of recovery after onset of acute herpetic pain during herpes zoster infection. The increased incidence of this disease in older people is thought to be due to the depressed cellular immunity that occurs with age [14].

Measurement of lymphocyte subsets is a key component in evaluation of immune function [26, 27]. Knowledge of lymphocyte subset numbers (cells/mL and percent of total) allows determination of relationships between immune functions and the number and percentage of cells responsible for

these functions. This can permit distinguishing between effects due to increased numbers of a particular subgroup of cells versus enhanced activity by the same number of cells. The latter could be related to antigen binding capacity per cell. Indeed, changes in antigen binding capacity per cell could be a mechanism by which micronutrients influence immune functions. However, the effects of changes in antigen binding capacity per cell on declining immunity with age are largely unexplored.

Other Immune System Changes with Aging

There is some evidence for a decline in B cell functions with age, although it is likely related at least in part to the T cell dependence of B cell functions. Older people vaccinated with tetanus toxoid, varicella-zoster, or hepatitis B antigens display reduced antibody production as well as a greater percentage of nonresponders compared to young adults. This may also be true after pneumococcal and influenza virus immunization, though the evidence is not as convincing [28].

Perskin and Cronstein [29] have reported that aging produces alterations in neutrophil plasma membrane viscosity that may result in compromised neutrophil function and increased susceptibility to infection with specific pyogenic bacteria. This is consistent with studies of Nagel et al. [30], Shoham-Kessary and Gershon [31], and Corberand et al. [32] that suggest compromised in vitro activity of neutrophils from older people. Depending on the microorganism studied, the neutrophil activity that was depressed was phagocytosis or intracellular killing.

A review by Makinodan et al. [33] suggests that although antigen-responsive cells such as B cells, monocytes, and killer cells are vulnerable to aging, T cells are clearly the most vulnerable. This is the reason that many of the studies of nutrition, immunity, and aging have focused on T cell functions.

Sen et al. [28] have published an insightful review that distinguishes between an increased incidence versus greater severity of infectious diseases in older people. For example, they report an increased case-fatality ratio for bacterial meningitis and pneumococcal pneumonia in older people, and an increased incidence of diseases such as urinary tract infections and varicella zoster. Other diseases such as influenza virus infection and gram-negative sepsis are both more frequent and more severe in older people. They suggest that in addition to changes in immunity with age, urinary tract, respiratory tract, and neurological changes may contribute to the increase in infectious disease morbidity and mortality in older people.

Relationships among the interleukins, their receptors, and immunity have been widely discussed in the recent literature. Of particular interest in the elderly is interleukin-2 (IL-2) because its production is decreased in older people [9]. Interestingly, soluble IL-2 receptor (IL-2R) levels are higher in older than in younger adults [34] and it has been suggested that this may be a factor in the decline of cellular immunity with age since high serum concentrations of soluble IL-2R may compete with and decrease IL-2 binding to T cell IL-2 receptors and thereby compromise immunity [35, 36]. We have previously found that serum IL-2R concentrations are relatively lower in physically active older people compared with sedentary seniors and that exercise/physical activity habits and multivitamin supplementation may interact to influence soluble serum IL-2R concentrations [37]. We have also verified the higher levels of soluble IL-2R in older people (unpublished data).

The immunity of the very old may in part explain their survival to an advanced age. The oldest people, including centenarians studied by Sansoni et al. [38], tend to have well-preserved immune functions, such as natural killer cell activity, that are often better than those age 50–80 years. In addition, those above age 90 tend to have lower serum autoantibody concentrations than those in the 60–80 year range [14, 39]. Thus, preserved immune functions and reduced autoimmunity appear to be associated with the ability to live to age 90 and beyond.

Micronutrients and Immunity

Nutrition, Immunity, and Aging

Scrimshaw and San Giovanni [40] have noted that infections, no matter how mild, can adversely affect nutritional status, which in turn can compromise immunity and exacerbate the effects of infection. They discuss evidence for the effects of various micronutrient deficiencies on immunity, including beta-carotene; pyridoxine; folic acid; pantothenate; vitamins A, B12, C, D, and E; and the trace elements iron, zinc, and copper, and magnesium. In general, cell-mediated and nonspecific immune functions are more sensitive to single micronutrient deficiencies than humoral immunity.

Fraker [41] has noted that the immune system is a large "organ," comprised of the blood, spleen, lymphatic system, thymus, and other components. In addition, millions of new immune system cells are produced daily. Its large size and high cellular turnover combine to make the immune system a major user of nutrients. Thus, it is not surprising that some aspects of immunity are very sensitive to nutritional deficiencies.

One key question is whether the decline in immunity with aging is due, at least in part, to nutritional deficiencies and/or increased requirements. Another question is whether micronutrient supplementation might improve immunity even in the absence of an underlying "deficiency," defined by factors such as low circulating nutrient concentrations or consistently low intakes.

Human studies of protein–calorie malnutrition (PCM) in underdeveloped countries or in hospitalized adults demonstrate a causal association between undernutrition and secondary immunodepression that results in diminished resistance to infectious diseases [14, 15, 42, 43]. This association is consistent enough to permit the use of DTH in medical and surgical patients as a predictor of clinical prognosis [19]. Thus, there appears to be little doubt that severe malnutrition has a major impact on resistance to disease that is mediated in part through the immune system. There is also evidence that moderate to marginal undernutrition may compromise immunity [44, 45].

McMurray [15] has noted that dietary deficiencies, both moderate and severe, of specific nutrients profoundly alter cell-mediated immune responses in humans and experimental animals. Diets with inadequate contents of calories, protein, vitamin A, pyridoxine, biotin, or zinc can result in depressed production of thymic hormones critical for T lymphocyte differentiation. Reduced numbers and depressed in vitro function of T cells have also been reported in experimental deficiencies of zinc, copper, iron, and vitamins A and E. Depressed DTH responses are a consistent result of dietary inadequacies of protein, pyridoxine, iron, zinc, and vitamins A and C.

A comprehensive review by Beisel [46] extensively examined the older literature up to 1982 on single nutrients and immunity. The water soluble vitamins that appear to be most critical for maintaining immunity are vitamin B6, folate, vitamin B12, and vitamin C. Among the lipid soluble micronutrients, vitamins A and E appear to exert the most significant impacts. Trace metals that exert substantial influences on immune functions are iron, zinc, selenium, and copper [15, 46].

Since the variability in immune responses increases with aging, subgroups that have impaired immunity because of nutrient deficiencies are more likely to be observed in the elderly than in other age groups. In addition, when episodes of nutritional vulnerability overlap with suboptimal immune function, an adverse synergistic interaction is possible [15]. These factors make it more rewarding to study nutrition/immunity relationships in older rather than in younger adults. In 1985 Beisel [47] noted that individual studies of immunity in humans have not been systematic or comprehensive; despite the passage of 30 years, this continues to be an accurate observation. The lack of comprehensive studies is no doubt related to the very considerable expense that would be incurred in studying multiple immune responses in a sizeable number of older people.

It can be useful to compare relationships between nutrition and immunity in healthy older people with these relationships in diseases in which immune functions are compromised. In the case of HIV

infection, we [48] have found that compromised nutritional and antioxidant status begins early in the course of infection and may contribute to disease progression. This observation can be compared to the decline in cellular immunity that begins in many older people in the fifth or sixth decade and is associated with a reduced life expectancy [2, 5, 20].

Cross-Sectional Studies on Micronutrient Nutrition and Immunity

Goodwin and Garry [49] compared immunological functions of healthy elderly New Mexico residents consuming higher than RDA levels (5× RDA or greater) of micronutrients with similar individuals not taking supplements. Micronutrients evaluated were vitamins A, C, D, and E, the B vitamins, iron, calcium, and zinc. There was no significant difference between the two groups in DTH responses or in vitro lymphocyte proliferative responses to mitogens. The authors suggested that the immune-enhancing properties of high doses of vitamins might be the result of a nonspecific adjuvant effect that does not persist with time.

More recently the same authors [50] studied 230 healthy older men and women to determine if subclinical micronutrient deficiencies could contribute to the depressed immunity found in many of the elderly. Immune functions studied included DTH responses, in vitro lymphocyte proliferative responses to PHA, lymphocyte counts, and levels of serum autoantibodies. Spearman correlation coefficients were calculated to assess associations between blood micronutrient concentrations and selected immune functions. The authors also compared subjects with the lowest responses to those with the highest. There were no significant associations between low serum micronutrient concentrations and immune functions, and the authors suggested that subtle nutrient differences did not appear to contribute to the immunodeficiency of aging. However, the population sample studied was relatively affluent and people taking prescription drugs or daily over-the-counter medications, as well as those with a serious medical problem, were excluded. Thus, the study may have excluded those subjects who might benefit most from micronutrient supplements.

Kawakami et al. [51] studied 155 healthy subjects aged 20–99 years and suggested that cell-mediated immunity was reduced as a result of malnutrition.

In a more recent study [52] we examined relationships between immunity and dietary and serum antioxidants, B vitamins, essential trace metals, and serum homocysteine in 65 older men and women aged 53–86 years. Subjects who had used vitamin or mineral supplements in the preceding 3 months were excluded. Soluble serum interleukin-2 receptor concentrations (sIL-2R) were positively associated with body mass index and serum concentrations of homocysteine, and negatively associated with serum beta-carotene and dietary lycopene. This is a logical result that suggests that systemic inflammation, for which elevated sIL-2R levels are a marker, is increased by obesity and factors that increase serum homocysteine, but reduced by dietary antioxidants. In a multiple regression model, the above 4 factors and serum vitamin B_6 concentrations explained 52 % of the variability in sIL-2R. The percentage of subjects with anergy to a panel of 7 recall skin antigens was 25 %, and these responses were negatively associated with T helper cell number, suggesting the reduced numbers of the latter as a factor that may have contributed to the anergy of the subjects. T helper cell numbers were positively associated with serum copper, and natural killer cell numbers were positively associated with dietary folate and vitamin B6. These results document significant relationships between micronutrient nutrition and immunity, and suggest that IL-2 may be influenced by dietary antioxidants and B vitamins, including those that modify homocysteine metabolism.

Sundaram et al. [53] studied the relationship between serologic responses to influenza vaccination and serum concentrations of retinol, alpha-tocopherol, and zinc in 205 men and women aged 65 or older. They hypothesized that lower concentrations of these three micronutrients would be associated with decreased hemagglutination inhibition responses to an influenza vaccine. However, they found no

significant associations between serum concentrations of the above three micronutrients and serologoic responses to influenza vaccination. This result might be explained by the fact that none of the men and women they studied had low serum retinol or alpha-tocopherol levels, and only 20 % had low zinc concentrations. However, in another study described by Barnett et al. [54] moderate zinc deficiency was associated with increased incidence and severity of pneumonia. No studies have addressed the hypothesis that mild to moderate zinc deficiency, which is common in older men and women in the USA and many other countries, can impair serologic responses to the various pneumonia vaccines.

The above studies were not attempts to intervene by provision of micronutrient supplements, but were assessments of associations between the subjects' usual intakes or blood concentrations and selected immune functions. Variables that cannot be controlled in cross-sectional studies may mask associations between nutritional factors and immunity, especially since immunity is likely to be dependent on a number of factors, only one of which is nutritional status. Such studies are valuable as a way to identify nutrients for more intensive study, but can only provide statistical associations that may not be cause/effect relationships. The latter can be assessed by standard placebo-controlled clinical trials.

Clinical Trials of Single Micronutrients

Several clinical trials have been conducted during the last three decades. These have included depletion/repletion studies in young volunteers and provision of micronutrient supplements to older people who did not appear to have preexisting deficiencies. Jacob et al. [55] studied the effects of short-term ascorbate depletion on immunity and other factors in young adult males confined to a metabolic ward. Ascorbate depletion was achieved using daily doses of 5-20 mg/day, while repletion was achieved with doses of 60 (the RDA at that time) to 250 mg/day. Although lymphocyte proliferative responses to mitogens were not affected by ascorbate depletion/repletion, delayed dermal hypersensitivity skin test (DTH) responses to a panel of 7 recall antigens were markedly depressed by ascorbate depletion. Repletion for 28 days at either 60 or 250 mg/day did not restore the mean antigen score to the predepletion level, though there was some improvement in induration in 3 of the 8 men studied. These results suggest that DTH is more sensitive to ascorbate depletion than mitogen responses. They further suggest that the repletion period was of insufficient duration to produce a return of DTH to baseline levels and/or the repletion doses were not large enough.

Fuller et al. [56] studied the effect of beta-carotene supplementation on the UV-radiation induced photosuppression of DTH in 24 young adult males, aged 19-39 years. They found that exposure to a UV–A/B light source over a 16-day period significantly reduced DTH responses in a control (placebo) group to 39 % of the initial values, but did not induce significant reductions in a group given 30 mg beta-carotene per day. This group repeated the study in elderly men and found similar effects of UV suppression that was prevented with beta-carotene supplementation, though, as could be anticipated, there was more variability in DTH responses in the older people compared to young adults [57, 58].

Watson et al. [59] investigated the effects of beta-carotene on lymphocyte subpopulations in male and female subjects with a mean age of 56 years. Beta-carotene was given at doses of 15, 30, 45, or 60 mg/day for 2 months. Using monoclonal antibodies to identify lymphocyte subsets, they found that the percentages of T helper and natural killer cells, as well as cells with IL-2 and transferrin receptors, were increased in a dose-related fashion. There were no significant effects of beta-carotene on T suppressor cells. However, the number of subjects in each treatment group was only 3–5; thus, further investigation is needed to confirm these findings.

Santos et al. [60] found that men participating in the Physicians' Health Study who consumed 50 mg of beta-carotene on alternate days for an average of 12 years had significantly greater natural killer cell activity than controls given placebos. Surveillance by natural killer cells is considered to be

protective against the development of cancer. However, two large intervention trials have found an association between high doses of beta-carotene and the development of lung cancer in cigarette smokers [61, 62]. The role of the immune system in leading to the development of lung cancer in these studies is not known.

Talbott et al. [63] in a pilot study investigated the impact of pyridoxine on lymphocyte responses in 15 older (aged 65-81 years) mostly female subjects and found that administration of a pharmacologic dose (50 mg/day) of pyridoxine hydrochloride significantly increased in vitro lymphocyte proliferative responses to phytohemagglutinin (PHA), pokewood mitogen, and *Staphylococcus aureus*.

Meydani et al. [64] have reported that vitamin B6deficiency impairs IL-2 production and lymphocyte proliferation in older adults. Each of these measurements was reduced by about 50 % by depletion, while repletion with near RDA levels of B6 eventually increased values to about the baseline levels. Although only 8 subjects were studied, this investigation supports a number of other studies that suggest that vitamin B6 may play a key role in immune responses [65].

In another study, Meydani et al. [66] gave older people 50, 200, or 800 mg of vitamin E daily for 4–5 months. This resulted in improved antibody titers to hepatitis B vaccine and enhanced DTH responses, especially in the group consuming 200 mg of vitamin E per day. This suggests 200 mg as a recommended dose, although lower doses may be equally effective when administered for longer periods of time. In a more recent study, Pallast et al. [67] investigated the effects of 6 months of supplementation of healthy older men (aged 65–80 years) with vitamin E at doses of 50 and 100 mg daily for 6 months. There was a dose-related trend of increased DTH responses, especially in those subjects with initially low responses, suggesting that there are subgroups of older people that might benefit most from vitamin E supplements.

There has been considerable interest in the potential for zinc to improve immune functions in older people. It is clear that severe zinc deficiency in animals and people, e.g., as found in the disease acrodermatitis enteropathica, can greatly compromise cellular immunity and lead to the development of life-threatening opportunistic infections [68]. There are also reports of significant associations between plasma or cellular zinc concentrations and immune functions such as DTH responses in older people [8, 69]. However, more recent studies of the impact of zinc supplementation on immunity in older people have not been encouraging. They have demonstrated either no beneficial effect of zinc supplements on immunity or an adverse effect even when the supplements contained modest doses of zinc in the range of 15–25 mg/day [70, 71]. In the absence of an underlying deficiency, use of zinc supplements by older people, especially at doses that exceed 15 mg/day, is more likely to adversely affect immunity than improve it. The effects of zinc on immunity in the elderly have been reviewed [72].

Doherty et al. [73] studied the effect of moderate (1.5 mg/kg) versus high (6.0 mg/kg) zinc supplementation on mortality in 141 young children in Bangladesh with protein–energy malnutrition, and weight-for-age z scores of about −4.6. Mortality was significantly greater in the high-dose group, with sepsis a frequent contributing factor. The results suggest that high-dose zinc supplementation may contribute to increased risk of sepsis and mortality in severely malnourished children. Although this study involved only very young children, aged 6 months to 3 years, it suggests caution in the use of high-dose zinc supplements by any age group.

In another study conducted in 56 *Shigella flexneri*-infected children in Bangladesh, Rahman et al. [74] administered 20 mg of zinc daily in combination with a multivitamin. Controls received only the multivitamin. They found that adjunct therapy with zinc during acute shigellosis significantly improved seroconversion to shigellacidal antibody responses and also increased the percentages of B lymphocytes and plasma cells in the blood. Given the widespread presence of zinc and other nutrient deficiencies in Bangladesh, the wider applicability of these results to other populations is uncertain.

Martineau et al. [75] studied the effect of a single high dose of 2500 µg of vitamin D on antimycobacterial immunity in 192 adults living in London who had been exposed to tuberculosis patients. The vitamin D supplement significantly improved in vitro antimycobacterial immunity in the study subjects.

Clinical Trials of Combinations of Micronutrients

The studies discussed above focused on the effects of relatively large doses of individual micronutrients on immune functions. There have been a limited number of published placebo-controlled trials of the effects of multivitamin/mineral supplements on immune functions in older people.

In the first of these studies, we investigated the effects of zinc given in combination with a multivitamin on immune functions in 63 older people [76]. All subjects received a standard multivitamin/mineral supplement that contained all the essential micronutrients except zinc. In addition, subjects received 15 or 100 mg of zinc, or a placebo. Daily consumption of the multivitamin/mineral supplement for 1 year was associated with enhanced DTH and mitogen responses, but these effects were reduced and delayed by ingestion of 15 mg and especially 100 mg of zinc each day. These data suggest that interactions among micronutrients may influence their effects on immunity, and that some individual micronutrients, even at modest doses, may have unexpected adverse effects. The adverse impact of zinc is consistent with other previously cited recent studies that indicate that zinc supplements in healthy older people either do not improve immunity or adversely affect it [68, 69].

Penn et al. [77] studied the effects on immune functions of a supplement containing vitamin C (100 mg), vitamin A (8000 IU), and vitamin E (50 mg); the supplement or a placebo were administered for 28 days to the 30 elderly subjects studied. All were patients who had been hospitalized for at least 3 months. The number and percent of CD4+ and CD8+ T cells were significantly increased in the supplemented group but not in a placebo group. Proliferative responses of lymphocytes to the mitogen PHA were also significantly increased in the supplemented group by 64-283 %, but were not affected by the placebo. There was biochemical evidence of deficiencies of vitamins A, C, and/or E in 5–47 % of the supplemented subjects at enrollment into the study. Thus, it is possible that the improvement in cellular immunity in these subjects with short-term administration of vitamins A, C, and E was due to correction of underlying deficiencies that are more likely to be present in hospitalized than in independently living older people. These results suggest that this group of micronutrients may be particularly important for enhancement of immune responses in older people.

In another study, Chavance et al. [78] enrolled 218 subjects aged 60 or older who were living independently and had not used any vitamin supplements for at least the prior 3 months. They were given a low-dose multivitamin or placebo for 4 months. No clinical or laboratory assessments of immune function were conducted. The authors found no significant effects of supplementation on the incidence of infections; however, effects on the duration of each infection or the total number of days of infection were not assessed. As suggested by the authors, the failure to find any significant effects on the incidence of infections may be due to the short duration of supplementation. This is consistent with our results, which suggest that periods of supplementation of more than 6 months are required before significant improvements in immune functions occur in older people.

We also conducted a randomized, placebo-controlled, double-blind trial of the effects of RDA-level micronutrient supplementation on plasma vitamin and trace metal concentrations and immune functions in independently living healthy older subjects [79]. The over-the-counter micronutrient supplement used in the study contained RDA levels of each of the essential vitamins and low to moderate doses of minerals. Of the 65 subjects enrolled, 56 (86 %) completed the 1 year study. About 2/3 were females. As expected, there were no statistically significant effects of the placebo on plasma micronutrient concentrations. In contrast, the data for the micronutrient supplement group show statistically significant increases at 6 and/or 12 months for plasma concentrations of ascorbate, beta-carotene, folate, vitamin B6, and alpha-tocopherol. These data verify compliance of the study subjects and demonstrate that supplementation with RDA levels of the latter micronutrients can increase their plasma concentrations in older people.

Table 28.2 contains the data on DTH for all study subjects combined and for males and females separately. For induration in the placebo group, there were no statistically significant differences

Table 28.2 Delayed-hypersensitivity skin test responses of placebo and micronutrient groups[a]

	Placebo group			Micronutrient group		
Subgroup and response type	0 month	6 month	12 month	0 month	6 month	12 month
All subjects						
Positive responses	1.65 ± 0.30	1.42 ± 0.25	1.73 ± 0.29	1.45 ± 0.25[b]	1.76 ± 0.27[b,c]	2.38 ± 0.33[c]
Total induration (mm)	5.37 ± 1.02	4.76 ± 0.93	5.80 ± 0.95	5.21 ± 0.98[b]	5.73 ± 0.94[b,c]	8.40 ± 1.25[c]
Males						
Positive responses	2.93 ± 0.60	1.93 ± 0.30	2.50 ± 0.78	1.64 ± 0.33[b]	2.59 ± 0.43[b,c]	2.86 ± 0.53[c]
Total induration (mm)	8.86 ± 1.91	6.36 ± 1.29	8.88 ± 2.51	6.23 ± 1.15	8.85 ± 1.58	10.91 ± 2.08
Females						
Positive responses	1.18 ± 0.29	1.24 ± 0.31	1.45 ± 0.27	1.33 ± 0.36[b]	1.25 ± 0.29[b,c]	2.08 ± 0.42[c]
Total induration (mm)	4.08 ± 1.09	4.17 ± 1.16	4.67 ± 0.83	4.58 ± 1.41[b]	3.83 ± 0.95[b]	6.86 ± 1.49[c]

Positive responses are the mean number of antigens eliciting a response from a total of seven antigens
Total induration is the sum of the indurations of all positive responses
Within groups, values in the same row with different letter (b, c) superscripts are significantly different, $P < 0.05$ (Wilcoxon signed-rank test)

[a]Mean ± SE; $n = 26$ for placebo group (7 males, 19 females), $n = 29$ for micronutrient group (11 males, 18 females)

between the 0 and 6-month results, 0 and 12 months results, or 6 and 12 month data. Similar results were obtained for the analyses of the data for the placebo group on the number of positive responses.

For the micronutrient supplement group, there was also no significant difference for the data on induration at 0 and 6 months. However, there was a statistically significant difference between the 0 and 12 month induration results ($p = 0.005$). There was an increase in induration between 6 and 12 months, but this did not achieve statistical significance ($p = 0.056$). Similar trends were observed for the individual skin test antigens.

Similar results were also obtained for the number of positive responses in the micronutrient treatment group. The mean number of positive responses in the placebo group increased by only 4.8 % between 0 and 12 months, and induration by 8.0 %. In contrast, in the micronutrient supplement group, the mean number of positive responses increased by 64 % and induration by 61 % between 0 and 12 months. These data provide strong evidence for the enhancement of DTH after 1 year of micronutrient supplementation.

The results also suggest that some enhancement of DTH responses occurred sooner (at 6 months) in the male subjects than in the females (Table 28.1). The male subjects had significantly greater DTH responses than the females at enrollment; this is consistent with previous data that suggest that DTH responses in males may differ from those in females [80]. The diets of the male subjects differed from the females, being higher in energy intake as well as intake of individual micronutrients, and it is possible that this factor may have interacted with micronutrient supplementation to influence DTH responses. There was an increase between 0 and 12 months in the number of subjects in the placebo group with low blood concentrations of some of the micronutrients measured, specifically beta-carotene, retinol, folate, and vitamin B6. This trend differed significantly from the micronutrient group, for which the number of low values changed very little between 0 and 12 months. Thus, the improvement in skin test responses in the micronutrient group is not due to the correction of underlying micronutrient deficiencies for the nine micronutrient concentrations that we determined in blood, at least as defined by current guidelines for low circulating concentrations. The increased number of low values in the placebo group suggests that older people who do not take vitamin supplements for a year may have an increased risk of developing one or more low concentrations, particularly for vitamin B6, folate, and beta-carotene.

Our data suggest that enhancement of immune functions in older subjects by low-dose micronutrient supplementation takes approximately one year. These results also suggest that the diets of older people are inadequate in one or more micronutrients and/or that the current RDAs for one or more

micronutrients may be too low to support optimal immunity in older adults. For optimal responses, they required the RDA level of the vitamins in the supplement in addition to the amounts in their food.

It could be argued that a 60 % increase in DTH responses over a 1 year period is only a mean increase of about 5 % per month. However, this increase far exceeds the decline in DTH responses per year that occurs with aging, and thus may completely prevent it. These results suggest that older subjects who take a "one-a-day" type multivitamin supplement faithfully for at least 6–12 months, may experience a substantial improvement in measures of cellular immunity such as DTH responses. It is possible that more rapid and/or larger increases in DTH responses would occur if higher doses of micronutrients were used.

Girodon et al. [81] studied the effects of trace element and vitamin supplementation on immunity and infections in institutionalized subjects aged 65 and older in France. Subjects ($n=725$) received daily for 2 years a placebo, a trace element supplement containing 20 mg zinc and 100 μg selenium, a vitamin supplement with 120 mg vitamin C, 6 mg beta-carotene, and 15 mg of vitamin E, or both the vitamin and trace element supplements. DTH responses were not significantly influenced by any treatment, but antibody responses to influenza vaccine were improved in the groups given zinc and selenium, and the incidence of respiratory tract infection was marginally lower ($p=0.06$) in these groups. The vitamin and trace element supplements also reduced the prevalence of underlying deficiencies of these nutrients. Since these were institutionalized subjects with a high frequency of low blood micronutrient concentrations, the applicability of these results to healthy independently living people is uncertain. Nevertheless, this large study provides the first evidence that selenium may be a key nutrient in the maintenance of immunity in older people.

Winkler et al. [82] found that daily ingestion of a micronutrient supplement containing minerals and vitamins reduced the incidence and severity of the symptoms of infection with the common cold. However, the reductions in symptom "scores" were only about 19 % and the duration of infection was not reduced. Because the study subjects also ingested a supplement containing "probiotic bacteria," the influence of the micronutrient supplement alone cannot be determined. The study subjects were young adults; thus, the applicability of the results to the elderly is uncertain.

Limitations of Current Knowledge

The above studies that focused on the effects of multivitamin/mineral supplements on immune functions, in combination with the short-term higher dose single nutrient studies such as those of Meydani [64, 66], Watson [59], and Talbott [63], provide solid evidence that micronutrient supplements can enhance immune functions in older people, but data on effects on the incidence and prevalence of infectious diseases are quite limited. Despite the evidence provided by these studies, we do not know if long-term daily use of multivitamin/mineral supplements will enhance immune functions and reduce the incidence and severity of infectious diseases in older people beyond the 1–2 year duration of the longest studies done to date. This is an unfortunate gap in our knowledge, because millions of older Americans currently consume a multivitamin/mineral supplement daily, either alone or in combination with one or more single nutrients at higher doses [83, 84]. This situation is in part the result of the limited objectives of all previously completed studies. All of the single nutrient studies have been of short duration, usually using high doses of one micronutrient, given to a relatively small number of subjects. Most of these studies have not assessed the impact of single nutrient supplementation on the incidence of infectious diseases, a limitation related to the small number of subjects enrolled in these studies and their short duration, with a consequent lack of statistical power to assess disease incidence. The studies on multivitamin and/or trace element supplements also have limitations:

1. The study of Chavance et al. [78] was of only 4 months duration. Although, this study assessed the impact of multivitamin supplementation on the incidence of infectious diseases, it did not include any measures of immune function.

2. The study of Penn [77] was only 1 month in duration and included only older people who had been hospitalized for at least 3 months.
3. Our studies [75, 79] assessed DTH responses, lymphocyte proliferative responses to mitogens and natural killer cell activity, but we could not examine other measures of immunity or clinical outcomes and the period of supplementation was limited to 1 year.
4. The 2-year study of Girodon et al. [81] included only institutionalized subjects.

Thus, additional studies of micronutrient/immunity/disease relationships are required, in particular studies that focus on clinical outcomes.

Factors That Can Influence Nutrition–Immunity Relationships

Factors that may influence micronutrient/immunity relationships in older people include gender, stress, disease, physical activity and exercise, obesity, and food choices.

In our 1994 study [79] of the effects of low-dose micronutrient supplements on immunity in older people, improvements in DTH responses occurred sooner in the males than the females. Although the reason for this is not known, one possibility is that the higher intake of micronutrients from food in the men results in a larger total micronutrient intake.

There are a considerable number of reports that psychological and physiological stress in experimental animals and people can depress cellular immune functions [85, 86], though it is beyond the scope of this review to assess these studies in any detail. As an example, death of a spouse has been associated with depressed immune functions [85]. However, virtually all studies of relationships between stress and immunity have not adequately assessed nutritional factors that may be altered by stress, and in most cases have completely ignored nutrition. Physical and psychological stress can modify food intake in animals and people, and thus studies of stress/immunity relationships are usually confounded by nutritional factors that have not been adequately evaluated.

There is considerable evidence that physical activity/exercise patterns can influence immunity [87–91]. In general, the data suggest that very strenuous exercise can acutely depress immunity. For example, various studies have found that participants in marathons have a significantly increased risk of respiratory infections in the 1–2 week period following the race [88, 89]. Chronic overtraining has also been associated with depressed immunity [89]. In contrast, regular moderate exercise appears to enhance immune functions [89]. One hypothesis is that regular exercise contributes to the maintenance of muscle mass, and muscle is the source of a key nutrient, glutamine, required by lymphocytes [92]. In addition, alterations in cytokine levels as a result of regular exercise may also be a factor [90, 91, 93, 94].

In a review, Nieman [95] concluded that infection risk following intensive exercise is likely related to acute nonpersistent changes in immunity. However, unless the athlete exceeds his or her usual training limits, immunocompromise is unlikely, though further research is needed to confirm this conclusion. In general, studies of macronutrient or micronutrient supplements in combination with exercise have shown no effects on immunity. For example, in a randomized trial of 112 elderly (mean = 79.2 ± 5.9 years) men and women, Paw et al. [96] reported that exercising twice per week improved DTH skin test responses to recall antigens, but consumption of micronutrient enriched foods (25–100 % of the RDA for various micronutrients) did not enhance the effect of exercise.

Stallone [97] has outlined studies that indicate that excess body weight in humans or experimental animals is associated with impairments in host defense mechanisms. Definitive studies have not been done, but there are data suggesting both beneficial and detrimental effects of weight loss on immunity. In experimental animals, it is well known that chronically reduced energy intake without malnutrition can profoundly ameliorate the detrimental effects of aging on immunity and can increase mean and maximum life span [98].

The well-established importance of some micronutrients such as zinc in the maintenance of immune function suggests that choices of foods high in these micronutrients may be beneficial, but this has not been validated in well-controlled studies.

Because of the evolutionary development of humans as hunter-gatherers who consumed foods but not supplements, it has been argued that appropriate food choices are sufficient to achieve optimal health, including optimal immunity. There is a substantial body of evidence that supports wise food choices, including diets high in fruit and vegetable intake and low in saturated fat, as key factors preventing some chronic diseases. However, arguments based on evolution are compromised by two factors: first, that evolution has programmed humans and other species to live through our peak reproductive years, but not necessarily beyond them, and second, that our preagricultural ancestors had intakes of some nutrients (e.g., calcium, iron, and zinc) much higher than those of modern humans [99]. Olshansky et al. [100] have used the term "manufactured time" to describe use of prescription drugs and other methods to increase the odds of living beyond our reproductive years. Thus, it should not be surprising that micronutrient supplements may be particularly beneficial to the immune and other organ systems of older people.

Goodwin [101] has suggested that the relationship between depressed cellular immune function and subsequently increased mortality may be due to compromised immunity being a marker for clinically latent diseases or poor overall physiologic function. However, impaired immunity may also contribute to a reduced ability to defend against infections, cancers, and perhaps cardiovascular heart disease.

Reviews

In a review article, Bogden and Oleske [102] concluded that adverse effects from high-dose micronutrient supplements may occur in HIV-1-infected patients, but that the combination of antiretroviral drug therapy, a good diet, and a safe low-dose multivitamin/mineral supplement may improve outcomes more than pharmacologic therapy alone.

In another review, Webb and Villamor [103] concluded that vitamins C and E and the carotenoids, either individually or when combined in supplements, can influence various measures of immunity, including lymphocyte proliferation and delayed-type hypersensitivity responses. They also suggest that there is good evidence that multivitamins that include the B-vitamin group have beneficial effects on immunity and clinical outcomes in HIV-infected patients.

The NIH sponsored a conference entitled "Multivitamin/Mineral Supplements and Chronic Disease Prevention" that occurred during May 15–17, 2006. The conclusion of a comprehensive report on the conference proceedings was that there is sufficient uncertainty in the data on this subject that precludes a recommendation either in favor of or against routine multivitamin/mineral supplementation [104].

The second edition of the monograph "Food, Nutrition, and Physical Activity and the Prevention of Cancer: A Global Perspective" occurred in November, 2007 [105]. Among the conclusions of this authoritative and comprehensive report are: that there is "convincing" evidence that high-dose beta-carotene supplements are a cause of lung cancer, that calcium "probably" protects against colorectal cancer, and that selenium in high doses "probably" reduces the risk of prostate cancer. Thus, supplements may have adverse or beneficial effects on the prevention of various cancers. However, the report concludes that "a general recommendation to consume supplements for cancer prevention might have unexpected adverse effects" in the general population in whom the balance of risks and benefits cannot be predicted with confidence.

The first two reviews described above focused on the outcomes of immunity and infection, primarily HIV-1 infection, whereas the latter two focus on cancers and other chronic diseases. Thus, it is not surprising that these reviews reach different conclusions about the use of supplements for disease prevention or management.

A topic of continuing interest and debate is whether or not vitamin D status influences immune responses to infections such as tuberculosis and influenza or serologic responses to vaccination. Most recent studies and reviews conclude that vitamin D status or supplementation, with or without supplemental calcium, does not alter the incidence or duration of influenza and other infectious diseases or serologic responses to influenza vaccine [106–109].

Research Needed on Micronutrients and Immunity in Older People

Several cross-sectional studies that assess relationships between micronutrient nutrition and immunity have been done in the past 20 years [49, 50], as previously discussed. In general, significant associations between serum micronutrient concentrations or use of micronutrient supplements and various measures of immunity were found in some studies but not others [49–52]. However, these studies compared micronutrient supplement users with nonusers, but did not evaluate use of specific supplements, and it is likely that some individual or combinations of micronutrients can improve immunity and others cannot.

The clinical trials of micronutrient supplementation and immunity done to date have usually involved healthy older subjects consuming their usual diets. In the case of some single nutrient studies, subjects lived in metabolic units and consumed standardized meals that contained about the RDA of all essential micronutrients. It is possible that the improvements in immunity found in some studies are due to correction of underlying deficiencies. However, it is also likely that micronutrient supplements enhance immunity even in the absence of underlying deficiencies, at least based on current concepts of "deficiency." This should not be surprising, since optimal immune function was not a factor in establishment of the current Dietary Reference Intakes, or in defining laboratory normal ranges for circulating micronutrient concentrations. In fact, daily intakes that optimize immunity may differ from both the current Dietary Reference Intakes and intakes that may prevent chronic diseases. For example, the current DRI/RDA for vitamin E (15 mg alpha-tocopherol equivalents for adult females and males) is substantially lower than amounts that optimize immune functions or have been associated with a reduced risk of cardiovascular heart disease [66, 67, 110, 111]. Similarly, the current RDA for vitamin C is adequate to prevent development of scorbutic lesions, but appears to be less than the intake that could optimize immunity or provide other health benefits [4, 112]. Recommendations for an optimal intake of any micronutrient will need to balance the impact of that nutrient on various health outcomes as well as consider possible adverse effects of relatively high doses.

Future studies that focus on clinical outcomes and have considerable statistical power are especially needed. An example is the investigation of Graat et al. [113]. They conducted a randomized, double-blind, placebo-controlled trial in 652 Dutch men and women older than age 60 who were given either a placebo, a multivitamin/mineral supplement, 200 mg of vitamin E as α-tocopherol, or both supplements. The multivitamin/mineral supplement included 23 essential micronutrients and one "possibly essential" trace element—silicon. The primary outcome measures were the incidence and severity of acute respiratory tract infections. The mean duration of participation was 441 days, and the percentage of subjects compliant with the protocol was 84 %. The incidence of acute respiratory tract infections did not differ significantly among treatment groups. Surprisingly, infection severity, measured as duration of infection, restriction of activities, number of symptoms, and presence of fever, was actually increased significantly ($p = 0.03$–0.009) in the groups ingesting vitamin E supplements. This study focused on a clinical outcome, but did not include laboratory evaluation of immunity. Thus, immune system assays that might explain the study results were not available. Only 0.2 % of study subjects had low plasma α-tocopherol concentrations at enrollment, and this may have precluded a beneficial effect of vitamin E supplements. The adverse effects on infection severity may be due to the long duration of high-dose supplementation in a cohort with normal plasma α-tocopherol concentrations at enrollment, and is consistent with previously cited studies on β-carotene [61, 62]

that demonstrate adverse effects after long-term high-dose supplementation. These studies suggest caution in the long-term use of high-dose single micronutrient supplements.

There is considerable evidence that patterns of physical activity and exercise can influence immunity both acutely and chronically, but very few studies have addressed interactions among physical activity, immunity and micronutrient nutrition.

It should be emphasized that the potential of micronutrient supplements to improve immunity or exert other beneficial effects must be considered in relation to their consumption from food. This is especially true for low to moderate dose supplements, for which the intake from food and supplements may be similar. Clearly, supplement use should be encouraged in conjunction with a sound diet that emphasizes fruits, vegetables, whole grains and other sources of micronutrients and limits the intake of saturated fats. However, it is likely that beneficial intakes of some nutrients such as vitamin E may not be possible in the absence of supplement use.

The promising but variable results of studies done to date suggest continued research on nutrition and immunity in older people. Such efforts should include:

1. A focus on long-term placebo-controlled double-blind clinical trials and prospective epidemiological studies that have sufficient statistical power.
2. Study of interactions among physical activity/exercise patterns, immunity, and nutrition.
3. Evaluation of effects of nutrition on both humoral (e.g., antibody responses to vaccination) and cellular (e.g., DTH responses) immunity using clinically relevant assays and on clinical outcomes, e.g., infectious disease incidence, duration, and severity.
4. Evaluation of dietary modification alone or in combination with low doses of supplemental micronutrients. Studies of older people consuming their usual diets are also needed.
5. Long-term studies that address the persistence of the effects of micronutrients on immunity both during and after micronutrient supplementation.
6. Use of appropriate inclusion and exclusion criteria in identification of subjects for study.
7. Study of both single micronutrients and multivitamin/minerals, with a focus on the antioxidant micronutrients and other widely used single or multiple micronutrient supplements.
8. Identification of host specific factors (e.g., gender, age range) that influence micronutrient/immunity interactions and the basis for these effects.
9. Identification of the molecular mechanisms and genes that determine the effects of micronutrients on immunity. This will become increasingly important as new genes that influence aging are identified.

About 50 % of adults living in the USA take multivitamin/mineral supplements, either alone or in combination with higher doses of the antioxidant vitamins [78, 79]. Well-designed studies that assess the health impacts of this practice are needed and should include evaluation of effects on the immune system.

Conclusions

Of course older adults and their health care providers want to know not only what they should do to improve immunity, but also which particular combination of micronutrients is able to reduce their risk of cardiovascular heart disease, cancers, and other major diseases of the elderly. Two types of recommendations can be made, those directed to the manufacturers of micronutrient supplements and those directed to individual patients and their health care providers.

Considerations that can guide the formulation and use of micronutrient supplements targeted to older people include: (1) consider all effects of the micronutrients, not just their effects on immunity, (2) adverse effects caused by micronutrient supplements can be anticipated in some individuals and

formulations should be designed to minimize these effects, (3) nutrient interactions should be considered, e.g., zinc/copper, (4) dose should be a key factor; more is not necessarily better and may be more risky, (5) the anticipated duration of supplementation should be considered; higher doses may be more appropriate for short-term use, (6) micronutrient supplements are not a substitute for a good diet and regular exercise, but rather are a complementary measure, (7) nutrient supplements should include only those substances for which there are adequate convergent data that document essentiality or substantial potential health benefits.

Physicians and other health care providers should advise their patients to eat diets low in saturated fat and high in fruits and vegetables. This can ensure consumption of significant quantities of the micronutrients and other phytochemicals that can favorably affect immunity. In addition older subjects, especially those with poor diets, should be encouraged to take a low-dose multivitamin/mineral supplement. Taking high supplemental doses of other micronutrients that can adversely affect immunity, for example, zinc, should be persuasively discouraged. High doses of supplemental beta-carotene are not recommended for smokers because of their association with the development of lung cancer and are unwise for other people, as concluded by the U.S. Preventive Services Task Force [114]. The favorable effects of regular exercise on immunity should also be mentioned to patients. Most of this advice (low fat diet, high intake of fruits and vegetables, regular exercise, and supplemental vitamins) may not only promote optimal immunity, but is also likely to reduce the risk of cardiovascular heart disease and some cancers.

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Part VI
Prevention of Major Disabilities: Adults and Children

Chapter 29
Gastric Acid Secretions, Treatments, and Nutritional Consequences

Ronit Zilberboim and Adrianne Bendich

Key Points

- Gastric acid provides an important selective advantage in the evolution of vertebrates.
- The use of the gastric acid-suppressive drugs, proton pump inhibitors (PPI), for the diagnosis and treatment of gastrointestinal disorders is effective.
- Certain gastroesophageal diseases require chronic acid-suppressive therapy.
- Long-term use of PPIs has been consistently associated with increased risk of bone fractures; the biological mechanism has yet to be fully understood.
- The negative effects of acid-suppressive therapy on both vitamin and mineral status is observed only after many years of therapy.
- Baseline assessment of nutritional status and nutritional intervention at the initiation of acid-suppressive therapy could help to prevent adverse nutritional consequences.
- Gastric acid's (and its suppression) effects on eating behaviors requires further investigation.

Keywords Gastric secretions • Acid-suppression therapies • Proton pump inhibitors (PPI) • Histamine receptor antagonists • Vitamin B_{12} • Calcium and iron metabolism • Achlorhydria • *Helicobacter pylori* • Gastroesophageal reflux disease

Introduction

The objectives of this chapter are to provide an overview of the roles of gastric acid in health and disease, to discuss the use of acid-suppressive therapies that are used to mitigate prevalent acid-related symptoms and/or diseases and to review potential detrimental nutritional and related consequences. Particular emphasis is placed on reviewing the body of evidence related to drug–drug, and drug–nutrient interactions, and further, the nutritional consequences of gastric acid imbalance as it is related to vitamin and mineral status. Finally, there is a brief discussion related to the consequences of reduced gastric acid with regard to disturbances in the natural bacterial population and balance (microbiome) throughout the gastrointestinal tract.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_29,

Gastric Acid

Gastric acid secretion developed during the evolution of vertebrates and is considered to provide selective advantages. Acid balance in the stomach is precisely regulated as acid is needed to facilitate digestion and absorption, and also to destroy certain food-borne pathogens; too much acid can damage the mucosa and cause ulcers. Stomach acid aids in protein digestion, by converting pepsinogen to pepsin, a proteolytic enzyme, and the acid is involved in the absorption of minerals such as calcium and iron, as well as vitamins such as B_{12}. In addition, acid in combination with pepsin and lipase (another enzyme that is involved in fat digestion) kills ingested microorganisms, viruses and prions. Therefore, acid has a central role in prevention of bacterial overgrowth and protection against enteric infection [1]. Gastric acid is also involved in upper gastrointestinal (GI) motility and finally may modulate eating behavior [2, 3].

Regulation and Secretion of Gastric Acid

The stomach is organized as vertical tubules and contains two functional elements, the oxyntic and pyloric glands (Fig. 29.1). The oxyntic and pyloric glands are organized in the stomach in three areas: the fundus, the corpus, and the antrum. The oxyntic glands are located in the fundus and the corpus which consists of the majority of the stomach's area (80 %). The remaining section of the stomach (20 %), the antrum, is covered with the pyloric glands. In terms of acid secretion, the parietal cells which are located only in the fundus and the corpus areas secrete hydrochloric acid via the hydrogen potassium adenosine triphosphatase pump (H^+K^+ATPase/ATPase; also known as the proton pump). The pyloric area contains gastrin cells (also called G cells) which produce gastrin, a gastric acid secretion stimulator. D cells secrete somatostatin, the main inhibitor of acid secretion, and are located throughout the stomach both on oxyntic and pyloric glands. Enterochromaffin-like cells (ECL) secrete histamine and are only located in the oxyntic area. It is estimated that human stomach contains 1×10^9 parietal cells and 9×10^6 gastrin cells [2].

Gastric acid secretions are stimulated both centrally and peripherally. Central stimulation occurs via peptides that are produced in the gut and can signal the brain directly and indirectly [4]. Peripherally, gastric acid secretion is regulated via hormonal, neural, and biological agents; the main stimulants include gastrin (hormonal), histamine (paracrine), and acetylcholine (neurocrine). In response to food, gastrin functions primarily by releasing histamine from ECL cells via cholecystokinin-2 (CCK2). The released histamine diffuses to the parietal cells where it stimulates acid secretion via activation of histamine H_2-receptors. In addition, gastrin acts directly on the parietal cell by binding to CCK2 and induces the release of cytosolic calcium which ultimately leads to the activation of the proton pump. Acetylcholine functions both by stimulating parietal cells and by inhibiting somatostatin release. The main inhibitor of gastric acid secretion is somatosatin which acts to attenuate acid secretion [2, 4]. Gastric acid secretions can be modulated by infection with *Helicobacter pylori* (HP).

The active parietal cell secretes hydrochloric acid (HCl) at a concentration of 160 mM/L or pH of 0.8 (highly acidic). The acid extrudes through the mucus layer based on the pressure generated during the secretion (~17 mmHg) [2]. Importantly, for acid to be secreted from the parietal cell, physiological processes that include exocytosis of the tubulovesicle followed by endocytosis after the termination must occur. The ATPase pump transforms from a sequestered state in the inactive cytoplasmic tubulovesicles to a docking stage followed by priming and fusion with the apical plasma membrane where it is in the active state. The cycle is completed once the stimulus has decayed and the proton pump is internalized via endocytosis and acid secretion is terminated. A detailed review of the early signaling events that ultimately result in acid secretion by parietal cells, recycling of the proton pump and the

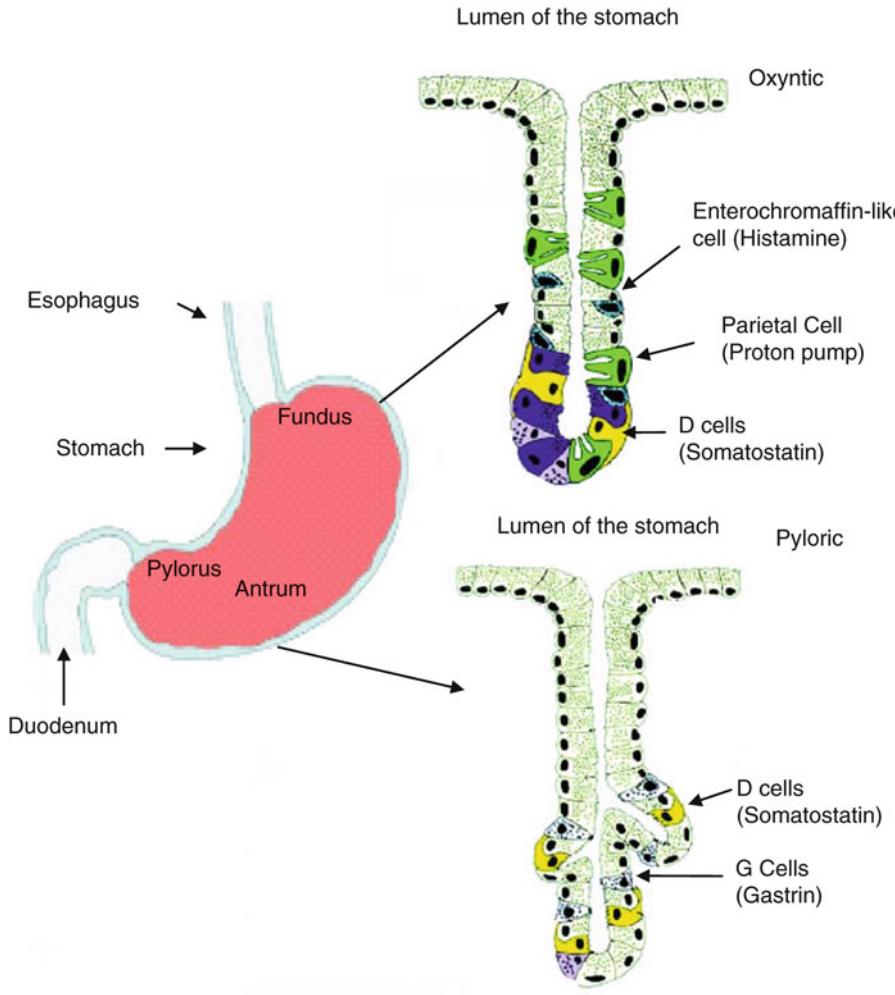

Fig. 29.1 Stomach anatomy gastric acid production and functional elements of the stomach's mucosa

role of the cytoskeleton in support of the active acid secretion can be found in Yao and Forte [5]. Gastric acid secretion is present at birth and secretion rate increases up to 2 years, and stabilizes at that rate, with no change with aging unless there is a co-existing disease [1].

Gastric Acid Imbalance

Several diseases disturb gastric secretions and both over- and under-secretion are observed in clinical practice. While hyposecretion is observed in atrophic and autoimmune gastritis, hypersecretion is manifested in the Zollinger–Ellison syndrome (ZES), and a related condition with ZES-like acid hypersecretion but with normal gastrin and absence of gastrinoma (pseudo-ZE) [6]. Individuals with HP infection also suffer from disturbances in acid secretion and interestingly colonization elicits both secretion extremes; hypersecretion and hyposecretion have been reported [7]. Importantly, hypersecretion causes acid-peptic ulceration of the esophagus, duodenum, and proximal small intestine. Table 29.1 summarizes diseases associated with both hyper- and hyposecretion, their prevalence and consequences.

Table 29.1 Diseases related to perturbation in acid secretion

Disease[a]	Acid secretion status	Prevalence/populations	Acid perturbation mechanism and consequences	References
Zollinger–Ellison syndrome (ZES)	Hypersecretion	Rare condition: 1–3 cases/year/million	• Gastrinoma, neuroendocrine tumor that causes ectopic secretion of gastrin leading to gastric acid hypersecretion • Chronic use of H_2RA and/or PPIs • Severe refractory peptic diseases and frequent complications • 60–90 % of tumors are malignant	[47, 178]
Chronic HP infection	Hypersecretion (see hyposecretion below)	10–15 % of HP cases; antral predominant (located in the antrum- lower part of the stomach)	• Increase in the production of gastric acid perhaps due to reduced somatostatin • Duodenal ulcer mainly due to the perturbations in acid secretion • Increased basal and simulated gastrin, primarily antral	[7]
Duodenal ulcer	Hypersecretion	In children the prevalence is 1 out of 2500 hospital admissions and only about 30 % was HP related [179] In US adults it is estimated that up to 20 % are idiopathic (HP negative; NSAIDs negative) [180]	• Idiopathic (HP negative; NSAIDs negative); increased basal and stimulated acid production and/or reduced somatostatin	[2, 179, 181–183]
Gastric ulcer	Hyposecretion to normal acid secretion	Very rare; may be related to other diseases	• Altered gastric mucosal defense • Oxyntic mucosa inflammation with reduced parietal mass cells	[2]
Autoimmune gastritis	Hyposecretion	General population about 2 % Prevalence increases with age (up to 12 % for 80 years old) In diabetes Type 1, 5–10 %	• High serum gastrin and low pepsinogen I concentrations • ECL cells undergo hyperplasia which may progress to dysplasia and gastric carcinoid tumor (4–9 % of patients) • Affect the partial cells in the corpus and fundus location of the stomach • Vitamin B_{12} deficiency (megaloblastic pernicious anemia) • Iron deficiency anemia (in 20–40 % of patients with autoimmune gastritis	[184]
Chronic atrophic gastritis/gastric cancer	Hyposecretion		• Chronic atrophic gastritis is a precursor of intestinal gastric cancer • Initiation of the carcinogenic process in most cases related to HP infection	[178, 185, 186]
Acute HP infection	Hyposecretion		• Decrease in acid secretion is thought to facilitate survival of the organism and colonization of the stomach • Nutritional consequences related to vitamin B_{12} and iron malabsorption • Direct inhibition of parietal cells probably due to a constituent from the bacteria • Indirectly changing hormonal, pancreatic or neural regulatory functions • Stimulation of somatostatin thus inhibition of gastrin, histamine and acid secretion	[2, 7, 187]
Chronic HP infection pan-gastritis	Hyposecretion		• Due to the inflammation products there is a functional inhibition of the parietal cells; alternatively products of the HP itself inhibit the partial cell from secreting acid. • Long-term infection of the gastric body and fundus (oxyntic mucosa) results in increased risk of gastric cancer • Chronic infection of eth gastric antrum is associated with duodenal ulcer disease	

[a]Other disease states that have been associated with hypersecretion such as renal failure are not be reviewed here

Diseases Associated with Gastric Acid

Gastroesophageal Reflux Disease: Signs and Symptoms

Under normal circumstances, food moves from the mouth through the esophagus into the stomach and continues downward through the small intestine, large intestine, and rectum. If the stomach (gastric) contents move inappropriately upward into the esophagus, this is termed gastroesophageal reflux. Gastroesophageal reflux disease (GERD) is a common disorder resulting from repeated contact between gastric contents and the esophagus epithelium. Gastric contents contain several factors including gastric acid and the enzymes that are secreted into the stomach in order to digest mainly proteins and starches in food. These biological factors can be damaging to the esophagus and cause reflux injury. Unlike the stomach, the esophageal tissues are not protected from strong acid and proteolytic enzymes that can break down the epithelial tissues of the esophagus. Acid has a major role in the development of GERD, and GERD symptoms respond acutely and quickly to antacids, and more slowly, yet more completely, to acid-suppressive therapy. Clinical data indicate that enzymes (gastric and pancreatic) and potentially bile salts contribute to the development of GERD. However, intervention to overcome the movement of these erosive factors from the duodenum into the stomach and then into the esophagus have not been successfully developed. The balance between contact time/frequency of exposure to gastric contents and the defense provided by the esophageal mucosal surface determines the potential for esophageal injury. Pepsin, a gastric proteolytic enzyme, together with gastric acid produces greater damage to the esophageal tissue relative to acid alone. Increased paracellular permeability due to the proteolytic activity allows for contact of refluxed gastric acid with nocioreceptors within the esophageal mucosa and transmission of this peripheral signal to the central nervous system for cognition resulting in experiencing a pain commonly called heartburn [8–10].

Prevalence of GERD

GERD was defined by the Montreal consensus as “a condition which develops when the reflux of the stomach contents causes troublesome symptoms and/or complications” [11]. This definition is accepted as the operational definition for GERD by the American Gastroenterological Association (AGA) [12], and was validated by an Italian consensus process that showed a high level of acceptability of the statements and terms used in the Montreal definition [13]. Further, the term “troublesome” was used to create a patient focused description. It has been acknowledged that GERD patients experience pain, disturbances in emotional well-being, and social function, all of which contribute to reduced quality of life. Sawaya and colleagues validated the Montreal definition and showed that the term troublesome indeed reflects reduced quality of life which is independent of pH data, frequency of reflux episodes, age, and gender. They confirmed that heartburn and regurgitation troublesome scores are highly sensitive; however, they also found that these are not specific symptoms for acid reflux which may limit their usefulness in design of clinical studies [14].

Based on survey data, GERD has been most prevalent in North America and in Europe with Asia trailing behind; however, the prevalence of GERD in the developed world [15] and developing world [16] is still increasing. Weekly symptoms of heartburn and GERD are a useful reference that is used in this review. Based on a US nationwide Gallup survey of a 1000 adults with heartburn at least once per week, Shaker and colleagues [17] reported that about 25 % experienced heartburn once or more daily, 43 % experienced heartburn one or two times per week and 20 % experienced it 3–6 times per week. In contrast, based on a random sample of 2200 Olmstead county residents, weekly GERD symptoms were reported in about 19.8 % and heartburn in 17.8 % of the population studied. A systematic review of prevalence data was reported by Dent et al. [18] based on data from

15 previous studies. They concluded that the prevalence for weekly heartburn is about 10–20 % in the Western world [18]. More recent US data show similar rates of prevalence based on a small population assessment of urban Black Americans. Over 17 % suffered from three or more incidences of heartburn per week [19]. In contrast, in a 2014 study, a much higher prevalence rate was reported in a US study conducted by Cohen and colleagues. They assessed GERD symptoms in the general population ($n = 1107$) relative to patients seeking GI care ($n = 707$). They found a prevalence of 59 %, and that there was no difference in the prevalence of heartburn between the two populations. They also found that regurgitation was higher in patients seeking GI care (46 % relative to 39 % in the general population) [20].

GERD prevalence is less common in Asian countries, perhaps due to limited reporting [21]. Unfortunately as the developing world is adopting a more Western lifestyle, it is expected that GERD prevalence will continue to increase and there are trend data to substantiate the increasing prevalence. Jung et al. [22] reviewed epidemiologic aspects of GERD and determined prevalence in geographical regions in Asia. In Eastern Asia (China, Japan, Korea, and Taiwan) prevalence of 5.2–8.5 % was reported while in Southeast (Malaysia, Singapore, and Thailand) and Western Asia (Israel and Turkey), it was 6.3–18.3 %, which was much higher than those in Eastern Asia. Jung and colleagues found that the prevalence of GERD in Western Asia was the highest among the whole Asian region as represented by prevalence of 20 % in Turkey. Based on a detailed questionnaire of hospital employees ($n = 2037$), 22 % reported heartburn and/or acid regurgitation at least once a week [23]. Interestingly, much lower values were reported in Taiwan recently; prevalence of 1.1 % was reported based on a cross-sectional study with 728,749 people from the national Health Insurance Database [24].

The GERD population has been sub-classified into esophageal symptomatic (with or without mucosal damage) and extraesophageal syndromes (with acid established association). The esophageal symptomatic group is further divided and includes a non-erosive reflux disease (NERD) group, which is the largest subgroup representing between 50 and 70 % of the GERD population [25]. Importantly, a large portion of the NERD population has normal physiological acid exposure, and acid exposure is not the sole etiology of the painful symptoms. Overall, this group is more sensitive to acid than those with erosive reflux disease [26, 27] and displays reduced pain or discomfort thresholds in response to visceral stimulation, resulting in increased sensitivity at a lower stimuli [27]. Notably, similar symptoms, in terms of frequency and severity, are experienced in both erosive and non-erosive patients. Regardless of status (NERD vs. GERD), symptoms significantly affect quality of life and work productivity is also negatively affected [15]. Further, GERD is associated with missing work and imposing financial burdens for both the healthcare system and employers [20].

Duration of GERD

With regard to duration, it was estimated, based on a survey of 1000 people that suffer from heartburn in the USA, that while about 15 % of those surveyed typically experience heartburn for less than 1 year, about 30 % experience it for over 10 years [17]. Based on calculations of prevalence and incidence it has been suggested that duration of reflux is likely to persist for at least 18 years. In an analysis of longitudinal studies, Armstrong and colleagues [28] concluded that most individuals with GERD suffer for several years, and the more severe the disease the more likely it is to be persistent and last longer and perhaps for the rest of the person's life [28].

GERD primarily affects the esophagus, and the major symptoms are heartburn and regurgitation. It tends to be a chronic, lifelong condition that may require continuous management including symptom relief and complication management, especially in patients with persistent symptoms [28, 29]. GERD's chronic nature can be confirmed based on the pathophysiology of the disease.

GERD and GI Motility Disorders

GERD is known to be related to motility disorders and in particular abnormal function of the lower esophageal sphincter, with reduced pressure and more frequent inappropriate relaxation and abnormal gastric clearance. These motility disorders are not transient and drugs used to manage GERD are not focused on addressing the underlying motility disorders. Drugs to treat heartburn are focused on the management of symptoms which can improve quality of life and on healing and preventing further esophageal injury. Importantly, although GERD is considered chronic, there are few long-term management strategies, and only a small number of patients receive counseling that reflect management for the long term [28]. Further, many patients continue to use intervention options that were not designed to be chronic as these may result in potential side effects.

Risk Factors Associated with GERD

Individuals with untreated GERD experience significant decrease in quality of life and higher risk of developing serious complications [30]. Reflux disease is one of the most frequent disorders seen in primary care, as well as in secondary referral centers with a high economic impact [14, 31]. GERD remains the most common gastroenterology-related outpatient diagnosis in the USA [32]. The origin of this chronic disease is not well understood; however, certain risk factors have been identified. The most important risk factors are obesity, smoking, and age. With regard to obesity there is evidence that central abdominal obesity (rather than BMI) is the most important factor associated with GERD. Importantly, this association has been demonstrated in the USA, Europe, and Eastern Asia [30, 33]. The increase in prevalence in both developing and developed countries has been attributed to the changes in lifestyle and in particular to changes in diet and exercise and the consequences of an increase in central adiposity [19].

Complications and Costs Associated with GERD Therapy

GERD complications include reflux esophagitis, esophageal strictures, esophageal adenocarcinoma, and Barrett's esophagus (BE). Nighttime and/or supine reflux are often associated with severe esophagitis or complicated GERD. Complications of long-term GERD such as BE and esophageal adenocarcinoma have been associated with obesity [33]. The economic impact of GERD is enormous. It has been estimated that the annual total costs of treating GERD exceed 10–14 billion dollars in the USA, and the majority is spent on proton pump inhibitors (PPIs discussed in detail below), the costliest among GI illness medications [14, 25, 34]. Based on 2012 USA data, PPIs ranked ninth among the most frequently used therapeutic classes based on all indications [35].

Peptic Ulcer Disease

Recent studies suggest that the pathogenesis of peptic ulcer disease (PUD) in the stomach is related to imbalance between aggressive and defensive factors. In addition to acid and pepsin, *Helicobacter pylori* (HP) infection (discussed in detail below) and chronic use of prescription and/or over the counter (OTC) nonsteroidal anti-inflammatory drugs (NSAIDs) are examples of offensive factors. The defensive factors include the protective mucus bicarbonate layer and mucosal blood flow in the stomach. Disruption in the balance between aggressive and defensive factors leading to PUD increases with aging. Reduced length of chromosomal telomeres seen during aging has been indicated as a possible explanation for the increased frequency of PUD with aging [1]. PUD can lead to complications such

as hemorrhage and duodenal/small intestine perforation. There are four types of gastric peptic ulcers based on location, acid secretion, and concomitant association with a duodenal ulcer. Most gastric ulcers are thought to be due to altered gastric mucosal defense despite normal to low basal and stimulated gastric acid secretions. Currently gastric ulcer is managed by inhibiting gastric acid secretion and, when possible, the removal of the assaulting agent (nonsteroidal anti-inflammatory drugs, NSAIDs or management of HP infection). In contrast to gastric ulcer, increased basal and stimulated acid production are often seen with the development of duodenal ulcers as the duodenum is the section of the small intestine that is attached to the stomach. Acid control is central to the management of these patients. HP infection is considered the root cause for many PUD cases; the incidence of PUD had declined in the past few decades, particularly in Western populations, most likely due to the decrease in HP and the use of PPIs [36]. HP was initially responsible for 95 % of gastroduodenal ulcers, and in more recent years there has been a slight decline in the prevalence of HP and there has been a 36–73 % decline in ulcer occurrence [37]. In addition, to HP infection, chronic NSAID use has been described as an independent risk factor for the development of PUD.

PUD can lead to several complications and it has been noted that the frequency of complications has increased in the female population; it has been suggested that this is due to their increased usage of NSAIDs [36]. In the USA, a small registry documented that the etiology of 53 % of ulcer cases was due to NSAIDs and only 26 % was due to HP infection [36]. In the elderly both HP infection and the use of NSAIDs are common, and there are some data to suggest that the risk of bleeding is increased when both factors are present [37]. Increased parietal cell mass due to suppression of somatosatin has been documented in HP-infected duodenal ulcer patients [2]. Patients with idiopathic PUD are usually maintained on PPI therapy, and higher doses may be required to control acid secretion and prevent ulcer relapse [38]. Prevalence of these diseases is summarized in Table 29.1.

Before the invention of the acid-suppressive therapies including histamine-2 receptor antagonists (H_2RAs) and PPIs, antacids were commonly used. However, since the ulcer was not cured by the antacids, recurrence and complications often resulted. Consequently, surgery procedures such as vagotomy that aimed to denervate the acid producing areas of the stomach or other more extensive ulcer surgeries such as subtotal gastric restriction were used. After the development of acid-suppression therapies especially PPIs, it was possible to use these medications to allow the duodenal ulcer to heal and further prevent recurrence. Importantly, healing is correlated with duration of acid-suppressive therapy, and longer periods of time are usually recommended.

Pharmacological Interventions Related to Excess Gastric Acid

Too much acid, and especially, acid in the wrong place is associated with several diseases that may require pharmacologic intervention, mainly in the form of acid suppression. There are three classes of drugs that can be used: antacids, H_2RAs, and PPIs; these are considered safe and effective. Currently, PPIs are leading the way in acid-suppressive therapies while H_2RAs, an older class of drugs, are used with decreasing frequency. Antacids, in contrast, act on acid that is already in the stomach and when dosed appropriately can provide rapid and effective acid neutralization; however, their use is limited as these do not control acid secretion and only provide temporary relief.

Proton Pump Inhibitors

PPIs are widely prescribed due to their beneficial therapeutic ability to reduce gastric acid secretion regardless of stimulus [2]. The PPI class is the standard drug currently used for acid-related gastrointestinal symptoms like heartburn, and in addition for the management of GERD, PUD, and erosive

gastritis [25]. The superiority of PPIs for the treatment of non-erosive reflux disease as well as erosive esophagitis (EE) has been proven in many randomized trials and is currently generally accepted [35]. In 2008, over 113 million prescriptions for PPIs were filled in the USA with sales of 13.9 billion dollars [39]. PPI use, in particular to manage heartburn symptoms, increased significantly in the USA after these were approved for OTC sale in addition to prescription sale in 2003 [40]. PPI therapy is popular among doctors and patients. Importantly, PPI use pattern has shifted from acute to chronic and patients are using PPIs for long periods and in some cases, on a continuous basis [41]. It is increasingly common for patients to take these drugs on a chronic basis to prevent recurrent gastroesophageal reflux, avoid potential complications such as the pre-cancerous condition, Barrett's esophagus, and to prevent complications due to chronic use of NSAIDs. PPIs are also used for the management of HP infection [42]. It has been reported that about two-thirds of PPIs prescribed in the UK are for long-term therapy [43].

Proton Pump Inhibitor Mechanism of Action

In the late 1980s, the first proton pump inhibitor (PPI) omeprazole was introduced. Several other related moieties later became available; all work on the same cellular mechanism. PPIs inhibit gastric acid secretion independent of gastric acid stimulators including histamine, gastrin, or acetylcholine. PPIs are powerful and the most effective acid secretion inhibitors due to their direct effect on the proton pump. PPIs provide a more sustained increase in gastric pH relative to H_2RAs which only affect the histamine receptors thus allowing for some acid secretion [42].

Most PPIs are taken in the form of prodrugs. To prevent protonation in the stomach prior to duodenum absorption, PPIs are formulated with an acid resistant coating. Once in the bloodstream, PPIs reach the stomach and inhibit acid secretion via the attachment of the active form to the proton pump ATPase. The bond between the PPI and ATPase prevents the final stage of acid secretion involving the proton's transport across the membranes from the parietal cell. PPIs are capable of raising intragastric pH by several units and reduce hydrogen ion concentration (acidity) by hundreds to thousands fold [44]. Upon absorption, most PPIs undergo biotransformation by cytochrome P_{450} (CYP) in the liver. In particular omeprazole and esomeprazole are primarily modified by CYP2C19 and CYP3A4. The PPIs also inhibit the activity of CYP2C19, and consequently their plasma levels are elevated. Further, this inhibition is the basis of their interactions with other drugs (see Sects. 4.3 and 5). Lansoprazole is also metabolized by both CYP2C19 and CPY3A4. In contrast, pantoprazole is primarily metabolized independent of cytochrome P_{450} by a sulphotransferase enzyme. Rabeprazole metabolism is primarily nonenzymatic. The differences in their metabolism confer differences in their antisecretory effect in part due to polymorphisms in CPY2C19, and to drug–drug interactions with clinical relevance [25, 45].

Chemically, PPIs are either substituted pyridlmethylsulfinyl benzimidazole or imidazopyridine derivatives. The pyridine and the imidazole are heterocyclic moieties forming the PPI prodrug that can be protonated as a function of the conditions in the acid space of the parietal cells. The protonation of both the pyridine and the imidazole is a prerequisite for their conversion to the active drug. Consequently, the activation of PPIs is dependent on the pH, and when the pH is below 2.5 there is greater activation. This delays the initiation of acid suppression due to the time needed to lower the pH [46]. The rate of activation, which differs across the PPI category, depends on the pH in the acid space and the chemical properties of the heterocyclic rings. The activated drug forms one or more irreversible disulfide bonds with the cysteine(s) in the proton pump. Therefore, the duration of their effects is longer than expected based on their blood levels. PPIs accumulate in acidic spaces of stimulated parietal cells where their concentration can increase up to 1000 times that of the blood. Following the first protonation, the PPI binds to the ATPase allowing for the second protonation to occur. Importantly, the second interaction location on the ATPase differs for the different PPI drugs

(dependent on the pH). These properties also highlight the need to consume the PPIs in proximity to a meal, when the proton pump is activated [47]. Uptake of PPIs by other cells such as vascular H^+ATPase (osteoclast proton pump) [48], colonic epithelial cells, and several other cells such as neurons, kidney, and bone cells (for rabeprazole) has also been reported [25, 42]. These cells may also contain ATPases that are slightly different than the parietal cell ATPase. The half time recovery of acid secretion is dependent on reversal of the sulfide bonds between PPI and ATPase, and by synthesis of new parietal cells. The PPI's binding location with cysteine to form the disulfide bonds determines the accessibility to repair by glutathione and reversal of the disulfide bonds. A range between 28 and 46 h is required for resumption of acid secretion for omeprazole and pantoprazole [25].

PPIs reduce acid secretion and this causes a decrease in secretion of somatostatin and consequent increase in gastrin secretion. Normal fasting gastrin is 30–50 pmol/L, and, in response to a meal, gastrin increases to 100–150 pmol/L. An increase of two- to sixfold in plasma gastrin is seen in long-term PPI users (80–100 % of omeprazole users). Some individuals may be hypergastrinemic and their gastrin level is higher than 500 pmol/L [49]. Importantly, hypergastrinemia produced by PPIs increases the size and number of ECL cells; when PPI therapy is discontinued, the presence of a greater number of ECL cells is responsible for the "rebound effect" of greater acid secretion than before PPI therapy was started, an effect that makes stopping PPIs extremely difficult. Another undesirable effect related to the hypergastrinemia is the potential development of gastric carcinoid, also called neuroendocrine tumors [1, 50].

Types of PPIs

There are currently seven PPIs that are available as prescription drugs in the USA, and several are available OTC. Marketed PPIs include omeprazole (Prilosec), omeprazole-sodium bicarbonate (Zegrid), S-omeprazole (Nexium), lansoprazole (Prevacid), pantoprazole (Protonix), rabeprazole (AcipHex), and dexlansoprazole (Kapidex). In development is tenatoprazole [46]. The dosage forms and the indications associated with the available PPIs are tabulated in Table 29.2.

PPIs and Gut Microbiota

The effect of PPIs on gut microbiota and weight loss patterns in patients undergoing gastric bypass was recently explored [51]. A small study with eight obese subjects determined the bacterial profile before and 6 months after gastric bypass. Interestingly, before the gastric bypass PPI users ($n=3$) had a nonsignificant higher percent of firmicutes compared to nonusers ($n=5$). Similar trends (higher percent of firmicutes) were observed after the gastric bypass. This is a new field of clinical research and more research into the effects of PPIs on the gut flora of individuals who have GERD or other gastric conditions is warranted.

Overutilization of PPI

In the past few years there has been increased awareness of the overutilization of PPIs in both inpatient and outpatient settings, perhaps due to their safe and effective profile. Unfortunately common clinical practices, that are not based on data, contribute to the misuse and/or overuse of PPIs in both settings which therefore contributes to potential risk and excessive economic burden [52]. An example of overutilization of PPI in an inpatient situation has been studied by Herzig and colleagues [53]. While acid-suppressive therapy may be needed in the intensive care unit (ICU), and there are data to

Table 29.2 Indications, dosage and elimination routes for PPIs

Brand name Chemical name Manufacturer	Indication/dose (mg/day) and duration recommended duration for specific indication	Dosage form/manufacturer directions	Half-life (hours) Elimination routes Protein bound (%)
Aciphex Rabeprazole sodium Eisai [95]	Healing of erosive or ulcerative GERD: 20 mg/day; for 4–8 weeks Maintenance of healing of erosive or ulcerative GERD: 20 mg/day Treatment of symptomatic GERD: 20 mg/day for 4 weeks Healing of duodenal ulcers: 20 mg/day for up to 4 weeks	Form: Delayed-release, enteric-coated tablets containing 20 mg of rabeprazole sodium Directions: Take with or without food	Half life: 1–2 h Elimination urine: 90 %; Remainder in feces Protein bound: 96.3 %
Prevacid Lansoprazole TAP [89]	Duodenal ulcer (Short-term treatment): 15 mg/day for 4 weeks Duodenal ulcer (maintenance of healed): 15 mg/day HP eradication to reduce the risk of duodenal ulcer recurrence triple therapy: 30 mg plus amoxicillin 1 g and clarithromycin 500 mg twice daily for 10 or 14 days HP eradication to reduce the risk of duodenal ulcer recurrence dual therapy: 30 mg plus amoxicillin 1 g 3 times daily for 14 days Benign gastric ulcer: 30 mg/day for up to 8 weeks NSAIDs-associated gastric ulcer, healing: 30 mg/day for 8 weeks NSAIDs-associated gastric ulcer, risk reduction: 15 mg/day for up to 8 weeks Short-term treatment of symptomatic GERD: 15 mg/day for up to 8 weeks Short-term treatment of EE: 30 mg/day for up to 8 weeks Pediatric (1–11 years) short-term treatment of symptomatic GERD and short-term treatment of erosive esophagitis: 15 mg/day for up to 12 weeks for ≤30 kg; 30 mg/day for up to 12 k for >30 kg Adolescent (12–17 years) Short-term treatment of symptomatic GERD Non erosive GERD: 15 mg/day for up to 8 weeks EE: 30 mg/day for up to 8 weeks Maintenance of healing of EE: 15 mg/day Pathological hypersecretory conditions including ZES: 60 mg/day	Form: Prevacid is supplied in delayed-release capsules, in delayed-release orally disintegrating tablets for oral administration and in a packet for delayed-release oral suspension. Each form is available in either 15 or 30 mg strengths. Directions: Take before eating	Half-life: 1.5 h Elimination urine: 1/3; Feces: 2/3 Protein bound: 97 %

(continued)

Table 29.2 (continued)

<table>
<tr><th>Brand name
Chemical name
Manufacturer</th><th>Indication/dose (mg/day) and duration recommended duration for specific indication</th><th>Dosage form/manufacturer directions</th><th>Half-life (hours)
Elimination routes
Protein bound (%)</th></tr>
<tr><td>Prilosec
Omeprazole (delayed-release capsules)
Omeprazole magnesium (for delayed-release oral suspension)
AstraZeneca [188]</td><td>Short-term treatment of active duodenal ulcer: 20 mg once daily for 4 weeks. Some patients may require an additional 4 weeks
HP eradication to reduce the risk of duodenal ulcer recurrence:
Triple therapy: Prilosec 20 mg, Amoxicillin 1000 mg, and Clarithromycin 500 mg. Each drug twice daily for 10 days
Dual therapy: PRILOSEC 40 mg (once daily for 14 days), Clarithromycin 500 mg (three times daily for 14 days)
Gastric ulcer: 40 mg once daily for 4–8 weeks
GERD: 20 mg once daily for 4–8 weeks
Maintenance of healing of EE: 20 mg once daily
Pathological hypersecretory conditions: 60 mg (varies with individual patient) once daily
Pediatric patients (1–16 years of age)
<table>
<tr><td rowspan="4">GERD and maintenance of healing of EE</td><td>Weight</td><td>Dose</td><td rowspan="4">Once daily</td></tr>
<tr><td>5 < 10 kg</td><td>5 mg</td></tr>
<tr><td>10 < 20 kg</td><td>10 mg</td></tr>
<tr><td>≥20 kg</td><td>20 mg</td></tr>
</table></td><td>Form:
Prilosec delayed-release capsules: 10, 20, and 40 mg
Prilosec for delayed-release oral suspension: 2.5 or 10 mg
Directions:
Delayed-release capsules should be taken before eating and swallowed whole.
Packets for delayed-release oral suspension should be emptied into a container containing water (5 mL water for a 2.5 mg packet and 15 mL of water for a 10 mg packet), stirred, allowed to thicken for 2–3 min, and stirred and drank within 30 min. If any material remains after drinking, add more water, stir and drink immediately.</td><td>Half-life: 0.5–1 h
Elimination urine: 77 %; Remainder in feces
Protein bound: 95 %</td></tr>
<tr><td>Protonix
Pantoprazole sodium
Wyeth [189]</td><td>Treatment of EE: 40 mg/day for up to 8 weeks
Maintenance of healing of EE: 40 mg/day
Pathological hypersecretory conditions including ZES: 40 mg twice daily for as long as clinically indicated. Doses as high as 240 mg/day have been administered.</td><td>Form:
Delayed-release tablet available in 2 strengths. Each delayed-release tablet contains 45.1 or 22.6 mg of pantoprazole sodium sesquihydrate (equivalent to 40 mg or 20 mg pantoprazole, respectively)
Directions:
Take with or without food</td><td>Half life: 1 h
Elimination urine: 71 %; Feces: 18 %
Protein bound: 98 %</td></tr>
</table>

Nexium Esomeprazole magnesium AstraZeneca LP [90]	GERD, healing of erosive esophagitis: 20 or 40 mg/day for up to 4–8 weeks GERD, maintenance of healing of EE: 20 mg/day Symptomatic GERD: 20 mg/day for 4 weeks Risk reduction of NSAIDs-associated gastric ulcer: 20 or 40 mg/day for up to 6 months HP eradication to reduce the risk of duodenal ulcer recurrence, triple therapy: 40 mg/day for 10 days plus amoxicillin 1000 mg twice daily for 10 days and clarithromycin 500 mg twice daily for 10 days Adolescent Use 12–17 year olds, short-term treatment of GERD: 20 or 40 mg/day for up to 8 weeks Pathological hypersecretory conditions including ZES: 40 mg twice daily	Form: Nexium is supplied in delayed-release capsules and in packets for a delayed-release oral suspension. Each delayed-release capsule contains 20 or 40 mg of esomeprazole. Each packet of Nexium. For Delayed-Release Oral Suspension contains 20 or 40 mg of esomeprazole, in the form of the same enteric-coated granules used in Nexium delayed-release capsules. Directions: At least 1 h before meals	Half-life: 40 mg dose: 1.5 h 20 mg dose: 1.2 h Elimination urine: 80 %; Remainder in feces Protein bound: 97 %
Zegerid Omeprazole sodium bicarbonate Santarus [91]	Short-term treatment of active duodenal ulcer: 20 mg/day for 4 weeks Benign gastric ulcer: 40 mg/day for 4–8 weeks Symptomatic GERD (with no esophageal erosions): 20 mg/day for up to 4 weeks GERD with EE: 20 mg/day for up to 4–8 weeks Maintenance healing of EE: 20 mg/day Reduction of risk of upper gastrointestinal bleeding in critically ill patients : 40 mg initially followed by 40 mg 6–8 h later and 40 mg/day thereafter for 14 days (oral suspension only)	Form: Immediate-release capsules and unit-dose packets as powder for oral suspension. Each capsule contains either 40 or 20 mg of omeprazole and 1100 mg of sodium bicarbonate. Packets of powder for oral suspension contain either 40 or 20 mg of omeprazole and 1680 mg of sodium bicarbonate. Directions: On an empty stomach at least 1 h before a meal.	Half-life: 1 h Elimination urine: 77 %; Remainder in feces Protein bound: 95 %

GERD gastroesophageal reflux disease, *EE* erosive esophagitis, *ZES* Zollinger–Ellison syndrome, *NSAID* nonsteroidal anti-inflammatory drugs, HP *H. pylori*

support its usefulness, current guidelines recommend against the routine use of prophylactic acid suppression in patients outside of the ICU. However, it was estimated that 40–70 % of inpatients receive unneeded acid-suppressive medications during their hospitalization [53].

Another recent example is based on the inappropriate utilization of PPI in patients receiving dual antiplatelet therapy (DAPT). It was suggested that prescribing habits are inconsistent with recent consensus recommendations, exposing patients to unnecessary adverse patient's outcomes. However, both lack of prescribing PPI when required and PPI prophylaxis when not recommended were documented [54]. There are also data to show that in ambulatory settings many patients are prescribed PPIs inappropriately. In one study, only 35 % were prescribed for an appropriate upper GI diagnosis [55]. Another report based on 100 patients with 24 h pH monitoring indicated that 39 % did not have objective measurements of GERD, and early referral to pH studies could prevent lengthy periods of unneeded PPI therapy. Interestingly, increased costs due to pH monitoring reached equivalence after 6.4–23.7 weeks of PPI therapy [34].

Proving causality of the side effects of PPIs is limited by lack of long-term randomized placebo-controlled trials. With respect to nutritionally related consequences, the best evidence available indicates that the long-term use of PPIs increases the risk of *Clostridium difficile* infection and bone fractures in susceptible populations. There is emerging data for an adverse effect on vitamin B_{12} status and hypomagnesemia [56].

Medical Therapy for Diseases Associated with Gastric Acid

Management of Gastroesophageal Reflux Disease

The cardinal symptoms of GERD are troublesome heartburn and regurgitation; however a number of additional symptoms/conditions may also exist. A presenting symptom may be chest pain, therefore to confirm GERD diagnosis, chest pain has to be distinguished from cardiac pain. Additionally because functional dysphagia (FD) and GERD symptoms overlap, FD diagnosis has to be ruled out due to potential complications. Once GERD diagnosis has been established, medical therapy is focused on inhibition of acid production with PPIs dominating the treatment algorithm. The recent Guidelines for the Diagnosis and Management of Gastroesophageal Reflux Disease [57] recommends empiric medical therapy with PPI after a presumptive diagnosis of GERD based on the typical symptoms. Further workup is recommended after failure of empirical PPI and it may be considered sooner in patients with extraesophageal GERD symptoms [34]. It has been acknowledged, that this empirical approach has some limitations, specifically with regard to detection of EE [58]. In the case of non-cardiac chest pain, there is some evidence of a high response rate to aggressive PPI therapy, suggesting efficacy and cost-effectiveness. At the same time, significantly higher levels of response to PPI therapy were observed when GERD diagnosis was supported by additional assessments (endoscopy and/or pH-measurement). There are consistent data to support treatment of GERD with antisecretory agents and PPIs are more effective than H_2RAs. PPIs provide superior healing for EE, including faster healing rate. In addition they provide more complete heartburn relief especially for erosive reflux.

Unfortunately, up to 40 % of GERD patients fail to achieve adequate control while using PPI therapy. This heterogeneous patient population has refractory GERD, and management is focused in the first instance on optimizing PPI regimen and compliance. Importantly, dosing time relative to the meal (30–60 min prior to a meal) is critical and appears to be ignored by both patients and physicians. Patients that do not respond after compliance optimization may require further workup. The lowest relief response rate was observed in patients with NERD. In the case of prolonged PPI use, a recent analysis indicates that prompt referral to pH monitoring is the cost-effective approach. Unfortunately, based on the pH monitoring results it has been determined that approximately a third of long-term PPIs users used these drugs for longer than 8 weeks [34].

There is only limited information to support other interventions as a first line of therapy including OTC antacids in combination with H_2RA or a PPI. For infrequent postprandial symptoms, a meta-analysis of OTC medicines concluded that antacids and antacids in combination with alginates that physically block the movement of acid into the esophagus are quite effective [59].

GERD Risk and Obesity

Lifestyle interventions including weight management and elimination of certain foods are also recommended as part of GERD therapy. Most dietary modifications are not supported by strong data and there are conflicting reports. The relationship between GERD and obesity is much stronger, and obese patients have increased reflux events and esophageal acid exposure based on pH studies, and increased risk of EE based on endoscopy [60, 61]. The positive effect of reduction in body mass index (BMI) is supported by multiple case–control studies [31, 57, 62]. Weight management is recommended for the management of GERD [57], although the validity of this approach as a long-term solution has not yet been demonstrated [63].

Helicobacter pylori *Infection and Management*

Helicobacter pylori (HP) is a human gram-negative pathogen that has been linked with sanitation and water quality in most but not all studies [64]. Successful eradication of the bacteria is associated with improved health outcomes including a decrease in peptic ulcer bleeding incidence [37], and reduced peptic ulcer recurrence rate [65]. HP affects half of the world's population and is considered the major cause of acute gastritis, chronic gastritis, gastroduodenal ulceration and is recognized as a carcinogen for its role in gastric carcinogenesis [66–68]. Further, HP infection is implicated in a number of extra gastric diseases, including autoimmune diseases as well as cardiovascular diseases [68–70]. Several recent studies suggest that HP infection is associated with some benefits to the host, and based on epidemiological studies, asthma and allergy may be positively influenced [37].

Infection with HP often occurs in childhood, and once established, can persist lifelong if untreated. Overall, the prevalence of HP infection is higher in developing countries when compared to developed countries, but within a region it can vary by ethnicity and socioeconomic factors. It is commonly understood that prevalence of infection is decreasing in many countries due to improvements in sanitation and living standards and the relatively recent movement of populations from rural to urban settings [64].

HP Mechanism of Action

HP specifically colonizes gastric epithelium and is able to survive within the gastric mucus layer by producing ammonia to protect itself. HP produces enzymes which play a role in the development of gastric mucosal damage. With regard to the bacteria, HP strains may differ in their motility, adherence to gastric epithelial cells as well as the chemicals that they produce to help their survival in the acidic environment or synthesis of the cytotoxins that they produce. Some HP strains especially those with vacuolating toxin A (Vac-A) and cytotoxin-associated gene A (Cag-A) are associated with greater pathogenicity [70]. Host factors also contribute to the degree and severity of the gastric inflammation (chronic gastritis), and the development of a more severe disease. Varied host immune responses to the pathogen have been documented [69], and in depth understanding of the interaction between host genetic factors and susceptibility to HP have not been fully elucidated [7]. Individuals infected predominantly within the corpus location suffer from reduced gastric acid secretion due to atrophy,

inactivation of ATPase, and direct inhibition of acid secretion. In contrast, antral predominant infection and decreased somatostatin is seen in a minority of chronic HP-infected individuals. They exhibit increased basal stimulated gastrin and acid hypersecretion, and these patients are predisposed to develop duodenal ulcer disease [7]. Eradication of HP restores somatostatin and gastrin and thus acid secretion.

HP and GERD

The effect of HP eradication on symptoms of patients with GERD is still controversial, perhaps due to the difference observed in the predominant infection location between Western and Asian populations [71], or due to lack of symptomatic differences [72]. Based on overall reduced acid secretion (corpus infection), it has been hypothesized that HP infection is one of the protective factors for GERD. There are a number of studies to support this perspective. A cross-sectional study conducted by Minatsuki et al. in Japan with 3472 subjects with chronic HP infection (positive antibody without history of eradication therapy) and 956 subjects who successfully eradicated HP (negative serum and history of eradication therapy) evaluated the effect of HP eradication on GERD [16]. They found statistical differences in the prevalence of reflux esophagitis (RE) between the two groups. Prevalence of RE was 2.3 % among the HP-infected group, and 8.8 % in the HP eradicated group. In contrast, there was no difference in terms of NERD prevalence between the groups, with about 20 % prevalence. Based on these results it was concluded that eradication of HP negatively affected RE but not NERD. Additional support is based on results of 24-h esophageal pH-metry; the Wu et al. study showed an increase in esophageal acid exposure corroborating the negative effect of HP eradication on the clinical disease [73].

In contrast there is a body of evidence to support a lack of a negative effect following HP eradication. There are a number of small studies that show some positive effects primarily when lower esophageal sphincter pressure, esophageal pH monitoring, or reflux index were evaluated rather than symptomatic relief [68, 72]. Additional data to support lack of a negative effect are based on quality of life assessment. Interestingly, significant improvement in quality of life was documented after HP eradiation only after a long period after the eradiation (1 year), while no positive effects were observed after 3 months [74]. Similar observations supporting the lack of negative effect due to HP eradication were recently reported in two large meta-analyses [75, 76]. Of importance, Saad and colleagues in a RCT study compared patients treated for HP infection with those treated with placebo. They showed significantly lower GERD symptoms in the eradicated group relative to the group that was not eradicated, providing additional evidence of the positive effects of HP eradication. Others concluded that HP has no effect on symptoms and treatment efficacy in patients with GERD [37]. Finally, based on the lack of association between HP eradication and GERD, the Maastricht III consensus report expanded their recommendations for eradication of HP for those who are chronic PPI users as well as those who use NSAIDs [36]. Table 29.1 describes the consequences of both hyper- and hyposecretion as related to HP infection.

HP Nutritional Effects

Recent focus has examined the effects of HP on the gastric environment and the possible effects on absorption of nutrients and drugs as well as the effects on hormones. Absorption of both iron and vitamin B_{12} are negatively affected by HP infection. HP-infected individuals have lower basal and fasting ghrelin and higher levels of leptin both of which potentially contribute to growth. Therefore, childhood infection, especially in malnourished children, may results in growth retardation [70]. HP infection lowers serum concentrations of vitamin C and reduces the relative amount of the

biologically active antioxidant form of vitamin C in the stomach. Inflammation of gastric mucosa tends to oxidize the antioxidant, ascorbic acid, to the non-antioxidant, dehydroascorbic acid. Further ascorbic acid is not stable in the higher pH that is associated with HP infection [77]. HP infection can also affect gastric microbiota, and species usually found in the lower GI tract can be found in the stomach of HP-infected individuals [70].

Management for HP Infection

Antibiotic Therapies

There is no universally effective regimen for the treatment of HP despite decades of efforts to eradicate this human pathogen. Due to the rising prevalence of antimicrobial resistance, previous therapies, which were complicated to begin with, are yielding unacceptable low levels of efficacy in most of the world [78]. Unlike other infections where susceptibility testing on the bacteria is performed prior to administration of antibiotic treatment, there is no convenient way to guide treatment for HP infection. Eradication has been difficult due to both recurrence and resistance to certain antibiotics in particular clarithromycin (resistance ranging between 11.1 and 29.3 %) but also to metronidazole (resistance ranging between 17 and 44.1 %). Therefore, novel regimens are becoming the new first line therapy with efficacy above 90 %. Currently the first line treatment for HP infection is a quadruple therapy which consists of bismuth + metronidazole + tetracycline + PPI; it is effective when resistance to metronidazole is low, and where clarithromycin resistance is high. Second line therapy includes levofloxacin-based regimens (amoxicillin together with levofoxacin). The amoxicillin acts by inhibiting bacterial cell wall synthesis and the PPI increases gastric pH which promotes active growth and division of the HP [78]. An emerging alternative strategy to the first line of therapy is sequential therapy where the antibiotics are administered in sequence. The initial treatment phase includes a PPI and one antibiotic, followed by a PPI and another antibiotic. In addition to sequential therapy, concomitant therapy as well as hybrid therapies exists. These emerging therapies are considered superior alternatives to the previous gold standard triple therapy for treatment of patients infected with resistant strains of HP. Recurrence rates of HP infection remain high in developing countries, and in certain populations in developed countries [64]. Based on a recent meta-analysis, it was concluded that in developing countries recurrence is related to infection with a new strain while in developed countries it is due to re-infection with the same stain [79].

Probiotic and Essential Vitamin Combination Therapies

Future therapies for HP infection may include combinations with certain probiotics. There are a number of potential mechanisms by which probiotics may help in the eradication of the HP including strengthening the mucosal barrier function as well as competition for adhesion and immunomodulatory effects. It appears that there is positive evidence with *Saccharomyces boulardii* and *Lactobacillus* spp. [78]. Another alternative therapy involves the use of standard triple therapy (PPI, amoxicillin and clarithromycin) combined with vitamin C and E for 30 days and quadruple therapy (PPI, amoxicillin and clarithromycin and bismuth) showed promising results in two RCT from Turkey. The mechanism by which vitamin C and E help increase the efficacy of the standard therapy is yet to be investigated [80, 81].

Management for Nonsteroidal Anti-inflammatory Drugs Complications

NSAIDs are used chronically for a number of conditions. Both prescription and OTC NSAIDs are commonly used on a daily basis for relief of pain and inflammation associated with musculoskeletal injury and arthritis. Additionally, NSAIDs are also used to reduce the risk of cardiovascular and cerebrovascular diseases, and in combination with clopidogrel (also called dual antiplatelet therapy DAPT), to reduce the risk of cardiac death, myocardial infarction, and stroke. It is well known that the

use of certain NSAIDs is associated with upper GI complications such as GI bleeding, ulcer perforation, and symptomatic peptic ulcer disease. It has been estimated that NSAID compilations develop in about 1–2 % of users per year, which is 3–5 times higher than nonusers [82], and further, there is a twofold higher risk of GI bleeding when used as co-therapy (DAPT), relative to NSAIDs alone [54]. Unfortunately, GI bleeding is associated with high mortality [83].

Several risk factors for potential GI complications while on NSAIDs have been identified. These include age (>65), history of prior complications, and co-medication with anticoagulants or corticosteroids. Other risk factors, such as HP infection, may be involved but the data remain controversial [83]. The most common cause of discontinuation of NSAIDs is upper GI injury, and several expert consensuses recommend discontinuation of NSAIDs in patients with GI bleeding [84]. However, this solution may be unacceptable to millions of patients who suffer from painful conditions such as rheumatoid arthritis or osteoarthritis who use NDAIDS on a daily basis. To overcome GI complications, several gastroprotective strategies including certain drugs and drug combinations have been used [85]. PPIs have been used extensively to reduce the risk of upper GI complications in NSAIDs users both as prophylactic and as therapeutic agents with significant positive results [83]. For example, in Japanese patients several PPIs have been shown to significantly reduce recurrence of peptic ulcers associated with NSAIDs and the Japanese health authorities approved PPIs for prevention of GI issues in NSAIDs users [86]. H_2RAs do not suppress acid secretion sufficiently and did not reduce the gastrointestinal complication risk while on NSAIDs [82], and in particular were not effective with gastric ulcers [83]. The role of COX-2 inhibitors is still controversial, and data assessing the benefits of COX-2 inhibitor usage in comparison to NSAIDs with PPI or misoprostol, or co-prescription of COX-2 inhibitors with PPI is conflicting. Additional gastroprotective therapies include co-therapy with PPIs or misoprostol, COX-2 inhibitors and vitamin C in combination with aspirin [85, 87].

PPIs and Drug–Drug Interactions

A number of drugs are affected by co-administration with PPIs, and at times readjustment of the PPI and/or the co-administered drug may be required. There are several mechanisms by which PPIs affect other drugs, including change in stomach pH and through interactions with liver enzymes. PPIs and drugs that are metabolized by the same liver enzymes (such as cytochrome CYP2C19) may be affected due to co-administration. PPIs may increase or decrease the effectiveness of the co-administered drug. An example of the effects of PPI through the increase in stomach pH was recently reported for aspirin. Increased risk of adverse cardiovascular events was reported when aspirin was used concomitantly with PPI. The increased risk is based on findings from all aspirin treated patients surviving 30 days after a first myocardial infarction in a retrospective nationwide propensity score in Denmark. The reduced efficacy of aspirin when used concomitantly with PPIs was attributed to its reduced bioavailability; all PPIs showed a similar magnitude and therefore considered a class effect. Aspirin absorption is dependent on stomach pH; when stomach pH is above 3.5 (less acidic than normal), it is effectively above the pK_a of the acetylsalicylic acid reducing its lipophilicity and hence its absorption. Due to the reduced aspirin bioavailability, there was a reduction in induced inhibition platelet aggregation, and therefore increased risk of cardiovascular events [88]. Another example of PPIs interfering with the bioavailability of drugs that are dependent on gastric pH is based on atazanavir, a potent antiviral drug. Lansoprazole, esomeprazole, and omeprazole plus sodium bicarbonate decrease plasma levels of the human immunodeficiency virus (HIV) protease inhibitor atazanavir, which depends on an acidic gastric pH for absorption [89–91]. Therefore, PPIs should not be co-administered with atazanavir.

Clopidogrel is a platelet inhibitor that reduces the risk of new ischemic cardiovascular events that is used in combination with aspirin in patients treated for coronary diseases. PPIs are often added to

reduce the risk of bleeding associated with the aspirin, and the American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) in partnership with the American College of Gastroenterology (ACG) issued in 2008 a consensus document recommending the use of PPI for all patients on DAPT. Hepatic P450 enzymes 2C19 and 3A4 metabolize most PPIs and also convert the clopidogrel prodrug into the active metabolite, raising concerns with regard to the efficacy of clopidogrel while on PPI. It was hypothesized that PPIs compete with the liver enzymes that metabolize clopidogrel resulting in enhanced platelet activity (reduced efficacy of the clopidogrel) [92]. It appears that this is not a class effect, as not all PPIs utilize the same liver enzymes as clopidogrel. In contrast to the reported negative omeprazole-clopidogrel drug interaction, the intake of other PPIs, such as pantoprazole, dexlansoprazole, or esomeprazole was not associated with impaired response to clopidogrel [93]. Despite conflicting results from clinical studies with regard to the risk of concomitant use, both the FDA and EMA have recently issued warnings against the concomitant use of clopidogrel and PPIs. In 2010 the consortium of ACCF, ACG, and AHA issued an update recommending that people with a history of GI bleeding or multiple risk factors for bleeding, PUD, and HP infection not use PPIs [54]. In addition to clopidogrel, other drugs in the class of thienopyridines such as ticlopidine are metabolized via the same liver enzyme system and may have the same issues as clopidogrel [94]. Another example of drugs interactions through liver enzymes is the doubling of esomeprazole exposure that results from concomitant administration with voriconazole (i.e., triazol, an antifungal medication) as both are metabolized through CYP2C19 and CYP3A4. Although not normally required, dose adjustment upward may be required in patients with ZES [90].

Increases in plasma concentrations of both PPI and antibiotics drugs have been observed when these are taken concomitantly. This is especially important as the standard treatment for HP infection may include the combination of antibiotic and PPIs. For example, increased plasma concentrations of rabeprazole and 14-hydroxyclarithromycin may be experienced by individuals administered combinations of rabeprazole, amoxicillin, and clarithromycin [95]. Similarly, increases in plasma levels of omeprazole, clarithromycin, and 14-hydroxyclarithromycin may occur when omeprazole plus sodium bicarbonate and clarithromycin are co-administered [91].

Patients receiving warfarin and PPIs (including rabeprazole, lansoprazole, esomeprazole, and omeprazole plus sodium bicarbonate) concomitantly may experience increased risk of bleeding due to increases in the international normalized ratio (INR) of platelets and increase in prothrombin time as the warfarin is not metabolized at the expected rate [89–91, 95]. In addition, PPIs should be taken 30 min prior to sucralfate, as absorption of Prevacid and omeprazole is delayed and bioavailability reduced when administered with sucralfate [89]. Omeprazole plus sodium bicarbonate may prolong the elimination of drugs that are metabolized by oxidation in the liver, including diazepam, warfarin, and phenytoin. Also, when administered concomitantly with the immunosuppressive drug, tacrolimus, (a macrolide), omeprazole plus sodium bicarbonate may increase serum levels of tacrolimus [91].

Gastric acid Suppression Anti-osteoporosis Therapy

Bisphosphonates (BP) are a class of medications that are used to reduce the risk of fracture in the elderly, and in particular postmenopausal women. They selectively inhibit bone resorption and thus increase bone mineral density (BMD). They are effective within the first year of treatment. The treatment with BP is associated with potential adverse upper gastrointestinal problems, such as dyspepsia, acid regurgitation, and esophageal reactions. Reduced compliance (adherence) to BP due to gastric side effects has been documented [96]. PPIs may be recommended for users of BP to help manage GI side effects. Further, it has been suggested that reduced stomach acidity can increase the bioavailability of BP and that the combination of BP and PPI would be more effective than BP alone [97]. The use of PPI and BP is prevalent in elderly who are at risk for osteoporosis and it is important to understand

the interactions between these classes of drugs. Overall, a high percentage of BP users are also PPIs users; between 8 and 30 % of BP users also take PPI [48, 98]. Therefore, it is important to understand the relationship between PPI and BP and the risk of fractures. To decipher potential dependency and timing effects (in terms BP new user relative to PPI new users), Fraser [99] separated new PPI users and found that only 15–17 % were also new BP users. Consequently it was concluded that the majority of new PPI users started the PPI independent of BP use.

PPIs and Fracture Risk With or Without BP Use

The recognition that PPIs might increase the risk of fractures encouraged research into the interactions between BP and PPI (Table 29.3). Although BP have been marketed for several decades the first study to evaluate the risk of fractures as a function of PPI and BP was reported in 2009. To assess effects, de Vries and colleagues used a retrospective cohort from the General Practice Research Database (GPRD) with patients 40 years and older starting PPI ($n=234{,}144$), or BP ($n=67{,}309$). For this analysis, patients were followed for up to about 3.5 years (PPI 3.5 and BP 3.3). In the PPI cohort, there were men and woman and the average age was 62, while in the BP cohort, there were 80 % women and the average age was 72 years old. In a time dependent regression, the risk of fracture while on PPI alone, as a function of daily dose, and as a function of cumulative duration was investigated. Fracture risk for current users was compared to past users. In addition, risk of fractures in concomitant users of BP and PPI versus users of BP alone were also compared. Current use of PPI was associated with a significant increase in fracture risk relative to past use [adjusted relative rate (ARR) 1.15–1.4]. Further analysis by dose showed some increased risk with increasing dose (ARR 1.05–1.61), and duration (ARR 1.18–1.83). Concomitant BP and PPIs users showed an increased risk of any fracture (ARR 1.08) and hip fractures (1.24), similar to that seen by PPI alone, with some data supporting PPI dose dependent observations for any fracture (ARR 1.23), and hip fracture (ARR 1.46). Fracture risk of PPI and BP users relative to BP alone was significant for hip (ARR 1.4) only for current users. This study indicated that the BP were not able to mitigate the increased fracture risk due to PPI use. The authors concluded that PPI is associated with an increased risk of fracture when taken alone or in combination with BP [100]. Similar observations were seen in a population-based Danish study with 38,088 men and women who were new BP users with a mean duration of follow-up of 3.5 years. They assessed the association of PPI use with 2071 hip fractures and 1110 major osteoporotic non-hip fractures in patients with a mean age 70.4 years old. The main results showed that BP alone significantly reduced risk of hip fracture by 39 %, while the reduction in hip fracture for the combination of PPI and BP was not significant at 19 %. A PPI dose dependent blunting of BP anti-fracture effects, with loss of about 50 % of their effect against hip fractures was observed. Age also played a major role in the interactions between PPI and BP taken in combination. However, this study failed to show similar effects for spine, humerus or forearm. It was concluded that the PPI use results in a loss of protection by the BP [48]. Increased risk for fracture due to PPI and BP combination relative to BP alone was also reported in a case–control study reported by Lee and colleagues. The adjusted odds ratio (AOR) for hip fractures on PPIs, and on the combination of PPIs and BP was evaluated. This study used patients with hip fractures (cases) and up to four matched controls from the Korean Health Insurance Review and Assessment Service database. A total of 24,710 hip fracture cases and 98,642 controls were identified; about 4 % of cases and 3 % of control subjects were users of PPIs and about 11 % of cases and over 13 % of the controls were using PB. Increased AOR for hip fractures was related to the use of PPIs, significant at 1.34 regardless of BP use. Further analysis indicated that while BP nonusers had a similar AOR as PPI users (AOR 1.3), BP users had a significantly higher AOR (1.71). The investigators concluded that the mechanism for increased risk of hip fracture by PPIs may arise mainly from interaction of BP and PPIs [101]. Additional data supporting the negative effect of PPI on BP therapy was obtained from a prospective Canadian cohort study with follow-up of over 10 years. The risk of

Table 29.3 Studies of the effect of proton pump inhibitors (PPI) PPI usage alone or with bisphosphonates (BP) on fracture risk

Author (year)	Study design	Population/country number of subjects	Measure (fractures studied)	PPI alone	PPI + BP	BP alone
de Vries et al. (2009) [100]	Retrospective cohort	GPRD, UK PPI $n=234,144$ BP $n=67,309$	Any, osteoporotic, hip, radius/ulna vertebral	Increased risk of fracture (any, osteoporotic, hip vertebral)	Increased risk of fracture, similar to PPI alone (any and hip)	Lower fracture risk
Abrahamsen et al. (2011) [48]	Population-based observational cohort	National register Denmark $n=38,088$ PPI $n=10,177$ No PPI $n=27,911$ Follow-up 3.5 years	Hip, osteoporotic non-hip	N/A	19 % non-significant decreased risk (HR = 0.81)	39 % significant decrease in hip fractures (HR = 0.61)
Roux et al. (2012) [98]	Post-hoc analysis of 3 RCTs	USA $n=2729$ BP $n=2725$ placebo $n=482$ PPI Follow up to 3 years	Vertebral	Fracture rate 16.1 % not different from no PPI (16.9 %)	No effect; same rate as BP alone RR = 0.43	Reduce the risk of new vertebral fracture relative to placebo. RR = 0.62
Lee et al. (2013) [101]	Case control	Korean Cases $n=24,710$ Control $n=98,642$	Hip	Increased fracture risk (AOR = 1.3)	Increased fracture risk relative to PPI alone (AOR = 1.7)	N/A
Fraser et al. (2013) [99]	Prospective cohort	Canada Multicenter Osteoporosis Study $n=9423$ follow-up 10 years	Non-traumatic	Increased fracture risk and decreased time to first non-traumatic fracture (HR = 1.75)	Same risk as PPI alone	N/A
Ioth et al. (2013) [97]	Prospective RCT	Japan $n=62$ BP $n=75$ BP + PPI 9 months	Bone bio-markers and BMD	N/A	Improved BMD and physical functioning beyond that seen with BP alone	Reference
Tanaka et al. (2014) [102]	Prospective RCT	Japan $n=49$ BP + PPI $n=47$ BP 24 months	Bone bio-markers and BMD	N/A	Improved BMD and physical functioning beyond that seen with BP alone	Reference

HR hazard ratio, *AOR* adjusted odds ratio, *RR* relative risk

fractures with PPI use while controlling for known fracture risks was evaluated using BP users. At the beginning of the study there were 9423 participants (6539 women and 2884 men), aged 25 years and older. At baseline 261 individuals were using PPI and out of which 88 (33.7 %) were BP users; there were 9162 PPI nonusers out of which 2200 (24.2 %) were BP users. After 10 years, there were 5569 participants with 1295 subjects experiencing one or more non-traumatic fractures with 158 hip fractures. After 10 years there were 675 PPI users. PPI users were characterized by being older, more likely to be female, higher BMI, and were less active. They were also more likely to use co-medications including corticosteroids and BP. The effect of PPI use was assessed as a time-dependent variable and was associated with shorter time to first non-traumatic fracture with a significant hazard ratio (HR) of 1.75, which held up after multivariate adjustments for age, gender, BMI, BMD, corticosteroids, smoking, and activity level (HR = 1.4). Importantly, adjustments for BP during the 10 years follow-up produced virtually the same results as when there was no adjustment for BP. The HR for BP use was significant at 1.51. Time to first hip fracture was also examined using time dependent PPI exposure yielded a significant HR (2.24) which was attenuated to HR = 1.75 (none significant). This study was designed to evaluate osteoporosis and therefore detailed information that included all known risk factors was collected and used in the multivariable assessment [99].

In contrast to the above studies, there are analyses of randomized, placebo-controlled studies in relatively small population groups that looked at vertebral fractures rather than hip or total fractures reported above in survey studies. PPI use did not make a difference when its effect was assessed in a post hoc analysis of three prospective randomized controlled studies that evaluated the efficacy of BP. The study populations included 2729 BP users and 2725 placebo patients with a follow up to 3 years. A total of 8.8 % of the study population were using PPI, with 240 subjects in the BP group and 242 in the placebo. In the placebo group, there were no differences in vertebral fracture rate between PPI users or nonusers (16.1 % PPI users and 16.9 % in PPI nonusers). Similarly, in the BP group, there were no differences in vertebral fracture rate between PPI users or nonusers (57 % PPI users vs. 38 % in PPI nonusers). BMD improved in BP users, and there was no difference between PPI users and nonusers [98]. In addition to the post hoc analysis, there were two prospective studies comparing bone marker and bone fracture effects of BP alone or in combination with PPI. In the first, Itoh and colleagues showed improvements with the combination of PPI and BP relative to BP alone (no PPI). A total of 180 women with low BMD were randomly assigned to BP with PPI and to BP. Bone biomarkers including N-terminal telopeptide of type I collagen corrected for creatinine, bone-specific alkaline phosphatase (BAP), PTH, BMD of the lumbar spine, and physical parameters were evaluated at baseline and every 3 months up to 9 months. At the end of the study, 62 subjects in the BP group and 75 subjects in the BP concomitantly with PPI were analyzed. There were no significant differences between groups. There was a significant increase in BMD and an improvement of physical functioning in the BP and PPI group, and a decrease in BAP. It was concluded that BP administration in combination with a PPI may be more effective not only for treating osteoporosis but also improving physical fitness than treatment with BP alone. This study highlights the negative effects of BP therapy on fitness, an observation supported by other studies [97]. In the second study, Tanaka et al. [102] used a very similar design and prospectively assessed the effects of co-administration of PPI (rabeprazole) with BP (risedronate) or BP alone on bone parameters after a 24 month intervention. Ninety-six Japanese women (>50 years old) with low BMD were eligible to participate; they were randomly divided into BP alone or a combination of BP and PPI. Biomarkers including BAP, PTH, BMD of the lumbar spine, and physical parameters were evaluated. Improvement in a number of measurements was observed in the group using co-administered BP and PPI; overall the results suggest that PPI does not adversely affect bone metabolism.

In summary, while the results are mixed, based on retrospective and case–control studies, the data suggest that there are interactions between PPI and BP, and consequently no decrease in bone fracture risk was observed in patients taking both PPI and BP. These studies show that BP use cannot mitigate the increased risk of fractures in PPI users, or that PPI use negatively affects risk of fractures in those

who are taking BP. In contrast, there was one neutral study based on a post hoc analysis of previous BP RCT studies that showed no effect of PPI and BP on risk of fractures. Finally, based on 2 RCT, positive effects on bone biomarkers and after BMD were, was observed while on co-therapy; however, there are no data on fractures. Study design, population, location, and duration of follow-up are some of the major differences that could be the reason for the differences in findings between these studies (Table 29.4).

Drug–Nutrients Interactions

Long-term decrease in gastric acid has been associated with several effects on nutritional status, with increased risk of vitamin and/or mineral deficiencies. Overall the risk of developing deficiencies in the general population may be low; however, increased risks exist in the elderly or malnourished sub-populations.

Vitamin B_{12}

Vitamin B_{12} (cobalamin) is an essential water-soluble vitamin that is primarily sourced from animal-derived foods. In foods, vitamin B_{12} is protein bound, and acid and pepsin are needed for the digestion of the protein and the release of the vitamin B_{12}. The free cobalamin attaches to protein R and passes to the duodenum where the R protein is removed and free cobalamin binds to the intrinsic factor (IF). The IF–cobalamin complex is absorbed in the distal ileum in a process that requires calcium. Reduced gastric acid may result in reduced B_{12} bioavailability by directly affecting the ability to release the cobalamin from the food. In addition to the direct effect, additional mechanisms that affect vitamin B_{12} absorption have been proposed [103]. One such potential mechanism is reduced IF, while another may be related to bacterial overgrowth due to hypochlorhydria.

Vitamin B_{12} deficiency is common in certain populations including the elderly and true vegans. In addition, those who suffer from acid imbalance, achlorhydric patients (gastric restriction or vagotomy), patients with atrophic gastritis, or those infected with HP may also be affected [104]. A newer classification related to vitamin B_{12} status, called "sub-clinical" vitamin B_{12} deficiency, was recently developed based on research suggesting that there are diseases that can develop when vitamin B_{12} levels are at "low-normal," levels that were previously considered adequate [105]. Further, there are data to show that levels higher than 350 pg/mL seem to be protective against symptoms of vitamin B_{12} deficiency [106].

Although there are multiple etiologies for vitamin B_{12} deficiency in the elderly, protein bound malabsorption is thought to be the most common cause [107]. Estimates of vitamin B_{12} deficiency vary greatly (3–40 %), and importantly only about half of those affected are clinically symptomatic [108–110]. Unpublished analysis conducted at the CDC of laboratory data from community-based samples of US adults 51 years of age or older, suggest about 1 of every 31 persons (3.2 %) has serum vitamin B_{12} levels below 200 pg/mL [111]. The negative consequences of reduced vitamin B_{12} absorption may take years to develop because the body is able to reutilize vitamin B_{12} by enterohepatic recirculation, and most patients will not manifest signs and symptoms of vitamin B_{12} deficiency until at least 3 years of no vitamin B_{12} absorption [110]. Consequently, a number of potential irreversible hematological and neurological consequences affecting sensory and motor function may develop if vitamin B_{12} deficiency is unrecognized and untreated. More subtle effects including osteopenia, neurocognitive impairment, and increased vascular disease risk associated with elevated homocysteine have also been described [112]. Other indicators of vitamin B_{12} status such as homocysteine and

Table 29.4 Studies of the effect of PPI usage on bone mineral density (BMD)

Study	Study type	Data source	% Women	Measure/Site evaluated	Number of PPI users/number of cases	Number of controls	Follow-up	Age (mean)	Conclusions
Ozdil et al. (2013) [150]	RCT	N/A	54	BMD lumbar spine and femur neck	114 GERD patients	110 healthy controls	6 months	18–56 (37.7)	Treatment with PPI results in significant reduction in bone density
Sharara et al. (2013) [145]	RCT	N/A	0	Biochemical markers of mineral and bone metabolism	29 Frequent heartburn sufferers	29 healthy controls	3 months	18–50 (33.2)	No measurable differences in calcium or bone metabolism
Jo et al. (2014) [149]	RCT	N/A	42	Bone turnover markers	13 gastric ulcer	13 gastric ulcer	2 months	55–85	PPI alter bone metabolism via vascular H^+ATPase
Yu et al. (2008) [134]	Prospective studies	Ongoing large cohorts in the US Osteoporotic Fractures in Men Study (MrOS)	0	BMD hip and femoral neck	487	4236	4.6 years	>65 79.5 nonusers and 79.1 users	Men using PPI had lower BMD (both hip and femoral neck) at baseline.
Yu et al. (2008) [134]	Prospective studies	Ongoing large cohorts in the US Study of Osteoporotic Fractures (SOF)	100	BMD hip and femoral neck	234	2856	4.9 years	>65 73.4 nonusers and 74.7 users	No significant differences were observed
Gray et al. (2010) [136]	Prospective studies	WHI hormone therapy; dietary modification; calcium and vitamin D; observational study	100	3 years change in BMD of hip, spine and total body	118 hip 111 spine 118 total body	6577 hip 6418 spine 6559 total body	3 years	50–80 (63.1 nonusers 64.8 user)	Use of PPI was associated with a marginal effect on 3 years BMD change in the hip but not in other sites.

Targownik et al. (2010) [151]	Longitudinal	Manitoba Bone Mineral Density Database	91	Total hip Spine	251	2298	2.3 years	59.6	Use of PPI did not have a negative effect on rate of BMD decline.
Solomon et al. (2014) [153]	Longitudinal	Study of Women's Health Across the Nation (SWAN) US	100	Spine Femoral neck Total hip	207	1676	9.9 years	50.2–50.7 (at baseline)	No difference in BMD in all sited between PPI users and nonusers.
Lewis et al. (2014) [115]	longitudinal	Calcium Intake Fracture outcome study (CAIFOS) Australia	100	BMD total hip, whole body Quantitative heel ultrasound	120	905	5 years	80 (at baseline)	Bone structure, as determined by total hip BMD, whole body BMD and heel ultrasound did not differ between long-term PPI therapy users and no long-term users.
Targownik et al. (2010) [151]	Cross sectional (case control)	Manitoba Bone Mineral Density Database	92	Total hip Spine	Cases (% PPI users) 2193 (23.2) 3956 (20.9)	Control (% PPI users) 5527 (25.3) 10257 (25.3)	5 years history	67–71	After multivariate analysis there was no association between PPI use and osteoporosis in the hip or lumbar spine
Targownik et al. (2012) [152]	Cross-sectional and longitudinal analysis (prospective)	Canadian Multicentre Osteoporosis Study CaMos	70	Total hip femoral neck lumbar spine	228 ($t=0$)	8112 ($t=0$), 4512 ($t=10$)	10 years	58–61 (at enrolment)	PPI users had lower BMD at baseline. There was no accelerated BMD loss in follow-up while on PPI.
Maggio et al. (2013) [147]	Epidemiological study of the elderly	InCHIANTI, Italy	48	BMD and bone geometry	36	1002	Self report PPI use in the past	74.7	PPI users showed lower BMD trabecular than nonusers. There was no differences in other measures between users and nonusers

methylmalonic acid levels could be used to signal potential increased risk of deficiency [108]. Due to the wide range of negative potential consequences, including irreversible neurologic damage which can impact quality of life, early identification and correction of modifiable risk factors for vitamin B_{12} deficiency is a significant public health target [111].

Several studies have demonstrated an inverse relationship between acid-suppressive therapies and vitamin B_{12} status, and most but not all, showed reduced vitamin B_{12} status with increased duration of acid-suppressive therapy. As with other nutrients, the effect of long-term PPI therapy on vitamin B_{12} has not be evaluated in randomized trials, and such studies will unlikely become available. Further, for vitamin B_{12} there are additional complicating factors including the long lag (5–10 years) between onset of malabsorption and clinical symptoms. Currently, there are no guidelines from medical organizations or task forces on screening for vitamin B_{12} deficiency in asymptomatic individuals despite identification of high risk populations [106]. Wilhelm and Kale-Pradhan proposed that it would be prudent to obtain serum vitamin B_{12} baseline concentrations prior to initiation of PPI therapy [113].

Only one prospective study evaluated the effect of short-term PPI therapy on vitamin B_{12} absorption. Vitamin B_{12} absorption was assessed using a protein bound vitamin B_{12} (cyanocobalamin) supplement with healthy individuals using PPI for 2 weeks [114]. There was a dose–response reduction in protein-bound cyanocobalamin absorption while using the PPI, omeprazole. About a fourfold reduction was observed after omeprazole ingestion, and a further reduction was observed after doubling the omeprazole dose [114]. Additional support for the effect of acid-suppressive therapies is based on lower vitamin B_{12} status from a number of case control, cross-sectional retrospective studies. In a recent large study with up to 15 years of exposure data, Lam and colleagues [107] evaluated the association between vitamin B_{12} deficiency and prior use of acid-suppressing medication. Using a nested case–control study within the Kaiser Permanente in the US database, they compared 25,956 patients diagnosed with vitamin B_{12} deficiency with 184,199 patients without vitamin B_{12} deficiency. Of the cases, 12.0 % were dispensed 2 years' supply of PPI while only 7.2 % of the controls were PPI users. They reported that a new diagnosis of vitamin B_{12} deficiency was more common among long-term PPI users (>2 years). Both duration of PPI use (among all users), and a higher dose (>1.5 PPI pills per day), were associated with an increased risk for vitamin B_{12} deficiency. Long-term PPI use was associated with doubling the risk of clinically diagnosed vitamin B_{12} deficiency. Similar observations were reported by Lewis and colleagues [115] who evaluated the effect of long-term ($\geq$1 year) PPI therapy in a post hoc analysis of a longitudinal population-based prospective cohort of elderly Australian postmenopausal women. They found that long-term PPI therapy users were more likely to have low vitamin B_{12} levels than non-users (50 % vs. 21 %).

Additionally, there are a number of small studies showing similar results. Lower serum vitamin B_{12} was confirmed in a cross-sectional study of elderly patients who were PPI users. Importantly, concomitant oral B_{12} supplementation slowed but did not prevent the decline in vitamin B_{12} status seen when long-term PPI therapy was used [108]. Finally, a retrospective case–control Medicaid drug database identified 125 patients who started vitamin B_{12} supplementation and 500 matched controls. The odds ratio for the initiation of vitamin B_{12} therapy was significantly higher for users of acid-suppressive therapy [110]. In heavy users of PPI with hypersecretion diseases, similar observations have been reported, again demonstrating the negative effects of PPI therapy on vitamin B_{12} status. A longitudinal study of PPI use and vitamin B_{12} status showed a significant trend towards reduced vitamin B_{12} levels with increased duration of PPI therapy. In another study, ZES acid hypersecretor patients with and without gastrinoma were treated with a PPI (lansoprazole) for an average of 140 months. Overall vitamin B_{12} went down about 46 % over 12 years. Ten percent had low serum vitamin B_{12} without signs of vitamin B_{12} deficiency and 31 % had normal vitamin B_{12} but had other symptoms related to vitamin B_{12} deficiency. Vitamin B_{12} therapy reduced vitamin B_{12} deficiency markers and confirmed the effect of PPI [116]. Importantly, as expected, evidence of vitamin B_{12} deficiency appeared only after many years on PPI therapy. Termanini and colleagues [103] evaluated yearly serum vitamin B_{12} status in patients with ZES who were treated with long-term (>4.5 years) omeprazole.

They found that vitamin B_{12} levels were significantly lower in patients treated with omeprazole and that the duration of omeprazole treatment was inversely correlated with vitamin B_{12} status. As ZES patients take PPI chronically, these results imply that ZES patients should have serum vitamin B_{12} levels monitored.

Based on the information available, causal association cannot be definitively ascertained; however, it appears that previous and current gastric acid inhibitor users had a significantly lower vitamin B_{12} status, and that PPI use was strongly associated with the presence of vitamin B_{12} deficiency. These findings should be considered before initiating PPI therapy, especially in certain subpopulations (i.e. elderly) that are known to suffer from more frequent vitamin B_{12} deficiency. As for all drugs, balancing the risks and benefits of using these medications should be evaluated on an individual basis.

Calcium

Calcium is the mineral with the highest concentration in the human body and 99 % of the body's total calcium is present in the skeleton and teeth. Calcium has many critical biological roles and is necessary to sustain life. Calcium's functions stem directly from its chemical properties including intermediate binding affinity, ionic radius, and coordination number which together allow flexibility in its interactions [117]. Interestingly, calcium deficiency does not manifest itself as shortage of calcium for cellular or physiological processes rather as a decrease in the calcium reservoir itself, the bones. Calcium is the most studied mineral in relationship to human health [118]. Calcium (Ca^{+2}) is absorbed in the intestine via two mechanisms: an active, transcellular and a passive, paracellular absorption. The active, classical epithelial transcellular transport occurs with the help of the calcium binding protein calbindin, and calcium ATPase pump. Active transport occurs primarily closer to the stomach, in the duodenum and upper jejunum. In this process, calcium is transferred across membranes into the extracellular space. The amount of calcium absorbed is dependent on the bioavailability of the calcium in the diet and the capacity for absorption in the intestine (which is regulated at least in part by 1,25-dihydroxy vitamin D). The second absorption mechanism is a passive, paracellular process that allows calcium to permeate across gap junctions. This process occurs throughout the length of the intestine. The amount of calcium transferred is dependent on the lumen's permeability which is influenced by 1,25-dihydroxy vitamin D and the bound calcium concentration [119], as well as the concentration of "free" calcium in the serum. The majority of ingested calcium is not absorbed and it is excreted in the feces.

Calcium Absorption and Gastric Acid

Stomach pH may affect intestinal calcium absorption by affecting calcium properties and both the active and passive absorption processes. For active transport, the pore structure may be affected by the level of acidity due to the changes in the confirmation of the proteins, and in particular, it is suspected that the ionic amino acids like glutamate act as a "pH sensor." Similarly, it has been suggested that pH affects the tight junctions that control the passive absorption of calcium from the lumen into the interstitial spaces [120].

The solubility of calcium salts is dependent on their physical and chemical properties and in particular the pH. However, although in vitro calcium solubility is dependent on pH, in vitro solubility does not truly represent human physiology as it relates to calcium absorption. Bo-Linn and colleagues demonstrated that calcium solubility was not a prerequisite for absorbability [121], and Heaney et al. [122] showed that when solubility increased by a factor of over 100, absorbability only doubled. Further, in the presence of a meal, there was bioequivalence in the calcium absorption from calcium

salts that are opposite in their neutral pH solubility including the most commonly used calcium salts in dietary supplements, calcium citrate and calcium carbonate. Therefore, it was concluded that calcium's in vitro solubility was not a prerequisite for efficient in vivo absorption [123]. Calcium absorption has been shown to be greatly enhanced by its consumption with a meal. Interestingly, ingestion of food is usually followed by a 2–3 unit increase in the stomach pH (from about 1 or 2 to well over 4), despite stimulation of acid secretion. The food's large buffering capacity results in a net increase in pH, an effect that is seen even with small meals such as breakfast [124]. Therefore, the role of food and gastric acid (or pH) in calcium absorption appears to be complex.

The effect of reduced stomach acid on calcium absorption was evaluated in one study with subjects suffering from achlorhydria [125], and in a number of small, short-term studies, with healthy individuals utilizing acid-suppressive therapy [126–130] with mixed results. Serfaty-Lacrosniere et al. [126] demonstrated in a placebo-controlled study with 13 healthy adults (59 years old), that PPI (omeprazole) did not negatively affect calcium absorption when the calcium was part of a meal (milk and cheese), despite significant differences in stomach pH due to the omeprazole intervention. Similarly, no effect of 30 days PPI intervention (omeprazole) on calcium absorption was observed in a sequential study with 21 postmenopausal women (58 years old). This study assessed calcium absorption after breakfast and utilized orange juice fortified with calcium citrate malate [127]. Finally, in a placebo-controlled study with 12 young adults who consumed esomeprazole or placebo, again no differences in calcium absorption were recorded despite significant increase in gastric pH (over 2 log pH units). Further, there were no differences in urinary calcium between the PPI and placebo groups [128]. All these studies concluded that short-term gastric acid suppression by PPIs (up to 30 days) does not attenuate intestinal calcium absorption in healthy young adults or postmenopausal women. In contrast, there were two studies where calcium absorption was negatively affected by the use of acid-suppressive therapy. O'Connell et al. [130] evaluated the effect of PPI (omeprazole) on calcium absorption in a placebo-controlled study. In this study, calcium was provided as isotope-labeled calcium carbonate, at 500 mg of elemental calcium without a meal. Omeprazole significantly impaired calcium absorption; in fact, calcium absorption was reduced by about threefold. Similarly, in a small placebo-controlled study with eight healthy male volunteers, Graziani and colleagues evaluated the effect of a high dose of PPI (omeprazole) on calcium absorption and urinary calcium excretion. Calcium was provided in the form of food (milk and cheese) in a single meal. The results indicated that after ingestion of the test meal, calcium plasma level increased in the placebo group, but did not change when volunteers were taking omeprazole. Calcium balance was also affected as was seen by urinary calcium excretion. In the placebo, urine calcium level was higher than after omeprazole where calcium excretion over 24 h period was 33 % lower [129]. The Recker [125] study, the only study with subjects with achlorhydria, showed the effect of the meal for different calcium salts (calcium carbonate or calcium citrate). Notably, without a meal, calcium from calcium carbonate was absorbed to a much smaller degree in achlorhydric patients compared to normal individuals, about 5× less. For calcium citrate, however, calcium absorption was not dependent on co-ingestion with a meal and the fraction absorbed was found to be doubled for the achlorhydric subjects. In summary, based on the results available, acid-suppressive therapy does not appear to affect calcium absorption especially if it is consumed as part of a meal.

Gastric Acid Suppression and Fractures

Since 2006, a number of epidemiological studies showed that PPI use increased the risk of fractures [100, 131–137]. In 2011, a systematic review and meta-analysis reported by Ngamruengphong et al. [39] reported combined findings supporting the association. Since the meta-analysis was published, four additional studies were reported to date [40, 99, 115, 138]. These studies support the original findings that PPI use increased the risk of fracture. Below is a brief review of the studies that establish the relationships between PPI use and fracture.

Gastric Acid Suppression and Fractures: Prospective Studies

A prospective study by Yu and colleagues examined the association between acid-suppressive therapy and skeletal outcomes using two large cohorts, one of 5755 men and the other of 5339 women over the age of 65. There were a total of 822 men and 753 women that were either PPI or H_2RA users. Baseline characteristics revealed that dietary elemental calcium intake was slightly over 700 mg, and that about 50 % of the women and 35 % of the men were taking calcium supplements. In terms of fracture outcomes, there was a 34 % significant increased risk of non-spine fracture in the women PPI users and a 20 % increase when looking at PPI or H_2RAs users. There was also a slight increased risk of non-spine fracture among men who were not taking calcium supplements. It is important to note that in the women's cohort, mean duration of PPI therapy was 1.8 years and that of H_2RAs was 3.6 years; there was a threefold increase in the number who used PPI therapy during the duration of the study (average 4.9 years). In the men's cohort there was a 50 % increase in PPI use during the study (average 4.6 years). Yu et al. estimated that one extra non-spine fracture would be expected for every ten women treated with PPIs for 5 years, and concluded that the use of PPIs in older women, and perhaps older men with low calcium intake, may be associated with a modestly increased risk of non-spine fractures [134]. In the second prospective study, Raux demonstrated increased risk of vertebral fractures in postmenopausal women recruited from five European centers. About 2400 women were recruited from 1999 to 2001 and were followed for a mean 6.1 years for the Osteoporosis and Ultrasound Study. There were 1211 vertebral fractures and 1346 non-vertebral fractures. In the multivariate analysis, PPI use (omeprazole) was a significant and independent predictor of fractures with a relative risk of 3.1, and importantly, it was independent of age [139]. A large prospective study used data from the Women's Health Initiative (WHI), to evaluate the effect of PPI use on both fracture risk and BMD. In terms of fractures, after a mean follow-up of 7.8 years, there was no increased risk for hip fracture; however, there was a significant increase in clinical spine fracture and forearm or wrist facture, and an increase in overall total fractures for PPI users. There was no consistent trend with regard to the duration of PPI intervention [136]. Khalili examined the association between chronic use of PPIs and risk of hip fracture in a prospective cohort study from the Nurses' Health Study. 79,899 postmenopausal women were followed for up to 8 years and had 893 hip fractures. This database contained detailed dietary and lifestyle information collected biannually. For example, in 2000, 16,478 participants provided information regarding their heartburn, use of PPI and reasons for its use. The risk of hip fracture among women who regularly used PPIs for at least 2 years was significant at 1.35 (compared to nonusers). The trend for duration was significant; longer use was associated with increased risk. Multivariable adjustment for risk factors did not materially alter this association (1.36). In this study, the risk of hip fracture was associated with duration of PPI use (1.36–1.55 with increase use from 2 years to 6–8 years). The effect of time since stopping PPI on fracture risk was also evaluated and while the risk persisted for 2 years, when women stopped for more than 2 years, the risk was similar to that of women who never used PPI. There was an interaction with smoking; among current and former smokers, PPI use was associated with greater than 50 % increase in risk of fracture (1.51). They concluded that chronic use of PPIs is associated with increased risk of hip fracture, particularly among women with a history of smoking [40]. In a prospective study with follow-up of over 10 years, a Canadian cohort was evaluated for their risk of fractures with PPI use while controlling for known fracture risks. There were 5569 participants at the end of the study with 1295 subjects experiencing one or more non-traumatic fractures including 158 hip fractures. PPI users were characterized as being older, more likely to be female, higher BMI, and were less active. They were also more likely to use co-medications including corticosteroids and BP. PPI use was assessed as a time-dependent variable and was associated with shorter time to first non-traumatic fracture with a significant hazard ratio of 1.75, which held up after multivariate adjustments. With regard to duration, the data indicate rapid divergence between PPI users and nonuser. This separation supports previous studies where short-term use of PPI has not been shown to be associated with fracture risk. However, this data set also suggests that increased duration of PPI use further strengthen the association. They concluded that PPI use is associated with increased risk of fragility

fractures [99]. Finally Lewis and colleagues [115] evaluated the effect of long-term (≥1 year) PPI therapy in a post hoc analysis of a longitudinal population-based prospective cohort of elderly Australian postmenopausal women. There were 905 participants that did not use PPI and there were 120 long-term PPI users with a mean age of 79.9 at baseline. Among the PPI users 23.3 % had injurious falls and there were 13.7 % injurious falls in the nonusers and the AOR was significant at 1.95. The AOR for fractures was in the PPI group at 2.17. Fracture sub-analysis by duration, showed that only long-term PPI use was significant and AOR was 2.04, while the AOR for short term and previous use was 1.2 and 1.12, not significant. The effect of dose on fracture was significant when the dose was higher than the standard dose with AOR of 2.46.

Gastric Acid Suppression and Fractures: Retrospective and Case–Control Studies

Vestergaard et al. [131] in a case–control study were first to demonstrate that PPI use was associated with increased risk of fractures. Subjects were sampled from the Danish population and eligible cases consisted of 124,655 individuals who suffered a fracture in the calendar year 2000. The control group of 373,962 individuals were matched by gender and age from the same database. Exposure and other variable confounders included antacids and acid-suppressive therapies and several other drugs that may interfere with calcium absorption or resorption. PPIs significantly increased the risk of fractures (OR 1.18, 1.45, and 1.60 for overall, hip, and spine fractures). Notably, due to the method by which the data was collected the population was not restricted by age [131]. Similar observations were also reported based on a very large nested case–control study with 13,556 cases of hip fracture and 135,386 controls in the UK. While studying the effects of long-term PPI use, Yang showed that both men and women PPI users over 50 years old were at increased risk of hip fracture and that the risk increased with increased duration, and dose of PPI. AOR was 1.44 for more than 1 year of PPI therapy, up to 2.65 for longer term and higher dose. Similarly when comparing duration of PPI therapy, AOR increased from 1.22 to 1.58 for 1–4 years, respectively [132]. The relationship between duration of exposure to PPIs and osteoporosis-related fractures was also explored by Targownik [133]. In a case–control study based on medical claims data in Manitoba, Canada, patients with hip, vertebra, and wrist fractures who were over 50 years old were matched with three controls based on age, sex and other co-morbidities and AOR were calculated. Overall, there were 15,792 fracture cases and 47,289 controls and follow-up continued for over 5 years for about a 1/3 of the cohort. Exposure to PPIs for over 7 years was associated with increased overall fracture risk; OR = 1.6 for hip fracture after 5 years and increased to 4.55 after 7 years of PPI use. Based on a retrospective cohort study using the GPRD in patients aged 40 years and older starting PPI (n = 234,144), there was increased risk of fracture with PPI use. In the PPI cohort, there were men and women and the average age was 62 and PPI users were followed up for about 3.5 years. In a time-dependent regression, the risk of fracture while on PPI alone, as a function of daily dose and as a function of cumulative duration was investigated. Fracture risk for current users was compared to past users. Current use of PPI was associated with a significant increase in fracture risk relative to past use (adjusted relative rate ARR 1.15–1.4). Further analyzing by dose showed some increased risk with increasing dose (ARR 1.05–1.61), and duration (ARR 1.18–1.83). They concluded that PPI is associated with an increased risk of fracture [100].

Moberg reported similar results while using a different way of looking at data based on a retrospective study from Sweden. Data strongly supported previous indications of increased risk of fractures with PPI. Mean follow-up of 14.4 years of 6917 postmenopausal women; history of a fracture before the follow-up was considered a separate risk factor; there were 903 women with 1137 fractures. Interestingly, women with fractures were taller, had a lower BMI and BMD, and had a shorter fertile period. Variables, including PPI use and other variables such as corticosteroids, were identified from the univariate analysis and were included in a multivariate logistic regression. The use of PPI was

significantly associated with increased fracture risk in all analyses and ranged from 2.01 to 2.53 as a function of the adjustments. Greater risk was observed (2.95) when women with previous fracture before the age of 40 were excluded. Based on outcomes, Moberg highlights the opportunity to initiate preventative measures for targeted populations with certain risk factors such as previous fractures [138]. The most recent prospective cohort published looked at PPI use for longer than a year, and risk factors including bone structure, balance-related function and falls in two cohorts: the first were women with a 5 year follow-up with assessed bone structure ($n=1025$) recruited from the Calcium Intake Fracture Outcome Study (CAIFOS), a study assessing the effect of daily calcium supplementation, and the second, the replication study, there were 686 women who participated in a 9 month intervention and were randomized to vitamin D supplementation [115]. In the first study, there were 120 (11.7 %) PPI users and the duration of PPI use was 3.35 years. The results indicate that 23.3 % of PPI users had related hospitalization and 17.5 % had fractures while there were 13.7 % incidence-related hospitalizations and 9.8 % fractures in the nonusers. For major osteoporotic fractures, the AOR was 2.07. Analysis indicates that falls accounted for the association between PPI therapy and fractures. There were significant effects related to duration and dose. In the replication study, 132 (19.2 %) used PPI. More PPI users, 35.6 %, had falls-related hospitalization, compared to 26.0 % of the nonusers, with AOR of 1.57.

Gastric Acid Suppression and Fractures: Meta-analysis

A recent meta-analysis included ten studies with 223,210 fracture cases concluded that PPIs were associated with increased risk of fractures [39]. Outcome measures were hip, spine, wrist/forearm factures. Subgroup analysis looked at study design, duration, dose, and risk of bias. Overall, there was an increase in risk of fractures in PPI users as compared to past or no users. For hip fractures, based on 9 studies, the risk was significant at 1.25, similar to previous observations. Based on duration analysis, the risk for short-term exposure was significant at 1.24, while it was not significant for the longer exposure duration. This result is in contrast to previous analyses and as more data will become available, this finding will be further explored. For vertebral fractures, based on four studies, the risk was significant at 1.5, and for wrist/forearm the risk was not significant at 1.09. Overall for hip and vertebral fractures, PPI significantly increased the risk of fracture. Although significant, the effect is small 1.3–1.5.

Gastric Acid Suppression and Fractures: Summary

Overall, most studies consistently show an association of low magnitude between long-term PPI use and increased risk of all fractures and/or hip fractures. Further, increased PPI dose and/or duration also contributed to the increased risk; however, it was not consistent. Although these studies may not be as conclusive as placebo-controlled intervention studies, they illustrate that more research is needed to elucidate the biological effects of long-term use of PPIs on bone. As summarized by Richards and Goltzman [140], clinicians should help patients understand risk-benefit and especially after long-term PPI use. There may be situations where reduced dose and/or finite duration should be considered [140]. In summary, the totality of the data indicate that the use of acid-suppressive therapy, and in particular PPIs consistently albeit to a small degree increase the risk of fractures.

Considering the data available in 2010, the US FDA issued an advisory warning regarding the high dose, and long-term usage of PPI, and required labeling warnings with regard to risk of fractures [141]. In 2011, the FDA determined that no label warning were needed for OTC PPI due to the short-term usage recommendations [142].

PPI and Fractures: Hypothesized Mechanisms of Action

A number of hypotheses have been proposed to explain the relationship between PPI usage and data-related risk of fractures; however, the exact mechanism is currently unclear. Involvement of bone's osteoclast, vascular ATPase (V-ATPase), modification of local acidification, and changes in the remodeling process has been suggested. While PPIs act on the gastric parietal cell ATPase, it was postulated that PPIs can potentially exert some activity on the bone's osteoclast V-ATPase activity. In vitro studies indicate, however, that much higher concentrations of PPI would be required to inhibit the osteoclast resorption activity relative to the gastric glands. Currently, there are no clinical data to support this pathway [48]. Localization of the PPIs activity may be related to the general structure of the ATPase as well as the different sub units of the parietal ATPase relative to the vascular ATPase [135].

Increased serum gastrin, a sign of hypergastrinemia, and the long-term omeprazole use were associated with increased fracture risk in a number of studies, and a two- to sixfold increase in gastrin has been reported [143, 144]. PPI induced hypergastrinemia, and consequently hyperplasia and hypertrophy of the parathyroid (also called primary hyperparathyroidism), may result in increased parathyroid hormone (PTH) which negatively affects bone mass [49, 145]. Only a small number of studies evaluated the effect of PPI on PTH; one small study showed a 28 % increase in PTH while on omeprazole [146]. Increased levels of PTH have not been associated with use of PPIs in an epidemiological study with a small percent of elderly PPI users [147], or in a prospective study with young adults [145]. Based on the information available, no conclusions can be made with regard to the effect of PPIs on bone through PTH at this time. Another potential effect of hypergastrinemia may be a result of the elevated gastrin and thus histamine production (H1RA) which can lead to bone loss. Indeed significant interactions between PPI and H1RA and fracture risk were documented in a case–control study of over 120,000 patients with hospital-related fractures in Denmark. It was demonstrated that H1 antagonists are not associated with hip fracture unless PPIs were used [148].

Gastric Acid Suppression and Bone Mineral Density

Among other mechanisms, a number of studies sought to investigate whether chronic use of PPIs is associated with differential changes in calcium metabolism, directly through calcium absorption and through effects on BMD. It was anticipated that these may provide insight into the biological plausibility of the association between PPI use and increased risk of fracture. The effect of acid-suppressive therapy on calcium absorption was discussed earlier (Section 6.2.1), and interestingly, PPI use does not seem to negatively affect calcium absorption. Summarized below is information related to changes in BMD with regard to PPI use. Several recent prospective studies assessed the relationship between PPI intervention and bone markers; these were short term (from 8 weeks to 6 months), studies with PPIs in "new users" [145, 149, 150]. These studies relied on bone markers and/or BMD and the results reported were mixed. Two studies showed an effect while the third did not. There are a number of prospective/case–control reports based on an analysis from large cohorts from the USA and Canada, and long-term (up to 10 years) follow-up, where annualized changes in BMD can be assessed [134, 136, 151–153]. Here again results with regard to bone metabolism and BMD are inconsistent. While short-term studies might show an effect, longer and larger prospective data do not support these findings. Overall, similar to calcium absorption, there does not seem to be an effect of acid-suppressive therapy on BMD.

Gastric Acid Suppression and Bone Metabolism and Density: RCT

Jo and colleagues assessed bone-related parameters after 8 weeks intervention with a PPI (pantoprazole), or an acid pump antagonist (revaprazan) in a parallel randomized controlled trial. Thirty-nine older Koreans with gastric ulcers (mean age about 63) were assigned to PPI ($n=20$) or revaprazan

($n = 19$) randomly. Several bone turnover markers (total serum and urine calcium, iPTH (ionized parathyroid hormone), urine deoxypyridinoline (DPD), and osteocalcin) were investigated. Twenty-six subjects completed the trial; serum calcium and urine DPD increased in the PPI group relative to baseline; in contrast, no such changes were observed in the revaprazan group. The multivariate analysis showed that being older (≥60 years) was an independent predictor for changes in serum corrected calcium and urine DPD. It was concluded that bone parameters underwent significantly greater changes in PPI users relative to the group taking revaprazan, and that short-term PPI intervention induces changes in bone metabolism [149]. Another study with a similar interpretation was reported by Ozdil using changes in BMD. The effect of intervention with several PPIs (esomeprazole, lansoprazole, or pantoprazole) or no intervention on BMD at the lumbar spine and femur neck was evaluated with 114 younger patients, and 110 age-matched controls (mean age 37.7 years old) after about 6 months of PPIs intervention. Overall, treatment with PPIs resulted in a significant reduction in bone density, both in lumbar spine and femur neck. Interestingly, although overall similar trends were observed with regard to the PPI class, different PPIs exerted significant differences in their overall effects [150].

Gastric Acid Suppression and Bone Mineral Density: Prospective Studies

No effect of PPI intervention on bone metabolism was observed in a prospective study that compared markers of bone metabolism in healthy young adults with heartburn who were given 12 weeks of PPIs (esomeprazole, rabeprazole, or lansoprazole) compared to healthy adults with no intervention. A total of 59 men with a mean age of 33 were included in the study. There were some significant differences between the groups at the initiation of the study including baseline calcium intake, BMI, and the bone biomarker, serum C-terminal cross linked telopeptides of type 1 collagen. There were no differences between the groups in terms of PTH, serum ionized calcium, or biochemical markers of bone remodeling. It was concluded that PPI intake for 12 weeks has no measurable effect on calcium or bone metabolism in healthy young men [145].

In a prospective observational study, Raux and colleagues [139] assessed the risk of vertebral fractures in 1211 postmenopausal women from the Osteoporosis and Ultrasound Study. Radiographs were performed at the beginning and after 6.1 years of follow-up, at the final visit. PPI, omeprazole exposure and potential cofounders were ascertained. At baseline patients using PPI had lower lumbar spine and hip BMD *T* score; 23 % had *T* score below 2.5 while only 14.2 % of the control group had *T* score below 2.5. In the multivariate analysis, lumbar spine *T* score at baseline was an independent predictor of vertebral fractures. Patients receiving acid-suppressive therapies have more comorbidities and their baseline status clearly show differences in BMD, an independent risk factor for future fractures [139]. Lewis and colleagues [115] evaluated the effect of long-term (≥1 year) PPI therapy in a post hoc analysis of a longitudinal population-based prospective cohort of elderly Australian postmenopausal women on bone structure. There were 905 participants that did not use PPI and there were 120 long-term PPI users. Bone structure, as determined by total hip BMD, whole body BMD and heel ultrasound did not differ between long-term PPI therapy users and non-long-term PPI users. In a prospective study, Yu et al. [134] reported on a secondary analysis on the effect of PPIs on BMD based on two US cohorts for men and women over the age of 65 in over 5000 individuals in each cohort. Using ongoing prospective studies, the analysis for women was based on repeated BMD and a follow-up exam following an average of 4.9 years ($n = 2856$), and for men, a follow-up exam after an average of 4.6 years ($n = 4230$). PPI users had higher BMI, used more NSAIDs and corticosteroids, were in poorer health (self reported) and were less active. At the initial visit, male PPI users had a lower BMD in total hip and femoral neck, and based on minimally adjusted models there was an increased rate of bone loss in PPI users, but no differences with the multivariable models. For female PPI users, there was a slight increase in one model which became insignificant in another model. A large prospective study used data from the Women's Health Initiative (WHI), to evaluate the effect of PPI use on both fracture risk and BMD. For BMD, there was a

significant reduction in hip BMD after 3 years; however, there were no differences after 6 years in BMD; no differences were seen for spine and total body at any time point. However, using repeated measures to analyze the effect of time showed significant reduction in hip BMD between PPI users and nonusers [136]. Another study with 8340 subjects (baseline) who were followed up for 10 years ($n=4512$) was used to assess the effect of PPI on BMD. Importantly, PPI users had lower BMD measurements at the initial assessment in all sites (lumbar spine, femoral neck, and total hip), higher BMI, suffered from other medical conditions and used concomitantly other medications including glucocorticoids. The same results were obtained after exclusion of osteoprotective medications and glucocorticoids, and when looking at subjects >50 years old. PPI use was not associated with rate of change in BMD as a function of all time points assessed [152].

Gastric Acid Suppression and Bone Mineral Density: Cross-Sectional and Cohort Studies

Targownik and colleagues in 2010 used the Manitoba Bone Mineral Density database to conduct several analyses related to the effect of PPI on BMD. In a cross-sectional study, they identified "hip cases" and "lumbar spine cases" based on low BMD and matched to normal BMD controls. There were over 2000 cases for hip (with over 5000 controls) and over 3000 cases for the lumbar spine (with over 10,000 controls). After adjustment for confounders, no association was identified between low BMD and PPI use in either bone location. When age >60 was analyzed, no association was identified either. In addition in the longitudinal assessment, they studied subjects with two BMD assessments separated by 1–3 years for yearly change in BMD in the total hip. For this analysis over 2000 subjects were identified, and there were no differences in the BMD decline based on PPI intervention, or PPI dose [151]. A cross-sectional epidemiological study with a small percent of elderly PPI users ($n=36$), showed a negative association between chronic PPI use (self-reported based on last 15 days) and trabecular BMD. This may be attributed to differences in density, porosity, three-dimensional structure of the bone. Further, the differences were found in the metabolically active part of the bone. No differences were found in cortical bone density or in bone geometry. The authors of this small study indicate that their data support the hypothesis that PPIs negatively affect bone and mineral metabolism [147]. No differences were found in PTH between PPI users and nonusers. This study excluded bisphosphonate users therefore the effect of PPI on bisphosphonate use cannot be evaluated.

To overcome limitations associated with studies where current PPI users are evaluated longitudinally and subjects who enter into the studies differ in their baseline profile, Solomon and colleagues designed a study that looked at a cohort prior to PPI intervention. Data from a longitudinal cohort from the Study of Women's Health Across the Nation (SWAN) compared annualized changes in BMD in new PPI users with subjects that did not use PPI. They identified 207 new PPI users and 1676 controls across their transitional years [42–52], with annual BMD measurements in most study sites. They were followed for a median 9.9 years. There was an opportunity to assess BMD prior to PPI intervention at baseline, and to then calculate the rate of bone loss with or without PPI use. Using adjusted models, no differences were found in annualized BMD changes in the lumbar spine, femoral neck, or total hip between PPI users and nonusers [153]. Maggio et al. [147] in an epidemiological study of the elderly in Chianti, Italy, looked at BMD and bone geometry in 36 PPI users and 1002 nonusers and showed lower trabecular BMD than in nonusers. There was no difference in other measures between users and nonusers.

Gastric Acid Suppression and BMD: Summary

A number of studies assessed the effect of long-term PPI use on changes in BMD with mixed results. Most prospective, retrospective and case–control studies showed no effect of PPI use on BMD. Weak effects were no longer significant after including additional modifications and adjustments in the

statistical models. In addition, there were a number of randomized studies that evaluated bone markers; these studies also showed no effect of PPI intervention on bone markers. Lower BMD of PPI users may contribute to differences found between PPI users and nonusers. Overall use of PPI does not appear to negatively affect BMD, yet does appear to increase fracture risk with long-term use.

Iron

Iron absorption is highly regulated and is primarily dependent upon the level of iron stores and erythropoietic activity. Iron in the diet can be divided into two forms, heme and nonheme. The heme iron is in the ferrous form (Fe^{+2}), whereas most of the nonheme iron is in the ferric (Fe^{+3}) form. There is a separate transporter protein for nonheme iron, and the ferric form requires reduction (in the presence of acid) to the ferrous form before it can be transported across the intestinal lumen and absorbed [154]. Heme iron is relatively more bioavailable and is only slightly influenced by dietary factors. In contrast nonheme iron absorption is dependent upon its solubility and interactions with other meal components. Gastric acid is needed to maintain nonheme iron in solution in the ferrous form and therefore decreased stomach acid may lead to impaired iron absorption [155, 156]. Ascorbic acid in the stomach enhances absorption of nonheme iron, primarily through reduction of ferric iron to the ferrous form [157]. Further, PPI therapy can potentially affect iron absorption through its effect on gastric vitamin C content, particularly in people with HP infection. Healthy volunteers using PPI (omeprazole) have significantly reduced fasting gastric levels of vitamin C, and the active form, ascorbic acid. Therefore, individuals that are on omeprazole therapy have a limited capability to absorb iron. This compromised capability may manifest itself in diseases such as iron deficiency and iron deficiency anemia [158, 159]. Other organic acids, such as citric, lactic, and malic acid, also enhance nonheme iron absorption. The level of gastric acid in the stomach also affects the potential to absorb iron; achlorhydria has been associated with increased risk of iron deficiency and is seen most frequently in the elderly [160–162]. There is limited and conflicting clinical data with regard to acid-suppressive therapy and iron absorption/indices. Several case reports indicate that PPIs negatively affect iron status [163–165]. A similar association can be made based on a recent small (~100), retrospective cohort study of adult patients who received PPI therapy for at least 1 year compared to matched controls [166]. A significant decrease in hematologic indices from baseline in patients receiving chronic PPI therapy was observed. Another small study supporting the adverse effect of acid-suppressive therapy on iron status used radioactively labeled iron (nonheme) and cimetidine (H_2RA) to show a reduction in the percent of iron absorbed that was dose dependent. While iron absorption was reduced by 28 % when 300 mg of cimetidine was administered, iron absorption was reduced by 42 and 65 % with the increase in cimetidine to 600 and 900 mg, respectively. Similarly, antacid (1.2 g aluminum hydroxide, 1.2 g of magnesium hydroxide, and 90 mg simethicone) also reduced iron absorption by 52 % [161]. In patients with abnormally high absorption of iron (hereditary hemochromatosis (HH)), reduced iron absorption is advantageous. Data indicate that long-term use of PPIs in patients with HH, reduced the requirement for maintenance phlebotomy. PPI-induced suppression of gastric acid reduced dietary iron absorption in the patients and there was a significant reduction in the volume of blood needed to be removed annually while on the PPI. It was concluded that administration of a PPI to patients with HH can inhibit the absorption of nonheme iron from a test meal and the habitual diet [167]. In contrast, in patients with Zollinger–Ellison syndrome taking PPI for at least 6 years, they showed no evidence of iron deficiency; however, this patient population is known to over-produce acid [168]. Similarly, no effect of short-term omeprazole administration on ferrous sulfate absorption, as measured by serum iron concentration, was found in a preliminary study with nine healthy individuals before or after administration of omeprazole for 4 days [169].

Magnesium

Magnesium homeostasis is determined principally by two processes, gastrointestinal absorption and renal excretion. Magnesium absorption occurs primarily by a passive, trans epithelial concentration-dependent gradient, and to a smaller degree by active transport. Only after decades of PPI prescription, the first association between symptomatic hypomagnesemia and PPI use was described in case reports [170]. Additional case reports supporting this association have accumulated and interestingly, improvement in magnesium status was observed upon discontinuation of PPI therapy. A number of cross-sectional and retrospective studies assessing this association have been published in the past 2 years [171–173], and Park and collogues conducted the first meta-analysis investigating the association between PPI and hypomagnesemia [174]. Nine studies all retrospective in nature, including 115,455 patients were analyzed. The median proportion of patients with hypomagnesemia on PPI was 27.1 % while for patients not taking PPIs, the median proportion of patients with hypomagnesemia was 18.4 %. The pooled odds ratio for hypomagnesemia while on PPI use was significant 1.775 [174]. This analysis had some limitations including heterogeneity of the studies and lack of randomized controlled trials.

The precise mechanism for hypomagnesaemia in patients taking PPI is unknown; however, based on measurements of renal excretion of magnesium, it was concluded that impaired intestinal absorption rather than renal excretion was the primary cause of PPI-induced hypomagnesemia [175, 176].

The US FDA recommended considering baseline magnesium levels be established prior to, and periodically while on chronic PPI therapy. The Food and Drug Administration issued a Drug Safety Communication in 2011 [177], emphasizing the importance of long-term use of prescription PPIs in this association. This is critical when PPIs are taken in combination with other medications that are known to impact or be impacted by magnesium status.

Effects on Vitamins and Minerals: Summary

Increasing evidence suggests that acid-suppressive therapies, and in particular long-term PPI use, is not without risk. Consequences include reduced mineral and vitamin absorption. In some cases, such as in the increased risk of fractures, the association between acid-suppressive therapies and adverse events is based primarily on observational studies [131–133]. While these observational studies use large databases that carefully document both the cases based on disease codes and medication usages, it is important to note that such associations are prone to confounders such as other conditions, lifestyle influences and other OTC medication intake. Further, no conclusive data are available based on short-term intervention studies that evaluate the effect of acid-suppressive therapies on calcium absorption. Finally, in the cases of vitamin $B_{12,}$ iron and magnesium, much smaller cohorts have been used to demonstrate the effect. Despite these limitations it is important to note that these adverse events have occurred and that risk-benefit analysis should always consider the potential for adverse nutritional effects.

Conclusions

The stomach is a critical organ in the digestion of food and protection from food-borne pathogens. Gastric secretion of highly acidic hydrochloric acid is a normal factor involved in food digestion. This chapter reviews the major results of hypersecretion of gastric acid as well as the abnormal exposure of esophageal or duodenal tissues to gastric acid. There is an in-depth review of PPIs, the major class

of drugs used to treat patients with gastric acid-related diseases including GERD and ulcers. Acid-suppressive therapies and in particular specific PPIs were reviewed in detail and their significant positive and specific negative effects in patients with GERD, gastric and duodenal ulcers, HP, as well as in patients using BP, NSAIDS, or DAPT were examined. Data indicate that long-term interventions, especially with PPIs, are associated with certain negative effects including bone fractures and certain negative nutritional consequences. Of particular note are the studies associating long-term PPI use with vitamin B_{12} deficiency, iron deficiency and perhaps magnesium deficiency; effects on calcium absorption have not been verified. The consistent association of long-term PPI use and increased fractures of the hip and other bones in patients with osteoporosis or taking BP are not supported by current finding in intervention studies that examine PPI's effects on BMD or biomarkers of bone metabolism. Thus, at present, there is no clear mechanism of action to explain the increased fracture risk. There are also potential drug–drug interactions because many classes of drugs, including the PPIs, use liver cytochrome enzymes. These important negative consequences of long-term therapy with PPIs have resulted in FDA changes in PPI labels to inform patients of the increased fracture risk. However, there are no well-controlled studies of PPI use beyond 10 years. Many patients use these drugs for several decades during the time of greatest bone loss. Older patients may also be using several drugs and have a number of dietary issues that can be adversely affected by long-term PPI use.

Recommendations

Long-term use of acid-suppressive therapy should be evaluated at least annually, and step down algorithms should be considered. Careful patient drug use histories should be obtained to prevent drug–drug interactions. Clinicians should monitor patients on chronic acid-suppressive therapy for vitamin and mineral status and initiate supplementation before deficiencies develop. Patients with osteoporosis and/or taking BP should be counselled about PPI use. HP infection should be ascertained and treated as there are numerous negative consequences associated with this infection. Research is needed to determine the potential effects of PPI on the microbiome as well as eating behaviors.

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Chapter 30
The Impact of Micronutrients on Inflammation and Health in Low- and Middle-Income Countries

Ian Darnton-Hill, Faruk Ahmed, and Samir Samman

Key Points

- Deficiencies of micronutrients (vitamins, minerals and trace elements) are common—up to a third of people in low- and middle-income countries are affected.
- Women and children, especially those living in poverty, are those most at risk often because of micronutrient-, protein- and energy-poor diets, increased metabolic demands of growth, pregnancy and lactation and repeated infections.
- A vicious cycle of undernutrition leads to reduced immunity that increases disease risk and then the disease itself causes further undernutrition and so on.
- Increasing challenges, in all countries, are overweight/obesity and non-communicable diseases—which have implications in management because they often are low-grade inflammatory diseases and frequently there are low micronutrient intakes of those affected.
- Immune systems are impacted by micronutrient deficiencies:
- Vitamin A deficiency impairs innate, cell-mediated and humoral antibody responses but probably not viral infection.
- Zinc deficiency affects both innate and cell-mediated immunity but effects of supplementation on antibody production in human less clear than in animals.
- Iron deficiency, and overload, impair both innate and cell-mediated immunity, with no effect on humoral antibody production.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_30

- Vitamin D deficiency impairs the regulation of innate immunity and other antimicrobial mechanisms and may be associated with cardiovascular disease risk.
- Vitamins B6, B12, folate and E deficiencies impair Th1 cytokine-mediated immune response through insufficient production of pro-inflammatory cytokines, shifting to an anti-inflammatory Th2 cell-mediated immune response thus increasing the risk of extracellular infections.
- Vitamin C deficiency in humans impairs leukocyte functions and decreases overall NK cell activity and lymphocyte proliferation.
- New avenues of immunomodulatory effect continue to be identified for these and other micronutrients.
- Supplementation with micronutrients generally reverses these impaired immune responses
- Micronutrient deficiencies can also be addressed by dietary improvement (if available and accessible), and by fortification.
- It is important to address other interventions such as control of infectious and chronic diseases, immunization, water and sanitation, breast-feeding and the reduction of social inequities.

Keywords Micronutrients • Immune system • Innate immunity • Cell-mediated immunity • Humoral immunity • Inflammatory mechanisms • Vitamin A • Zinc • Iron • Public health interventions • Public health nutrition • Women and children • Low- and middle-income countries (LMIC) • Chronic diseases

Introduction

The global under-five mortality rate has declined by nearly half (49 %) since 1990, dropping from 90 to 46 deaths per 1000 live births in 2013 (UNICEF 2014). Nevertheless, nearly 6.3 million children under 5 years of age continue to die unnecessarily in low- and middle-income countries (LMIC) [1, 2]. Undernutrition is the direct cause of almost half of these deaths [3] aggravated by infectious diseases and detrimental environments. Undernutrition contributes to over 3.5 million child deaths [3] and micronutrient deficiencies (vitamin A and zinc deficiencies in particular) have been estimated to account for one million of these deaths per year, or 9 % of global childhood burden of disease (under 5 years) [3]. At the same time, iron deficiency is a risk factor for maternal mortality, responsible for 115,000 deaths per year, or 20 % of global maternal deaths [3]. FAO estimates over 800 million people are hungry and at risk of food insecurity [4], with all the consequences on ill-health and impaired development and subsequent reduced productivity [5].

Malnutrition, both undernutrition and the more recent epidemic of overweight, obesity and related non-communicable diseases, impacts the development and function of the immune and inflammatory responses [6]. The relationship between poor nutritional status and impaired immunity and inflammatory responses is bidirectional [6]. Protein-energy malnutrition and micronutrient deficiencies, based on both animal and human studies, impact on immunity and inflammation through a variety of possible mechanisms: epithelial barrier function, innate physiological barriers, macrophage function; neutrophil function; NK cell function; APC function; T-cell function and B-cell function [6]. The global epidemic of overweight, obesity and non-communicable diseases in all countries, LMIC and more affluent [7], has also considerable implications due to the low-grade inflammatory nature of most of these conditions and the often accompanying micronutrient deficiencies [8]. The importance of micronutrient deficiencies on immunity, inflammation and infection is a critical piece in the global effort to address child and maternal mortality and global health. Vitamin A, zinc and iron will be discussed primarily, along with the other relevant vitamins and minerals such as folate and vitamins D and B12, selenium and others which are known to have an impact on immune function and status. These micronutrients will be discussed only in terms of their immune functions in humans and the

public health implications of deficiencies, as there are many other sources of information on structure, dietary sources, bioavailability, clinical manifestations, pathophysiology and the epidemiology of micronutrient deficiency.

Even just 40 or so years ago, it was generally assumed that, as far as the human was concerned, each vitamin served one particular main function, e.g. vitamin C preserved connective tissue; vitamin D, bone; vitamin B1, the nervous system; nicotinic acid, the skin; folic acid and vitamin B12, the blood and vitamin A, the eye [9]. This is now known to be clearly not the case and many vitamins, and minerals, are involved in many physiological actions, often, directly or indirectly, impacting on the immune system [6]. This is more apparent with vitamins A, D and folate and zinc, iron and selenium, and less so vitamins C, D and E although the specific mechanisms are not clear. In the seminal book from WHO [10] 40 years ago, it was noted that the formation of specific antibodies is inhibited by many severe nutrient deficiencies, including protein, tryptophan, vitamins A and D, ascorbic acid, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folate and vitamin B12.

This chapter examines the cycle of deficiencies, and sometimes excesses of micronutrients, leading to impairment of the immune system and leading to infectious diseases and inflammation, as well as their relationship to chronic diseases. The public health implications of these interactions and public health interventions in the control and prevention of micronutrient deficiencies will be evaluated. Although there is a considerable literature on micronutrients and growth, that will not be considered here, although obviously of considerable public health importance. Although much of the investigative work on micronutrients and immunity has been carried out in animal studies, it has been found that the mechanisms in humans may differ and so the emphasis has been on the sparser literature on humans [10] and on public health aspects.

Dietary factors that impact on inflammation and immunity, and thus on health, operate throughout an individual's lifecourse, probably even before birth. It has been suggested that maternal supplementation might affect the newborn's immune development. A trial in SE Asia followed infants in the first 6 months of their life after maternal supplementation with beta-carotene and zinc (as well as iron and folic acid) [11]. There was no effect of beta-carotene on the infants' morbidity but maternal zinc did reduce infant morbidity and significantly reduced diarrhoea and a higher interleukin (IL)-6 production, and beta-carotene leading to lower interferon-γ (IFN-γ) production [11]. Early postnatal nutrition may lead to an inadequate gut microbiota composition and function in early life, which seems to partly account for the deviant programming of later immunity and overall health status [12]. There is increasing evidence that epigenetic mechanisms that regulate gene expression during immune differentiation are directly affected by dietary factors or indirectly through modifications in gut microbiota induced by different dietary habits [13]. With increasing age, the inflammatory response becomes dysregulated, with excess production of inflammatory cytokines, and these processes advance with age. The variation in immune protective function is one factor in differential risk of cancer in the elderly, and an individual's risk to autoimmune diseases also increases [14]. This is important because the world's population of those aged 60 years and over is predicted to almost double by 2025 (from the 672 million in 2005). Nearly two-thirds of older persons (64 %) already lived in LMIC in 2012 and this figure is projected to increase to 71 % by 2025 [15]. Nevertheless, the main emphasis here is on young children and women in countries with the developing and transitional economies of LMIC which have generally higher risks of micronutrient deficiencies, reduced immunity and increased incidence of infectious diseases. It will also briefly consider the specific impact of the inflammatory response on the selection, use and interpretation of nutrient biomarkers. The last is exhaustively and ably discussed in the record of a meeting ('INSPIRE' Project) coordinated by the USA National Institute of Child Health and Development (NICHD) [6] and the Biomarkers of Nutrition for Development (BOND) Initiative [16]. Lastly, public health implications of micronutrients and infectious diseases and inflammation are outlined, and methods of addressing micronutrient deficiencies are briefly addressed.

Inflammation and Immune Function

Inflammation is an integral part of the innate immune response to infection. The acute inflammatory response is initiated upon detection by cellular sensors and other pattern recognition receptors of inducers, mainly infections, but also reactive oxygen species (ROS) and tissue damage. In typical cases, the inflammatory response is localized to the site where the inflammatory trigger is present; however, an increasing number of inflammatory conditions have been described where the initiating factor is not infection, and inflammation appears to be chronic [17, 18], characterized by elevated levels of cytokines [19], such as in obesity.

Inflammation is usually associated with increased oxidative stress due to the production of ROS, which draws on available antioxidants such as vitamins C and E, and triggers cellular antioxidant responses. Antioxidative enzymes require the presence of nutrients such as zinc, iron and selenium at active sites or as structural components. This suggests that in chronic inflammation, the utilization and requirement of some vitamins and minerals is increased. The presence of low grade systemic inflammation in chronic diseases such as obesity and Type 2 diabetes mellitus (DM), has highlighted the challenges in managing the double burden of disease, with nutrition interventions required for not only the management of traditional risk factors for metabolic disease but also to decrease inflammation.

Optimal immune function is dependent on the availability and balance of nearly all macro- and micronutrients. For instance, diets that are high in saturated and *trans* fatty acids, and high glycaemic index carbohydrate, have been linked to elevated concentrations of C-reactive protein (CRP) [20]. Non-traditional diets are also typically high in *n*-6 relative to *n*-3 polyunsaturated fatty acids. *n*-6 Arachidonic acid and *n*-3 eicosapentaenoic acid are essential for the production of inflammatory modulating eicosanoids. *n*-6 Derived eicosanoids are powerful inflammatory agents compared to those derived from *n*-3 fatty acids [21, 22]. Nutrient deficiencies, such as protein-energy malnutrition, are a major cause of immunodeficiency because of high requirements for amino acids and energy for immune cell proliferation and synthesis of protein-mediators. Undernutrition, including of micronutrients, impairs immune function and increases susceptibility to disease. Understanding the mechanisms of action are complicated by the fact that stimulation of the immune system can also impair nutritional status. Mounting an immune response requires energy and amino acids, but also demands micronutrients [23]. The generation of energy itself requires vitamin B coenzymes, such as thiamin, riboflavin and niacin. Minerals such as iron and copper are essential at active sites of proteins involved in oxidative phosphorylation and the generation of ATP.

In the context of obesity, adipose tissue provides a source of circulating cytokines derived from adipocytes and resident immune cells. Cytokines and other mediators signal between immune cells to coordinate the inflammatory response in a manner specific to the particular inflammatory insult. Cytokines function synergistically, and are produced most commonly from T-lymphocytes and macrophages (Table 30.1). Many cytokines have both pro- and anti-inflammatory roles and the net inflammatory response depends on a range of factors, including the local environment in which they are released and the presence of synergistic or competing factors [24].

Adipose tissue recruits pro-inflammatory macrophages that contribute to chronic inflammation [25]. Studies in humans have demonstrated the ability of adipose-derived cytokines to inhibit insulin signalling and induce insulin resistance [19]. Furthermore, inflammatory status is improved following weight loss in obese patients and is associated with enhanced insulin sensitivity [26]. The morphogenic role of retinoic acid (RA) in regulating immunity shows regional concentrations of RA and commensurate RA signalling in CD8(+) cells within the tumour microenvironment. This intrinsic RA signalling is required for tumour associated antigen (TAA)-specific CD8(+) T-cell survival and hence for tumour surveillance [27].

T helper (Th)1/Th2 immune response has been linked to obesity-related immune disorders and retinoid-active derivatives improve immunity via regulating Th1/Th2 balance [28]. The decline seen in

Table 30.1 Selected cytokines and examples of their functions in inflammation

Cytokine	Primary sources	Key functions in inflammation
IL-1	Macrophages	Synthesis of acute phase proteins; local and systemic inflammatory effects
	Endothelial cells	
IL-2	Activated T-cells	Proliferation of T-cells, B-cells; proliferation and activation of NK cells
	Th1 cells	
IL-6	Macrophages	Synthesis of acute phase proteins; proliferation of B-cells; regulation of IL-1 and TNF production; activation of immune cells, osteoclasts, endothelial cells; hypothalamic pituitary axis—fever and hormone release
	Endothelial cells	
	Adipocytes	
	Myocytes	
IL-10	Macrophages	Resolution of inflammation; inhibition of inflammatory cytokine synthesis; inhibition of activated macrophages and dendritic cells
	Th2 cells	
IL-12	Macrophages	Promotion of Th1 differentiation; stimulation of IFN-γ production by T-cells, NK cells
	Dendritic cells	
CRP	Hepatocytes	Acute phase response; activation of innate immunity; macrophage phagocytosis; complement cascade; oxidative stress
	Adipocytes	
CAMs	Endothelial cells	Cell adhesion by interaction with other CAMs or extracellular matrix; cell binding and anchorage; transmembrane signal transduction
	Smooth muscle cells	
	Immune cells	
TNF-α	Macrophages	Synthesis of acute phase proteins by hepatocytes; recruitment and activation of neutrophils and monocytes at sites of infection; stimulation of CRP release from liver; activation of NF-kB pathway; induction of insulin resistance
	T-cells	
	NK cells	
	Lymphoid cells	
	Endothelial cells	
	Adipocytes	
	Neuronal cells	
TGF-β	Macrophages	Resolution of inflammation; inhibition of proliferation/activation of B-cells, T-cells, macrophages; limit production of IL-2, IFN-γ, and TNF
	T-cells	
IFN-γ	Th1 cells	Activation of macrophages; suppression of Th2 cell activity; promotion of leukocyte migration
	NK cells	

Adapted from [24]
CAM cell adhesion molecule, *CRP* C-reactive protein, *IFN* interferon, *IL* interleukin, *NK* natural killer, *NF-kB* nuclear factor-kappaB, *Th* T helper, *TGF* transforming growth factor, *TNF* tumour necrosis factor

serum concentrations of IL-1 beta and IL-1 beta/IL-4 ratio in obese women suggests that vitamin A is capable of regulating the immune system and possibly reducing the risk of autoimmune disease [28].

It is well documented that circulating levels of many micronutrients decrease rapidly due to inflammation [29]. The mechanisms by which this occurs are not clear, but likely are contributed to by the utilization of micronutrients for immune activities. Micronutrients such as zinc and iron may influence inflammatory signalling pathways at different levels and in a variety of ways, including via the modulation of cytokine production. In infection, serum vitamin A concentrations decline rapidly but are able to recover without vitamin A supplementation when the infection is resolved. This supports the notion that the coordinated response to inflammation includes the redistribution of micronutrients to tissues or cellular compartments. In contrast, the redistribution of micronutrients in non-resolving inflammation may contribute to the pathogenesis of a range of chronic conditions, including obesity, atherosclerosis and DM. It is known, for example, that impaired iron utilization in chronic disorders compromises many functions of iron, which further exacerbates the processes of disease.

Undernutrition and Disease: A Vicious Cycle

Interactions between malnutrition and infection contribute directly to the health of individuals and communities, and particularly so in lower socio-economic groups and less economically developed areas and countries (Fig. 30.1) [10, 30]. Infections and immunity can be synergistic in bidirectional ways: infections are likely to have more serious consequences among persons with clinical or subclinical undernutrition, including micronutrient deficiencies; and infectious diseases have the capacity to turn borderline nutritional deficiencies into severe clinical manifestations of undernutrition, e.g. marginal vitamin A deficiency into xerophthalmia [31]. One of the issues to be discussed, because of the public health aspects, is this concept of synergism, and antagonism (i.e. where aspects of undernutrition appear to limit infectious disease). In humans, the authors of an earlier seminal review concluded, however, that 'interactions between malnutrition and infection are regularly synergistic' [10]; a view more recently also concluded by Caulfield et al. [32] and Prentice [33].

Nutritionally induced determinants of synergism (between nutrition and infection) may include [10]:

1. Reduced capacity of the host to form antibodies
2. Decreased phagocytic activity of microphages and macrophages
3. Interference with production of non-specific protective substances
4. Reduced non-specific resistance to bacterial toxins
5. Alterations in tissue integrity
6. Diminished inflammatory response and alterations in wound healing and collagen formation
7. Effects originating in alterations of intestinal flora
8. Variations in endocrine activity

In response to infection, both innate and then acquired host defences are brought into play, and both processes involve activation and propagation of immune cells and synthesis of an array of molecules. These processes require DNA replication, RNA expression, and protein synthesis and

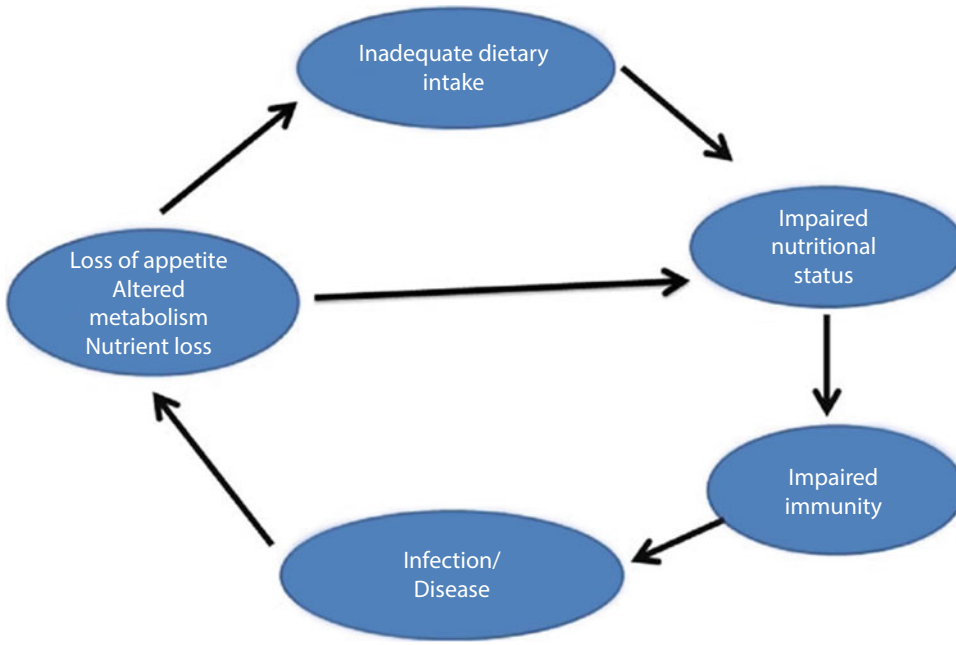

Fig. 30.1 Undernutrition/disease cycle

secretion, all consuming anabolic energy. Mediators of inflammation further increase the catabolic response [34]. The nutritional status of the host critically determines the outcome of infection and includes deficiencies in single nutrients such as micronutrients, fatty acids and amino acids, with general protein-energy mal(under)nutrition greatly increasing susceptibility to infection, particularly in LMIC, and particularly in children. Ultimately, productivity and well-being are affected at the community level which perpetuates what has beencalled the 'alarming spiral of malnutrition, infection, disease and poverty' [34].

As will be seen below, many of the micronutrients have the potential to have an association with impaired immune responses. Conversely, infectious disease adversely influences the nutritional state in several indirect ways, including loss of appetite and intolerance for food that result in metabolic effects, and an often-increased utilization of nutrients. Cultural factors can lead to substitution of less nutritious diets on the assumption of therapeutic effect and sometimes as purgatives, and antibiotics and some other drugs also can reduce appetite or digestion or absorption of specific nutrients [10]. An increased loss of body nitrogen is characteristic of all infectious disease. Among resource-poor societies the premature death of a mother and the lower income-generating capacity of iron-deficient and anaemic workers translate into greater rates of disease and overall undernutrition [35]. Women and girls are often discriminated against in terms of nutrition and health, including, in South Asia, the intrahousehold distribution of micronutrient-rich foods [36]. Disease can also affect the ability of populations to grow and harvest food if widespread enough, e.g. endemic malaria, onchocerciasis and more recently HIV/AIDS. Consequently the cycle can lead to poor nutrition leading to impaired immune systems leading to increased incidence of infectious diseases which in turn leads to further deterioration of nutritional status (Fig. 30.1).

Micronutrients, Immunity and Infectious Disease

Undernutrition, as noted above, can interfere with any body mechanism that interposes a barrier to the multiplication or progress of infectious agents [10] and that formation of specific antibodies is inhibited by many nutrient deficiencies. Severe protein depletion and folate deficiency are particularly important in reducing response and activity of phagocytes, both microphages and macrophages. The integrity of skin, mucous membranes and other tissues is important in preventing entrance of infection. Such changes associated with nutritional deficiencies include (1) alterations in intercellular substances; (2) reduction or absence of secretion of mucus; (3) increased permeability of intestinal and other mucosal surfaces; (4) accumulation of cellular debris and mucus to produce a favourable culture medium; (5) keratinization and metaplasia of epithelia surfaces; (6) loss of ciliated epithelium of the respiratory tract; (7) nutritional oedema, with increased fluid in the tissues; (8) reduced fibroplastic response and (9) interference with normal tissue replacement and repair (10).

Effect of Deficiencies on Immunological Status

> As elements of the antioxidant system, cofactors of enzymes, components of transcription factors, and epigenetic modulators, micronutrients influence various metabolic processes that are directly associated with immune functions [37].

All infectious diseases have direct adverse metabolic effects that, among other things, influence the amount and kind of food consumed and nutrients absorbed. Infectious disease nearly always makes co-existing undernutrition worse while the consequences of infection are more likely to be more serious in a malnourished host than a well-nourished one [10]. The possible importance of this

continues to be debated, especially in relation to iron supplementation in areas endemic for malaria [33] but the increasing body of evidence suggests that as long as malaria prophylaxis and treatment are available iron-deficiency status is better avoided [38].

The immune system can be broadly categorized into two groups: the innate immune system and the acquired immune system.

1. *The innate immune system*, the first line of defence, is naturally present and it is not influenced by previous contact with infectious agents. It includes epithelial barriers, the complement system, circulating phagocytes (neutrophils and macrophages) and other cytotoxic cells (natural killer (NK) cells). Innate immunity is regulated by two types of cytokines: pro-inflammatory cytokines such as IL-1, IL-6, IL-12 and tumour necrosis factor (TNF)-α, and anti-inflammatory cytokines such as IL-10, produced by neutrophils and macrophages.
2. On the other hand, *the acquired immune system* is antigen specific, where antibodies are produced by the B-lymphocytes, known as humoral-immunity, and cell-mediated immunity which depends on the T-lymphocytes system [39]. Acquired immunity involves the identification of an antigen by antibody or T-cell receptor on CD4+ T-helper (Th) cells or CD8+ effector T-cells. The antigen presenting cells carry the antigen to regional lymph nodes, where naïve Th cells are exposed to the antigen, and proliferate and mature to form memory T-cells. Memory T-cells then follow either of two pathways, Th1 or Th2 memory cells. In response to an intracellular pathogen, Th1 memory cells produce IFN-γ and IL-2, which in turn stimulate a response by cytotoxic T-lymphocytes (CTLs), activation of macrophages, response of delayed-type hypersensitivity (DTH) and provide limited help to stimulate B-cell development and antibody production. Th2 memory cells act in response to a pathogen produce IL-4, IL-5 and IL-10, which stimulate B-cells to produce antibodies, eosinophil and mast cell development and deactivation of macrophages.

It has long been accepted that malnutrition, in particular undernutrition, impairs immune function and increases the risk and severity of diseases. There are instances where the immunomodulatory effect is independent of any nutritional value, e.g. canthaxanthine, which does not have any provitamin A activity, has been shown in rodents to have the same ability to enhance immune responses as β-carotene [40].

Micronutrients and Their Immunological Roles in Disease and Inflammation

Vitamin A

Vitamin A deficiency was the contributing cause of over a million premature deaths each year in children globally in 2009 [41], as well as the commonest cause of childhood blindness, and remains a serious public health problem in 122 LMIC countries [42]. It is also likely to be a factor in the aetiology of several cancers [31]. Xerophthalmia was a recognized public health problem in much of Europe until early last century. The public health significance of vitamin A deficiency has been redefined beyond xerophthalmia in the last 35 years or so, to include its impact on deaths from infectious diseases in LMIC where vitamin A deficiency is frequently endemic. There has been tremendous progress in reducing the prevalence of the most severe manifestations of the disease (xerophthalmia and blindness), which has been on the decline in all regions of the world [43]. Subclinical vitamin A deficiency (serum retinol <0.07 μmol/L in children under 5 years), resulting from a chronic, dietary insufficiency of vitamin A, either preformed or from precursor carotenoids, and its impact on immunity and childhood infectious disease, however, is still a problem of considerable public health

significance [31, 42, 44, 45]. A deficiency state may arise with prolonged inadequate intake, often coupled with the high, normal demands imposed by rapid growth during childhood, pregnancy or lactation, or by excessive utilization and loss during infection [46]. The relative frequent occurrence in women during pregnancy in LMIC, and the possible consequences of that, have only relatively recently been widely recognized, which much increases the magnitude of the problem including possibly an impact on maternal mortality, at least in deficient populations such as Nepal, although apparently not in less severely deficient populations in Bangladesh [45, 47–49].

At least since the time of the Pharaohs were reigning in Egypt, vitamin A has been mostly known for its role in xerophthalmia and night blindness, and cases of xerophthalmia have been described since those times and especially throughout the eighteenth and nineteenth centuries [50]. A series of scientists, Hopkins, along with McCollum and Davies and Osborne and Mendel found that animals, fed only fats, protein, starch and inorganic fats not only failed to grow normally but became also more susceptible to infection and frequently died of overwhelming sepsis [50]. Bloch (cited in [50]), studying the growth and development of children in a Danish orphanage, found that when they were given butterfat and whole milk they were less susceptible to infections of the urinary and respiratory tracts and middle ear (and less likely to develop xerophthalmia). By 1928, Green and Mellanby had declared vitamin A as an 'anti-infective factor' [9, 46]. Ellison administered daily vitamin A and reduced by half the case-fatality rates due to measles. By 1930s, it was accepted that besides the ophthalmologic manifestations of vitamin A deficiency, there was also reduced resistance to some microbial infections [50].

With Wolbach and Howe's classic description in 1923 of widespread metaplasia and keratinization of epithelial linings of the respiratory and genito-urinary tracts and glandular ducts in vitamin A-depleted animals, loss of the 'barrier function' of epithelial linings became one plausible explanation for the associated decreased resistance to infection (cited in [31]). While animal experimentation continued, clinical studies in humans from the 1920s through the 1940s revealed associations between vitamin A deficiency or xerophthalmia and infectious diseases [51]. The inverse relationship between febrile illness and plasma vitamin A concentration, now understood as part of the acute phase response to infection, and the potential therapeutic efficacy of vitamin A in reducing childhood measles fatality, puerperal fever in women and other clinically relevant conditions were recognized [31, 51]. The regulatory roles of vitamin A in maintaining epithelial cell differentiation and function and immune competence have provided biologic plausibility to its importance in decreasing severity and mortality of infectious diseases [46, 52–54].

Vitamin A Deficiency and Immune Function

Vitamin A is one of the most extensively studied nutrients in relation to immune function. A review of the results from the supplementation of vitamin A in human studies was published in 2005 [55]. More recently, several lines of evidence have converged to show that retinoic acid (RA), a major oxidative metabolite of vitamin A, plays a key role in the differentiation of T-cell subsets, the migration of T-cells into tissues, and the development of T-cell-dependent antibody responses. Conversely, in a state of vitamin A deficiency, inflammatory T-cell reactions may be inadequately opposed and therefore become dominant. Although more data from human studies are still needed, the framework now developed from studies in rodent models suggests that adequate vitamin A status, whether derived from ingestion of preformed retinol or beta-carotene, is important for maintaining a balance of well-regulated T-cell functions and for preventing excessive or prolonged inflammatory reactions [56].

Vitamin A elicits a broad array of immune responses through its metabolite, RA and loss of RA leads to impaired immunity, whereas an excess of RA can potentially promote inflammatory disorders. Most of the effects of vitamin A on immune or inflammatory responses can be explained via binding of the vitamin A metabolite, all-*trans* retinoic acid, to one of three Zn-finger containing members of

the nuclear receptor superfamily (retinoic acid receptor (RAR)α, RARβ and RARγ). Recent estimates, in various cell lines, indicate that RAR are constitutively bound to around 500 genomic sites and all-*trans* retinoic acid treatment induces RAR binding to 500–600 DNA sites [6]. RA at basal levels is required for immune cell survival and activation. During immune responses, enzymes metabolizing vitamin A are induced in certain types of immune cells such as dendritic cells (DC) and tissue cells for induced production of RA. RA regulates gene expression, differentiation and function of diverse immune cells. The cells under the influence of RA in terms of differentiation include myeloid cells such as neutrophils, macrophages and DC. Also included are lymphoid cells such as effector T-cells, regulatory T-cells and B-cells [57]

Vitamin A maintains and restores the integrity and function of all mucosal surfaces, including a very sophisticated bidirectional mechanism that takes place in the digestive system and leads to immune tolerance across the entire gut lining. RA provides an intestine-specific environmental cue to differentiating immune cells. When T-cells and B-cells are activated in the intestine and associated lymphoid tissues, gut homing receptors are induced on the cells in a retinoic acid and antigen-dependent manner [58]. RA, produced by gut dendritic cells, is also an important signal that induces IgA-producing B-cells. The gut homing T-cells and B-cells play essential roles in protecting the digestive tract from pathogens.

The intestine is exposed continuously to complex environments created by numerous injurious and beneficial non-self antigens. The unique mucosal immune system in the intestine maintains the immunologic homeostasis between the host and the external environment. Crosstalk between immunocompetent cells and endogenous (e.g. cytokines and chemokines) as well as exogenous factors (e.g. commensal bacteria and dietary materials) achieves the vast diversity of intestinal immune functions in moderating the fine balance between physiologic and pathologic conditions of the intestine [59].

Innate Immunity

Vitamin A deficiency is associated with impaired innate immunity. Animal studies have shown that vitamin A deficiency is significantly associated with altered mucosal epithelial barriers in the conjunctiva of the eye [46, 60], respiratory [61], gastrointestinal [62] and genitourinary tract [63]. Vitamin A deficiency can result in a loss of microvilli, mucus-producing goblet cells and mucin in the small intestine [64–66]. Mucins are glycoproteins, secreted into the lumen, found on cell surfaces and serve as a first line of defense. Changes that occur due to vitamin A deficiency include squamous metaplasia of the conjunctiva and cornea, loss of goblet cells, and abnormal keratinization [46, 67] of the epithelium. In humans, using the lactose/mannitol urinary excretion test as an indicator of gut integrity in vitamin A supplementation trials in children suffering from severe infections, a rapid increase intestinal integrity was shown [68]. A few studies have failed to show a consistent effect on the mucosal anti-infective or inflammatory markers in milk, saliva or general fluid [55].

Animal studies suggest that vitamin A deficiency may lead to an increased total number of macrophages [69]. In addition, vitamin A deficiency leads to increased IL-12 produced by macrophages, with IL-12 promoting the development of Th1 cells, which produce IFN-γ. Increased IFN-γ leads to increased macrophage activation [70]. Although data from human studies are limited, clinical trials suggest that vitamin A supplementation may diminish the production of pro-inflammatory cytokines (TNF-α and IL-6) by macrophages, but only in response to infections [55]. Vitamin A supplementation was found to be associated with increased production of the anti-inflammatory cytokines IL-10 [71]. All these data suggest that vitamin A deficiency can lead to increased inflammation mediated by cytokines from macrophages, while impairing the ability of macrophages to ingest and kill bacteria.

NK cells are one of the components of innate immunity which work by killing virus-infected cells, as well as tumour cells. Studies in animals have shown that vitamin A deficiency impairs both the NK

cell number and its lytic activity [72, 73]. In a clinical trial among HIV-infected children in South Africa, vitamin A supplementation showed increased number of cells with the CD56 receptor expressed by the NK cells [74]. Vitamin A deficiency impairs normal neutrophil development, which can lower the capacity of phagocytosis to kill bacteria [74]. However, the evidence on the association of vitamin A and neutrophil function in humans is limited [55].

Acquired Immunity

Cell-mediated immunity can be affected also by vitamin A deficiency. Studies in animals have shown that vitamin A deficiency is associated with reduced weight of the thymus [66] and decreased lymphocyte proliferation in response to mitogens [69, 75]. In murine T-cells, all-*trans* RA has been shown to stimulate the expression of RA receptor-α and increased antigen-specific T-cell proliferation [76]. Vitamin A supplementation to infants has been shown to significantly increase total lymphocyte count [77], especially the CD4 subpopulation [78]. Similar findings have been observed in HIV-infected children [74], while when vitamin A was supplemented in HIV-infected women, no significant effect on CD4 T-cell counts was observed [79, 80]. Human study findings on Th1-mediated response are equivocal. One study showed increased DTH response in infants following high-dose vitamin A supplementation [81] whereas another study found no difference by treatment groups in the proportion of children with DTH response in a non-placebo-controlled trial of intramuscular vitamin A [82]. Further, in a study among children with measles, vitamin A supplementation apparently diminished the proportion of children with DTH response [83].

Human studies indicate that vitamin A can regulate the production of IL-10 from Th2 cells: vitamin A deficiency impairs secretion of IL-10 [84, 85], while supplementation of vitamin A increases the IL-10 secretion in vitamin A deficient subjects [71]. The cytokine IL-10 plays a role in the inhibition of the synthesis of pro-inflammatory Th-1 type cytokines, such as IFN-γ and IL-2, in both T and NK cells. In vitro lymphocyte stimulation to various mitogens was higher in vitamin A deficient rats, with higher IFN-γ and IL-2 production, indicating that vitamin A deficiency increased Th1 responses [86]. The results from animal studies suggest that modulation of the balance between Th1 and Th2 responses by retinoids may be influenced by the type of pathogens [87]. Results from human studies that examined the effect of vitamin A on either a Th1 or a Th2 responses also suggest that the immunological mechanisms through which vitamin A exert an effect are pathogen specific [55].

The growth and activation of B lymphocytes requires retinol [88]. The growth of B lymphocytes is also known to be mediated by the metabolites of retinol [89]. B lymphocytes are responsible for the production of immunoglobulins (antibodies). All-*trans* retinoic acid was found more active than retinyl acetate, retinaldehyde or retinol in restoring IgG responses in a murine model [90]. Vitamin A deficiency typically impairs antibody response to T-cell-dependent antigens [53, 69, 86] and in some T-cell-independent antigens [91]. Studies with a vitamin A deficient animal model have shown impaired serum IgG1 antibody response to purified protein antigens [54, 69], impaired serum IgG1 and IgE responses to the intestinal helminth *Trichinella spiralis* [92] as well as the intestinal IgA response to cholera toxin [86]. Most animal studies showed no impairment of serum antibody response to viral infection in vitamin A deficiency [73]. The evidence for an effect of vitamin A supplementation on T-cell-dependent antibody response in humans is equivocal. Administration of a large dose of vitamin A in children, aged 1–6 years, did not result in any significant effect on the antibody response against tetanus toxoid [82]. Another study compared the effect of different doses of vitamin A supplementation on the antibody responses against both tetanus and diphtheria toxoids in children, 1–6 years, and also found no effect [93]. In contrast, others have shown significantly higher antibody response against tetanus toxoid following vitamin A supplementation in tetanus-naïve 3–6 year old children [40, 94]. The effect of vitamin A in infants on the antibody response against diphtheria toxoid

was found positive, while there was no effect with tetanus toxoid [95]. Current consensus suggests vitamin A supplements can increase the antibody response to tetanus toxoid particularly in vitamin A deficient children who have not been exposed to tetanus. Effects of vitamin A supplements on antibody response against measles infection or measles immunization were found to be either positive [77, 96] or negative [97] or no change [60, 96]. The serum antibody response to polio vaccine showed no effect by vitamin A supplementation when given at routine immunization time [51, 98]. In a study when vitamin A was administered to both mother and children, a significantly higher proportion of children had protective titres against type 1 poliovirus than in the placebo group [99]. The differential effect of vitamin A supplementation observed could to be attributed to doses of vaccines, time of supplementation or baseline vitamin A status of the population studied [55, 94].

Public Health Implications

It is now well accepted that all-cause mortality among children 6 months to 5 years of age is reduced by about a quarter when supplementation with vitamin A capsules takes place as recommended by WHO [46]. A national cross-sectional survey, a large, population-based, prospective study, and several hospital-based clinical studies of xerophthalmia among Indonesian children by Sommer and colleagues in the late 1970s built on earlier work and demonstrated aspects of causation, progression, risk factors and health consequences of childhood xerophthalmia and vitamin A deficiency in LMIC [46]. Reports from this work, in the early 1980s, showed that non-blinding, mild xerophthalmia (night blindness and Bitot's spots) was associated with markedly increased risks of preschool child mortality [100]. Presumably vitamin A supplementation increased resistance to the severity of infection (measles and diarrhoeal diseases) by reducing the functional degree of vitamin A deficiency. In contrast to evidence relating vitamin A deficiency to respiratory tract compromise and infection [46], vitamin A supplementation has not had a consistent effect in reducing the incidence, severity or mortality of acute lower respiratory infection in children, and vitamin A supplementation of infants under 6 months of age has generally not shown a survival benefit in early infancy.

This considerable public health effect (the reduction of childhood mortality by an average of 23 %) can be partly explained by an ability of vitamin A to lower case fatality from measles by almost half, as observed in field trials and hospital-based measles trials [46], mortality from severe diarrhoea and dysentery, by approximately 40 % [50] and, based on morbidity findings from a recent supplementation trial, possibly falciparum malaria [32]. Vitamin A deficiency and infection interact within a 'vicious cycle' [10], whereby one exacerbates and increases vulnerability to the other. The bidirectional relationship complicates frequent cross-sectional evidence of depressed plasma retinol levels with diarrhoea, acute respiratory infections, measles, malaria, HIV/AIDS and other infectious illnesses [31]. Combining mortality effects with data on the prevalence of vitamin A deficiency, it has been estimated that 1.3–2.5 million early childhood deaths each year can be attributed to underlying vitamin A deficiency [46].

Zinc

Low zinc intakes, through an effect on immune function, reduce resistance to infection. Conversely, zinc supplementation reduces the morbidity and mortality of common childhood diseases, including diarrhoea, lower respiratory tract infection, and probably malaria [101]. Zinc was used topically as calamine lotion as far back as 1500 BC by the Egyptians. Its current name probably originates from an early German word meaning 'tooth-like, pointed or jagged' (presumably referring to the

needle-like metallic zinc crystals). Zinc mines near Udaipur in the Indian State of Rajasthan were active during 400 BC and there are references to medicinal uses in *Charaka Samhita* (300 BC). Pure zinc was not isolated in China until the seventeenth century although the smelting and extraction of impure forms was being undertaken around 1200 AD in India. There is a record however of the metallurgist Andreas Libavius receiving in 1597 from Asia a quantity of pure zinc metal, unknown in the West before then, although several different Englishmen and Germans probably isolated zinc independently in the late first half of eighteenth century.

Cellular zinc concentrations are maintained by two classes of zinc transporter families (ZnT and Zip). ZnT transporters promote cellular zinc efflux or its sequestration into intracellular organelles, whereas Zip transporters facilitate extracellular or organellar zinc influx into the cytoplasm. Metallothionein plays a central role in the maintenance of zinc homeostasis. Inflammatory cytokines have been reported to both up- and down-regulate the expression of specific transporters—with the net effect thought to increase the intracellular zinc in response to an increased demand for zinc in inflammatory conditions [24]. Although advances have been made towards understanding cellular zinc metabolism, the identification and quantification of zinc deficiency is hindered by the lack of a suitable diagnostic test. Under the EURopean micronutrient RECommendations Aligned (EURRECA) consortium, Lowe et al. [102] used meta-analysis to examine the usefulness of biomarkers of zinc status in humans. They showed that plasma zinc concentration responded in a dose-dependent manner to dietary manipulation in a range of population groups. Data on urinary zinc excretion, though limited, appeared to respond in the same manner. Further analysis by Lowe's group [103] revealed that for every doubling in zinc intake, the difference in serum or plasma zinc concentration is 6 %. The small magnitude of this relationship places further emphasis on the technical aspects of sample collection and analytical processing.

The effects of zinc on immune function have been demonstrated by intervention trials showing an impact on infectious diseases, more frequently diarrhoea, and to a lesser extent respiratory tract infection. There is a consensus now that zinc deficiency is a problem in many countries with high child mortality rates [104–106]. The impact on growth and maturation has been long recognized where the deficiency is more florid and has a clinical effect on growth and failure to thrive, immune effects and delayed sexual maturation are clinical manifestations of zinc deficiency. It has also been observed as an inborn error of zinc metabolism, acrodermatitis enteropathica, in patients fed incomplete parenteral solutions, in patients with Crohn's disease and occasionally in infants. Zinc-responsive night blindness has been observed in alcoholism and Crohn's disease [107].

Zinc Deficiency and Immunity

An initial consequence of zinc deficiency is an impairment of immunological functions. Zinc is crucial for the normal development and function of cells mediating both innate and acquired immunity. Several reviews in the available scientific literature have summarized the effect of zinc deficiency and immune function and the possible mechanisms [24, 108, 109] which appear to be multifaceted, from the physical barrier of the skin to gene regulation within lymphocytes. Even with mild zinc deficiency, multiple aspects of the immune system are impaired [110–112].

Innate Immunity

Zinc deficiency may impair epithelial linings of the gastrointestinal and pulmonary tracts [112, 113], and also damage epidermal cells, resulting, e.g. in the skin lesions of acrodermatitis enteropathica [114]. In both human and animal studies, NK cell activity has been found to be depressed [110, 115],

and treatment of human peripheral blood NK cells with exogenous zinc has been found to stimulate production of IFN-γ [116]. Rajagopalan et al. [117] have suggested that zinc is required for killer cell inhibitory receptor on NK cells and so zinc deficiency results in the inhibition of the killing activity.

Zinc deficiency impairs chemotactic responses of neutrophils, while absolute numbers of neutrophils are not affected [114, 118, 119]. The chemotactic response of monocytes is impaired and can be rapidly restored by the in vitro addition of zinc [114, 118]. Macrophage phagocytosis in zinc deficient animals (both mice and rats) has been found to be reduced [120], enhanced [121] or to remain unchanged [122]. High concentrations of zinc in vitro inhibit macrophage activity [123]. Information regarding the effects of zinc on macrophage function in humans is limited and more studies are needed to confirm the role of zinc on macrophage phagocytosis.

Acquired Immunity

Zinc plays an important role in cell-mediated immunity. In their review article, Shankar and Prasad [124] summarized the available data from both animal and human studies of subjects showing thymic atrophy, a reduction in the size of thymus, due to zinc deficiency. Thymus is the main organ for T-cell development, and the reported atrophy would confirm the role that zinc plays in the early stages of T-cell maturation. Zinc deficiency causes depleted numbers of T-cells in the spleen, lymph nodes and peripheral blood in animals [110, 125], and in the blood and peripheral lymphoid tissues in humans [124]. Studies demonstrate that zinc supplementation reverses these conditions [115, 125, 126].

Delayed hypersensitivity response and cytotoxic activity of T-lymphocytes are impaired in zinc deficiency and are reversed by zinc supplementation [127]. Additionally, zinc supplementation in malnourished children restores their delayed hypersensitivity responses [128]. Besides maintaining the proliferation, there is a role of zinc in lymphocyte homeostasis by suppression of apoptosis [129]. Several studies have reported that apoptosis of T-lymphocytes induced by in vitro treatment of toxins and other agents can be prevented by adding high doses of zinc [130, 131]. The thymic atropy seen in zinc deficiency, mentioned above, is accompanied by apoptosis of lymphocytes [132].

Thymulin, a thymus-specific hormone, binds to the high affinity receptor on T-cells and promotes T-cell functions, such as allogenic cytotoxicity, suppressor functions, and IL-2 production [133–135]. Thymulin regulates the cytokine release by peripheral mature T-cells [136] and induces the proliferation of CD8+ T-cells that function as cytotoxic cells able to recognize and kill pathogens [137]. Thymulin requires zinc for its biological activity to be expressed [133, 134]. Experimental zinc deficiency decreases the activity of serum thymulin, which is required for the maturation of T-helper cells [109], leads to an imbalance of T-helper 1 (Th1) and T-helper 2 (Th2) functions, decreases the recruitment of T-naive cells [138], and reduces NK cell lytic activity [139]. Zinc modulates the oxidative burst that is generated by polymorphonuclear leucocytes as part of their microbiocidal activity [140]. Although the activity of thymulin in serum has been found to be significantly impaired in zinc deficiency, this was able to be corrected by both in vivo and in vitro zinc supplementation [141, 142].

A T-cell subpopulation study showed a significant decrease in the ratio of CD4+ to CD8+ during zinc deficiency that was corrected by zinc supplementation [138]. It has been suggested that zinc is required for regeneration of new CD4+ T-cells [109]. Studies on experimental human models have shown a decreased proportion of CD73+ in the CD8+ subset of T-lymphocytes in zinc deficiency [143]. The CD73 molecule on CTLs is required for antigen recognition, proliferation and cytolysis [143]. Zinc deficiency is known to affect the production of a variety of cytokines, such as IL-1, IL-2, IL-4 and IFN-γ [24, 144], by influencing the functions of T-lymphocytes and macrophages [145]. IL-1β production is higher in zinc-deficient adults [146, 147] and, compared to zinc-sufficient individuals, production of IL-2 is lower [148]. Inconsistent results are reported for IL-6. Zinc deficiency in Indonesian infants was accompanied by lower production of IL-6 [85], while no significant

differences were observed in IL-6 between zinc-sufficient and zinc-deficient adults [146]. The addition of zinc to human peripheral blood mononuclear cells was found to induce the release of IL-1, IL-6, TNF-α and IFN-γ [149]. Studies in the experimental human model and in patients with sickle cell disease suggest that the impaired cell-mediated immunity of zinc deficiency is caused by the imbalance between Th1 and Th2 cell functions [138, 143, 146]. While there was a decrease in the production of IFN-γ, and IL-2 (Th1 response), the production of IL-4, IL-6 and IL-10 (Th2 response) were not affected during zinc deficiency [138, 143, 146].

Human intervention studies measuring the effects of zinc on plasma cytokine concentrations or cytokine production in primary human blood cells have been reviewed previously [24]. Increased cytokine concentrations have been shown in stimulated mononuclear cells isolated from populations supplemented with ≤20 mg zinc/day [150], suggesting a zinc dose–response. Measurements of plasma cytokine concentrations in response to zinc supplementation support a difference in effect depending on zinc dose; plasma concentrations of IL-6 have been shown to decrease with zinc supplementation of 45 mg/day [151] but to increase with 10 mg zinc/day [24]. The significance of these changes is unclear but the ability of zinc supplementation to influence cytokine concentrations in humans is consistently reported.

Effects of Zinc Supplements on Infection and Inflammation

Otitis media is inflammation of the middle ear, which is common in young children in LMIC, and may lead to hearing loss. Gulani and Sachdev [152] evaluated the evidence on whether zinc supplementation can reduce the incidence of otitis media in healthy children living in LMIC and found the outcomes to be inconsistent. The authors identified limited signs of benefit in children being treated for marasmus.

Singh and Dass [153] assessed RCTs to determine the efficacy of zinc supplementation in reducing the incidence and symptoms of the common cold. Zinc supplements were associated with a significant reduction in the duration, by approximately one day, but not the severity of symptoms. Very high heterogeneity was observed in the included trials, suggesting that the estimates must be viewed with caution.

The effect of zinc supplementation on the prevention of pneumonia in children aged 2–59 months of age was investigated by Lassi et al. [154]. Their analysis showed that supplementation reduced the incidence and prevalence of pneumonia by 13 % and 41 %, respectively. Brown et al. [155] conducted a meta-analysis of zinc supplementation in infants, preschool, and older prepubertal children, and showed that supplementation reduced the incidence of acute lower respiratory tract infections by approximately 15 %. The authors reported significant heterogeneity among the studies, with the magnitude of reduction in infection being greater in children who were stunted.

Zinc supplementation for treating children with diarrhoea was evaluated by Lazzerini and Ronfano [156]. In their Cochrane review they included 24 eligible trials, with the majority of the data obtained from Asia, and from individuals at high risk of zinc deficiency. In children aged greater than 6 months with acute diarrhoea, zinc supplementation may shorten the duration of diarrhoea, and as reported previously [155], the improvement was greater in malnourished children. For children with persistent diarrhoea, zinc supplementation shortened the duration of diarrhoea by around 16 h although the trials were considered of moderate quality evidence. As reported by Brown et al. [155], age is an important contributor to the outcome: zinc supplementation reduced the incidence of diarrhoea by approximately 20 %, but the impact was limited to studies that enrolled children age greater than 12 months. In children with initial age >12 months, the relative risk of diarrhoea was reduced by 27 %.

One study investigated the effects of zinc supplementation for 6 months in HIV-infected children [157]. Children supplemented with zinc had fewer clinic visits in which watery diarrhoea was observed, and there was more weight gain in the zinc group, but the difference was no longer significant 3 months after supplement cessation.

Public Health Implications

Supplementation under experimental conditions has shown reduced poor growth rates and possibly an association with an increase in energy and protein intake. More recent field trials in Bangladesh, India, Pakistan and sites in Africa have shown conflicting results in growth, reduction in disease, severity of disease, and varied results depending on the disease. Nevertheless, results of two recent meta-analyses of such studies seem to indicate a definite role for zinc supplementation (probably with other micronutrients), especially in growth and diarrhoeal disease [104]. Consequently, zinc deficiency and its impact on reducing child mortality has come much more to the fore, with sufficient evidence that the accepted treatment of diarrhoea is now oral rehydration therapy with a 2 week course of zinc supplementation [158] and is a recommendation by WHO [159]. Effectively, the guidance recommends that mothers, other caregivers and health workers should provide children with 20 mg per day of zinc supplementation for 10–14 days (10 mg per day for infants under the age of 6 months) [159]. There is considerable debate now on whether this also applies to respiratory disease and the public health prevention of zinc deficiency, as opposed to therapeutic use.

Zinc and the Double Burden of Disease

In Type 2 DM, patients exhibit an impaired immune function as part of their pathogenesis that ultimately results in a decreased functional pancreatic β-cell mass; while the failure of β-cells in Type 2 DM occurs over a prolonged period and involves the chronic activation of the innate immune system [160]. The aberrant expression in DM of a number of important immune mediators that are zinc-responsive, including the proinflammatory cytokines, suggests a potential interaction between zinc status, impaired immunity, and DM. A systematic review and meta-analysis of randomized placebo-controlled trials was conducted to determine the effect of zinc supplementation on markers of glycaemic control, with the trials carried out mainly in LMIC. A reduction in fasting glucose concentrations was observed following zinc supplementation and in those with underlying chronic metabolic disease zinc supplementation produced a greater reduction in glucose concentrations compared to the effect in healthy participants [161]. The results suggest that zinc could play a role in the management of hyperglycaemia and may serve as co-adjuvant therapy for DM, particularly in LMIC [162].

Part of the need for low-dose zinc supplementation is the difficulty of increasing intakes of zinc through dietary methods, especially poor diets low in animal-source foods [163]. Large variations in zinc content can be found between otherwise nutritionally similar food sources but tend to be high in meat, cheese, lentils and cereals. These tend to be components of more expensive diets with cereals being the major source of energy and zinc in large parts of the world. As the zinc is mainly located in the outer layer of the grain, a low extraction rate means that the majority of the content of zinc, as well as other minerals are removed, although this should also reduce the phytates that affect bioavailability. Use of zinc-rich galvanized cooking pots and canning may contribute zinc in the diet. Unrefined cereal-based diets present the largest risk for low zinc absorption [164]. Contributing factors to poor zinc intakes may be geophagia and large zinc losses due to intestinal parasitic infections [164]. Lower zinc intakes than in western type diets have been described in Brazil around the Amazon where the diet is fish based, and where signs of zinc deficiency were also observed [163]. Similarly low intakes have been described in other parts of the developing world including Papua New Guinea and South Asia. Nevertheless, Gibson and others have demonstrated the theoretical possibility and the feasibility in West Africa of increasing the bioavailability of micronutrients in plant-based diets [165].

Iron Deficiency, Iron Deficiency Anaemia and Other Nutritional Anaemias

Conservative estimates indicate that 1500 million people are anaemic worldwide, with perhaps over 90 % of these in LMIC, mainly South Asia and Africa [166]. All the figures suggest that over half of all women in LMIC are anaemic. Iron deficiency, the main cause of anaemia, is a major contributor to low birth weight, prematurity and maternal mortality [167, 168]. Iron deficiency anaemia (IDA) is even more prevalent in infants and young preschoolers, and while there are only very recently global data on prevalence of IDA in infants and children, in some sample populations prevalence reaches 70 % or more [169]. Nutritional anaemia, largely because of iron deficiency, remains the major nutritional problem facing the poorer nations, although even in more affluent countries, it remains a significant problem in certain, usually disadvantaged, groups. Earlier WHO estimates give prevalence data for preschool-age children, non-pregnant and pregnant women, according to information available and that were included according to pre-specified criteria [169]. For infants and young children, the range is from 3.4 % in North America to nearly two-thirds (65.4 %) in Africa. In women, the range is 7.6 % in North America to 44.7 % in Africa (non-pregnant), and for pregnant women, 4.7–55 %. However, individual studies have identified far higher prevalences for infants and women, especially in South Asia that show, e.g. 84.9 % of pregnant women anaemic (Hb < 110 g/L) with 13.1 % having severe anaemia (Hb < 70 g/L). In India, adolescent girls had levels of 90.1 % with 7.1 % having severe anaemia, in the 16 Districts of India surveyed [170].

Iron Deficiency and Immunity

In both humans and animals, the effects of iron deficiency on immune response have been studied extensively. Overall, there is little evidence that shows any effects of iron deficiency, especially in humans, on B-cell-mediated immunity and antibody production. On the other hand, specific defects in several components of both innate immunity and cell-mediated immunity have been well documented [171]. Overall though, from a public health perspective there has been limited appreciation of a role. An earlier review in the Lancet, besides a mention of increased susceptibility to upper respiratory infections associated with iron deficiency anaemia [172], the only mention was that 'the effect of iron status on immune function and cognition in infants and children needs to be clarified' [173].

Much progress has been made in the understanding of iron metabolism. Hepcidin, a peptide that prevents the efflux of iron across the intestinal mucosa and from macrophages [174], has attracted much attention in the last few years, partly because infection and inflammation increase hepcidin synthesis and this discovery has enhanced understanding of the association between iron and innate immunity [175]. Hepcidin appears to be regulated by body iron status, and is synthesized by hepatocytes, macrophages, neutrophils and adipocytes [175].

Innate Immunity

In both human and animal studies, several components of nonspecific immunity have been found to be impaired by iron deficiency. NK cell activity was found to be depressed in iron deficiency [176, 177], presumably because the NK cell needs iron for its differentiation and proliferation. Macrophage phagocytosis in general appears to be unaffected by iron deficiency, while bacteriocidal activity of these macrophages has been reported to be impaired [176, 177]. In iron deficiency, neutrophils have reduced activity of myeloperoxidase, which is involved in the killing process of pathogens [176].

Acquired Immunity

Iron plays an important role in cell-mediated immunity [178, 179]. In most but not all studies, the number of T-cells was found to be reduced with thymic atrophy during iron deficiency [178, 179]. In addition to a reduced number of T-cells, more reports than not on iron deficiency show an impairment of lymphocyte blastogenesis and mitogenesis in response to a number of different mitogens [181, 182]. This change is largely correctable with iron repletion [180]. Further, studies on iron deficient patients have reported either an absent or diminished DTH response, compared with control subjects, to a variety of antigens such as Candida, mumps, diphtheria, trichophyton and streptokinase-streptodornase. Following iron supplementation, the impairment of the DTH responses, including tuberculin reactivity, were found to be reversed [182].

Studies have shown a reduced in vitro production of IFN-γ by spleen cells taken from iron deficient mice [183]. In a study in hospitalized children with iron deficiency, they were found to have a lower percentage of lymphocytes producing IFN-γ in vivo (spontaneously), while they had a higher percentage of lymphocytes producing IFN-γ following in vitro stimulation [184]. The IFN-γ is a potent macrophage activating lymphokine and an important mediator of the DTH response and cellular cytotoxicity [185]. Cellular iron availability modulates the differentiation and proliferation of Th cells subsets, with Th1 cells being more sensitive than Th2 cells to iron deficiency [186]. Further, the ratio of CD+ to CD8+ T-lymphocytes in blood was found to be reduced in iron deficiency, whereas the number of cells remained unchanged [187].

Humoral immunity on the other hand, appears to be normal in iron deficient individuals. In iron deficient patients, the serum IgG, IgA and IgM concentrations were either normal or elevated [188]. Antibody production in response to specific immunization with most antigens was found to be well preserved in iron deficient humans [189].

Infection and Iron Supplementation

It is well established that serum iron concentrations decrease markedly in response to systemic inflammation or infection [190]. In patients with tuberculosis (TB), supplementation with iron increases mycobacterial growth [190] and is associated with increased morbidity and mortality [190]. Similarly, the deleterious impact of iron supplementation in parasitic disease such as malaria is well documented [33]. The provision of iron supplements in endemic malaria regions could increase morbidity and mortality with the most likely explanation being the appearance of non-transferrin-bound iron (NTBI) in the plasma. NTBI forms when the rate of iron influx into the plasma exceeds the rate of iron binding to transferrin. Malaria decreases iron absorption in single-meal studies, but there is no evidence of decreased efficacy of iron-fortified foods, and no significant increase is observed in NTBI on consumption of iron-fortified food. Therefore strategies such as fortification of staple foods and condiments, or the use of micronutrient powders for home fortification, could be effective for improving iron status in susceptible groups such as women and children [191, 192].

Thus, based on the existing literature, it can be concluded that iron deficiency impairs both innate (reduce bactericidal macrophage activity and NK cell activity) and cell-mediated immunity (reduce T-cell proliferation, DTH response, decrease in the ratio of CD4+ to CD8+ cells). It also impairs a variety of cytokines (IFN-γ, TNF-α, IL-2, IL-10), and suppresses Th-1 cells response with a small decrease in Th2 response.

On the other hand, iron overload affects various components of the immune system. Several relatively recent reviews have summarized the effects of iron overload on the immune system [193, 194]. Iron overload, as seen in hereditary haemochromatosis patients, enhances suppressor T-cell (CD8) numbers and activity, decreases the proliferative capacity, numbers, and activity of helper T-cells (CD4)

with increases in CD8/CD4 ratios, impairs the generation of cytotoxic T-cells, and alters immunoglobulin secretion and increased levels of IL-4, IL-6 and IL-10 [193, 194]. Thus iron overload may result in increased susceptibility to infection by impairing Th1 cytokine-mediated response through diminished activity of regulatory cytokines (IFN-γ, IL-2 and IL-12), and by increasing Th2 response, by impairing the killing of intracellular pathogens by macrophages.

Public Health Implications

The overt physical manifestations of iron deficiency include the generic symptoms of anaemia, which are tiredness, lassitude and general feelings of lack of energy. While neuromaturational delays and reduced productivity, physical activity and work performance are the most important clinical features, reduced immunocompetence, thermoregulatory function, and energy metabolism are also consequences [195, 196].

Iron and the Double Burden of Disease

Increased inflammation and iron stores have been correlated with established risk factors of DM, obesity and the metabolic syndrome [190]. A link between obesity and iron deficiency was first made over 40 years ago following the publication of a study reporting significant associations between obesity, CRP and iron deficiency [190]. With the increasing incidence of overweight and obesity in virtually all parts of the world, there has been renewed interest in the area of iron status in the obese. More recently a number of studies have reported an association between obesity and iron deficiency or 'hypoferremia' in adults [190]. A recent systematic review reported a tendency for lower transferrin saturation and higher ferritin concentrations in obese populations consistent with the inflammation hypothesis in which hepcidin plays a central role [197].

As obesity is known to be a state of chronic low-grade inflammation, a mechanistic link between inflammation in obesity and hypoferremia is plausible. In obese populations circulating hepcidin concentrations are reported to be higher than in non-obese subjects [198]. The underpinning mechanisms that lead to higher hepcidin levels in obesity are likely to be driven by higher concentrations of IL-6 [199]. One in vitro study involving leptin, which is markedly increased in obesity, suggests that leptin also may stimulate hepcidin expression [200]. Therefore iron deficiency adds further to the burden of obesity and complicate weight management. Iron deficiency has been associated with fatigue, depression and reduced exercise capacity and this may impact negatively on the efficacy of behavioural weight management programmes aimed at increasing physical activity and improving motivation and psychological well-being [201]. Restricted energy diets used for weight management may be low in iron, particularly for women during reproductive years where requirements are high.

In a cross-sectional survey in Moroccan adults, where iron deficiency is prevalent, biomarkers of inflammation were linked significantly with serum ferritin concentrations and with body mass index (BMI). The prevalence of iron deficiency was underestimated by not adjusting for serum ferritin concentrations, and the difference increased with increasing adiposity. This suggests that in LMIC where the double burden of disease is increasing, markers of inflammation should be used to correct biomarkers of iron status even if infectious or parasitic diseases are no longer widespread [202]. Aderibigbe et al. [203] undertook a review of the literature with the aim of elucidating the link between iron status and adiposity in women in LMIC. They showed that the studies had inconsistent outcomes, and factors such as infection, alcohol consumption, dietary intake and genetics were significant confounding factors.

In obese young women in Australia, where anaemia (haemoglobin <120 g/L) and iron deficiency (serum ferritin <15.0 μg/L) are less prevalent than those in LMIC, BMI was shown to be a significant predictor of serum iron, transferrin saturation and CRP. When the study participants were investigated based on their BMI, those with BMI ≥ 35 had significantly higher CRP than those in lower BMI categories but with no apparent effect on hepcidin. The authors concluded that obesity per se was not sufficient to induce clinically significant disturbances to iron metabolism, possibly due to the lack of co-morbidity in this cohort [204].

A relatively recently published review volume on nutritional anaemia gives considerably expanded information from a largely public health perspective [167]. Iron deficiency anaemia has been re-recognized as an important cause of cognitive deficit in this age group [205], including in the very recent and potentially influential *Lancet* series on early child development [206]. Iron deficiency also has a profound effect on productivity and hence has economic implications for countries in which it is a significant public health problem [207, 208] with physical work capacity being reduced even in moderate anaemia [209, 210].

The greater understanding of factors in the control and prevention of nutritional anaemias for public health interventions are still being evaluated but are likely to be important. For example, if populations have high levels of infection, then they will also have high levels of hepcidin—this may then block the uptake of iron that is in the diet from fortification and supplementation [211]. However, hepcidin has not been linked to any effect on dietary haem uptake, thus lending support for promotion of animal-source foods in poor diets [212]. Therefore it is becoming more apparent that treatment strategies encompass all the health concerns of a population—nutritional anaemia can only be completely addressed if other diseases are concurrently treated.

Selenium

Selenium plays a pivotal role in maintaining the functions of the immune and antioxidant systems as well as affecting the networks of genes that are central to anti- and pro-inflammatory mediators [213]. Low selenium status is associated with increased risk of mortality and poor immune function, with demonstrated adverse effects on immune cells during activation, differentiation and proliferation [214]. This is related to increased oxidative stress, but additional functions such as protein folding and calcium flux may be impaired also in immune cells under deficiency conditions [214].

Animal models and human studies with supplementation have been shown to enhance immune competence and resistance to viral infections. However, while the influence of selenium on immune responses is generally to enhance them, it may not always be beneficial, e.g. on antiparasitic responses or allergic asthma suggest the levels of selenium may affect different types of immunity [215]. While micronutrients can influence the ability of the host to respond to a viral infection [216], the virus itself may respond to the nutritional status of the host [217]. For example, a deficiency of selenium influences the expression of mRNA for the chemokine monocyte chemo-attractant protein-1, which may contribute to the development of myo-carditis in the selenium-deficient host [217]. In selenium deficiency, benign strains of Coxsackie and influenza viruses can mutate to highly pathogenic strains, and increasing selenium intake or supplementation alone or in combination with other micronutrients, may improve outcomes in patients infected with HIV and/or TB. Selenium promotes the acute cellular immune response [218]. Several trials assessed the effects of multi-micronutrient supplements, which also contained selenium in small doses but there was only low-level evidence in support of a beneficial effect on mortality in patients with tuberculosis, but little or no effect on mortality in those with TB and HIV. Plasma levels of selenium are improved by supplementation during the early stages of tuberculosis treatment, but a consistent benefit on tuberculosis outcomes has not been demonstrated [219]. Supplementation with Selenium beyond the upper tolerable limit can impinge on immune cell

function, with some types of inflammation and immunity particularly affected [214]. A crucial factor that needs to be emphasized is the U-shaped link with status in that selenium supplementation may benefit people with low status, those with high status might be affected adversely [220].

Iodine

The public health importance of iodine deficiency is that it is the most common cause of preventable intellectual impairment in the world. It is important in terms of women's reproductive outcomes and probably infant mortality. The fact that infants born to mothers who are iodine deficient are likely to suffer impaired intellectual impairment, even when there may be no clinical manifestations of cretinism- the most extreme manifestation, makes this extremely important both in community terms but also for national economic development. An estimate of iodine deficiency, or those suffering from iodine deficiency disorders as assessed by goitre prevalence, was estimated globally at around 740 million in 1998 [43]. More reliably (with urinary iodine the global prevalence is around 35.2 % (or 1,988,700,000 people) [221]. It is unlikely to have an impact on immune status and so will not be further considered in this chapter.

Other Micronutrients of Less Current Public Health Significance

Deficiencies of vitamins A, C and D, the B group of vitamins, especially B6, B12, riboflavin and folate, have all been associated with poorer health outcomes, although the pathways are not all clearly established and likely do not all work through impaired immunity [10, 205]. Besides vitamin A, iron and zinc, in public health terms, there are other micronutrients that are important through having either low or deficient status in certain population groups, such as pregnant and/or lactating women, and the elderly. In this section, the roles of these micronutrients on immune response are briefly discussed. Allen [222] has identified, in addition, riboflavin, vitamins B6 and B12, calcium, and depending on local variations in inadequate diets of poorer populations, β-carotene, folate and vitamin C. Similarly, Zimmermann [223] notes that single micronutrient deficiencies do not occur in isolation and that overlapping deficiencies affect more than 50 % of children and women in many LMIC.

The framework used here of micronutrients only of current public health interest is used to justify, in an already very brief background, why micronutrients such as niacin, thiamin and calcium are not addressed more explicitly. Historically and even now in certain geographic areas, these are, or have been of important public health interest. Niacin was widespread in many maize-consuming areas, including the south of the USA in the early nineteenth century but the deficiency disease *pellagra*, is considerably less often seen because of widespread fortification of flour with niacin amongst other B vitamins and iron [224, 225]. Thiamin deficiency, expressed as the disease *beriberi* was widespread throughout rice-eating populations [226] and is again now less often seen in rice-consuming populations due to generally improved diets (so that there are other sources of thiamin in the diet) or by fortification, as in Japan. However the contribution of thiamin deficiency to Wernicke–Korsakoff's syndrome in alcoholics means it is being addressed in a public health manner (in this case fortification of flour) in Australia. The report of the FAO/WHO meeting in Bangkok in the late 1990s gives useful information on other micronutrients [226], especially those now of somewhat more historical interest. Many of the B vitamins continue to be of interest as fortificant pre-mixes being added to flour (both wheat and maize) along with iron, and sometimes other fortificants such as zinc and vitamin A [227, 228]. Fortification is discussed in more detail below.

Vitamin D

Vitamin D is known to be essential to immune function [229, 230]. Relatively little is known about vitamin D status in equatorial populations but a recent study in Tanzania showed that hypovitaminosis D is common among pulmonary tuberculosis patients (and is not explained by the acute phase response) [230]. At the turn of the last century, ultraviolet light was successfully used to treat TB of the skin but it has only been in the last decade or so, that the understanding of vitamin D beyond its role as a determinant of mineral metabolism and rachitic bone disease, skeletal homeostasis and prevalent bone disorders such as osteoporosis, has emerged [231]. It is now clear that vitamin D has an important anti-infective role, involved in the production of defensins and cathelicidin (antimicrobial peptides) [232] and the induction of antimicrobial peptides and autophagy in cells of the monotype/macrophage lineage [233].

Calcitriol, or 1,25-dihydroxyvitamin D3, is well known as an endocrine regulator of calcium homeostasis. It is now known that local calcitriol production by immune cells also exerts autocrine or paracrine immunomodulating effects. Immune cells that produce calcitriol express the vitamin D receptor (VDR) and the enzymes needed to metabolize vitamin D3 (1 alpha-, 25- and 24-hydroxylases). These immunomodulatory effects may explain the reported epidemiological associations between vitamin D status and a large number of autoimmune and inflammatory diseases such as rheumatoid arthritis, lupus, inflammatory bowel disease, and Type 1 DM, as well as infections, malignancies, transplant rejection and cardiovascular disease [234]. Induction of the vitamin D-activating enzyme CYP27B1 in monocytes via pathogen recognizing receptors has highlighted an entirely new function for vitamin D as a potent inducer of antibacterial innate immune responses [235]. Vitamin D deficiency may affect Th17 responses and microvascular function, which may protect against IL-17-mediated inflammation and vascular dysfunction [236].

Vitamin D levels are independently and inversely associated with IL-6 in older populations suggesting a potential anti-inflammatory role for the vitamin [237]. Treatment with high dose vitamin D3 reduces CD4+ T-cell activation, a clear human example of influence of cell-mediated immunity [238], confirming the potential role of vitamin D in chronic inflammation. However, different inflammatory biomarkers have been shown to be differently associated with vitamin D with beneficial effects of increasing 25(OH)D for fibrinogen and WBC. In contrast, the U-shaped association between vitamin D and CRP indicates that increased vitamin concentrations may also be related to pro-inflammatory states [239].

Impaired vitamin D status is common to many populations across the globe with associations with chronic health problems including autoimmune and cardiovascular diseases, hypertension and common cancers [231]. Adequate vitamin D status now appears to be protective against a variety of conditions: musculoskeletal disorders (muscle weakness, falls, fractures), infectious diseases, autoimmune diseases, cardiovascular disease, Type 1 and Type 2 DM, several types of cancer, neurocognitive dysfunction and mental illness, and other diseases, as well as infertility and adverse pregnancy and birth outcomes. Vitamin D deficiency/insufficiency is associated with all-cause mortality [240]. The long-term effects of low vitamin D status remain somewhat unclear but increasingly of the opinion that optimization of vitamin D status in otherwise healthy individuals may potentially have lasting beneficial impacts on the immune system [241]. Optimal vitamin D levels and appropriate dosing schedules have yet to be determined [232] and guidelines for supplementation are urgently needed.

Folate

Folate (or its most common supplemental form of folic acid) has come to prominence recently as the flour in the USA, and now other countries, is being fortified with folic acid. Folate is required for DNA synthesis and so its deficiency is clinically expressed in tissues with high rates of cell turnover.

The principal sign is megaloblastic anaemia. However, its current public health importance is as a cause of anaemia, a cause of neurological tube defects (NTDs) and a possible role in cardiovascular disease. The recent fortification with folic acid in an increasing number of countries [228] is to prevent NTD. The public health importance of folic acid in fortified cereals has increased and has had a dramatic effect on the incidence of NTDs. At current fortificant levels, there is emerging some concern around unintended effects, e.g. some cancers. There is increasing awareness of the public health importance of folate deficiency in immune function, including its association with impaired cell-mediated immunity. Blood folate status and the expression of over 60 proteins that are involved in immune function, inflammation, and coagulation are both affected in deficiency. In response to long-term synthetic folic acid supplementation the protein response can be categorized into metabolic pathways related to complement fixation (e.g. C1, C3, C4, Factor H, Factor 1, Factor B, clusterin), coagulation (e.g. antithrombin, alpha-1-antitrypsin, kininogen) and mineral transport (e.g. transthyretin, haptoglobin, ceruloplasmin) [242]. Low folate status is associated with lower levels of proteins involved in activation and regulation of immune function and coagulation [242].

Folate deficiency has been associated with reduced cell-mediated immunity by reducing the proportion of circulating T-lymphocytes and their proliferation in response to mitogen activation [243]. Folate deficiency has been demonstrated to be associated with increased ratio of CD4+ to CD8+ T-lymphocytes due to decreased CD8+ T-lymphocytes proliferation, and which was reversible by in vitro addition of folate [244]. It has been suggested that the reduction in CD8+ cell replication in folate deficiency may be related to the finding of an increased carcinogenesis due to reduced cytotoxic activity [244]. Studies among post-menopausal women aged 50–70 years with diets low in folate showed an increased NK cell activity following folate supplementation [245]. All these findings indicate that folate deficiency is associated with impaired Th1 response.

Vitamin B12 and Other B Vitamins

Studies on vitamin B12 deficiency and immune response are limited. In patients with vitamin B12 deficiency (with pernicious anaemia or post-gastrectomy megaloblastic anaemia) a significant decrease was found in the number of lymphocytes and CD+ T-cells and a reduction in the proportion of CD4+ T-cells. Further, there was an abnormally high CD4+/CD8+ ratio and reduced NK cell activity [246]. Following treatment with methylcobalamin, CD8+ T-cells were restored and NK cells activity improved [246]. In an elderly population with low serum vitamin B12 concentrations, a reduction in antibody response to pneumococcal polysaccharide vaccine was observed suggesting an impaired synthesis of specific immunoglobulins [247].

Possible mechanisms that link obesity/visceral fat to DM and cardiovascular complications include inflammation and increased oxidative stress. Measures of plasma antioxidant vitamins status, markers of oxidative damage (malondialdehyde (MDA) and protein carbonyls), and inflammation (CRP, IL6 and TNF alpha) are part of an increased effort to find appropriate biomarkers, including of vitamin B12 itself, and its physiological activity [16]. Using these measures, antioxidants supplementation with B-group vitamins enhances antioxidant capacity, and may have an anti-inflammatory effect on obese diabetic patients [248].

A variety of inflammatory disease conditions have been found to be associated with low levels of plasma pyridoxal 5′-phosphate (PLP), the active form of *vitamin B6*. The inverse association between plasma PLP and inflammation may be the result of mobilization of this coenzyme to the site of inflammation, for use by the PLP-dependent enzymes of the kynurenine pathway of tryptophan degradation, metabolism of the immunomodulatory sphingolipids, ceramide and sphingosine 1-phosphate, and for serine hydroxymethylase for immune cell proliferation [249]. Vitamin B6 (pyridoxine) deficiency in humans has been found to be associated with reduced lymphocyte maturation, growth and proliferation, impaired NK activity, decrease in pro-inflammatory cytokines IL-1-β, IL-2, IL-2

receptors, and a decreased antibody response of DTH [250]. Thus vitamin B6 is associated with suppressed Th1 response and increased Th2 response, which is reversed following repletion of the vitamin [251].

Other Vitamins

In animal models, *vitamin C* (ascorbic acid) deficiency has been associated with decreased neutrophil function and impaired delayed cutaneous hypersensitivity [252, 253], decreased T-cell proliferation and abnormal complement concentrations [254]. In humans, vitamin C deficiency was associated with decreased DTH response to several antigens, which could be reversed by high dose supplementation [255]. Administration of vitamin C in humans has been described as resulting in improvement of anti-microbiocidal and NK cell activities [256]. Supplementation of vitamin C has been found to enhance neutrophil chemotaxis in adult healthy volunteers [257], and an increase in the proliferative response of T-lymphocytes to PHA and concanavalin A in the elderly [258]. Thus vitamin C deficiency in humans can impair leukocyte functions, and decrease overall NK cell activity and lymphocyte proliferation. A study looking at associations between circulating ascorbic acid, alpha-tocopherol, 25-hydroxyvitamin D and plasma cytokine concentrations in young adults concluded that alpha-tocopherol (vitamin E), but not ascorbic acid or 25(OH)D, is inversely associated with inflammation in healthy young adults [259]. One review, however, found that one of the consequences of vitamin C deficiency is impaired resistance to various pathogens, whereas an enhanced supply increases antibody activity and infection resistance [260].

Vitamin E is a fat-soluble vitamin important for normal function of the immune system. In the few rare cases of vitamin E deficiency in humans, impaired T-cell function and DTH response were observed [261, 262]. Dietary vitamin E may play a protective role a protective role in the development of allergic sensitization [263]. Supplementation of vitamin E in healthy adults showed a significantly increased T-cell proliferation in response to PHA, an improved CD4+/CD8+ ratio and decreased parameters of oxidative stress [264]. In general, the elderly are at a greater risk for lower vitamin E intake. A review by Meydani et al. [265] presented a comprehensive coverage of the role of vitamin E and immunity in humans, especially in the elderly. Vitamin E supplementation above currently recommended levels has been shown to improve immune functions in the aged including DTH skin response, increased mitogen-stimulated lymphocyte proliferation and increased production of IL-2, enhanced NK cell cytotoxic activity, and increased phagocytic activity by macrophages [265, 266]. Antibody production in response to vaccination was shown to be significantly associated with the nutritional status of vitamin E, which was mediated through increased production of IL-2, leading to enhanced proliferation of T-cells [267]. Thus higher vitamin E intake is associated with enhanced Th1 response and decreased Th2 response. Besides its protective role as an antioxidant, the possible mechanism for the improved immune function due to vitamin E supplementation is because of the reduced production of the T-cells suppressive factors, such as PGE_2 by macrophages [265]. Low vitamin E concentration and vitamin E have both been associated with obesity, and in a further link to non-communicable diseases, low concentrations of zinc, vitamins A and E in children who were overweight and obese were associated with lipids, inflammation and insulin resistance [268].

Vitamin K derivatives attenuate T-cell-mediated immunity by inhibiting the proliferative response and inducing apoptosis in activated cells [269]. Data from the Framingham study show that vitamin K status is inversely associated with concentrations of inflammatory markers, including CRP, suggesting a possible protective role for vitamin K [270].

To briefly summarize, the above section shows how little some understandings have changed; however, it is the mechanisms and the understanding of the incredible complexity of micronutrients, immunity and inflammation that has expanded so dramatically in the last decade or so. But in 1968, Scrimshaw et al. [10] concluded that vitamin A is regularly synergistic with infection; vitamin D deficiency commonly fails to show evidence of an interaction but synergism has been demonstrated;

deficiencies of the vitamin B-complex and some individual B vitamins behave variably, sometimes showing synergism and at other times antagonism, depending on species, the agent and host; vitamin C deficiencies are usually synergistic, but antagonism has been demonstrated; and finally, lack of minerals may result in either synergism or antagonism, depending on agent, host and species [10].

Impact on Infectious Diseases

The evidence for the impact of protein-energy undernutrition on immune status, in humans, has been stronger than in micronutrients but the evidence, and complexity, continues to expand. The causal line between undernutrition, including micronutrient deficiencies, to impaired immunity and then to increased incidence and/or severity of diseases leading further to a cycle of poor intakes leading to poor nutrition and so on, can continue until death or resolution. However, the direct evidence of the actual mechanisms linking micronutrient deficiencies to increased disease is sometimes less clear, e.g. the inadequacy of vitamin A and subsequent diseases, especially with respiratory tract infection [31]. Some of the stronger evidence comes from the role of micronutrient deficiencies in ageing and infectious disease. Of course, ageing is itself associated with impaired regulation of the immune system contributing to a higher incidence of morbidity and mortality from infectious, inflammatory, autoimmune and neoplastic diseases [229]. Subtle subclinical deficiencies of micronutrients such as zinc, selenium and vitamin E and inadequate macronutrient intake contribute to the decline in immune functions in the elderly [229]. Nevertheless, there is considerable more evidence in the last 10 years on the role of the many and complex roles of micronutrients in disease, inflammation and immunity. There is, for example, the much-increased understanding around vitamin D [6] and the associations of micronutrient deficiencies and metabolic syndrome signs and symptoms, even in (overweight) children [268].

What are the public health impacts of addressing compromised immune function through improving micronutrient status? The first section showed that the public health impact of micronutrient deficiencies extends far beyond their impact on infectious diseases, therefore it is important, when addressing micronutrient deficiencies, to go beyond a medical model. Micronutrient deficiencies affect both intellectual development and potential, and as has been graphically demonstrated in the recent *Lancet* nutrition series in early 2008 [2] and 2013 [3] and elsewhere, including also impacts on individual earning capacity and the economic development of whole countries [208]. Similarly the correction of the deficiencies needs to be far more than supplementation, especially in terms of sustainability.

The challenges of getting expensive foods, the ones that are usually higher in iron and zinc and preformed vitamin A, into the diets of the poor are discussed below. Both the problem and the solutions need to be perceived broadly. The challenge is bigger than just impaired immunity and inadequate diets but is one of wider development and reduction of inequities. This must include broad solutions, not least the improvement of women's status and education and other opportunities for female children, adolescents and women [271]. It is encouraging, that child deaths under 5 years of age each year in poorer communities and countries has been reduced from over 12 million children in 1990 to 6.3 million in 2013, a drop of 49 % [272]. The average annual reduction has accelerated—in some countries it has tripled—but overall progress remained short of meeting the MDG global target of a two-thirds decrease in under-five mortality by 2015. Nevertheless, most of the nearly 17,000 child deaths a day are entirely preventable and existing limited programmes need to be scaled-up nationally [273] along with considerably greater efforts on improved water and sanitation measures [274].

Consequently the following section is about the prevention, control and treatment of micronutrient deficiencies rather than about treating diseases or infectious diseases control, and improving hygiene and sanitation measures that would be expected to have an impact on immune status. Another whole area that is not addressed is the increased immunity over time of children, and adults, continuously

exposed to disease and the likely compromising of this defence in affluent populations where exposure is usually delayed and reduced. In this context, a study with pregnant women in Indonesia showed that when they were supplemented with zinc or β-carotene (along with the routine iron and folic acid), the mothers having zinc in pregnancy had a better ability to produce IL-6, and those receiving β-carotene, produced less IFN-γ, independently of nutritional status or birthweight [275]. So the authors suggest that giving mothers improved antenatal nutrition might even have the unintended consequence of an increase in the incidence of allergy and atopy in their offspring. A further aspect is the possibility that vaccinations and immunization outcomes may be compromised if the child is inadequately nourished, e.g. vitamin A and triple antigen, as briefly discussed earlier.

Infectious disease and the inflammatory response also present a number of challenges to an individual's nutritional status. Not only can infection result in poor intake, but for many nutrients it affects the body's natural homeostatic processes. This latter effect can have implications both in terms of nutrient physiology and function as well as the ability to assess nutrient status [6]. These challenges are perhaps best exemplified by iron particularly in the context of malaria. Although a need exists for a clear determination of the relative risks versus benefits of the most prominent iron interventions strategies (i.e. supplements vs. multiple micronutrient powders vs. fortification), the increasing body of evidence suggests that at least in the context of malaria, interventions to improve iron nutrition appear to be safe and effective in conjunction with malaria prophylaxis [191, 192].

Prevention, Control and Treatment Interventions to Improve Micronutrient Status

The recognition of the magnitude of the prevalence and impact of micronutrient deficiencies, and the knowledge of the possibility of doing something about them on a large scale, has resulted in a series of international goals. A meeting in Ottawa in 1991 reviewed and recommended ways to reach these goals [276]. These built on experience gained over previous decades (since the early 1960s in the case of iron-fortified cereal flour and iodized salt). As more experience has been gained, and funding increased, these have been continuously refined, and expanded. However, there were no goals or targets for micronutrients in the MDGs (to be achieved by end 2015) and there is unlikely to be in the Sustainable Development Goals that will replace them. The prevention and control of micronutrient deficiencies has become a higher global priority over the last couple of decades, but the extent of the programmes and the level of funding remain vastly under-resourced. This section briefly examines the currently most commonly used interventions.

A suggested categorization of such interventions is seen in Table 30.2 and is broadly:

1. Food-based approaches, including dietary diversification, nutrition education and fortification of staple and value-added foods.
2. Supplementation with vitamin A capsules, iron-folic acid tablets and iodized oil with increasing interest in a multi-micronutrient supplements and weekly low-dose supplements.
3. Public health interventions such as immunization, adding vitamin A supplementation to other programmes such as national immunization days and child health days, promotion of breast-feeding, and treatment of infectious diseases.
4. Change in the possibilities that are available to people by modification of the political, socio-economic and physical environment. As with so much of public health, those most vulnerable are those who are poorest.

The important point about these different approaches is that they are complementary, and should be started in concert, as they may have different time-frames, and differing feasibility, depending on local circumstances. Behaviour change to improve the intake of micronutrients is an essential part of whatever method is being used; through communications, social and political facilitation, social

Table 30.2 Public health approaches to modifying micronutrient intake used in the prevention and control of micronutrient malnutrition

Food based
Dietary diversification
Home gardening
Nutrition education
Development of high micronutrient content varieties of staple foods ('bio-fortification')
Fortification
Staples, e.g. flour, noodles
Fats and oils, e.g. margarine, edible oils
Condiments, e.g. salt, sugar, soy sauce, fish sauce
Complementary foods for infants 6 months and older
Home-based fortification, e.g. 'sprinkles'
Beverages, e.g. fortified juices, condensed milk and other dairy products
Supplementation
National distribution to all preschool children
National immunization days
Through health system centres, including maternal and child health programmes, and routine treatment
Outreach, e.g. with E.P.I. and other programmes
Post-partum supplementation
'Life cycle' distribution to adolescents and young women through schools and factories
Home-based supplementation, e.g. 'foodlets'
Public health measures
Improved antenatal and obstetric care
Immunization
Appropriate prevention and control of diseases such as diarrhoea, respiratory tract infections and malaria
Promotion of exclusive breast-feeding
Appropriate complementary feeding
Water and sanitation measures
Appropriate birth spacing
Global equity corrections, poverty reduction and socio-political change
Increased availability and accessibility of micronutrient-rich foods
Improved health systems
Improved status and education of women

marketing, and nutrition education. The overall strategy is to reduce the size of the most vulnerable group (to the left of the curve in Fig. 30.2) by improving the coverage of the middle group by fortification, dietary diversification and reduction of the disease burden [277]. The most at-risk group is likely to continue to need supplementation for many years to come. The factors listed in Table 30.2 have all been shown, to a greater or lesser degree, to have an evidence-based impact on micronutrient deficiencies prevention and control programmes [278].

In the following section, the prevention, control and treatment of micronutrient deficiencies are described. Improving immune response is not directly addressed as it is presumed to be a function of improved micronutrient status where that is the cause of the impaired immune function and increased risk of infectious disease. Ways in which improving nutrition may reduce the negative impact of infections on growth by the following actions can be seen in Table 30.3 [279].

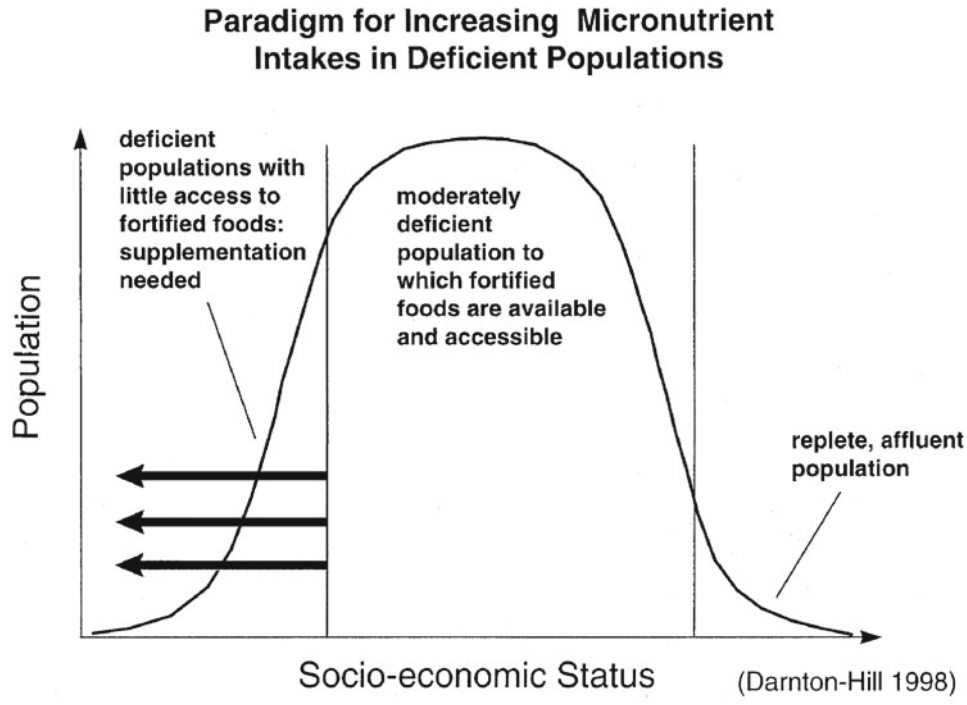

Fig. 30.2 Paradigm for increasing micronutrient intakes in populations by socio-economic status

Table 30.3 Improved nutrition may reduce the negative impact of infections on growth by the following actions

	Interventions
1	Strengthening the child's immune system, thereby reducing the severity and duration of infections and their impact on growth
2	Providing extra amounts of nutrients to compensate for those that are not well absorbed during infection, lost during diarrhoea, reallocated elsewhere in the body due to immune system activation or consumed in lower amounts than usual because of reduced appetite during infection
3	Providing the required amounts of nutrients for catch-up growth following infection, particularly the nutrients that are needed to build lean body tissue such as protein, potassium, magnesium, phosphorus, zinc and sodium
4	Preventing poor appetite caused by micronutrient deficiencies, thereby facilitating catch-up growth
5	Favouring the growth of beneficial bacteria in the gut that enhance gut function and immune defences

Adapted from [279]

Food-Based Approaches and Fortification

Dietary and Horticultural Interventions

With the exception of iodine in certain ecological settings, micronutrients are found abundantly in many plant foods and animal products. However, many families in resource-poor settings simply do not have enough to eat—over 800 million people according to FAO [4]. In the Indian sub-continent, nearly half of all women are categorized as underweight, e.g. Bangladesh [280]. But it is even more the quality of the diet, as diets characterized by poverty are less likely to include many micronutrient-rich foods

which are in any case generally more expensive and often less accessible, and so diets are likely to be low in vitamins and minerals, as well as energy [281]. This low accessibility to food sources is aggravated by the usually low bio-availability of micronutrients in the diets eaten by poor families, and it is poor dietary quality, rather than quantity, that is considered to be the key determinant of impaired micronutrient status [282]. In the current environment of high food price cycles, the accessibility of the poor to all foods is critically affected. The resulting shift to increased cereal staples such as rice, as other more micronutrient-rich animal-source foods becomes priced out of poor households' ability to purchase, the changes in household food expenditure patterns have a negative impact on the clinical vitamin A status of women of child-bearing age [283] amongst other micronutrients [284].

Food-based approaches have been categorized as (1) increasing small-scale production of micronutrient-rich foods, by community fruit and vegetable gardening, school gardening and/or small animal, poultry or fish production; (2) increasing community production of micronutrient-rich foods, such as horticultural products, oil seeds, palm oil, beverages and natural nutrient supplements; (3) maintaining micronutrient levels in commonly eaten foods with food storage and preservation techniques, improving food safety, and better food preparation; (4) plant breeding to increase micronutrient levels, including through genetic engineering and (5) community strategies to increase consumption of micronutrient-rich foods [212, 285].

Improving dietary diversification through increasing variety and frequency of micronutrient-rich food sources through nutrition education and horticultural approaches has been shown to be effective in many settings. Measuring effectiveness should use indicators of outcomes that go beyond increased serum levels of micronutrients, to clinical outcomes (reduction in night blindness) to social outcomes such as women's empowerment [286–288]. Food preparation interventions to achieve dietary diversification can include nutrition education concerning available foods and their more effective utilization; horticultural approaches such as home gardens; and improved methods of food preparation, preservation and cooking that better conserve the micronutrient content. There is increased interest in the genetic manipulation and breeding of staples and other foods to increase micronutrient content ('biofortification') [289–291].

While home gardening is a traditional family food production system widely practised in many LMIC [285, 292], anecdotal experience suggests home gardening (as an intervention method for improving nutrition) has been generally successful at the pilot or local phase, but often not been scaled up successfully. Recent experience in Bangladesh has demonstrated a successful example where it has, now reaching 800,000 families [292], and some of the lessons learned are being tried, with apparent good acceptance in Cambodia, Nepal [280], and parts of Africa such as Ethiopia [293]. An evaluation has shown that food gardening programmes also strengthened the capacity of local non-government organizations as a contribution towards sustainability of improvements in the community [294]. They have been found to increase income and empowerment of women and that can result in increased intake of micronutrient-rich foods such as eggs and meat as well as other foods such as oil, and improved caring practices [280, 287, 295]. Where home gardening is traditionally practised, using such an approach to increase micronutrient intake is more likely to be successful. In Indonesia, ownership of a home garden appears to indicate long-term vitamin A intake from plant foods, which explains its relationship with vitamin A status [296]. In the Bangladesh national survey, young children who had not received a vitamin A supplement were half as likely to be night blind if the family had a home garden [295].

Biofortification, also a food-based approach, uses traditional plant-breeding methods such as identifying plants that have cereal seeds naturally high in zinc or iron, or low in phytates, and then breeding for these, and more recently transgenic methods [289, 297]. Effectiveness of the resultant grains to raise micronutrient status in humans has been shown in one study to date of a successful feeding trial in the Philippines (using Catholic nuns to ensure adequate control conditions) [297]. The use of genetic engineering is expanding the possibilities, and a relatively recent alliance among the International Rice Research Institute (IRRI) and the International Maize and Wheat Improvement

Center (CIMMYT) has increased both efforts and coordination of research efforts on rice, wheat and maize aimed at 'improving the lives of poor farmers' [289, 290, 298]. Poor farmers are a group that has not much benefited from transgenic food research up to this point, which has mainly benefited horticulture for western markets, despite much of the rhetoric [291]. Probably the best known micronutrient example of this research approach, at least in terms of micronutrients, is the 'golden rice' where four different genes from the daffodil (*Narcissus pseudonarcissus*) and two from a bacterium (*Erwinia uredovora*) have been introduced to allow a non-biologically active precursor of beta-carotene, to proceed to the next three biological steps to become beta-carotene [290, 299]. However, it is not anticipated nutrigenetics will be a significant source of micronutrients in population terms within the next decade [291, 300].

Fortification

Probably the most cost-effective food-based approach to improving micronutrient availability and accessibility is fortification, with the proviso that the fortified foods must reach those who most need them. Not infrequently, those most at risk are outside established market systems that provide many of the 'value-added foods' most likely to be fortified. It has also not been shown that the fortification of staples will be able to provide adequate micronutrient content in most of the complementary foods given to young children (due to the small volumes involved), and so commercially processed and fortified foods will generally be necessary where available and accessible. There does appear to be increasing evidence that some animal sources in the diet are necessary for adequate micronutrient status [212, 294, 301]. Nevertheless, for the majority of many populations, fortification of foods with micronutrients has been shown to be a technologically, programmatically and economically effective method of increasing micronutrient intakes in populations [277]. Food fortification is likely to have played a significant role in current nutritional health and well-being of populations in industrialized countries [225]. Starting in the twentieth century, fortification was used to target specific health conditions: goitre with iodized salt; rickets with vitamin D fortified milk; beriberi, pellagra, and anaemia with B vitamins and iron enriched cereals; and more recently in the USA and other western countries, but also lately Pacific Island Nations and South Africa, risk of pregnancy affected by NTD by adding folic acid to fortified flour and cereals.

A relative lack of appropriate centrally processed food vehicles, less developed commercial markets, and relatively low consumer awareness and demand has meant that nearly 50 years have passed since its recognized successful impact in industrialized countries [225]. However, fortification is now increasingly seen as a viable option for the less developed and industrializing countries to increase micronutrient intakes [41, 302], including more recently in Africa [303]. As many of the previous constraints to widespread accessibility are minimized and with an increasingly global market, there is a great deal of current investment in fortification as an approach to the prevention and control of micronutrient malnutrition in LMIC [227, 302]. Fortification is but one arm of a micronutrient deficiency prevention and control strategy, but by becoming commercially viable, can reduce the size of the at risk population needing other measures such as supplementation (Fig. 30.1). Where the costs are passed onto the consumer, and the food industry routinely fortifies, sustainability is potentially high [304].

Globally 82 countries currently have legislation to mandate fortification at least one industrially milled cereal grain: 81 countries plus the Punjab province in Pakistan have legislation to fortify wheat flour; 12 countries have legislation to fortify maize products; and six countries have legislation to fortify rice [305].

A single micronutrient addition to an appropriate food vehicle is increasingly an uncommon approach in food fortification programmes, except iodine in salt and vitamin A in sugar. Even with iodine there is now considerable work in double fortification of salt with iodine and iron [41] and even triple fortification with vitamin A as well [306]. As Huffman et al. [307] and others have described,

women in LMIC often are consuming diets of poor bio-availability and limited micronutrient content, leading to concurrent deficiencies of iron, vitamin A, zinc, folic acid, B6, B12 and occasionally other vitamins and minerals [308–310]. Such deficiencies have important consequences for women's own health, pregnancy outcomes and their breast-fed children's health and nutritional status [307], and increasingly it seems on the birthweights of their children [310, 311]. Mason et al. [43] have estimated that nearly a quarter of children have multiple deficiencies. Consequently, it is now generally recommended that fortification be with a mixture of micronutrients, often in a pre-prepared fortificant mix of iron, folic acid and other B vitamins [227].

Supplement-type home fortification, e.g. 'Sprinkles', are microencapsulated micronutrients, including usually ferrous fumarate, which are available in a single dose sachet, and can be sprinkled onto complementary and weaning foods and other foods. In a randomized, controlled trial in Ghana, they were found to be as efficacious as iron drops in the treatment of anaemia [312], and have extensive efficacy experience such as those carried out in Bangladesh, Benin, Bolivia, Canadian First Nations and Inuit areas, China, Haiti, India, Nicaragua, Pakistan, Sri Lanka and Vietnam [313]. Although there was initially some concern about the levels of iron being given, these have now been reduced and there seems no doubt about their efficacy. Cure rates from anaemia have ranged from 55 to 90 % in children in the studies conducted [312, 313]. While the effectiveness applications need further demonstration, their use is already gaining considerable experience in the post-Tsunami disaster areas in South Asia [314] and non-emergency settings such as Mongolia [312].

In the more affluent industrialized countries, micronutrient deficiencies have been, and continue to be, addressed by food fortification, as well as by overall economic growth and general improvements in health, sanitation and nutrition that have contributed to the prevention and control of these deficiencies. These same aspects must be addressed in any prevention and control programmes in non-industrialized countries. Fortification, supplementation, other food-based approaches, and complementary public health measures are all necessary. This will only be done by partnerships with government, industry, and the consumer. There is a need to assess more widely the impact of interventions, not least for advocacy. Ultimately the success, impact, and sustainability of food fortification, like other interventions, rest with educating the consumer, developing consumer demand and demonstrating impact.

Supplementation

Supplementation has often been characterized as a short-term approach, criticized as an example of medicalization of a public health intervention, and presumed to have difficulty with likely sustainability, especially when supplements are supplied by foreign donors. Nevertheless, iron supplementation with folic acid, has been the method of choice to address anaemia in pregnant women despite little evidence of its effectiveness and likely limited impact [43, 210, 315], although efficacy has been repeatedly shown [205, 316]. Vitamin A supplementation has now been in place for over 40 years in countries such as Bangladesh and so hardly merits being seen as short-term, and many would argue that the need will be there for many years yet [31, 46, 317] although others are increasingly questioning this as the infectious diseases situation globally is so different now to what it was 35 years ago [43]. Consequently the effectiveness of vitamin A supplementation to preschool children to continue to reduce the risks of mortality and morbidity from some forms of diarrhoea, measles and malaria is thought to be decreasing, especially with the decline in measles rates. Nevertheless the observed effects in the earlier studies should be continued where vitamin A deficiency remains a serious public health issue. It is presumed these positive effects are the result of the actions of vitamin A on immunity [31]. Some of the immunomodulatory mechanisms of vitamin A have been described in clinical trials and can be correlated with clinical outcomes of supplementation, despite serum levels staying elevated for only a couple of months at most. The effects on morbidity from measles are related to

enhanced antibody production and lymphocyte proliferation. Benefits for severe diarrhoea could be attributable to the functions of vitamin A in sustaining the integrity of mucosal epithelia in the gut, whereas positive effects among HIV-infected children could be related to increased T-cell lymphopoiesis. The colostrum of women supplemented with retinyl palmitate has higher levels of SIgA, which suggests that the production of antibodies is modulated by vitamin A [318].

Zinc supplementation is now the recommended treatment for diarrhoea in children in LMIC [156], and while not used, at least as yet, in prevention, does reduce the risk of recurrent attacks of diarrhoea for some months after treatment. Since the release of the UNICEF-WHO Joint Statement on Clinical Management of Acute Diarrhea with new oral rehydration solutions and zinc, at least 54 countries have changed national child health policies to include zinc for treatment of diarrhoea. The experience of zinc treatment for diarrhoea has been instructive in terms of demand being generated before supply was assured. However the actual roll-out of zinc at country level has been slow for a number of reasons, including the need for changes to national policy and treatment guidelines, as well as adequate supply of zinc supplements.

Iron and folic acid supplementation has been the traditional approach for preventing and treating iron deficiency, particularly during pregnancy [168, 319] but logistics continue to be an issue, it is relatively expensive (for better quality iron/folic acid tablets that have far better compliance) and coverage is often poor [210]. Compliance is usually blamed but it is likely that distribution and logistical problems are every bit as important [320]. The efficacy of intermittent dosages, once or twice a week, has been demonstrated, suggesting that this may be a possibility for prevention, although not to treat anaemia in pregnancy [321]. However it does appear appropriate to recommend a dosage regimen of one or two times per week before pregnancy, e.g. to adolescents and young women in schools and factories [322]. It is presumed this approach would encourage compliance and reduce side effects and would certainly reduce costs [315, 316, 323]. Logistic constraints in many settings would still be a potential problem, although work in four Asian countries has shown promise with a social marketing approach [323]. With iron supplementation, gains in productivity and take-home pay have been shown to increase 10–30 % [318]. Consequently there are important reasons, in addition to the already compelling health, cognitive development and reproduction consequences, to accelerate programmes to prevent and control iron deficiency anaemia.

Nevertheless, there is increasing consensus that new approaches to scaling-up supplementation coverage are required [205, 210]. Anthelmintics treatment improved the haemoglobin and serum ferritin concentrations of Tanzanian schoolchildren [324], growth, appetite and anaemia [325] with similar positive synergies in other settings [326]. As the strategy for improving micronutrient status moves more towards integrated approaches as a way of helping to improving child survival [327, 328], reducing micronutrient deficiencies will be increasingly seen as an approach to increasing child survival and development in general.

Interactions are also a potential issue in other multiple micronutrient intervention settings. Most of the research to date has focused on the effect of single nutrient deficiencies on immune response and few studies have examined the simultaneous association of multiple nutrients, or their status, with immune function [6]. However, it is known that four micronutrients at least (vitamin A, vitamin D, zinc and folic acid) have specific points of convergence on the regulation of two major regulators of inflammation, NF-κB activity and the induction, and maintenance of Treg cells [6]. Up until now, much of the work on multimicronutrient supplementation has been in the relatively affluent elderly in western societies, with a considerable amount of self-medication as immunologic function, particularly cell-mediated immunity declines with age, this probably contributes to the increased incidence of infectious diseases in the elderly [14, 329]. High [329] has concluded that ‘multivitamin/mineral supplements or specific micronutrients such as zinc and vitamin E maybe of value … oversupplementation may be harmful’. Nevertheless women in North America, with generally micronutrient replete diets, are recommended to take multiple micronutrient supplements during pregnancy. On the hand, many women in less affluent economies survive on diets of poor quality and micronutrient

deficiencies are common in LMIC [309]. As these authors note, the ability of the newborn to maintain health, withstand disease, grow and develop normally is influenced by the gestational nutritional experience, and replacing likely deficient micronutrients would presumably correct these deficiencies. However it is not known exactly how such micronutrients interact in depleted/infected populations, or against habitually compromised diets [309] but positive evidence is accruing [278, 330].

An independent systematic review and meta-analysis of 12 randomized, controlled trials in LMIC, comparing multiple micronutrient supplementation with iron-folic acid supplementation found that both supplements were equally effective in reducing anaemia (even though iron content was often lower in the multimicronutrient supplement) and resulted in a small, significant increase in mean birthweight [330]. Following these findings, it was suggested that replacing iron-folic acid supplements with multiple micronutrients in the package of health care, including improved obstetric care of health and nutrition interventions, would improve the impact of supplementation on birthweight, small-for-gestational age neonates, and perhaps child growth and development [330]. Despite some initial concern in some settings of (non-significant) risk of increased neonatal mortality (not found in other reviews), the conclusion to recommend antenatal multiple micronutrients was subsequently endorsed by the second *Lancet Series on Maternal and Child Nutrition* following further evidence supporting the approach [3]. Trials are underway in a number of countries at present. A meeting to review nutrition as a preventive strategy against adverse pregnancy outcomes concluded that effective interventions with micronutrients are likely to be required at an earlier stage than happens in public health programmes at the present time, certainly before mid-pregnancy, and for some interventions, probably during the pre-conceptual period [331]. There is already an existing WHO/WFP/UNICEF joint statement on preventing and controlling micronutrient deficiencies in populations affected by emergencies [332], which includes both women and young children, but not for prevention in non-emergency settings.

Control, Prevention and Treatment of Infectious Diseases by Strengthening Immunity

Once an infection is established, a host can do one of three things to minimize the agent's impact on its health. The immune system of the host can directly attack the growing pathogen population to contain or eliminate it (*resistance*); or it can attempt to minimize the harm caused by a given number of pathogens by increasing tissue repair or by detoxifying pathogen by-products (*tolerance*); or some combination of both [333]. Traditionally, it is suggested that immunologists, microbiologists and parasitologists have focussed on the ability to limit parasite numbers or on the overall ability to maintain health irrespective of parasite burden (resistance plus tolerance), with less emphasis on just tolerance although there are good examples of that such as the presence of α-thalassaemia and the resulting reduced life-threatening episodes of malaria [333].

With micronutrient deficiencies, the line between prevention, treatment and control is often blurred except in serious deficiency, e.g. xerophthalmia and serious anaemia (Hb < 7 g/dL). Children with any stage of xerophthalmia should be treated with vitamin A according to WHO treatment guidelines, as should pregnant women with such life-threatening levels of anaemia. But, in a population with say 50 % prevalence of a particular deficiency and the resultant clinical outcomes, such as may occur with anaemia in pregnant women in resource-poor settings, is giving iron a treatment or prevention [334], especially in the case of the relatively recent recommendation by WHO of weekly preventive iron and folic acid supplementation [335]. In the majority of the programmes, it is clear that to address impaired immunity, integrated programmes that also address food security, care, the health services, community measures and the water, sanitation and hygiene environment will all be necessary [273].

As there are standard guidances for treatment of the clinical outcomes of micronutrient deficiencies and these are usually well tried and efficacious, they will not be discussed further. WHO is the technical agency of the United Nations system that takes a normative role in developing these and coordinating available research information on an evidence-base and the consensus of experts and are available through their electronic library of recommendations. The BOND Initiative (Biomarkers of Nutrition and Development) is assisting WHO and others in updating six of the micronutrients of public health concern [16]. The prevention of micronutrient deficiencies, although undoubtedly efficacious, remains challenging in terms of effectiveness, especially in hard-to-reach populations.

Improving the Immunological Status and Resistance to Disease Through Related Public Health Interventions

Despite the recognized bidirectional interactions between nutrition and immunity [336], it is clear that while important, improving the nutritional status, and micronutrient adequacy, will alone not be enough to improve immunological status of an individual. Most children at risk of increased risk of undernutrition and early child death from infectious diseases live in unhealthy and deprived environments. The World Bank increased the definition of people living in poverty as those with under US$1.25 a day, and so in 2005, 1.4 billion people were defined as living in poverty (a quarter of LMIC)—although an improvement on the 1.9 billion in 1981. It is hard for most people not living in such conditions to have any idea of what this means in terms of inadequate food security, impossibly unhygienic conditions and increased risk of maternal and child death. Five years ago, over 80 % of all children stunted lived in just 20 countries [337] and 90 % of the global burden of under 5 mortality is borne by 36 countries [2, 3]. Consequently there is a need to address other issues as well, quite apart from the need to reduce inequities both within and between countries. Also there will need to be a scaling-up of water and sanitation measures, infectious disease prevention and treatment, improved measures to improve household food security and nutrition security and a reduction of parasite infections, as well as social measures. *The Lancet* has recently reported the estimate that a tenth of the global disease burden could be addressed by properly tackling water and sanitation issues [338], almost certainly now considered a conservative figure [274].

For maximum impact other public health interventions are essential. These integrated interventions include, amongst other locally appropriate actions, control of infectious diseases, expansion of measles and other childhood immunization interventions, deworming for intestinal parasites (hookworms), malaria control, promotion of breast-feeding, and proper health care such as oral rehydration therapy, all of which have an impact on micronutrient status [326, 339, 340], and hence, in many cases, immune status. Multiple factors are associated with immune response to vaccines administered during childhood including the timing of antigen exposure, age, concurrent infections, undernutrition, particularly micronutrient deficiencies of vitamin A, iron and zinc [341]. Breast-feeding is identified in the Bellagio child survival reports as the most important intervention providing 13 % of the total impact on potential young lives saved [2, 3]. An earlier study from rural Ghana showed epidemiological evidence of a causal association between early breast-feeding and reduced infection-specific neonatal mortality [342]. Vitamin A and iron supplementation of pregnant Indonesian women benefited the vitamin A status of their infants, but still, the authors concluded in that study, the infants may need vitamin A supplementation or increased dietary intake after 6 months [343]. Human milk ascorbic acid levels can be doubled or tripled by increased intake of ascorbic acid in women with low human milk ascorbic acid content with the impact far more evident in African women compared with European women [344].

The International Food and Nutrition Policy Research Institute (IFPRI) has identified the four main factors contributing to infant and child undernutrition: food accessibility and availability; mother's

education; women's status relative to men in the society; and the health and sanitation environment [271]. Bendich [345] has demonstrated the roles of nutrients in optimizing women's health and immune function, as well as other roles of micronutrients in women's health [216]. While critical, the actual interventions are outside the scope of this chapter but there is increased recognition that parallel, vertical programmes are no longer enough (although the funding community finds them easier to manage); but as it is the same families and communities that need investment in all these areas, and usually the same inadequate health systems trying to support them, and a lack of nutrition capacity, there are increasing attempts to recognize and implement these realities on the ground.

Among the major remaining constraints, as the recent re-analysis of the Child Survival approach has reminded the international health community, are amongst other things, poor health systems and inadequate resources [2, 3, 327]. They demonstrate convincingly that it is not that cost-effective interventions for both child survival and development and young child undernutrition are not known about, but that they are not being implemented on a sufficient scale [327, 337, 340]. Largely based on this series, but drawing on years of often poorly documented experience, international agencies, with national governments, are directing their efforts more towards an integrated approach: reduction of the diseases of childhood, reduction of the vaccine-preventable diseases, neonatal causes of death, undernutrition, water and sanitation-related disease and national policy making to strengthen health systems and coordinate funding through the various political and policy mechanisms.

Micronutrient deficiencies prevention and control, largely through mechanisms discussed in this chapter, will need to be scaled-up to be a more important part of overall public health approaches in resource-poor settings [346]. Noting that potential investments appear under-resourced, Behrman and colleagues have also noted the high rates of benefit-to-cost ratios and that the 'gains appear to be particularly large for reducing micronutrient deficiencies in populations in which prevalences are high' [347].

Conclusion

Micronutrient deficiencies are a recognized problem in as many as a third of people living in LMIC and in disadvantaged sub-populations in more affluent countries. The impact of such deficiencies, and more uncommonly excesses, on immune status and disease incidence, growth, development and survival is now well appreciated although some of the underlying mechanisms are still not clear. What is often less appreciated is the impact of micronutrient deficiencies on immunological status. As has been described, there is good evidence that vitamin A, vitamin D, other vitamins, and iron and zinc and other mineral and trace element deficiencies all impact on the incidence and prevalence of infectious diseases through an impairment of the immune system resulting from these same deficiencies.

At the same time, there are many environmental, societal and cultural reasons, all aggravated by poverty, that are increasing the risk of these same populations of a heightened risk of contracting diseases. As in undernutrition in general, there is a vicious cycle that is set-up with micronutrient deficiencies further reducing resistance to infection with increased disease and further reduced appetite and absorption which then re-enforces the underlying micronutrient deficiency. Because of the broader environmental determinants of increased disease through undernutrition and impaired immunity, the interventions need to be broad ranging across health, nutrition, environment, water and sanitation and reduction of social inequities. The relatively recent recognition of the role of inflammation in overweight and obesity and associations between many non-communicable diseases and impairment of immunity, adds another dimension of complexity to addressing these issues.

The efficacy of micronutrient prevention and control, and treatment, is well established. The big challenge is effective national or sub-national intervention programmes being adequately scaled-up. Transition from vertical to more integrated programmes, and getting donor support for these, are a

current major challenges. As is frequently quoted, the cost-effectiveness of most micronutrient interventions continues to need advocacy to policy makers; overall, it has been estimated by the World Bank that for 'less than 0.3 % of their GDP, nutrient deficient countries could rid themselves of these entirely preventable diseases, which now cost them more than 5 % of the GDP in lost lives, disability and productivity'. Given the comparative success of many of the micronutrient deficiency prevention and control programmes in many parts of the world, because of the known interventions, the challenge is now to scale-up such programmes to a more comprehensive national level. This would achieve results in improving the survival and development of children and women, through improving immunity and reducing micronutrient deficiencies in integrated, community-based programmes supported by adequate resources at district, national and international levels.

Acknowledgements The authors would like to acknowledge and thank Dr. Dan Raiten Ph.D., Program Director-Nutrition in the Pediatric Growth and Nutrition Branch of NICHD, NIH, USA, for his continuing wise and informed advice; Mr. Hossein Khosravi Borujeni, PhD student, School of Medicine, Griffith University, Australia, for his assistance with an updated literature search; and Anne Morrison, Department of Human Nutrition, University of Otago, New Zealand, for her assistance with the formatting of the document.

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Chapter 31
HIV and Nutrition

Murugi Ndirangu and Kevin A. Sztam

Key Points

- The interaction of HIV and nutrition is incompletely understood.
- Immune manifestations of protein-energy malnutrition overlap with immune manifestations of HIV.
- Protein-energy malnutrition and micronutrient deficiencies are prevalent in areas where HIV is also prevalent.
- As antiretroviral therapy becomes more accessible and life expectancy for those infected with HIV increases, there will be an increased need for research involving nutrition and metabolism in conjunction with medical treatment in developing settings.
- The impact of micronutrient supplementation in different settings is beginning to be elucidated.
- Generalized recommendations of macronutrient supplementation in HIV cannot be made given very scant data.
- Breastfeeding is the recommended mode of feeding where formula feeding is not acceptable, feasible, affordable, sustainable, and safe.

Keywords Breastfeeding • HIV • Resting energy expenditure • Protein-energy malnutrition • Nutrition • Food insecurity • Micronutrient and macronutrient supplementation • Resource limited settings • Children • Adults

Introduction

Human immunodeficiency virus (HIV) and nutritional status maintain a critical and important relationship that is incompletely understood. Consequences of chronic viral infections may have organ-specific effects as well as general effects on health and function. HIV infection is a nutritionally progressive disorder with major metabolic changes in nutrient utilization as the balance of viral replication, immune response, and inflammation changes over time. HIV treatment has its own specific metabolic effects which have been discussed elsewhere [1–3]. Worldwide, nutrition and HIV are important determinants of clinical outcomes including survival, with special importance in areas of food insecurity like Sub-Saharan Africa. States of normal and abnormal nutrition may affect the

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*, Nutrition and Health, DOI 10.1007/978-3-319-22431-2_31

progression of HIV and response to treatment in different ways. Poor nutrition in the patient infected with HIV not only has implications for the individual, but in conjunction with progressive immune dysfunction also has significant implications for community and global health.

HIV Background and Statistics

Global Burden, Current Trends

An estimated 35.3 million people were living with HIV worldwide in 2013 with about 2.3 million of those being new infections (Fig. 31.1). Africa continued to bear the burden of the epidemic with an estimated 70 % of all new infections in 2012.

The primary mode of HIV transmission in this region is mainly heterosexual contact.

However gains have been made in this region since 2001 with the annual number of new HIV infections among adults in Sub-Saharan Africa declining by 34 % in the same time period, the decline in new infections has been even greater in the Caribbean region, with a reduction of 49 %. In contrast, new infections have been on the rise in Eastern Europe, Central Asia, Middle East, and North Africa [4]. Currently, Sub-Saharan Africa accounts for nearly 71 % of HIV-positive persons in the world and remains most severely affected, with nearly 1 in every 20 adults living with HIV (Fig. 31.2). These data clearly show that a significant number of individuals are infected and prevalence continuous to be fairly high. Clinical care issues related to treatment and chronic disease, including the interactions of nutritional status and HIV, will therefore continue to be important.

Due to the effects of the HIV pandemic, many countries in Southern Africa had experienced a decline in life expectancy in contrast to historical gains since the 1950s in other parts of Africa and the world. However due to the scale up of antiretroviral this trend is being reversed in many countries. Life expectancy was 11.3 years higher in KwaZulu-Natal province of South Africa in 2011 compared to 2003 [5].

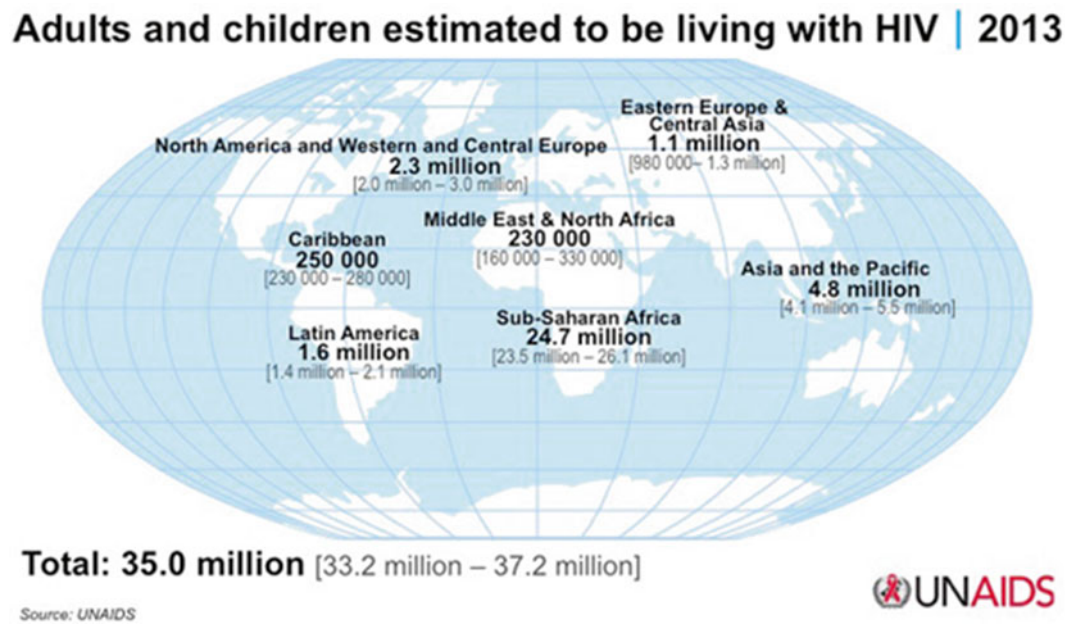

Fig. 31.1 Adults and children estimated to be living with HIV 2013

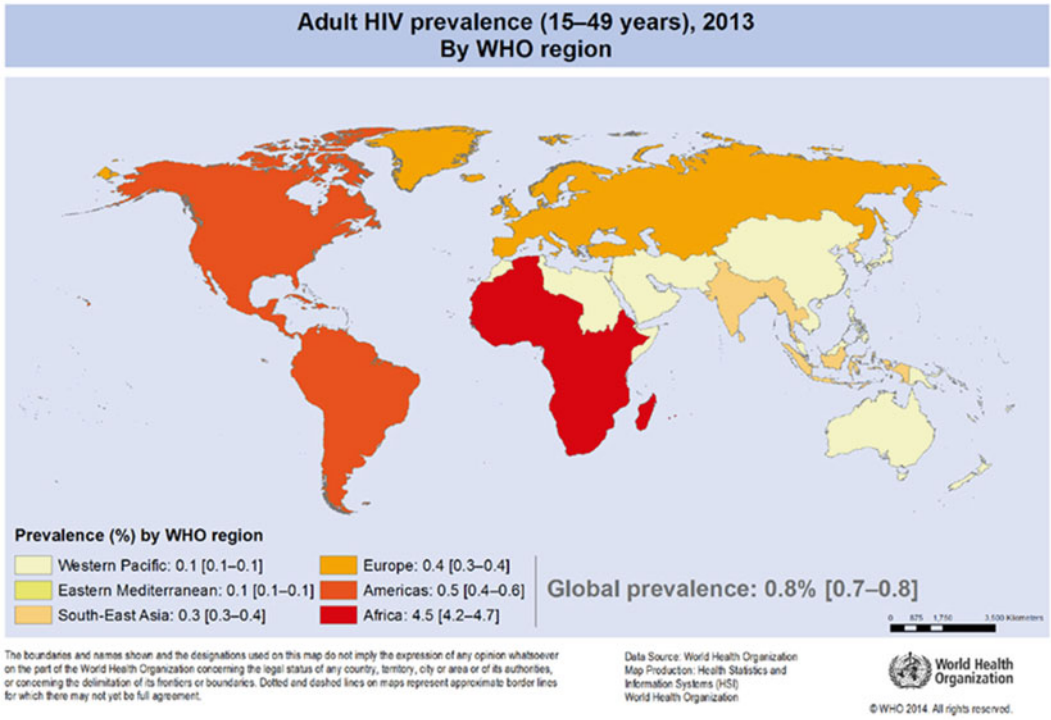

Fig. 31.2 Adult HIV prevalence (15–49 years), 2013 By WHO region

Chronicity of HIV and Epidemiology of Long-Term Treatment, Increasing Survival

The availability of antiretroviral therapy (ART) has increased, with the number of people receiving ART tripling from 2009 (Fig. 31.3). Since 2005, the number of people receiving treatment has increased in all regions except Eastern Europe, Central Asia, the Middle East, and North Africa [4]. One program alone, the President's Emergency Plan for AIDS Relief (PEPFAR), provides over 6.7 million infected people worldwide with ART [6].

As a result of increased global availability of medications, many HIV epidemics have been moderated regionally. Between 1995 and 2012, ART averted 6.6 million AIDS-related deaths worldwide, of these, 5.5 million deaths in low- and middle-income countries (Figs. 31.4 and 31.5). ART interrupts the cycle of infection by controlling viral loads, increasing T-cell numbers and improving function and decreasing opportunistic infections. Treatment with ART also dramatically increases life expectancy [7]. Viral loads are linked to transmission rates in a dose-response relationship, with higher viral load increasing infection rates [8]. Other risk factors for HIV transmission include the presence of sexually transmitted diseases with ulceration, number of sexual partners, possibly genetic HLA type I alleles and stage of infection [9–11]. Interventions such as education and condoms [12–14] help decrease the incidence; however, ART itself still has the greatest potential to decrease new infections [15].

With significant advances in treatment modalities and survival, and increased life expectancy in the patient treated with ART (Figs. 31.6 and 31.7), the approach to HIV has changed to a model of chronic disease rather than acute immune dysfunction and clinical deterioration [15, 16]

In this model of chronicity, support of the immune system apart from ART has become critical, and the interplay of nutritional status and HIV infection with or without medication has gained focus. Food and nutrition support programs are possible strategies to reduce vulnerability to HIV infection,

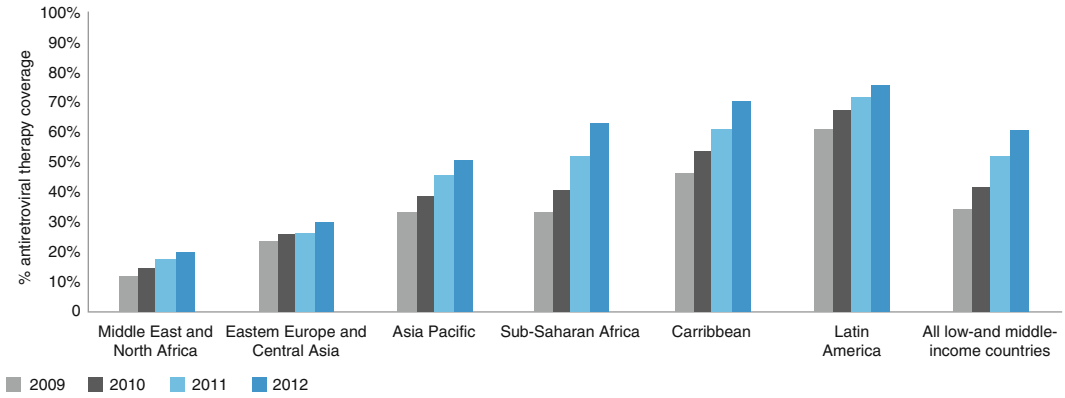

Fig. 31.3 Percentage of people eligible who are receiving antiretroviral therapy (based on 2010 WHO guidelines) in low- and middle-income countries, by region, 2009–2012, from GLOBAL REPORT: UNAIDS report on the global AIDS epidemic 2013. Geneva, Switzerland (Accessed at http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf)

especially among women and girls [17]. Adequate nutritional status can help maximize functionality at all stages of disease, minimize side effect of therapies, and could have additional roles in improving outcomes of patients in developing settings before and during ART [18, 19]

Epidemiology of Malnutrition

Geographic Coexistence of Malnutrition and HIV

The HIV epidemic disproportionately affects countries already burdened by acute and chronic malnutrition. High levels of malnutrition are prevalent in many parts of Africa, for example, linked to food insecurity (Fig. 31.8). Protein-energy malnutrition and micronutrient deficiencies are common as a result of poverty, environment determinants, and economic instability.

Areas of traditional food insecurity are also areas affected by HIV. The HIV epidemic which has taxed social, economic, and cultural systems also affects food security [20]. Effects of the HIV epidemic on families and communities change food access and availability leading to decreased intake of micronutrients and macronutrients [21, 22]. Decreased intake by the individual leads to a cycle of nutrient deficiency causing associated pathologies and immunodeficiency, increased risk of infections, increased metabolic demand and losses which ultimately results in decreased intake thus exacerbating preexisting malnutrition [23].

In areas like Africa and Southeast Asia affected by high burdens of both HIV and malnutrition, multiple factors may affect food availability, access, and utilization. Food security is linked to war and displacement, productivity, geography, proximity to arable land, storage techniques, location of central markets, and pricing [24–27]. Food availability is affected by seasonality and cyclical effects of rains or drought [28, 29] and affects dietary diversity. Food access is determined by socioeconomic status. Notably in Sub-Saharan Africa, over 50 million individuals are undernourished [30]. Utilization of food involves choices of foods to consume, food preparation, food additives, and sources of water.

Limited-resource areas such as parts of Sub-Saharan Africa and Southeast Asia tend to have less dietary diversity than more affluent areas [31]. Maize and rice, two global staples, are relatively inexpensive compared to other sources of energy and are thus often the majority if calories consumed in

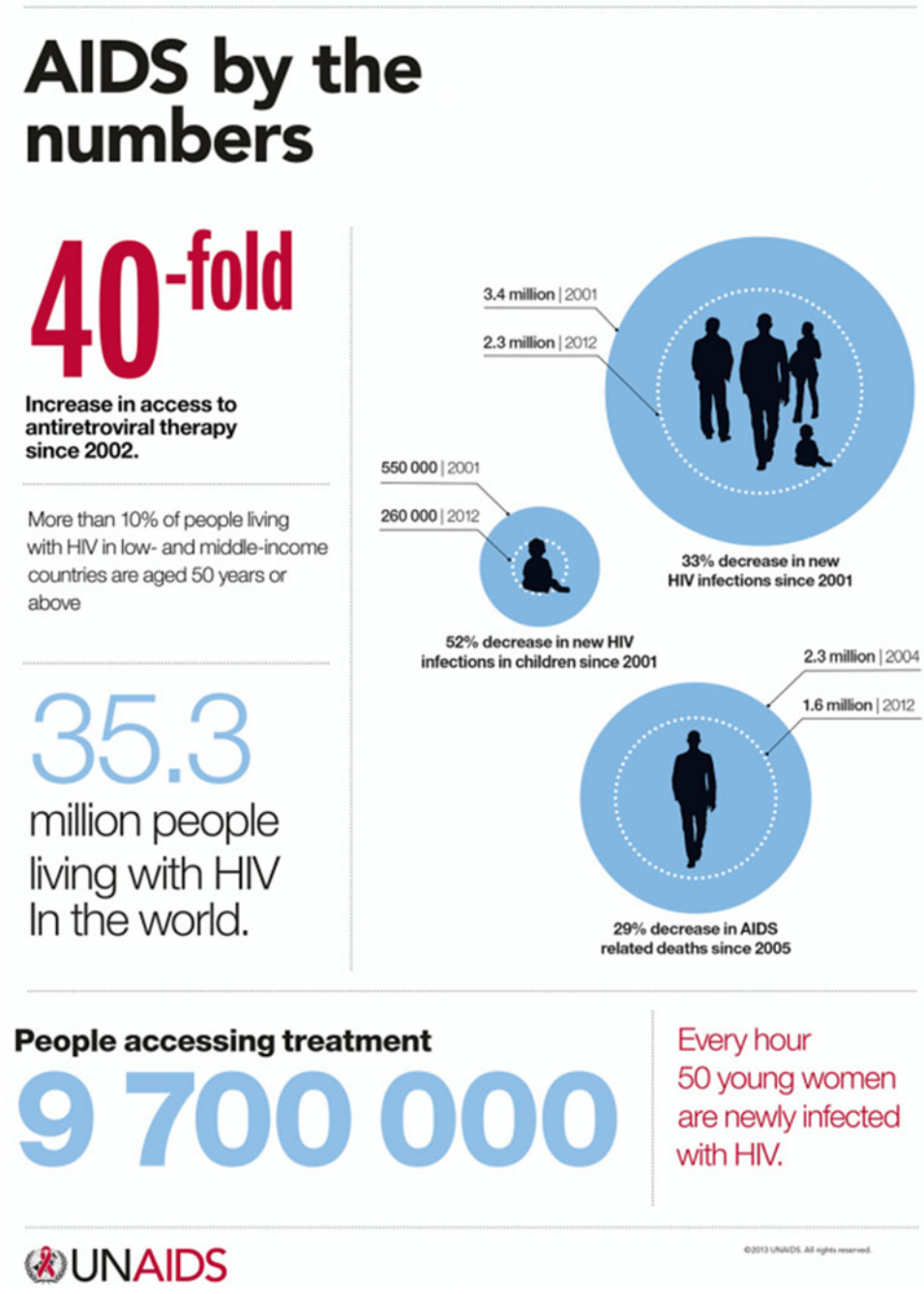

Fig. 31.4 AIDS by the numbers. Assessed at http://un.org.au/files/2014/01/Untitled.jpg

developing areas. While price may be a prime determinant of dietary choices, food availability and cultural influences shape what an individual considers healthy or desirable food [32]. Disproportionate use or choice of these foods may cause dietary inadequacy, even when animal and other source proteins may be available.

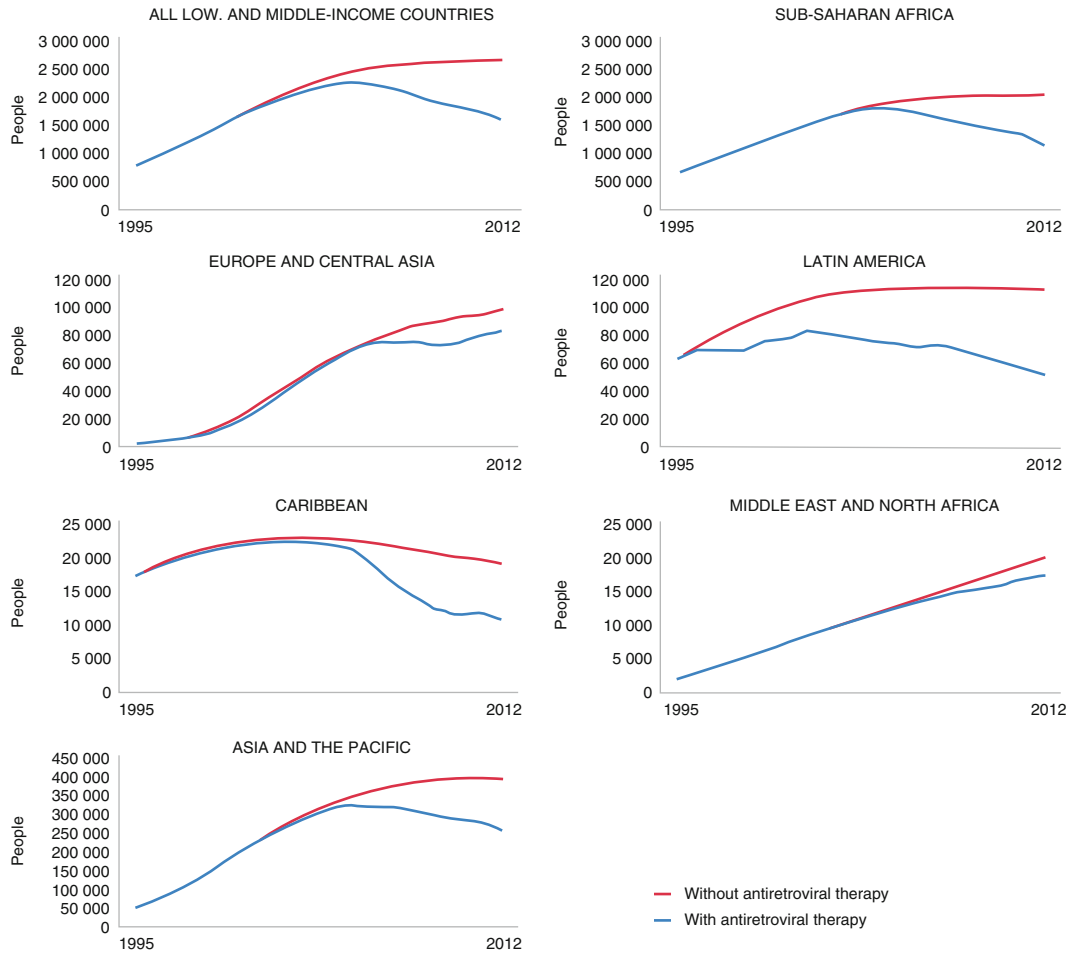

Fig. 31.5 Estimated number of AIDS-related deaths, with and without antiretroviral therapy, in low- and middle-income countries, and by region, 1995–2012 from GLOBAL REPORT: UNAIDS report on the global AIDS epidemic 2013. Geneva, Switzerland (Accessed at http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf)

Food insecurity inhibits effective prevention and treatment [33]. For an individual patient infected with HIV, weight loss may be related to HIV infection itself or to co-infections in the immunocompromised host. In geographic areas with baseline malnutrition such as Sub-Saharan, the cause of weight loss may be due to food insecurity. Due to an increased frequency of concomitant illness, HIV infection contributes to malaise, inactivity, muscle loss, and deconditioning. This can reduce the ability to perform physical work. This is especially important in resource-limited countries where large portions of the population depend directly on small-scale labor-intense farming for their sustenance and livelihood. The impact on communities of decreased productivity due to death and illness is significant as HIV primarily affects those of reproductive age, also the most economically productive portion of the population [34–36]. If individuals are too ill to engage in food production, they are likely to be food insecure [26]. In turn, lack of adequate food intake further escalates the cycle of untreated infections and malnutrition and can have community-wide effects. AIDS-affected households experience a decline in income as costs related to managing illness and the wider and epidemic rise [37]. With a decline in income, the ability of a household to obtain sufficient healthy foods and adequate food quantities also declines.

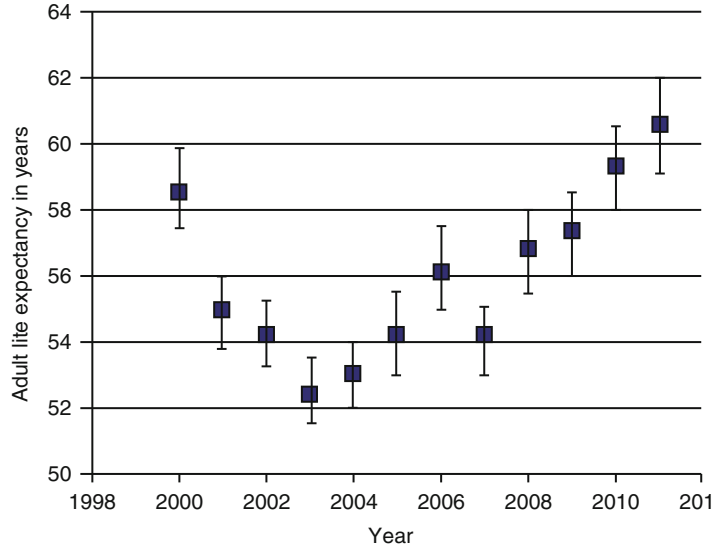

Fig. 31.6 Average adult life expectancy, rural South Africa, 2000–2011 (Bor, J., Herbst, A. J., Newell, M. L., & Bärnighausen, T. (2013). Increases in adult life expectancy in rural South Africa: valuing the scale-up of HIV treatment (*Science*, *339*(6122), 961–965)

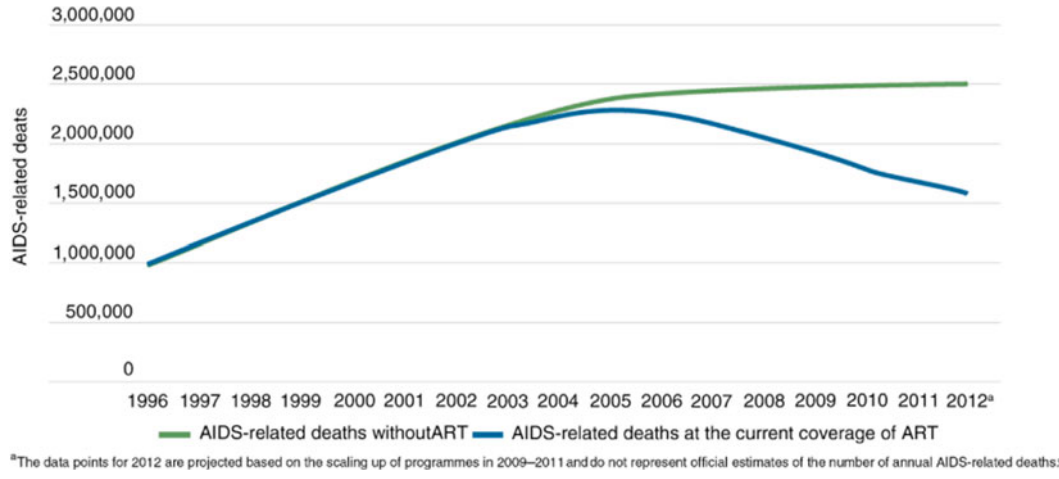

Fig. 31.7 Annual number of people dying from AIDS-related causes in low- and middle-income countries globally compared with a scenario of no antiretroviral therapy, 1996–2012 (Ford, N., Vitoria, M., Hirnschall, G., & Doherty, M. (2013). Getting to zero HIV deaths: progress, challenges and ways forward *Journal of the International AIDS Society*, *16*(1))

The 57th World Health Assembly resolved to encourage countries and agencies such as the World Health Organization to integrate nutrition into the comprehensive response to HIV/AIDS as part of the scale-up treatment and care [38]. Nutrition support programs are cited as a critical tool to help HIV-infected individuals cope with the effects of the treatment in resource-limited settings holds promise of making a substantial impact on morbidity and mortality. With the availability of ART in areas of high prevalence of malnutrition, investigation must focus upon the interaction of treatments such as medications and nutritional status.

The aim of HIV programs is to address malnutrition in areas of food insecurity simultaneously to treating HIV is ambitious. In contrast to supplementation as adjunctive treatment, food replacement is required to counter food insecurity, as patients do not have access to or availability of local food

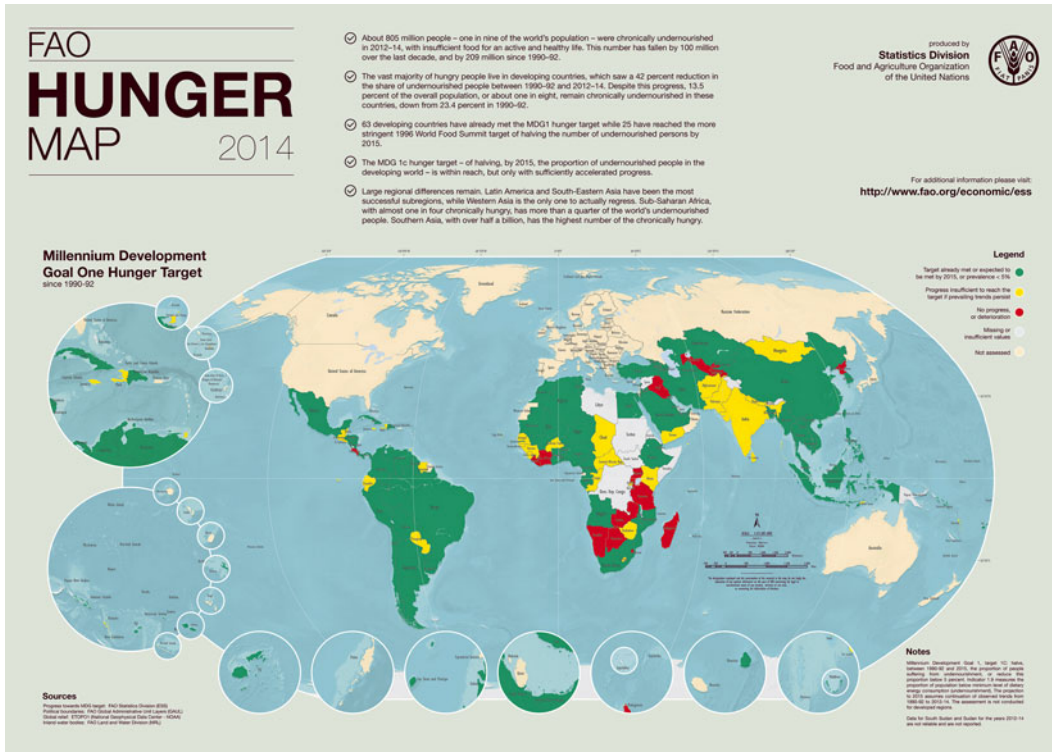

Fig. 31.8 FAO Hunger Map 2014

resources. This strategy differs somewhat from targeted supplementation designed to meet time-specific or patient-specific nutritional goals. Indeed some HIV treatment programs function in areas with a high prevalence of malnutrition. Not attending to malnutrition leads inevitably to poor clinical outcomes for the individual patient. Addressing food insecurity as the cause of this malnutrition through HIV treatment programs may be insufficient to improve long-term outcomes for patients at the community-level; however, the dual goals of clinical care and improving food access and food intake are intertwined. In advocacy for access to HIV care, broader population-based approaches, including efforts at economic stability, support of livelihoods, and the development of income generating activities will likely play a role. A more comprehensive approach will be needed in areas where both HIV and malnutrition are prevalent.

HIV and Nutritional Effects

The Cycle of Malnutrition and Infection

Nutritional status is closely associated with the survival of HIV-infected persons and with disease progression [39, 40]. Helper T-lymphocytes number and cellular immunity are affected by HIV, and they are secondary effects on humoral (antibody) immunity. Changes in immune function increase the risk of a variety of co-infections including those termed "opportunistic" because of this decreased host immune response [41, 42]. Each co-infection has its own pathology and organ-specific manifestations, which in turn may affect nutritional status by increasing metabolic demand, causing nutrient losses, and reducing dietary intake [43–45].

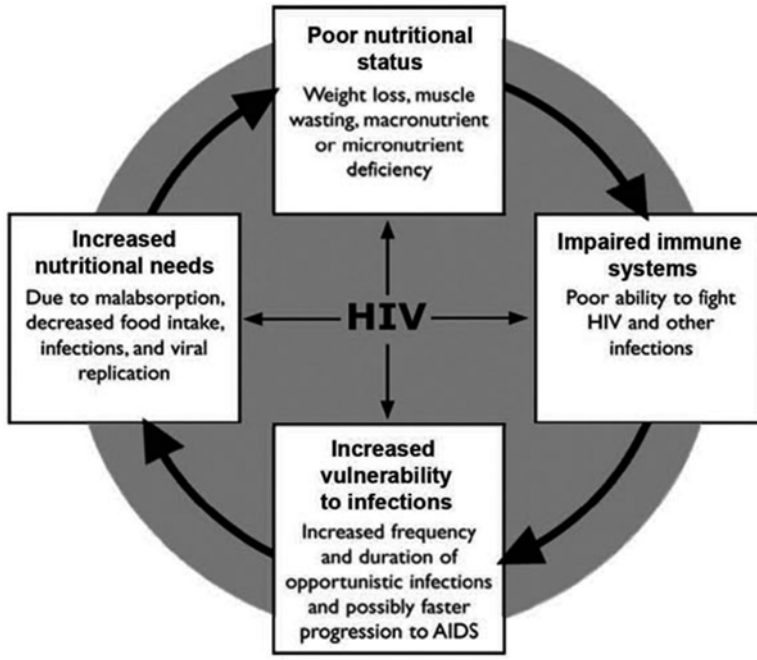

Fig. 31.9 The cycle of malnutrition and infection in the context of HIV (Source: Federal Ministry of Health, 2008, *Ethiopian Guide to Clinical Nutrition Care for Children and Adults with HIV*)

In this setting weight loss can be rapid. Loss of both fat mass and fat-free mass is a common manifestation of advanced HIV infection, where decreased metabolic reserves and decreased intake together lead to additional infections by directly impairing the immune system. This interplay completes the continual cycle of malnutrition and infection in HIV (Fig. 31.9).

Weight loss is more than a symptom of HIV infection. Rather loss of body weight reflects an imbalance of nutritional inputs and metabolic demands and is prognostic. Many HIV-infected persons become nutritionally deficient in association with HIV disease and wasting syndrome is a common complication preceding death. The issue of underlying malnutrition is very important in the context of HIV infection as a body mass index less than 18.0 kg/m^2 is strongly predictive of death (adjusted hazard ratio 2.5, 95 % CI 2.0–3.0) [36]. Weight loss in patients with HIV infection predicts clinical outcomes with or without ART. In HIV-infected adults, a baseline body mass index of less than 18.5 kg/m^2 at the initiation of ART is significantly associated with mortality in univariate analysis [hazard ratio 2.9 (95 % CI 1.04–8.01)]. In fact weight loss during the first 4 weeks of ART is also associated with death ($p=0.009$) [37], and this effect persists even when patients have access to ART [46, 47]. Higher body mass index is protective, and is associated with higher CD4+ T-lymphocyte count responses [48]. Thus, especially at the beginning of therapy, general nutrition status as measured by BMI is prognostic.

HIV and nutritional status interact in complicated ways which are not fully understood. Resting energy expenditure (REE) has been examined in multiple studies. Most studies demonstrate an increase in REE approximately 10 % in the asymptomatic patient, and up to 34 % in the patient with co-infections [46, 49, 50]. Energy expenditure was found to be higher in HIV-infected women compared to HIV-negative women [51], even when adjusted for lean body mass. In the setting of co-infections, resting energy-expenditure increased and energy intake decreased. Despite these changes in REE, intake remains the prime determinant in weight loss. In fact total energy expenditure may decrease due to activity levels; however, intake is still often less than energy expenditure in HIV-infected patients with rapid weight loss [52, 53].

The demands of maintaining viral replication and supporting the inflammatory immune response appear to require significant metabolic resources in both the asymptomatic state and with co-infection [54]. Reflecting demands against growth, disease activity in children as measured by viral loads is inversely related to energy intake, growth velocity, and fat-free mass [55]. Energy destined for deposition into muscle, bone, fat, and other tissues of a child is directed toward the maintenance of infection and inflammation.

A redirection of metabolic resources to HIV infection may cause wasting [47]. According to the WHO wasting is defined as unexplained weight loss of 10 % of total weight [56]. Wasting patterns include loss of lean body mass and muscle in men, and initial fat loss in women followed by loss of muscle [57]. There is preferential loss of lean body mass, particularly in men [47, 58]. While malabsorption, inflammation of HIV infection, metabolic demand of co-infections, and food insecurity all contribute to weight loss, decreased intake appears to be the largest single contributor [59]. Decreased intake may be related to gastrointestinal symptoms, thrush, esophagitis with pain, and disease-related anorexia from effects on eating mechanics, perception of hunger, poverty, or psychosocial problems [60–62]. A comprehensive nutritional approach is warranted in all HIV-infected patients as weight loss "remains a major problem and may be the best predictor of mortality in [people living with HIV/AIDS]" [19].

Weight loss may be reflective of overall nutritional status, but protein and fat malabsorption are specifically affected in HIV infection. Patients infected with HIV have increased rates of protein turnover [63, 64] and increased losses through the gastrointestinal tract [65]. If goal protein intake is not met, inflammation from viral infection persists, and physical activity during disease is limited, the result is muscle wasting. HIV infection may also cause specific patterns of fat loss (lipoatrophy) and redistribution (lipodystrophy), which are associated with metabolic abnormalities including dyslipidemia [66]. These changes may be augmented by ART, especially regimens containing protease inhibitors. Chronic dyslipidemia may increase atherosclerosis and in turn cardiovascular risk in both adults and children [67, 68].

Aside from specific changes in metabolism, there are generalized anthropometric changes that can occur in HIV infection. The sum of the effects of losses, changes in intake, and increased metabolic demand is often protein-energy malnutrition (PEM). PEM is a state of persistent nutritional deficiency, but during which the body still actively compensates to maintain lean body mass and visceral organ function. Further malnutrition causes uncoupling from this compensated state and rapid wasting may result. Nutritional recovery then becomes less likely [19]. The cause of wasting is especially important in management decisions for HIV-infected individuals. If weight loss is due to the metabolic effects of HIV and co-infections, nutritional supplementation must be accompanied by treatment of infections and the initiation of ART in the appropriate patient. ART is critical in restoring helper T-cell numbers and function, which offers a window for recovery and improves innate protection from further infectious insult. If the wasting is due to poor intake, however, and the patient has been screened and treated for opportunistic infections and tuberculosis, primary treatment may not include antiretroviral medications. PEM itself may decrease T-cell numbers; therefore, in certain patients low CD4 T-cell counts may be partly attributable to PEM, not solely HIV. Additionally ART can complicate medical nutritional issues such as refeeding syndrome, exacerbate anemia, and cause an immune reconstitution syndrome associated with tuberculosis [69] and other infections such as *Cryptococcus*, cytomegalovirus, JC virus, hepatitis B and C, and varicella.

In contrast, a case of malnutrition may be related to poor intake from lack of food access, where replacement feeding is indicated. In practice, patients in developing settings with CD4 counts that qualify them for ART are often stabilized with enteral supplements as the first treatment. In the time before ART initiation, repletion of some nutritional stores may lessen the likelihood of metabolic complications and promote anabolism. The reversal of the catabolic state can also result in improved immune function. According to the WHO, target caloric intakes should increase by 10 %, but should not be more than 15 % of total caloric intake to provide sufficient non-protein calories to avoid protein catabolism [68]. In cases of extremely low CD4 counts and malnutrition where mortality is high,

however, ART is usually indicated despite the potential risks of reconstitution syndromes and drug toxicity during simultaneous treatment for viremia and malnutrition.

Immunologic Effects of Nutrient Deficiencies

While clinicians may be concerned about a variety of effects of malnutrition, undernourished patients infected with HIV have a considerable immunologic burden. Helper T-cells, or CD4+ T lymphocytes, are a primary infective target for HIV, where the virus causes death. As numbers of CD4+ cells decline with natural progression of HIV, cell-mediated immunity is greatly affected. Given the central signaling role played by CD4+ T-helper cells, other immune mechanisms such as innate and humoral immunity may also be affected. This leads to a repetitive cycle of infection, inflammation, and increased metabolic demand.

In a patient with immunodeficiency related to decreases number and function of t-helper lymphocytes, malnutrition plays a synergistic role. Protein-energy malnutrition, or undernutrition (PEM) is the state of energy deficiency due to chronic insufficiency of macronutrients. PEM has long been recognized as a primary cause of immunodeficiency and notably has manifestations similar to those seen in progressive HIV infection. While PEM can be accompanied by other micro- and macronutrient deficiencies, PEM itself is a cause of T-cell dysregulation and may affect other immune processes (Table 31.1). Both the quantity and the function of T-cells are affected in PEM, and thymic maturation of nascent T-cells is decreased due to thymic atrophy [68]. T-cell dysfunction resulting from PEM coupled with decreased T-cell number and function from HIV is a potent combination. This synergy of immune deficiencies is likely to play a role in the early mortality seen at ART initiation for those with lower BMIs. In addition, both immune function and malnutrition seen in HIV and superinfections like measles may deprive the immune system of needed building blocks for appropriate reaction to new antigens and impair B-cell memory response to antigens such as vaccines [68].

Even in the setting of low CD4+ that might otherwise qualify a patient to receive ART, the catabolic state of malnutrition in HIV may first need to be reversed with direct nutritional therapy. Before starting ART, some hospitals in developing settings treat malnourished patients with micronutrient and macronutrient supplements such as porridge mixtures and F75, a food mixture designed specifically for the initial treatment phase of severe malnutrition in children [70, 71]. Delaying ART specifically allows for treatment of PEM and micronutrient deficiencies, permits diagnosis of refeeding syndrome in the severely malnourished patient, and ultimately promotes anabolism. Treatment of malnutrition can itself support lymphocyte production. Addressing metabolic reserve and aiding in the production of blood cells prior to ART initiation also helps to minimize medication-related adverse effects including reconstitution syndrome and anemia. Specifically diagnosed deficiencies of micronutrients should be treated appropriately. Common deficiencies in malnourished HIV-infected patients mirror those seen in malnourished non-HIV infected, such as fat-soluble vitamins A, C, D, E, K and B vitamins such as riboflavin, thiamine, and pyridoxine. Most anemias are best treated with transfusion in the acutely ill patient and with enteral iron or micronutrients if non-urgent. Medications for worms and malaria also play a role. Knowledge of the regional prevalence of nutritional deficiencies and treating these deficiencies, especially in the case of PEM, are both critical in optimizing therapy for HIV-infected patients.

Superinfections, HIV, and Malnutrition

One consequence of HIV infection is the destruction of CD4+ T lymphocytes, important as helper cells to conduct cell signaling in the normal immune response. Decimation of T-helper lymphocytes

by HIV causes increased susceptibility to fungal, parasitic, and other infections. Individual infections have specific and additive effects on nutritional status. In resource-limited settings, baseline malnutrition contributes to the development of infections like tuberculosis, pneumonia, and diarrhea and impairs recovery. Multiple infections are important to consider in the nutritional assessment of the patient infected with HIV.

Tuberculosis (TB)is a leading cause of death among HIV-infected people causing roughly 200,000 annual deaths among people living with HIV, most in Africa [43]. People who are infected with both HIV and TB are likely to be at a greater risk for malnutrition compared to those infected with HIV alone [72–74]. The percentage of men and women with a BMI less than 18.5 kg/m^2 was higher in those subjects co-infected with HIV and TB than individuals with only HIV infection, and individuals who were HIV-uninfected [73]. Co-infection is a common occurrence in developing settings.

Tuberculosis (TB) is a frequent co-infection with HIV and has significant effects itself upon nutritional status. Chronic, untreated TB infection causes weight loss, increased inflammation and organ-specific manifestations. Co-infections such as TB have significant systemic effects, thus nutritional status may only improve when these infections are properly diagnosed and treated. Screening is important, but not always comprehensive. Even in one US cohort, approximately 54 % of newly diagnosed individuals had a tuberculin skin test following HIV diagnosis [75]. There is a need for improved diagnosis of co-infections, and a need for further investigation to evaluate the possible outcomes of nutritional interventions for patients with co-infection of HIV and tuberculosis once diagnosed [73] Superinfections, like tuberculosis and others, may themselves be immunosuppressive, complicating diagnosis and treatment decisions. Disease manifestations are often apparent because symptoms are caused by the immune response, rather than by the pathogen itself. In the setting of low CD4+ T-lymphocyte counts, many infections go undiagnosed, as typical symptoms may not be apparent to a clinician. Symptoms of TB may be unmasked with treatment, however, causing clinical worsening of symptoms. Similarly, treatment of HIV virus with ART may be revealing for a variety of preexisting infections, including TB. In a patient with PEM, the outcome from this inflammatory condition, a reconstitution syndrome, can be disastrous as there is little reserve to support the proliferation of new cells. For this reason, a high degree of suspicion of infection and aggressive screening must be employed in the undernourished patient. This includes assessment of nutritional status and consideration of nutritional therapy when either ART or TB medications are initiated.

In addition to TB, other superinfections may have significant nutritional effects. Co-infections may cause bone marrow suppression, weight loss, malabsorption and diarrheal disease, and chronic inflammation. Bone marrow suppression due to viral infections, tuberculosis, and other infections may have nonspecific effects on immune response and their treatment can modestly improve HIV viremia [76, 77]. Co-infections may increase nutrient losses [24] and affect intake via nausea, vomiting, pain, and anorexia. Other superinfections include fungal and bacterial infections of the gastrointestinal tract. Mucositis, esophagitis, and gastroenteritis can cause pain in the mouth, chest, and abdomen; nausea and vomiting; bleeding; malabsorption; and may increase the frequency of other infections of the gastrointestinal tract after causing mucosal inflammation and damage. Protein-energy malnutrition in otherwise healthy individuals can make a patient susceptible to *Pneumocystitis jirovecii* (formerly

Table 31.1 Estimated risk and timing of mother-to-child transmission of HIV in the absence of interventions

Timing	Transmission rate (%)
During pregnancy	5–10
During labor and delivery	10–15
During breastfeeding	5–20
Overall without breastfeeding	15–25
Overall with breastfeeding to 6 months	20–35
Overall with breastfeeding to 18–24 months	30–45

P. carinii) pneumonia, a superinfection common in HIV [43]. This infection may manifest as coughing interrupting feeding and increased work of breathing requiring additional energy expenditure.

Parasites such as cryptosporidia and giardia may cause chronic diarrhea and malabsorption. Co-infections also affect host immunity and can result in higher viral loads. Levels of viremia correlate with HIV transmission risk, and treatment of various concurrent infections including viruses, mycobacteria, and parasites not only may improve nutritional status via decreases in inflammation, but also may decrease viral load via effects on immune cell production [76, 77]. Micronutrient deficiencies via effects on natural killer and T cells and B cells can exacerbate the immunodeficiency of HIV and increase risk of concomitant infections. To maximize nutritional status, interrupting the cycle of new infections is critical to prevent ongoing nutrient losses and to moderate metabolic demands.

Women, Children, and Breastfeeding

Women make up about half of people infected with HIV globally. In some resource-limited settings such as Africa, however, women are disproportionately affected by the epidemic. There are almost 12 million HIV-infected women in Africa in 2007, representing about 60 % of all adult infections in this region (Fig. 31.6). Not only do women suffer the metabolic effects of HIV infection and its treatment, but also there is an interaction between gender discrimination and HIV itself. Violence and discrimination in accessing education, property, prevention programs, and care and treatment exacerbates the risk of HIV/AIDS for women and girls [78, 79].

Women are the usual caretakers for HIV-infected children, who are disadvantaged by prevalent malnutrition, a large burden of infectious disease including diarrhea and pneumonia, and the lack of treatment resources especially in Africa. In 2007, an estimated 1500 children were infected with HIV daily, most of which occurred during pregnancy, birth, or breastfeeding [80]. By 2013, 3.2 million children were living with HIV worldwide, 240,000 of them infected during that year alone. Most infections occurred through pregnancy, birth, or breastfeeding. The effects on families and communities is enormous, with approximately 17.8 million children worldwide being orphaned due to AIDS in 2011, 15.7 million of whom were from Sub-Saharan Africa (aids orphans). However, progress has been made in the reduction of new infections among children. By 2012, the number of children newly infected with HIV was 35 % lower than in 2009 [4]. This was as a result of sustained interventions to provide ART to pregnant women living with HIV and programs to prevent mother to children transmission (PMTCT).

The impact of HIV on child growth, morbidity, and mortality is severe. HIV-infected children have a fourfold higher risk of death compared with those uninfected. However, preventable conditions including contaminated water supplies, child under nutrition, and anemia contributed significantly to infant and child mortality independent of HIV infection [78].

These conditions are present in many areas at baseline, even without HIV. However, with prevalent HIV and the lack of trained pediatric personnel and appropriate medications, the impact is stunning. In the absence of ART, HIV-infected children suffer significant stunting, under nutrition, and wasting compared to uninfected children. In a study conducted in Tanzania, maternal schooling, immunological status, and infant infections were important predictors of early growth in children born to HIV-positive women [81]. Prevention of new pediatric HIV infections through treatment of mothers, provision of medications to those children already infected, and support of children and orphans affected by HIV has progressed significantly in industrialized countries, but access to care and treatment is still lacking in developing countries, especially Africa [82]. Treatment has direct positive effect on child survival and growth. To improve early mortality outcomes, the WHO has adopted new recommendations advising the initiation of ART in children at diagnosis, rather than delaying ART

for the potential benefits of decreased resistance, delayed metabolic consequences, and burden to caretakers. These expanded recommendations will affect the nutritional status of infants infected with HIV worldwide and decreased the nutritional consequences of wasting in children [83]. The long-term nutritional impact of widespread pediatric treatment has yet to be studied.

One risk factor for pediatric HIV infection is exposure to virus in breast milk. The World Health Organization recommends early initiation of exclusive breastfeeding for all newborns; exclusive breastfeeding up to 6 months of age; safe complementary feeding from 6 months of age; good maternal nutrition; vitamin A supplementation; and counseling and support for optimizing nutrition; and infant and young children feeding With ART, mother-to-child transmission (MTCT) of HIV is approximately 5 % prenatally and up to 45 % after 2 years of breastfeeding (Table 31.1). The WHO recommends exclusive replacement feeding if it is acceptable, feasible, affordable, sustainable, and safe (AFASS). If not, then exclusive breastfeeding is recommended during the first months of the baby's life [71, 84]. Exclusive breastfeeding to 3 months or longer was associated with a significantly lower risk of infection (hazard ratio 0.52 [95 % CI 0.28–0.98]) and never having breastfed carried similar risk of infection to mixed feeding (0.85) [85, 86].

However, the risk of morbidity and mortality from replacement feeding in resource-limited settings is substantial. Contaminated water supplies, unsanitary preparation formula, and over dilution exposes children to morbidity and mortality from diarrhea, vomiting, and malnutrition. Successful replacement feeding has been found to depend on certain criteria, including availability of piped water, electricity, gas or paraffin for cooking fuel, and early disclosure of HIV status [87, 88]. Infants of South African mothers who met the three household criteria and chose to formula feed had the best outcomes in terms of HIV-free survival. The risk of formula feeding to infants in Sub-Saharan has been demonstrated. Thior et al. compared the efficacy and safety of breastfeeding plus infant zidovudine prophylaxis for 6 months to formula feeding from birth plus 1 month of infant zidovudine for reducing postnatal transmission of HIV [89]. Breastfeeding with zidovudine prophylaxis was not as effective as formula feeding in preventing postnatal transmission, but was associated with a lower mortality rate at 7 months. Both strategies had comparable HIV-free survival at 18 months. Other work is examining nevirapine prophylaxis for the breastfeeding infant.

The WHO also recommends that all HIV-infected mothers receive counseling on infant feeding including information on the general benefits of various infant feeding options and guidance on selecting the option best suited for their situation [84]. However, this recommendation is not always practical because there is very little communication with health-care providers as to how to translate this into practice [90, 91]. Also, HIV-infected women in many societies face challenges when making infant feeding choices due to stigma against formula feeding. Even when mothers are provided with infant feeding counseling to enable them to make an informed choice, the intent and the actual practice may be different [92]. Social expectations by family and the community may force a mother to breastfeed to avoid identification as a person infected with HIV. Social concerns, including reputation and stigma, traditional advice and authority, compete with the medical concerns and risks making it difficult for women to adhere to the feeding options be they exclusive breastfeeding or replacement feeding [93–95].

There may also be a relationship between mode of infant feeding and factors such as fear of HIV/AIDS stigma, male partner reactions, lack of disclosure to family members, and knowledge of prevention of mother-to-child transmission (PMTCT). Mothers who had hospital delivery, knew of their HIV-positive serostatus, and disclosed their HIV-positive status to family members were more likely to practice exclusive breast feeding [96]. However, Omwega et al. found that maternal MTCT knowledge influenced the chance of alternative infant feeding options but not breastfeeding practices [97]. Women with high MTCT knowledge tended to be more receptive and considered feeding alternatives. Clearly cultural influences in breastfeeding practices are strong and must continue to be considered when designing interventions to limit HIV incidence, but education may have a direct impact on infection rates.

Despite cultural and community pressures for breastfeeding, or perhaps because of them, mixed feeding with breast milk plus other foods is common in many places. Mixed feeding may be most dangerous as it exposes to HIV as well as numerous pathogens that can disrupt the protective gastrointestinal mucosal layer permitting easier access to the bloodstream and immune cells. Although exclusive breastfeeding is recommended [84, 94], the method of infant feeding counseling is vital to the success of preventing MTCT. While prevention programs for MTCT may contribute to mother's knowledge and understanding of feeding practices, there are many instances where the choice of exclusive breastfeeding is still being undermined by the impression that HIV transmission through breast milk is a certainty rather than a risk [94]. There are still many gaps in the evidence, particularly around the pathogenesis of HIV transmission through breastfeeding [4]. In addition to the risk of HIV, health workers sometimes have some difficulty convincing HIV-infected mothers that breast milk is sufficient as infant food [94]. Therefore accepting some risk through breastfeeding is difficult to justify. Adejuyigbe et al. reported that HIV-infected mothers in southwestern Nigeria counseled on infant feeding according to the WHO recommendations preferred exclusive breastfeeding and that mixed feeding was more common among mothers who planned to use exclusive replacement feeding [98]. In addition a study in Southwest Nigeria reported that despite women in the study having a high level of awareness of HIV/AIDS, and good knowledge about MTCT and PMTCT of HIV/AIDS, they still had poor attitude towards PMTCT [99]. Independent predictors of adherence to exclusive breastfeeding include knowledge of exclusive breastfeeding as a method of preventing mother-to-child transmission of HIV, getting support from the family, especially the male partner, and the socioeconomic status of the mother [4, 77, 100]. Indeed counseling on infant feeding may be more helpful for less educated mothers [77]. The choice to initiate at least some breastfeeding was more common among women with less education, who lived in homes without electricity, and who had no water source in their home or on their property [82]. Feeding counselors need to be sensitized to client issues and must be aware of the context in which clients make choices [94].

Nutritional counseling on breastfeeding is critical to promoting benefits of breast milk even in the context of HIV infection. Counselors need to be adequately trained and prepared for the task [84]. Stress and frustration may be prevalent among nurses who perform counseling [79]. However, breastfeeding counseling, even more than traditional nursing, requires time and fundamental knowledge of the sociocultural environments within which health and illness are addressed. The authors recommended that to improve counseling, pre-service and in-service training is fundamental. Furthermore, culturally appropriate counseling tools can be developed as a way to improve the standardization and routine of infant feeding counseling [84].

HIV-infected mothers in resource-limited settings need support to make realistic and feasible infant feeding choices. To promote and support optimal feeding practices, it is important to understand the barriers to exclusive breastfeeding and the reasons that mothers mix the feed. Promotion of exclusive breastfeeding for at least 6 months, followed by a nutritious complementary diet, and early weaning to an animal milk formula may be the most appropriate option for the poor in countries with high levels of MTCT [74]. Food-based dietary guidelines should complement and strengthen policies to prevent MTCT [86]. Infant feeding counseling should therefore be culturally appropriate and tailored to individual situations [75, 94]. To encourage women to adhere to good infant feeding practices, involvement of their partners, family members as well as the community for support should be encouraged [97, 100]. Methods to reduce transmission and optimal breastfeeding choices will continue to change as new and increased therapeutic options for babies and mothers emerge.

When available, HAART dramatically reduces mother-to-child transmission of HIV. One of the current approaches to reduce the risk of mother-to-child transmission of HIV is triple antiretroviral prophylaxis during pregnancy and breastfeeding [101]. Updated WHO guidelines on PMTCT recommend that mothers or babies receive antiretroviral prophylaxis during breastfeeding, if the mother is not already receiving antiretroviral treatment for her own health [102].

HAART should be delivered as part of a comprehensive strategy to prevent mother-to-child transmission of HIV. Some comprehensive and supportive strategies that have proven successful in the prevention of MTCT in resource-limited settings include routine antenatal voluntary counseling and testing; maternal HAART during the last trimester of pregnancy, at labor, and for up to 6 months following delivery with a goal of minimizing maternal viral load in plasma and breast milk; PCR testing of infants of seropositive mothers at 6 weeks of age; combinations of 3–6 months of exclusive breastfeeding; perinatal administration of ART; and provision of affordable and safe infant replacement feeds [87, 103].

However, there may be barriers that hindered the effective implementation of these interventions, including low acceptance of HIV testing because of fear of being stigmatized by spouse, family, or community, poor compliance with complex drug regimens, the high cost of antiretroviral drugs, inadequately resourced health-care systems, and unavailability or poor acceptance of safe breast milk alternatives [87]. The Drug Resource Enhancement against AIDS and Malnutrition (DREAM) initiativein Mozambique, Malawi, and Tanzania has achieved an overall reduction of HIV-1 transmission rates to levels very similar to those reported in high-income countries reflecting the effectiveness of a comprehensive approach. The program provides supplementary formula and water filters for use during the first 6 months after delivery and HAART [103]. Infectious diarrhea with or without diarrhea continues to be a major killer of children worldwide. Indeed, a large impact may be made upon HIV transmission by employing interventions that are outside of the normal treatment modalities for HIV itself.

Medical Treatment of HIV and Nutritional Effects

Antiretroviral Therapy

Medications aimed at interruption of HIV function and replication are collectively termed *antiretroviral therapy*. ART in current use aims to interrupt viral replication or activity by interfering with reverse transcription needed for turning RNA into stable DNA or by inhibiting an enzyme that cleaves viral pre-proteins in the cytosol into functional viral proteins. ART has multiple nutritional effects, some being specific to drugs or drug classes.

ART is itself a nutritional therapy, associated with an increase in weight [104, 105]. These changes may be greater for those who begin ART with more profound immunodeficiency at baseline [106]. Reversing inflammation and immunodeficiency is key in promoting anabolism and weight gain. Although nutritional status often improves with ART alone, diagnosis and treatment of co-infections and baseline nutritional status clearly play roles and must be addressed simultaneously for optimal outcomes.

Several notable nutritional effects have been observed particular to a medication or to a class of medications. Zidovudine (AZT) can suppress bone marrow causing anemia and may cause nausea and abdominal pain. Protease inhibitors are associated with dyslipidemias and insulin resistance [107]. This effect may be potentiated by ritonavir, which inhibits cytochrome P450 complex and prolongs the serum half-life of protease inhibitors. HIV itself is associated with redistribution of body fat stores and atrophy in a variety of patterns, termed lipodystrophy. Some patients have a pattern of lipoatrophy of peripheral fat accompanied by accumulation of central and visceral fat. These patients may be predisposed to metabolic abnormalities during treatment including dyslipidemia most often associated with the use of protease inhibitors [108]. Cardiovascular risk as measured by 10 year estimates of coronary heart disease were significantly higher for HIV-infected patients with lipodystrophy from the Framingham Study [109–113]. Several medications can even exacerbate renal or liver disease [114].

Side effects of medications may also interfere with intake, a major cause of wasting. Lactic acidosis, a potential side effect of nucleoside analogs, can cause gastrointestinal symptoms like nausea, vomiting, and right upper quadrant abdominal pain all leading to weight loss. Pancreatitis can occur with didanosine and stavudine, both nucleoside analogs. Nausea is a well-recognized effect of multiple protease inhibitors and has been associated with newer medication classes such as CCR5 and integrase inhibitors. Effective management of nausea and timing of meals may help minimize the effect of these symptoms on nutrient intake.

The WHO recommends that all adolescents and adults, including pregnant women, with HIV infection and CD4 counts of ≤350 cells/mm should start ART even if clinical symptoms are absent. Those with worse clinical status (WHO clinical stage 3 or 4) are recommended to start ART regardless of their CD4 cell count. First-line therapy consists of an NNRTI + two NRTIs, one of which should be zidovudine (AZT) or tenofovir (TDF). It is recommended that Clinicians progressively reduce the use of stavudine (d4T) in first-line regimens because of its toxic effects. The Second-line ART is a ritonavir-boosted protease inhibitor (PI) plus two NRTIs, one of which should be AZT or TDF, based on what was used in first-line therapy. Ritonavir-boosted atazanavir (ATV/r) or lopinavir/ritonavir (LPV/r) are preferred [115].

Interventions for Nutrient Deficiencies in Patients Infected with HIV

Nutrition support is an essential component of comprehensive HIV care and treatment programs, but is not always available. In a survey conducted in 128 sites in 41 countries including sites in the Asia-Pacific region, Latin America and the Caribbean, North America, Central Africa, East Africa, Southern Africa and West Africa, nutrition support was provided in 82 % of the sites [116].

Nutritional Counseling

Given that intake is a prime determinant of weight and nutritional status for people living with HIV, a comprehensive approach to improving intake includes nutritional counseling, treatment of HIV disease, treatment of co-infections, and increased food access where needed. Nutritional counseling is important to ensure clients have a well-balanced diet, and country-specific counseling materials have been developed as part of national HIV care programs [114]. The value of nutrition counseling was identified early and can improve nutritional status of HIV-infected individuals [117–120].

Nutrition counseling, with or without oral drink supplements, was shown to be a feasible intervention at low cost in HIV-infected patients with recent weight loss [121]. Fifty-three percent of South African HIV-infected patients who were counseled gained weight compared to 21 % of matched controls without counseling [122]. Weight loss occurred in 27 % of counseled and 43 % of controls. Patients with the lowest CD4 count had the best response to nutritional counseling. Nutritional counseling appeared to help prevent the adverse effects of GI tract or infection, especially in patients with CD4 counts less than 200 cells/μL. The authors suggested that in areas with little resources nutrition education and dietary counseling are a simple yet effective means of stabilizing or increasing body weight in HIV-infected patients. Intake is a prime determinant of weight loss and gain. Counseling may improve intake in developing area as well, thus food availability is not the only determinant of intake. This has obvious implications for the design of macronutrient supplementation programs on a large scale.

Nutritional counseling by itself may not always improve the nutritional status of persons infected with HIV. Without ART, body weight, serum cholesterol levels, and CD4 counts may decrease despite dietary counseling and continued maintenance of energy intake for 6 months [123]. Oral nutrition

supplementation has been shown to be more effective in improving weight gain compared to nutrition counseling alone [124]. Because weight loss may progress despite dietary counseling, those who fail counseling should be identified early. Interventions should be considered to increase energy intake by both counseling and supplementation when indicated [123].

Micronutrients

Several nutrition studies in the context of HIV have focused on the effects of micronutrient supplementation. Results generally have been mixed. A review was performed on 30 trials involving 22,120 participants. Twenty trials evaluated the effects of single supplements such as vitamin A, vitamin D, zinc, and selenium, and ten trials evaluated of multiple micronutrients. Multiple micronutrient supplements were associated with reduction in morbidity and mortality in HIV-infected pregnant women and their offspring. Children born to mothers taking multiple micronutrients during pregnancy had improved early child growth in one large randomized controlled trial in Africa. There is need for additional research to determine if these findings apply in other settings. Vitamin A supplementation has been found to be beneficial and safe in HIV-infected children, but it is not known if it offers similar benefits to adults. Zinc is safe in HIV-infected adults and children and has the potential to be protective in the control of diarrhea in HIV-infected children as it does in HIV uninfected children [125, 126].

There is more evidence in children to support certain micronutrient supplementation. Periodic vitamin A supplementation in children reduced all-cause mortality and improved growth in one trial and reduced diarrhea-associated morbidity in another [127]. Whether this effect is mediated through impact on HIV or is a reflection of improvements in immunity via another mechanism is unknown. Villamor et al. found that vitamin A supplementation given every 4 months to children less than 5 years of age improved growth in infants infected with HIV and malaria and decreased risk of stunting from diarrhea [128]. A large positive effect on linear growth was evident among infants with HIV infection, while weight gain was favored among children with malaria infection at baseline. Vitamin A could constitute a low-cost, effective intervention to decrease growth retardation in settings where infectious diseases are highly prevalent and antiviral therapies are not readily available. In both trials population sizes were small.

A randomized clinical trial with 878 HIV-positive patients in Botswana of supplementation with either daily multivitamins, selenium alone, or multivitamins with selenium vs. placebo for 24 months found that patients that received multivitamins supplement plus selenium compared to placebo had a significantly lower risk of reaching CD4 cell count 250/μL or less. Multivitamins plus selenium compared to placebo also reduced morbidity. There was no effect of supplementation on HIV viral load [129].

Higher dietary intakes of vitamin A and D were positively correlated with CD4 count [130]. However Wiysonge in a Cochran review of five randomized studies conclude that regardless of outcomes of micronutrient supplementation at the community level, access to ART remains the prime intervention to restore immune function, improve nutritional status, and decrease morbidities and mortality for children infected with HIV.

Wiysonge et al. assessed the effect of antenatal vitamin A supplementation on the risk of MTCT of HIV as well as infant and maternal mortality and morbidity [131]. The authors found that evidence does not support the use of vitamin A supplementation of HIV-infected pregnant women to reduce mother-to-child transmission of HIV. They also noted that currently available data do not exclude the possibility that supplementation could be beneficial or harmful; there was need for an appropriately powered randomized controlled trial to assess the additive effect of the intervention on the risk of MTCT of HIV in antiretroviral-treated women.

While there are some data concerning the supplementation with vitamin A, many micronutrients have yet to be evaluated in the same fashion. Individual patients with severe malnutrition and specific deficiencies should be treated appropriately. Complicated interactions exist between infection and nutritional status making general recommendations of supplementation for all HIV-infected patients challenging. For the best clinical care, more data are needed from the most common clinical settings, namely developing countries.

Macronutrients

Macronutrient support has been shown to support HIV treatment adherence [21, 22] and have been designed as part and parcel of the HIV comprehensive care programs [132]. Given that intake plays a central role in weight loss, and intake correlates with CD4 counts [133] increasing intake would seem to be an appropriate treatment. In a Cochrane review of 14 trials, Grobler et al. [134] found that no firm conclusions could be drawn about the effects of macronutrient supplementation on morbidity and mortality in people living with HIV. The role of increasing calorie and protein intake in all HIV-infected patients is controversial, however, as supplementation may only increase weight but not fat-free mass. Treatment of muscle wasting is a goal of nutritional therapy in HIV because regaining fat-free mass is a surrogate for overcoming increased protein turnover and improving the capability to perform physical work. Supplements for those with nutritional depletion, additional calories, and protein are indicated and recommended [135–137].

Several studies have been conducted in developed countries evaluating the effects of macronutrient supplementation with and without nutritional counseling. The results of these few studies have been mixed, with some studies indicating positive change in body weight, macronutrient intake, and immunological factors. Other studies do not support these findings. In a Cochrane meta-analysis of 14 small randomized controlled trials, evaluating different macronutrient supplements in different populations at different stages of HIV infection and with varying treatment status found that no firm conclusions could be drawn about the effects of macronutrient supplementation on morbidity and mortality in people living with HIV.

A systematic review that evaluate 14 small studies on the effectiveness of various macronutrient interventions, given orally, in reducing morbidity and mortality in adults and children living with HIV infection concluded that no firm conclusions could be drawn about the effects of macronutrient supplementation on morbidity and mortality in people living with HIV [134]. The type of nutritional supplement also matters. Energy dense ready-to-use fortified spreads were more resulted in a greater increase in BMI and lean body mass than feeding with corn-soy blend [138]. Oral nutrition supplementation with a whey protein supplement did not increase weight or lean body mass in HIV-positive subjects who were eating adequately, but it did increase CD4 cell counts [139, 140]. Oral nutritional supplements for a 3 month period were well tolerated and resulted in body weight gain in HIV-infected patients. Supplement-enriched formula, with peptides and *n*-3 fatty acids, increased CD4 count [123, 141]. Body weight was also maintained or increased with a high energy, high protein nutrition supplement in conjunction with nutrition counseling among patients with HIV in the early stages of HIV without secondary infections. However, most patients who developed a secondary infection lost weight despite nutrition supplementation and counseling [142]. Six months of oral supplementation with arginine and *n*-3 fatty acids did not change CD4 and CD8 lymphocyte counts or total energy intake. However, there was an increase in body weight and fat mass [143]. Normal intake amounts may have been supplanted by the supplement itself, resulting in no change in net intake. Two randomized, double-blind controlled trials examined the effects of glutamine on AIDS-related wasting. In one trial, the subjects received glutamine with select antioxidant over a 12-week period, while the other trial prescribed glutamine, beta-hydroxymethylbutyrate, and arginine for 8

weeks. Both trials found an increase in body mass in their respective trial groups when compared to those subjects on the placebo [144, 145]. In yet another study L-glutamine 30 g/day significantly ($p < .01$) reduced the severity of nelfinavir-associated diarrhea and produced improved quality of life compared with placebo this population of HIV-positive participants [146]. While supplementation may address decreased intake, the addition of calories may itself increase energy expenditure and protein turnover in the fed state and may have implications for the type of macronutrient supplement and for patient selection [147, 148]. This hypermetabolic effect may be mediated by antiretroviral treatment with protease inhibitors [149]. Confounders in all of these studies include baseline nutritional status, co-infections, control of viremia, and adherence to medical therapy for which each was only sometimes controlled in analysis.

Despite mixed results in studies from developed settings, the WHO recommends a 10 % increase in energy intake for asymptomatic results, a 30 % increase in symptomatic adults, and up to 100 % increase in symptomatic children [68]. The WHO does not offer recommendations on protein intake, rather general recommendations about dietary diversity [150]. More research is required to understand the effects of macronutrient supplementation on HIV and AIDS.

Programs designed to treat malnutrition have considered the use of modified nutrient-rich foods or food combinations to address specific nutritional needs. Table salt with iodine is one important example. Whole foods with supplemental vitamins such as A, E, as well as iron have been bred or engineered into common staples such as rice sorghum, millet, or cassava [151–153]. There are other groups using unmodified but nonnative foods. Recently there have been efforts to introduce such foods to communities to whom the foods may be foreign [154]. Acceptance of such foods requires experience of taste and knowledge of preparation and the ability to instruct patients on their use. These are tasks that may not easily fall under the rubric of publicly funded HIV treatment programs. However, increasing exposure to new nutritious foods may become easier as farming techniques change over time, and seeds and growing techniques for new crops become more available with support of governments, organizations, and increased global trade.

Given increased protein turnover in HIV [63, 64, 155, 156] accessing sufficient animal protein in diets dominated by carbohydrates can be a challenge. In addition, geographic access to protein sources may be limited by terrain, climate, violence, and disease itself. Certain communities and food scientists are working on new ways to access animal protein, by growing and using local dried fish, rabbits, or other animal source proteins [127, 157]. Natural plants and insects can be used when food access is limited or as a cultural practice [158]. Coping mechanisms for food insecurity overlap with the search for optimal methods to ensure adequate intake in HIV. New methods and foods will be needed to address both issues.

The need for food-as-medicine programs in food insecure and resource-limited settings is urgent. Several governments, international agencies, and community groups have responded in part to this need by establishing food and macronutrient supplementation programs in Africa and elsewhere. These programs are increasingly present in the context of HIV care and treatment programs. Existing programs range from prevention of malnutrition to counseling and palliative nutritional care for HIV-infected individuals [132, 159, 160]. In the context of HIV treatment programs, the aim of supplementation must be considered. Not only are metabolic demands increased in the HIV-infected patient, but also in the developing settings decreased intake from HIV disease and co-infections is exacerbated by food insecurity. Large-scale efforts to address food security may be helpful and ultimately needed, but targeting the patients for nutritional therapy via individual or family support may be a manageable goal for HIV treatment programs operating in resource-limited settings. While there may be a clear indication for treatment of malnutrition for an individual patient, and while the wide scale integration of nutritional support may be viewed by many as good policy, data are only slowly increasing the evidence-base to support benefit in clinical setting [161]. Clearly additional evidence is needed to support the compulsory adoption of supplementation programs by ministries of health and to provide the best nutritional therapies for patients.

Multiple questions exist concerning how best to treat patients infected with HIV. Questions remain as to the optimal choice of macronutrients, how much supplement to provide based on resting energy expenditure or other parameter, targeting the supplement to the patient himself or the whole family, duration of therapy for maximal and lasting effect, and lastly, the timing of the intervention in the course of infection. As a result, work to date has been heterogeneous in approach.

A group in Malawi examined patients who received an individual ration of corn-soy blend and vegetable oil, with primary household earner also receiving maize and beans [162]. There were no differences in weight gain or CD4 counts at 6 or 12 months, though there was a significant increase medication possession. Patel et al. examined the rate of weight gain and percentage of children reaching weight-for-height greater than 90 % ideal who received RUTF or CSB, finding that patients with less than 85 % weight-for-height but greater than 80 % of the international standard, and who consumed RUTF, had improved growth [163]. A Cochrane review of eight randomized controlled trials in low- and middle-income countries concluded that both lipid-based nutrient supplements and blended foods are effective in treating children with moderate acute malnutrition [164]. Another Cochrane review that included 32 studies, assessed the effectiveness of supplementary feeding interventions, for improving the physical and psychosocial health of disadvantaged children aged 3 months to 5 years concluded that supplementary food was generally more effective for younger children (less than 2 years of age) and for those who were poorer/less well-nourished [165].

Community- and home-based therapies are increasingly employed to improve outcomes for children and to alleviate burden on health-care facilities. Caretakers may then stay near the patient, in order to provide more accessible care, instead of leaving a patient at a health facility while the caretaker works or cares for others in the household. This home-based strategy has been used in HIV care to address clinical nutrition problems. Ninety-three HIV-infected children from Malawi with wasting were stabilized in the hospital and then allocated to full calories via RUTF, supplemental calories via RUTF, or full calories via maize soy flour. Seventy-five percent of children receiving full calories of RUTF reached 100 % of weight-for-height, while 53 % of those receiving maize/soy flour reached the same goal. Lower initial weight-for-height *Z* score was associated with a poor outcome [163]. One hundred and six HIV-infected children who received a 4-week supply of amino acid-based formula had higher hemoglobin and weight compared to a separate group who received soy/skim-milk preparation. Weight was significantly greater in the group receiving amino acid-based compared to control. Mortality, diarrhea frequency, and recovery scores did not differ between groups. It was unclear from this study if the groups received the same calories, and analysis by HIV serostatus was incomplete ([166]. Food supplementation did not improve survival but effects on nutritional status trended to significance in a descriptive longitudinal study in Malawi examining the introduction of food supplementation to HIV-patients in various stages of disease [167]. Communities are often resourceful when faced with issues such as food insecurity. One type of local support for HIV-infected individuals are community-based programs that provide a variety of services including food supplementation, dietary advice, counseling, and palliative care [132, 152]. These approaches will likely be important in future interventions to address malnutrition.

Another type of support is via HIV programs such as multiple PEPFAR-supported programs that function within the governance of national ministries of health throughout the world. Certain programs like the World Food Programme, the US Agency for International Development, United Nations Children's Fund, and Food and Nutrition Technical Assistance project, among others, have coordinated to offer some nutritional supplementation via HIV care and treatment. A more unique example of nutritional support within the context of HIV care is the Academic Model for Prevention and Treatment of HIV/AIDS (AMPATH) based in western Kenya. Patients are recruited for supplementation based on criteria including food security, socioeconomic status, and anthropometrics, in the same setting in which HIV care is provided. Despite questions of sustainability AMPATH administers its own agriculture activities growing food and purchasing milk and other items for program participants [152].

There is a small but growing literature concerning macronutrient supplementation for HIV-infected patients. This includes alternative means of nutrient delivery, such as ready-to-use therapeutic foods (RUTF), fortified blend foods (FBF), and fortified spreadable paste. RUTF is most commonly a peanut (groundnut)-based paste (or butter), vegetable oil, fortified with dried cow's milk, micronutrients, and sugar for taste. Its most common application is in home-based treatment of childhood malnutrition (community therapeutic feeding), as children accept the taste, dosage can be titrated to weight, and this form of RUTF does not require a cold chain and resist bacterial growth. Some observational data from Malawi and other locations have examined use of RUTF for children in program settings [164, 168]. The most beneficial intervention for patients infected with HIV is still undetermined.

Adding whey and skimmed milk powder in FBF is used to improve protein quality in vulnerable patients has renewed interest in HIV, including in the setting of diarrhea, a major cause of nutrient loss in developing settings [169, 170]. Fortified blended foods are an evolution of common supplementation methods used by the World Food Programme, where culturally acceptable maize- and rice-based products are mixed with other calorie, protein, and micronutrient sources to achieve a more balanced macronutrient supplement. FBF aims to treat deficiencies and to maintain normal physiology in food insecure areas; however, it is not available everywhere. The use of combinations of RUTF and FBF as part of medical treatment in conjunction with pre-ART counseling and with ART is emerging.

As new food technologies and products emerge, evaluations of each in the setting of HIV treatment will be required. Adjunctive fortification therapies for protein and fat are now being postulated for use in developed settings. Re-evaluation of the best measures to determine the success of interventions is also needed. A range of anthropometric and clinical measurements may be needed to identify patients that might benefit from interventions, in addition to BMI. While BMI is a surrogate physiologic homeostasis including the immunologic ability to handle infection and inflammation, and absolute cutoff for BMI that indicates clinical risk for an individual patient is not clear. A cutoff of 18.5 is somewhat arbitrary and in fact is not the same weight-for-height percentile in each sex. These rough tools need refinement. Indeed, the clinical profile of the patient most likely to benefit from supplementation remains unanswered. Multiple strategies and new ideas will be required to identify appropriate patients to address nutrient deficiencies in HIV, and to optimize combinations of macronutrient that improve clinical outcomes for all patients infected with HIV.

Other Therapies

To promote intake and anabolism, other therapies have been examined. Prolonged combination therapy with either the appetite-stimulant megestrol acetate, the anabolic steroid oxandrolone, or dietary advice may reverse weight loss associated with HIV and improve physical health [171, 172]. Testosterone, anabolic steroids, and human growth hormones can help increase lean body mass in individual with HIV/AIDS [173]. Additional work investigating mechanisms of wasting in HIV infection has recently involved the cytokine inhibition (thalidomide) and adipokines such as leptin in the pathway of satiety [174]. More work is needed to understand the role of medical and nutritional treatment in various stages of HIV disease.

Conclusions and Recommendations

HIV-infected patients should begin nutritional care at the diagnosis of the disease to assess baseline nutritional status and provide dietary counseling [175].

In considering food-as-medicine in resource-limited settings, there is need for more comprehensive programs no simply to address individual deficiencies when diagnosed but also to address

malnutrition and food insecurity in communities. Answers will involve more than medical personnel. Given the importance of nutritional status of communities for health and productivity, it is surprising that there has been little specific research on the use of macronutrients in HIV, particularly in resource-limited settings. This is clearly the future application of knowledge in HIV, whether treating malnutrition or understanding metabolic changes during ART treatment. Basic questions about macronutrient supplementation remain including What are the nutritional characteristics of patients who would most benefit? What type(s) of foods are optimal? How long should patients receive support? and When in the course of HIV disease or treatment is support most beneficial? This will require substantial mental and economic capital and require training of researchers and the integration of research into implementation programs. While there are substantial challenges to performing clinical research in general in resource-poor settings with nutritional research in particular, findings from such could provide information for new guidelines in the management of individual patients and for national policy. Perhaps because of the difficulties and insufficient data, nutritional interventions and specific guidance are not recognized as a component of national HIV treatment guidelines until recently [175, 176]. Providing supplemental food for HIV-infected patients remains expensive and logistically difficult and may contribute to lack of evidence and macronutrient policy [177]. Even specific micronutrients like vitamin A, which is more widely researched, have weak evidence to support their use in guidelines [178–181].

Addressing stigma through education and community involvement is likely to improve access to all types of HIV services, including nutrition. As noted above, lack of information seems to be a common barrier of access to treatment, either with nutritional supplements or with medication [182]. A review article examining the barriers of access to antiretroviral treatments (ART) in developing countries [183] found that the most commonly cited barrier was lack of information. This study also found that lack of information took the form of a stigma associated with HIV/AIDS, which ultimately discouraged people from seeking ART. Education and reduction of stigma will continue to be important in the success of nutrition programs from breastfeeding counseling to supplementation campaigns.

Communities should also be involved when making policies aimed at improving participation in programs. Investigators may be able to involve communities more directly in the design and implementation of research and in policy development [184, 185]. There are benefits to HIV-infected individuals being involved in policy developments and may improve outcomes. The resource of the community should not be overlooked in addressing the dual epidemics of HIV and malnutrition.

A comprehensive approach to managing HIV and AIDS is needed for success of programs targeting prevention, care, and treatment [186–188]. This access was publicly funded to all those in need and provided access to 397 accredited hospitals, 79 day-care hospitals, 58 home-care centers and 422 outpatient facilities, 82 lymphocyte phenotyping, 71 viral load laboratories, 18 genotyping centers, and access to 17 drug pharmaceutical compounds for diagnosis, prevention, and treatment. These policies demonstrate how a developing country can develop systems of equity, independent of factors such as race, gender, or financial means which will likely be of increasing importance in future program design [189].

In the USA, the CARE Act provides for a comprehensive care model in HIV care and treatment, ensuring access for vulnerable populations [190, 191]. This is echoed in developing settings where advocacy for comprehensive care models continues to gain momentum. The expansion of HIV care and treatment programs has engendered new discussion about development and strengthening of primary health-care systems worldwide [192–194] and as nutrition is critical to health status of communities, addressing nutrition via these systems will continue to be possible and necessary.

Nutritional consequences of HIV infection can be significant. While some advances have been made in understanding nutrition in the context of this chronic infection and its treatment, our knowledge is nascent and will continue to evolve as more attention is given to optimizing medical therapies and improving clinical outcomes for those affected by this global epidemic.

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Chapter 32
Folic Acid During Pregnancy May Reduce the Risk of Certain Types of Severe Congenital Heart Defects: Time for Action?

Andrew E. Czeizel

Key Points

- Structural birth defects, i.e., congenital abnormalities represent a special category of disorders due to the very early (prenatal) onset and the limited opportunity for complete recovery.
- Congenital heart defects are the most common and one of the most severe congenital abnormalities.
- The possible role of environmental factors in the origin of congenital hear defects is unclear.
- Previous Hungarian intervention trials showed that periconceptional folic acid-containing multivitamin supplementation significantly reduced the occurrence of neural-tube defects (NTD). In these intervention trials, we also noted that supplementation was also associated with a significantly reduced risk of congenital heart defects, although these outcomes were not the primary indication examined in our studies. These associations were also noted in some observational studies in the USA (multivitamins) and in a separate study in China (folic acid).
- The objective of the recent observational population-based Hungarian case–control study was to estimate the possible preventive effect of medically documented and recorded folic acid supplementation taken during pregnancy. Specifically we examined the association between supplementation during the critical period when it is known that certain congenital heart defects will form in the embryo/fetus. Diagnosis of the congenital heart defects were confirmed either at autopsy, or following surgical intervention with either catheterization or operation.
- There was a significant reduction in the birth prevalence rate of cases with ventricular septal defect (OR with 95 % CI: 0.57, 0.45–0.73), Tetralogy of Fallot (TOF) (0.53, 0.17–0.94), D-transposition of great arteries (0.47, 0.26–0.86), and atrial septal defect secundum (0.63, 0.40–0.98) in the infants born to mothers who had taken higher doses of folic acid during the critical period when the congenital heart defects would have formed.
- Our data suggest that about 40 % of major congenital heart defects may be preventable with folic acid supplementation if appropriate doses are used at appropriate times during pregnancy.
- In conclusion, the preliminary data strongly suggest that the risk of the development of certain types of congenital heart defects may be significantly reduced in pregnant women who are supplemented with higher doses of folic acid. Thus, the documentation of congenital heart defects should be considered as a secondary assessment in future neural-tube defect preventive programs.

Keywords Congenital abnormalities • Congenital heart defect • Ventricular septal defect • Atrial septal defect secundum • D-Transposition of great arteries • Tetralogy of Fallot • Folic acid • Population-based case–control study

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_32

Introduction

Over the past 15 years, we have included chapters in the each of the four Editions of Preventive Nutrition [1–4] that reviewed and updated the results from our seminal intervention trials proving that periconceptional multivitamin supplementation that included 800 μg of folic acid significantly reduced the risk of NTD [5–10]. We have also reported on results from our case–control study that has followed pregnant women who were given high doses (3–9 mg with the mean of 5.6 mg) of folic acid during pregnancy and have seen similar degree of NTD reduction as well as reductions in some other classes of congenital abnormalities (CAs) [11].

The objective of this chapter is to present the main results of a recent population-based, case-matched control study of congenital heart defects (CHD) to estimate the possible preventive effect of folic acid supplementation for these CAs. There have been a number of methodological weaknesses in previous epidemiological studies that examined the possible etiological factors in the origin of CHD and possible preventive effect of folic acid for CHD. We will review the classification of CAs including CHD and the methodologies used in our study to document the use of folic acid as well as the determination of the cardiac defects.

Our study has attempted to avoid the methodological problems of previous epidemiological studies by undertaking the following protocols:

1. Different CHD-entities with different etiopathogenesis were evaluated separately.
2. Only isolated CHD-entities were included in the study, i.e., syndromic cases and unclassified multiple cases including CHD as component CA were excluded.
3. Only isolated CHD-entities with validated diagnosis (either autopsy or surgical intervention: catheter and operation) were evaluated.
4. Finally only prospectively and medically recorded folic acid use in the prenatal maternity logbooks were analyzed in order to limit maternal recall bias.
5. Folic acid use (exposure) was evaluated mainly in the critical period of different CHD-entities/groups.

General Evaluation of Birth Defects/Congenital Anomalies

The expert groups of the World Health Organization (WHO) suggested the term congenital anomalies for the special category of disorders includes “morphologic, functional and/or biochemical-molecular defects that develop from the onset of organogenesis until birth present at birth, are present at birth detected at that time or not” [12]. This definition is used for the same category of disorders in the USA but with a different name, birth defects. Birth defects/congenital anomalies have two main characteristic: very early (prenatal) onset and limited opportunity for complete recovery after medical intervention. Thus the prevention of such birth defects is the only optimal medical-social choice if the child is to be born and/or live without the defects.

Several categories can be differentiated/classified within birth defects/congenital anomalies [13] (Table 32.1).

This review focuses on congenital abnormalities (CAs) with the following definition: morphological/structural defects of an organ, part of an organ, or a larger region of the body [12]. Previously an expert group suggested the following subclassification of CAs [14]:

1. Malformation: resulting from an intrinsically abnormal developmental process (e.g., cleft lip)
2. Disruption: resulting from the extrinsic breakdown of, or an interference with, an originally normal developmental process (e.g., amniogenic limb deficiency)
3. Deformation: caused by mechanical forces (e.g., clubfoot)

Table 32.1 Categories of birth defects/congenital anomalies

Genetic disease with early manifestation (e.g., hypothyroidism)
Congenital abnormalities (i.e., structural birth defects)
Congenital tumors (e.g., teratomas)
Idiopathic intrauterine growth retardation (i.e., newborns with small for gestational age)
Fetal diseases (e.g., fetal varicella disease)
Immunological disease (e.g., Rh maternal-fetus incompatibility)
Mental retardation
Behavioral deviation

Table 32.2 Approaches to evaluate CA

Occurrence	Public health importance
Severity	Clinical practice
Phenotypic manifestation	Help for diagnosis
Etiology	Prevention

Table 32.3 CA evaluation based upon occurrence rates

Magnitude	Rate per 1000 births	No. of CA-groups[a]
Common	1‰ or more	10
Medium	0.1–1.0‰	32
Rare	Less than 0.1‰	~200

[a]The number of CA-entities in Hungary

Table 32.4 Evaluation of CAs based upon severity

Degree	Total prevalence per 1000[a]
Lethal = CA causes stillbirth or infant	6.15
Death in more than 50 % of cases	
Severe = without medical intervention CA	19.30
Causes handicap or death	
Lethal + severe = major	
Mild = CA requires medical intervention	39.87
But life expectancy is good	
Total	65.32

Minor anomalies or morphological variants without serious medical consequences are not CAs [51, 52]

[a]In Hungary between 1980 and 1996

4. Dysplasia: caused by an abnormal organization of cells into tissue(s): dyshistiogenesis (e.g., osteogenesis imperfecta), but this reasonable recommendation was not followed by the international scientific community.

There are many approaches for the evaluation of CAs [13–16] (Table 32.2).

If we evaluate CAs according to their occurrence (e.g., prevalence at birth) the following three are differentiated (Table 32.3).

From clinical and public health aspects, the estimation of the severity of different CAs is important (Table 32.4). The total prevalence means the number of cases affected with CA in live-born newborn infants, stillborn fetuses and electively terminated malformed fetuses after prenatal diagnosis; the total of informative offspring. At present we cannot evaluate fetal defects in miscarriages.

Table 32.5 Phenotypic manifestation of CAs

Isolated	Multiple (a concurrence of two or more different component CAs in the same person)
Single	CA-syndrome
E.g., ventricular septal defect	E.g., Down
Complex	CA-association
E.g., Fallot tetrad	E.g., VACTERL
Sequence	Random combination
E.g., spina bifida + hydrocephalus + clubfoot	E.g., cleft lip + hypospadias

Unfortunately the etiology of some CAs is not known but their phenotypic evaluation can help the estimation of diagnosis [13, 15] (Table 32.5) because most isolated CAs with common and medium prevalence have multifactorial origin [17] while most multiple CAs are caused by chromosomal and gene mutations [15, 13], and in addition may be caused by teratogens [18].

It is expected that classification will be based on the etiology of CA in the near future

Genetic

Gene mutations (monolocal-monogenic Mendelian inheritance), e.g., Holt–Oram
Chromosomal aberrations, e.g., Down syndrome
Environmental

Teratogens, e.g., rubella virus
Maternal, e.g., hyperthermia
Gene–environmental interaction (multifactorial origin), e.g., NTD or most CHD

Finally it is worth mentioning the different level of prevention of CAs [19]:

Primary

(a)Avoiding the causes of CAs, e.g., rubella vaccination
(b)Inhibition of the pathogenesis of CAs, e.g., NTD by periconceptional folic acid/multivitamin supplementation

Secondary

(a)Early postnatal detection and medical treatment, e.g., neonatal screening of congenital dysplasia of hip or drug treatment of undescended testis and persistent ductus arteriosus
(b)Diagnosis of fetal defects followed by elective termination of pregnancy, however, recently international organizations, including WHO, excluded this approach from the term of prevention, because it is not prevention, it is avoidance of the birth of affected fetuses.

Tertiary

(a)No residual effect after early pediatric surgery, e.g., congenital.
(b)Hypertrophic pyloric stenosis or certain types of ventricular septal defects (VSDs).

Based upon the above review, it is clear that primary prevention is the optimal medical intervention for CAs. Many CAs can be prevented, thus CAs should not be considered an irreducible component of infant mortality and late disability. However, there is no single strategy of prevention because CAs vary so greatly in cause and course.

Congenital Heart Defects

CHD are the most prevalent and serious structural birth defects, i.e., congenital abnormalities (CAs). Among 1000 live births, 4–50 are affected by CHD [20], and these defects have profound medical, psychosocial, and economic consequences. The range of affected births is great due to the fact that in different countries the birth prevalence rate depends on the age at examination, the sensitivity of the examination technique, the case definition, and the types of CHD included in the studies [20, 21]. The pediatric cardiologic examination and/or the evaluation of the autopsy report of each individual child resulted in 10.2 ± 2.1 per 1000 live birth prevalence of CHD in a Hungarian population-based study [22].

There has been significant progress in the care of infants/children with CHD during the last decades [23]; nevertheless CHD are responsible for a significant percentage of childhood mortality [24]. For example, in Hungary between 1970 and 1981 the average number of annual live births was 166,064 and the total years of lost life per 10^4 live births was 1839 due to CHD while the total years of potentially impaired life per 10^4 live births was 3722 due to CHD [25]. About one-third of infant mortality was caused by CHD in Hungary during the 2000s. The cumulative cost of CHD in dollars or euros is in the hundreds of millions annually [26]. Recent progress in human genetics has helped to understand better the genetic background of CHD [27, 28]; however, the role of possible environmental factors in the origin of most CHD is unclear [29].

The Critical Evaluation of Previous Epidemiological Studies

1. CHD have heterogeneous manifestations and origins [30]. Nevertheless different CHD-entities/groups were evaluated together in many previous studies.
2. The diagnosis of CHD needs time and great expertise nevertheless the first clinical diagnosis without confirmation later or unspecified CHD cases were used for evaluation in some of the earlier studies [18].
3. The spectrum of some CHD is very wide including spontaneous closure of ventricular or atrial septal defects (ASDs), ductus arteriosus, etc., but the different manifestations of CHD-entities were not evaluated separately.
4. The so-called time factor is very important because we have evidence that the exposure to environmental agents, e.g., certain prescription drugs, can induce CA only during the organogenesis of the given organ or part of the body [31]. Previously this critical period of CAs was considered to be during the first 3 months of gestation, i.e., in the first trimester. However, we calculate *gestational time/age* from the first day of the last menstrual period therefore the first month of gestation is before the organogenesis. The first 2 weeks are before conception while the third and fourth weeks comprise the pre- and implantation period of zygotes and blastocysts including omnipotent stem cells. Thus CAs cannot be induced by teratogenic agents such as drugs with relatively short half-lives that are taken for a short period of time in the first month of gestation. This timing helps to explain the "all-or-nothing effect" rule, i.e., total loss or normal further development of zygotes/blastocysts. Thus the first trimester concept is misleading and unscientific [32, 33], except at the evaluation of exposures to a drug with a long half-life such as isotretinoin, or drugs used for chronic maternal diseases with the onset of use before conception and continuing during pregnancy. The critical period of most major CAs is in the second and third gestational months; however, human teratogens may induce CAs such as cleft palate, hypospadias, undescended testis, and many CHD-entities after the third gestational month. Thus we recommend the evaluation of exposures during the critical periods of different specified CAs separately [34].
5. In general the exposure data were based on retrospective maternal information in previous studies with significant recall bias mainly in the mothers of controls [35, 36].

There are two main sources of exposure data regarding medication: maternal self-reported information and medical records. In addition, it is important to differentiate the time of data collection: retrospective (i.e., after the birth) and prospective (before the birth). However, retrospective maternal information is burdened by recall bias because the birth of an infant with birth defects is a serious traumatic event for most mothers who therefore try to find a causal explanation, such as drug use during pregnancy, for birth defects seen in their offspring. This type of bias does not usually occur after the birth of a healthy newborn infant. Thus recall bias might inflate an increased risk of birth defects for certain drugs. Using the data of the Hungarian Case–Control Surveillance of Congenital Abnormalities (HCCSCA), we were able to estimate the recall bias of drug exposures during pregnancy on the basis of comparison of prospective medically recorded drug use with retrospective maternal information and recall bias was increased in the mothers of offspring with CAs by a factor of 1.9 in the OR [36].

Avoidance of Previous Methodological Weaknesses in Our Study Protocol

We followed the classification of CHD used in The Baltimore-Washington Infant Study [37, 38], thus different CHD-entities were evaluated separately. The diagnosis of CHD was reported to the Hungarian Congenital Abnormality Registry (HCAR), but it was confirmed and/or completed in the HCCSCA 3.5 ± 2.1 month later. Finally there was a follow-up of cases with CHD and unspecified CHD cases were reduced significantly. Only patients with diagnosis based on catheter examination, surgical correction, or autopsy were included in our study. We evaluated the exposure (i.e., folic acid use) in the critical periods for the different specified CHD and evaluated separately for each CHD. We have attempted to limit recall bias by evaluating exposure during the critical period for different CA-entities/groups because we expected an underreporting of treatment in both the critical and noncritical periods of CAs in the control group. We used only prospectively evaluated and medically recorded exposure data in prenatal maternity logbook as our gold standard.

The objective of our study was to estimate the efficacy of folic acid during pregnancy for the prevention of different CHD-entities.

Summary of Results of Previous Studies That Examined Folic Acid Use During Pregnancy and Prevention of CHD

Both the Hungarian randomized controlled trial (RR with 95 % CI: 0.42, 0.19–0.98) [5–9] and cohort controlled trial (OR with 95 % CI: 0.60, 0.38–0.96) [10] of maternal periconceptional supplementation with a folic acid (0.8 mg)-containing multivitamin resulted in a significant reduction in the birth prevalence of newborns with CHD in addition to the well-accepted prevention of the first occurrence of NTD. The combined results of these two intervention trials suggested a 43 % reduction in the risk of CHD, mainly ventricular septal and conotruncal heart defects.

The population-based observational studies from Atlanta [39–41] showed that the use of periconceptional folic acid-containing multivitamins was associated with a 24 % reduced risk of CHD. This association was also strongest for ventricular septal and conotruncal defects. The data of another US observational study of periconceptional multivitamin use was associated with some reduction in CTD of the heart (OR with 95 % CI: 0.7, 0.4–1.1) [42].

However, two other US observational studies did not find associations between periconceptional multivitamin supplementation and the reduction of VSD and/or outflow tract defects [43, 44].

The meta-analysis of both observational case–control studies (OR with 95 % CI: 0.78, 0.67–0.92) and randomized or cohort controlled trials (OR with 95 % CI: 0.61, 0.40–0.92) confirmed the associations between the use of maternal folic acid-containing multivitamins and the significant reductions in CHD [45].

A previous population-based observational study based on the Hungarian Case–Control Surveillance of Congenital Abnormalities showed an association of high dose (3–6 mg with the mean of 5.6 mg) folic acid use by pregnant women with a significant reduction in the risk of CHD (OR with 95 % CI: 0.86, 0.77–0.96) but the different CHD-entities/groups were not evaluated separately [11]. An observational Dutch Study [46] reported the potential CHD protective effect of folic acid intake (doses were not measured but 0.4 mg was the presumed dose) in early pregnancy on the risk of CHD (OR with 95 % CI: 0.82, 0.68–0.98) with the main effect due to sepal defects. A hospital-based case–control study analyzed 358 cases with CHD and 422 controls in China [47]. Compared with a mother who reported no folic acid supplementation, mothers who reported FA supplementation were less likely to have offspring with isolated CHD (AOR: 0.52, 0.34–0.78). The longer use of folic acid supplementation before pregnancy was associated with a lower risk of CHD (less than 1 month: 0.80, 0.38–1.68; 1–3 months: 0.37, 0.18–0.76; more than 3 months: 0.28, 0.17–0.48). The risk reduction was explained by lower occurrence of septal and CTD. This study did not show apparent difference in the strength of association with CHD between folic acid alone and folic acid-containing multivitamins. However, the study does not address how many doses of folic acid are most effective in preventing CHD because the most simple folic acid tablets used by the mothers contained about 0.4 mg of folic acid.

The risk of severe CHD (TOF, endocardial cushion defects, univentricular hearts, truncus atreriosus, or transposition complexes) declined by approximately 6 % in the years following folic acid fortification in Quebec (administrative databases of Canada analyzed birth data between 1990 and 2005) [48]. In contrast, another Canadian study from Alberta showed that only the birth prevalence of cases with left ventricular outflow tract obstruction declined after fortification when comparing birth data between 1995–1997 and 1999–2002 [49].

The Recent Population-Based Case–Control Observational Study

Material and Methodology

Cases and Controls

Children born with CA including CHD in the HCCSCA [50] were selected as cases from the HCAR [51, 52]. Reporting of cases with CA to the HCAR is mandatory for physicians, and most are reported by obstetricians (in Hungary practically all deliveries occur in inpatient obstetric clinics and birth attendants are obstetricians) and pediatricians (who are working in the neonatal units of inpatient obstetric clinics and various general and specialized, e.g., cardiologic inpatient and outpatient pediatric clinics). Autopsy is mandatory for all infant deaths and pathologists send a copy of the autopsy report to the HCAR if defects were identified. Since 1984 prenatal diagnostic centers were also asked to report malformed fetuses diagnosed prenatally with or without elective termination of pregnancy to the HCAR. The recorded total (birth + fetal) prevalence rate of cases with CA was 35 per 1000 informative cases (live-born infants, stillborn fetuses, and electively terminated malformed fetuses) between 1980 and 1996 [50] and about 90 % of major CAs were recorded in the HCAR [19].

Only those CA cases were selected from the HCAR for the HCCSCA that were reported to the HCAR during the first 3 months after birth or elective termination of pregnancy. In addition cases with CA-syndromes caused by gene mutations or chromosomal aberrations with preconception origin were excluded [50].

Controls were defined as newborn infants without CA. Controls were selected from the National Birth Registry of the Central Statistical Office including all Hungarian births for the HCCSCA on the basis of case lists for each quarter of the year from the HCAR. In general two controls were matched to every case according to sex, birth year/week, and district of parents' residence. If controls were twins, only one of them was randomly selected for the HCCSCA [50].

Data Collection

There were three sources of exposure data and confounders [50]:

1. Prospective recorded data in medical records. An explanatory letter and a printed informed consent were mailed continuously to the address of mothers of cases and controls immediately after their selection for the HCCSCA. Mothers were requested to send us the prenatal maternity logbook, discharge summary of their delivery and all medical records related to conditions during the study pregnancy and related to their child's CA. (These medical documents were returned within 4 weeks.) Prenatal care was mandatory for pregnant women and to encourage registration for prenatal care, the pregnant woman does not get a maternity grant and approved leave from her employment without her prenatal maternity logbook. Thus nearly 100 % of pregnant women visited prenatal care, on average seven times between the sixth gestational week and delivery. The task of obstetricians in prenatal care was to record all maternal diseases and medication products used by women during the study pregnancy in the logbook.
2. Retrospective maternal self-reported information. A structured questionnaire with a list of medications and diseases was also mailed to the mothers of cases and controls. This questionnaire contained questions regarding folic acid and multivitamin use and mothers were asked to read an enclosed list as a memory aid before they filled in the questionnaire. The mean ± S.D. time that elapsed between the end of pregnancy and the return of the "information package" (including logbook, discharge summary, questionnaire, and informed consent) in our prepaid envelope was 3.5 ± 2.1 and 5.2 ± 2.9 months in cases and controls, respectively.
3. Supplementary data collection. Regional district nurses were asked to visit all nonrespondent case mothers and to help them complete the questionnaire used in the HCCSCA and to evaluate the available medical documents. District nurses could visit only 200 nonrespondent [53] and 600 respondent [54] control mothers in two validation studies because the ethics committee considered this follow-up to be disturbing for the parents of all healthy children.

The final analysis of our study was based on prospective medically recorded data of folic acid use. Information regarding folic acid use was available for 96.3 % of cases (84.4 % from replies and 11.9 % from visits) and 83.0 % of controls (81.3 % from replies and 1.7 % from visits), i.e., 22,843 cases and 38,151 controls were evaluated; the latter represented 1.8 % of 2,134,714 live births in Hungary. There was only one kind of folic acid tablets containing 3 mg in Hungary during the study period.

The informed consent was signed and sent back by 98 % of cases; personal identifiers were deleted from the records of the remaining 2 % [50]. This paper evaluates the data set of the HCCSCA from 1980 to 1996 because the method of data collection was changed in 1997 and unfortunately recent data have not been validated.

Statistical Analysis

SAS version 8.02 (SAS Institute, Cary, North Carolina, USA) was used for statistical analysis of data. Three maternal confounders: age, birth order, and employment status, as the indicator of socioeconomic status [50] were considered. Other maternal variables, e.g., diseases and related drug

treatment, were considered without difference in case and control group, therefore were neglected as confounders. Conditional logistic regression model was used to estimate the relative risk/protection (odds ratio: OR) with 95 % confidence intervals (CI) of folic acid use in the mothers of cases with different types/groups of CHD and their matched controls.

Evaluation of Cases with CHD

The main objective of our study was the estimation of the possible association of folic acid supplementation with the reduction in the formation of certain CHD during the critical periods of embryonic development [31, 34].

In general, cases with CA were reported immediately after birth to the HCAR and about 50 % of cases with CHD were reported as unspecified CHD because the exact diagnosis of CHD needed further time-consuming examinations. The collection of medical data from cases with CA in the HCCSCA was 3.5 ± 2.1 months after birth, thus we were able to get specified CHD diagnoses in a further 20 % of cases. However, the rest, i.e., 30 % of our CHD cases had no specified diagnosis in the HCCSCA. Most cases with CHD were cared for or had surgical intervention in the pediatric cardiologic institutions in Hungary, therefore the relevant coworkers of the HCCSCA visited all cardiologic in- and outpatients clinics in 2008–2009. Medical records were reviewed and the previous diagnosis of specified CHD was checked (and corrected if necessary) and unspecified CHD were modified to specified CHD diagnoses. Several new cases with CHD were found, but patients without data in the HCCSCA could not be evaluated. If cases were not found in the records of pediatric cardiologic institutions, we had a correspondence with the case mothers to clarify the fate and/or diagnosis of these cases in 2009 and 2012. However, if these cases with unspecified CHD were not found or mothers refused collaboration, they were excluded from the study.

There were three selection steps in the evaluation of cases with isolated CHD:

I. Cases with syndromic CHD due to major mutant genes (e.g., Holt–Oram) or chromosomal aberrations (e.g., Down) were excluded from the HCCSCA, and unclassified multiple CAs including CHD were also excluded from the study.
II. Cases with isolated CHD were classified according to the system of The Baltimore–Washington Infant Study [38]. Figure 32.1 shows anterior view of normal heart and great vessel, while we can see the interior view of normal heart in Fig. 32.2. There are a number of developmental errors during the very complicated and long development of heart and great vessels. In the study CHD-entities/groups with homogeneous structural defects and sufficient number of cases were evaluated.

The selection of cases for analysis needed the knowledge of the diagnostic criteria of isolated CHD-entities/groups [38] and the basic characteristics are summarized below.

Ventricular septal defect (VSD) is the most frequent CHD with 2.0 per 1000 birth prevalence [22], though when echocardiography was used in the diagnostic algorithm, a prevalence of up to 3.9 per 1000 patients has been recorded [20]. VSD is also heterogeneous group of CHD including different errors of ventricular septum development because it is composed from four parts: septum membranaceum, anterior septum, posterior smooth septum, and posterior trabeculated septum. These four components of the ventricular septum are derived through three developmental processes: (a) the posterior septum forms from the ventricle, (b) anterior septum from the conotruncal crest, and (c) the septum membranaceum from the endocardial cushions. VSD due to the failure of the union between the endocardial cushions causes a defect of the interventricular muscular ridge and septum bulbi, and these defects are the consequences the defective growth and position of different components of the heart. Obviously VSD caused by the muscular or junctional defects have different embryonic and possible

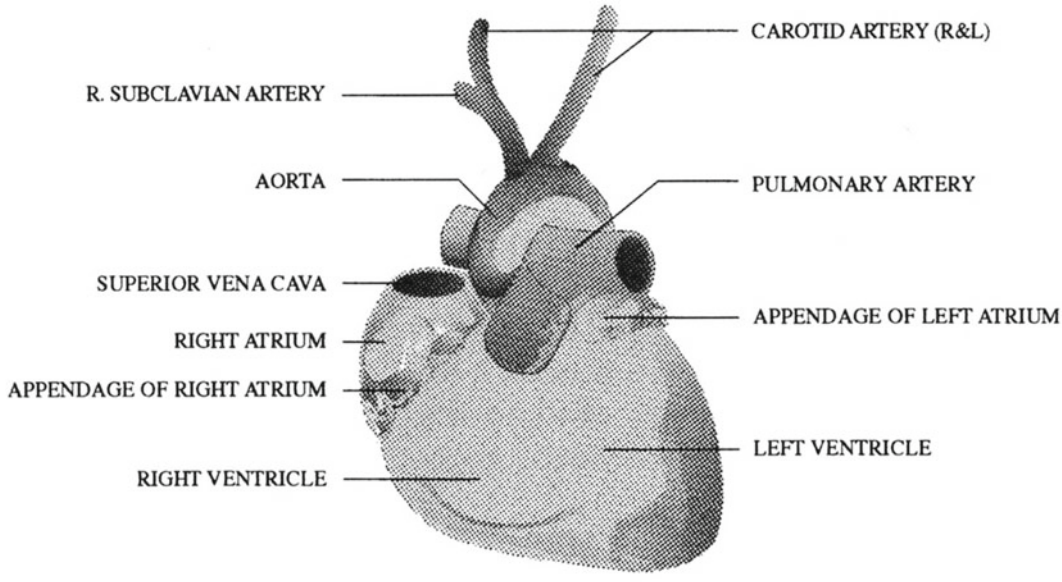

Fig. 32.1 The normal heart and great vessels, anterior view, with identification of the chambers, the great arteries (aorta and pulmonary artery) and the superior vena cava (the inferior vena cava is not visible in this view). From Ferencz et al. [38, p.16]

etiological origin, thus only the membranous (Fig. 32.3) and muscular types (Fig. 32.4) of VSD were included in our study. However, cases with single ventricle complex (SVC) were also evaluated, but separately in the study.

Single ventricle complex (SVC) is relatively rare CHD. SVC is a univentricular heart characterized by the entire flow from the two atria being carried directly through the left and/or right atrioventricular valves into the single ventricle. This anatomic structural defect has also been called as a common ventricle or cor triloculare biatriatum.

Atrial septal defect (ASD) is known in different anatomic types: (a) Defect of the ostium secundum is in the position of the foramen ovale, thus this CA represents true absence of the septal tissue. This CHD is called ASD secundum (ostium secundum: fossa ovalis secundum) and its abbreviation is ASD-II. (b) The ostium primum defect of ASD results in communication between the right and left atria in the lower part of the septum; this endocardial cushion type of ASD is called septum primum type of ASD and considered a partial form of atrioVSDs. (c) ASD may occur posteriorly between the right atrium and the sinus venosus, some of the right pulmonary veins may carry arterialized blood directly into the right atrium in this sinus venous ASD type. (d) The complete absence of the entire atrial septum may also occur and it is called a single or common atrium. ASD-II is the most frequent type of ASD, representing 85 % of all ASD and we evaluated cases with ASD-II in this study. Among CHD, ASD-II belongs to the most frequent CHD-group with the birth prevalence 0.74 per 1000 in Hungary [22]. Studies in other countries estimated the birth prevalence of ASD as 1 per 1500 live births explaining about 10 % of CHD [20, 21].

Common atrioventricular canal defect (CACD) occurs in about 3 % of infants and children with CHD; however, about half of these patients have Down syndrome and these cases were excluded from the study. CACD is characterized as CAs in isolation or combination including an ASD in the lower most part of the atrial septum (ostium primum), a cleft of the mitral valve (either alone or in combination with a cleft of tricuspid valve), or VSD. Complete and partial types of CACD were differentiated.

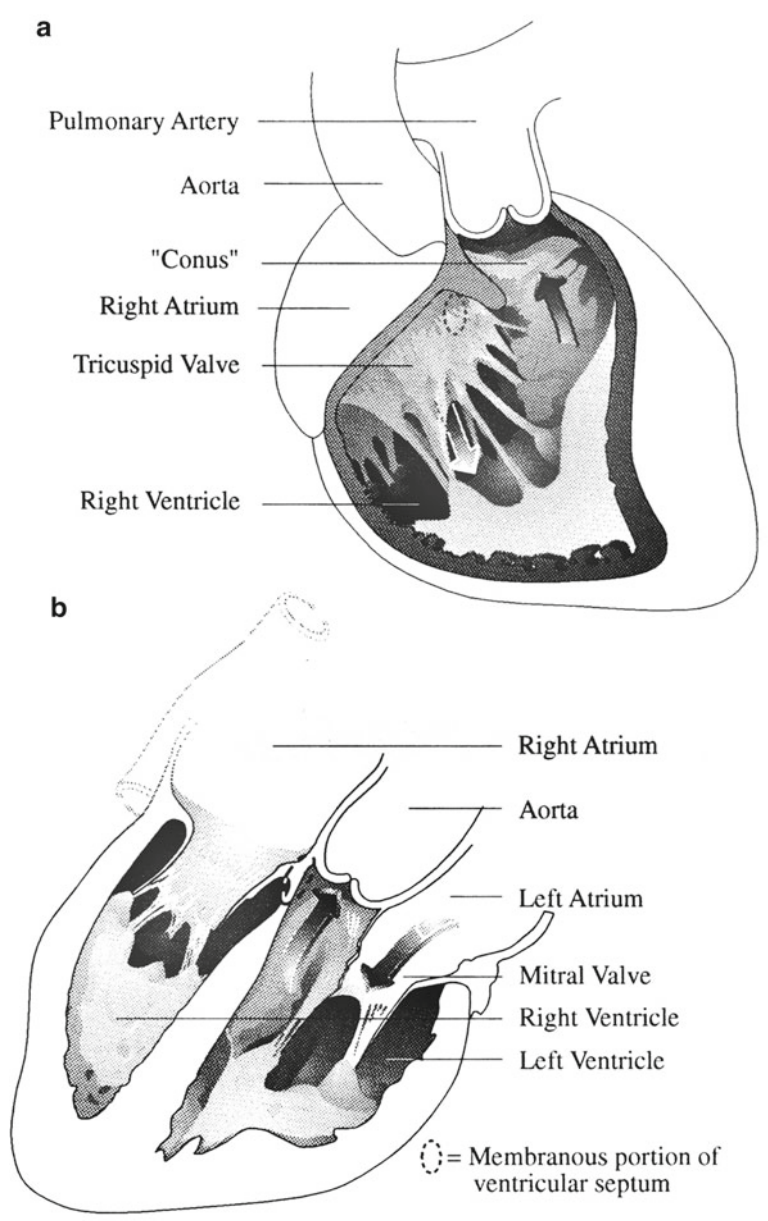

Fig. 32.2 The normal heart, interior views. (**a**) Right ventricle. This anterior view shows that blood that enters through the tricuspid valve is ejected into the pulmonary artery (*dark arrow*) through the muscular outflow portion of the right ventricle called the infundibulum or "conus." The membranous portion of the ventricular septum lies behind the tricuspid valve. (**b**) Four chambers view of the heart. This longitudinal posterior section shows the septum between the right and left ventricles and the relationship of the membranous part of the septum to the right atrium, tricuspid valve, and aortic valve. Blood entering through the mitral valve is directed into the aorta and membranous continuity of the mitral and aortic valves. From Ferencz et al. [38, p.17]

The complete type of CACD is characterized by the failure of partitioning of the primitive canal into separate atrioventricular orifices. The orifice between the atria and the ventricle is guarded by a common valve with the anterior leaflet derived from the ventral atrioventricular endocardial cushion and represents the anterior halves of the anterior mitral and septal tricuspid leaflets. The posterior leaflet originates from the dorsal atrioventricular endocardial cushion and represents the posterior halves of

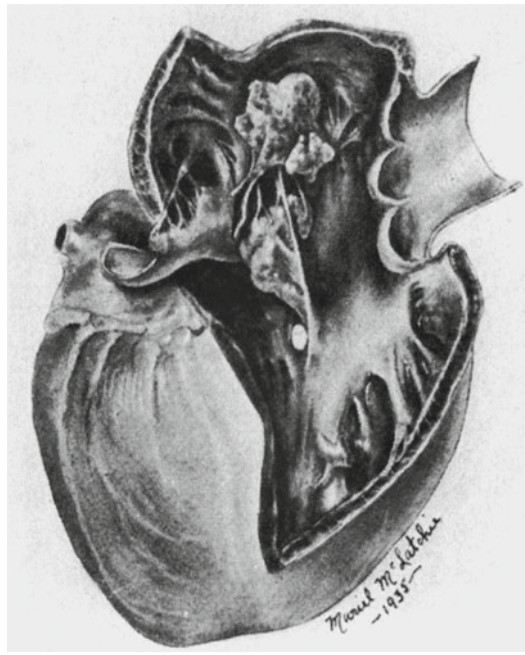

Fig. 32.3 Membranous ventricular septal defect, lying behind the tricuspid valve. From Ferencz et al. [38, p.19]. Originally form Abbott [73]

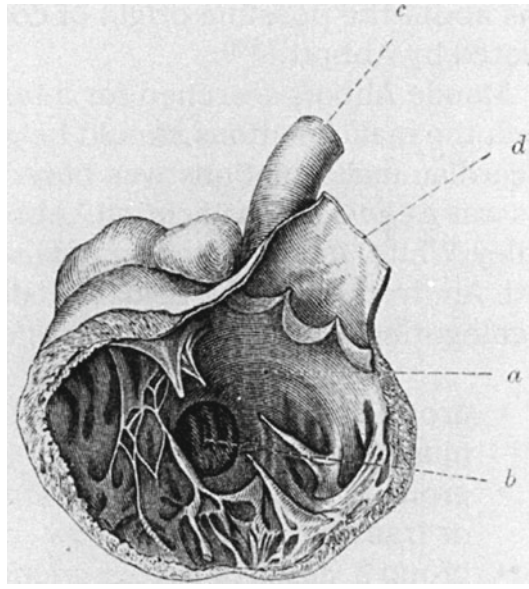

Fig. 32.4 Muscular ventricular septal defect (the large defect of the muscular septum seen from the right ventricle. From Ferencz et al. [38, p.23]. Originally from Rokitansky [74]

the anterior mitral and septal tricuspid leaflets. Usually there is a free communication between the ventricles in embryos due to considerable space between the anterior and posterior leaflets above and the ventricular septum below. Partial type of CACD or the ostium primum ASD is associated with a "cleft" in the anterior mitral leaflet or, probably a septal commissure between the superior and inferior leaflets of the left atrioventricular valve. However, we could not follow this classification because

partly surgical/autopsy description was not clear in all CACD cases, partly we were not able to classify the complete and partial types of CACD at the ascertainment of cases in the medical records. Thus finally all cases with CACD without noncardiac CAs were included in the study.

Persistent/patent ductus arteriosus (PDA) needs special diagnostic criteria. The closure (obliteration) of ductus arteriosus is usually complete by the third postnatal day, thus the diagnosis of PDA is recorded only after the third postnatal day in the HCAR. However, obliteration of ductus arteriosus is occurs more slowly in preterm babies; therefore the diagnosis of PDA in preterm babies is accepted only after the third postnatal week in the HCAR. According to the Hungarian treatment protocol, first the closure of PDA is attempted by the administration of indomethacin and/or ibuprofen. If pharmacologic closure is unsuccessful, the second step of PDA treatment is surgical ligation because the failure of ductus arteriosus closure is associated with increased mortality in preterm infants.

Conotruncal defects (CTD) of CHD represent the major anatomic phenotypes of outflow tract abnormalities, i.e., disturbances in the ventriculo-arterial portion of the ascending limb of the primitive S-shaped cardiac loop (the so-called conus or bulbus cordis) which will become septated by ridges derived from the endocardial cushions and by the aorticopulmonary septum, respectively, to form the divided arterial outflow from the right and left ventricles and of the pulmonary artery and aorta. There are four CTD-types with well-defined diagnostic criteria:

1. *Truncus arteriosus communis* (TAC) is a CHD in which truncus arteriosus is not properly differentiated into the two great arteries. One large single artery receiving blood from both right and left ventricles has one semilunar valve and distributes blood to both systematic and pulmonary circulations. The pulmonary artery may arise either as a single vessel or as two separate vessels from the trunk. A VSD is present in all cases.
2. *Transposition of great arteries* (TGA) with or without ventricular defects and pulmonary or tricuspid atresia, the aorta arises from the right ventricle in the anterior position and the pulmonary artery from the left ventricle in a posterior position (Fig. 32.5). This complete transposition creates two parallel circulations; this situation obviously is incompatible with life, thus only surgical intervention can

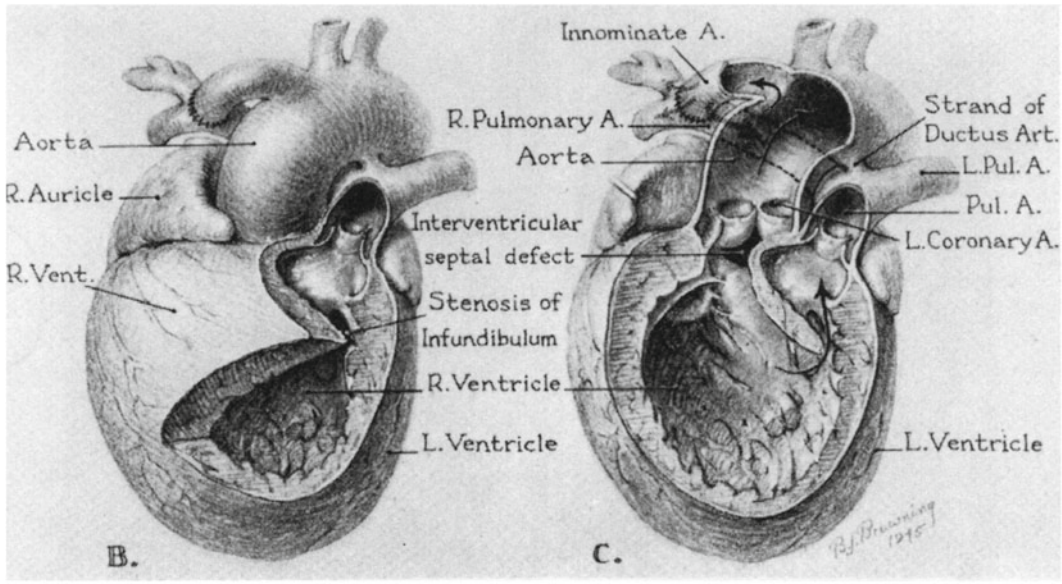

Fig. 32.5 Transposition of great arteries. The aorta arises from the right ventricle and pulmonary artery from the left ventricle. A defect in the ventricular septum is shown to have a bidirectional shunt. From Ferencz et al. [38, p.23]. Originally from Taussig [75]

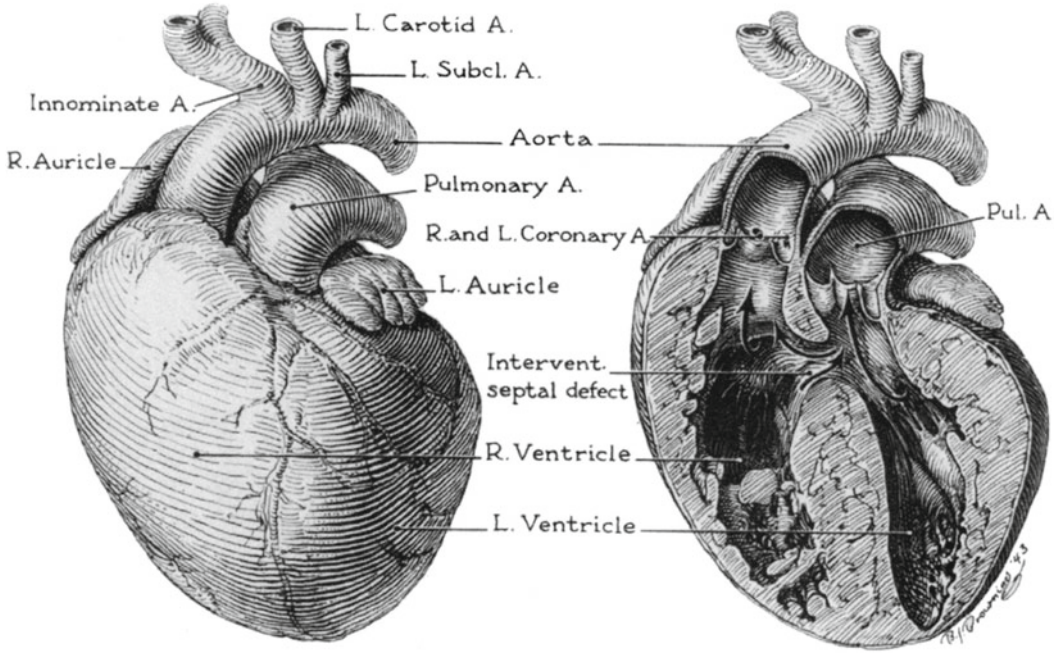

Fig. 32.6 Tetralogy of Fallot: a large right ventricle, severe stenosis of the infundibulum, small pulmonary valve and pulmonary artery, and a large aorta which "overrides" a ventricular septum, The innominate artery has been anastomosed to the right pulmonary artery (Blalock-Taussig shunt). From Ferencz et al. [38, p.23]. Originally from Taussig [75]

protect the life. Complete transposition of great vessels may exist with intact ventricular septum, VSD, double-outlet right ventricle, and pulmonary/tricuspid atresia.

3. *Tetralogy of Fallot* (TOF), classically this CHD comprises four components (Fig. 32.6): large VSD, an aorta overriding the VSD, severe infundibular pulmonic stenosis (small pulmonary valve and pulmonary artery) or atresia, and right ventricular hypertrophy. Thus TOF is characterized by biventricular origin of the aorta above large VSD.
4. *Double-outlet right ventricles* (DORV). In this rare CHD (about 1 % of cases with CHD), more than 50 % of the semilunar valve orifices of both great arteries arise form the morphologic right ventricle. In most cases, the ventricles display a D loop, and the pulmonary arterial origin is normally positioned, arising from the conus above the right ventricle. The aorta also arises from the right ventricle above conal tissue. In most cases, the aortic origin is to the right (D-malposition) of the pulmonary arterial origin, with the two vessels in a side-by-side relationship. Rarely, the aortic origin is distinctly anterior to the pulmonary origin or the aorta arises to the left (L-malposition) of the pulmonary artery.

Congenital right-sided obstructive defects of CHD (RSOD) consist of four groups:

1. *Congenital atresia/stenosis of pulmonary valve* (CAPV) is characterized by the thickened and dome-shaped pulmonary valve. Only cases with intact ventricular septum were included in the study, but in general the usually normally formed right ventricle is frequently hypertrophied. This group of RSOD cases covers a wide spectrum of clinical severity, most infants are asymptomatic and therefore this CA is one of the most common CHD. However, a small percentage of these cases have very severe obstruction with obvious symptoms such as fatigue and shortness of breath with exertion. The prognosis of cases with severe obstruction without intervention is poor, especially in

infants, thus surgical management (balloon valvuloplasty or valvectomy) is necessary. Complex CAPV cases associated with absence of pulmonary valve, pulmonary valve regurgitation, infundibular and supravalvular pulmonary valve stenosis, and these cases were excluded.

2. *Congenital atresia/stenosis of tricuspid valve* (CATV) is a failure of communication from the right atrium to the right ventricle. This group of RSOD cases has normal great vessels and may occur in mid and late pregnancy. Morphological studies have distinguished two forms: (a) tricuspid valve is replaced by a diaphragmatic structure at the site of the external atrioventricular connection and (b) complete failure of the atrium to appose the right ventricle. Tricuspid atresia with VSD or transposition of the great vessels is a complex CHD and these cases were excluded from this study.
3. *Ebstein's anomaly* (EbA) is a CA of the tricuspid valve, first described by Wilhelm Ebstein in 1866 [55]. EbA is characterized by a downward displacement of the attachment of the tricuspid valve into the inflow portion of the right ventricle. The abnormal leaflets and attachments are such that the tricuspid valve hangs like a curtain into the right ventricle, incorporating a greater or lesser portion of it to the right atrium and diminishing the trabecular outlet portion of the right ventricle, resulting in obstruction of blood flow into the pulmonary artery. The severity spectrum of EbA is very wide from severe disturbances in fetal and neonatal life to virtually symptomless survival throughout a long and active adult life.
4. *Congenital atresia/stenosis of pulmonary artery* (CAPA) with intact ventricular septum was evaluated in this study. This CA with an additional VSD is classified as a complex CHD, and these cases were excluded from the study. In addition, this peripheral stenosis of the pulmonary artery is sometimes associated with an abnormal right ventricle due to variable degrees of hypoplasia and endocardial fibroelastosis, or with sinusoidal communication that connect the high-pressure right ventricle to the coronary arteries. Other rare complex CCVA cases were also excluded from this study.

Congenital left-sided obstructive defects of ventricular outflow tract (LSOD) consists of different anatomic phenotypes, but obstructive CAs of the left heart and aorta represent a major group of CHD. This group of CHDs is morphologically and etiologically heterogeneous, as some CAs of the left atrium and mitral valve are associated with primary developmental disturbance of the atrioventricular region. CAs of the aortic arch system such as double aortic arch and interrupted arch are the defects of the branchial system. Recent research showed that the remaining "core" of LSOD includes four types/groups:

1. *Valvular aortic stenosis* (VAS) (congenital stenosis of aortic valve, aortic valve stenosis) is defined as subtotal obstruction of varying severity in the channel of left ventricular outflow. According to the sites of obstruction, (a) valvular, (b) subvalvular, and (c) supravalvular aortic stenosis are differentiated. Only cases with VAS are evaluated in this group. The typical VAS means two cups of aortic valves (i.e., bicuspid) instead of the normal three cups. Thus the stenosis (partial obstruction) of aortic valve results in the fusion of valve cusps. The vast majority of cases with VAS are asymptomatic, but about half of the infants with severe VAS need surgical intervention.
2. *Hypoplastic left heart syndrome* (HLHS) is a complex severe obstructive CHD encompassing atresia (complete obstruction) or severe stenosis (partial obstruction) of the aortic valve (orifice) and/or mitral valve associated with hypoplasia (underdevelopment) of the left ventricle and of the ascending aorta with or without VSDs. The vast majority of these cases died after birth.
3. *Coarctation of the aorta* (COA) is a discrete narrowing of the distal segment of the aortic arch. Approximately half of these cases had heart failure within the first month or two of life thus prompt correction of COA is recommended for all infants. Complex CHD cases including COA and VSD, hypoplasia of the aortic arch, and aortic stenosis (valvular and/or subvalvular) were excluded from this group of LSOD.
4. *Other CAs of aorta* (OAC). Beyond the previously mentioned VAS, HLHS, and COA, subvalvular aortic stenosis, bicuspid aortic valve, interrupted or double aortic arch, aortic atresia, and aortic

hypoplasia were included in this group. Recently bicuspid aortic valves can be diagnosed in infants with two-dimensional echocardiography. Mitral valve abnormalities were excluded from this group of LSOD.

Only isolated CHD cases were evaluated in the study. However, isolated CHD cases included single and specified complex CHD-entities, but complex CHD cases without specification were excluded from the study.

III. Some types of CHD have a wide spectrum of manifestations including spontaneous closure of ventricular or ASDs, ductus arteriosus, etc. Thus finally only cases with *lethal outcomes verified by autopsy record or with documented catheter examination and/or surgical correction* were included in the study. Our cases therefore represent homogeneous groups of severe manifestations of different CHD-entities.

Results

The flow of cases with CHD from the HCAR to the HCCSCA and the formation of the study cohort in the HCCSCA are shown in Fig. 32.7.

After the exclusion of cases with misdiagnosis, the plan was to check the diagnosis of 8103 cases with CHD in cardiologic clinics. Of these 8103 cases, 7415 cases (91.5 %) were evaluated, but 2275 cases (30.7 %) were excluded due to inappropriate diagnosis. Of 5140 cases with confirmed isolated CHD, the diagnosis was based on autopsy or catheter/surgical documents in 3838 (74.7 %) cases. Fifteen CHD (0.4 %) were diagnosed in stillborn fetuses, they were excluded. Prenatally diagnosed and electively terminated fetuses affected with CHD did not occur in the HCCSCA during the study period. Some isolated types of CHD (e.g., defects of laterality) with too low a number of cases and cases with complex CHD consisting of two or more different CHD without specification were excluded from the study. Thus, finally 3567 live-born cases with different CHD and 5395 matched controls were evaluated.

The objective of the study was to estimate the possible preventive effect of folic acid alone during pregnancy for different CHD-entities/groups; therefore, pregnant women who were taking folic acid-containing multivitamin supplements with or without additional folic acid were excluded from the study. Multivitamins have many different components and folic acid doses.

The main socio-demographic data of the mothers of cases and matched controls with or without folic acid supplementation are shown in Table 32.6. Mothers of matched controls were somewhat younger with lower mean birth order compared to the mothers of cases with CHD. Folic acid users appear to be the same age as nonusers and to have similar birth order, but were somewhat older with higher mean birth order than nonusers in both study groups. Mothers of cases with folic acid use had lower socioeconomic status than folic acid user mothers of matched controls mainly due to the lower proportion of professional-managerial women (27.3 % vs. 34.9 %) in the control group and the higher proportion of semi- and unskilled workers, housewives, and others in the case mothers (45.8 % vs. 32.7 %).

The use of folic acid alone was differentiated into three time periods (Table 32.7): (a) anytime during pregnancy, (b) during the critical period of the given CHD-entity based on either medical records in prenatal maternity logbook or maternal self-reported information in the questionnaire, and (c) during the critical period of the given CHD based only on medical records. Of 3567 cases with CHD, 1759 (49.3 %) had mothers with folic acid use anytime during pregnancy based on two sources of data, compared to 2952 (54.7 %) pregnant women in the group of 5395 matched controls. Folic acid use was medically recorded in 805 (22.6 %) mothers of cases and in 1953 (36.2 %) mothers of matched controls in the prenatal maternity logbook. Folic acid use was known in 954 (26.7 %) case mothers and 999 (18.5 %) control mothers through only maternal information. Medically recorded

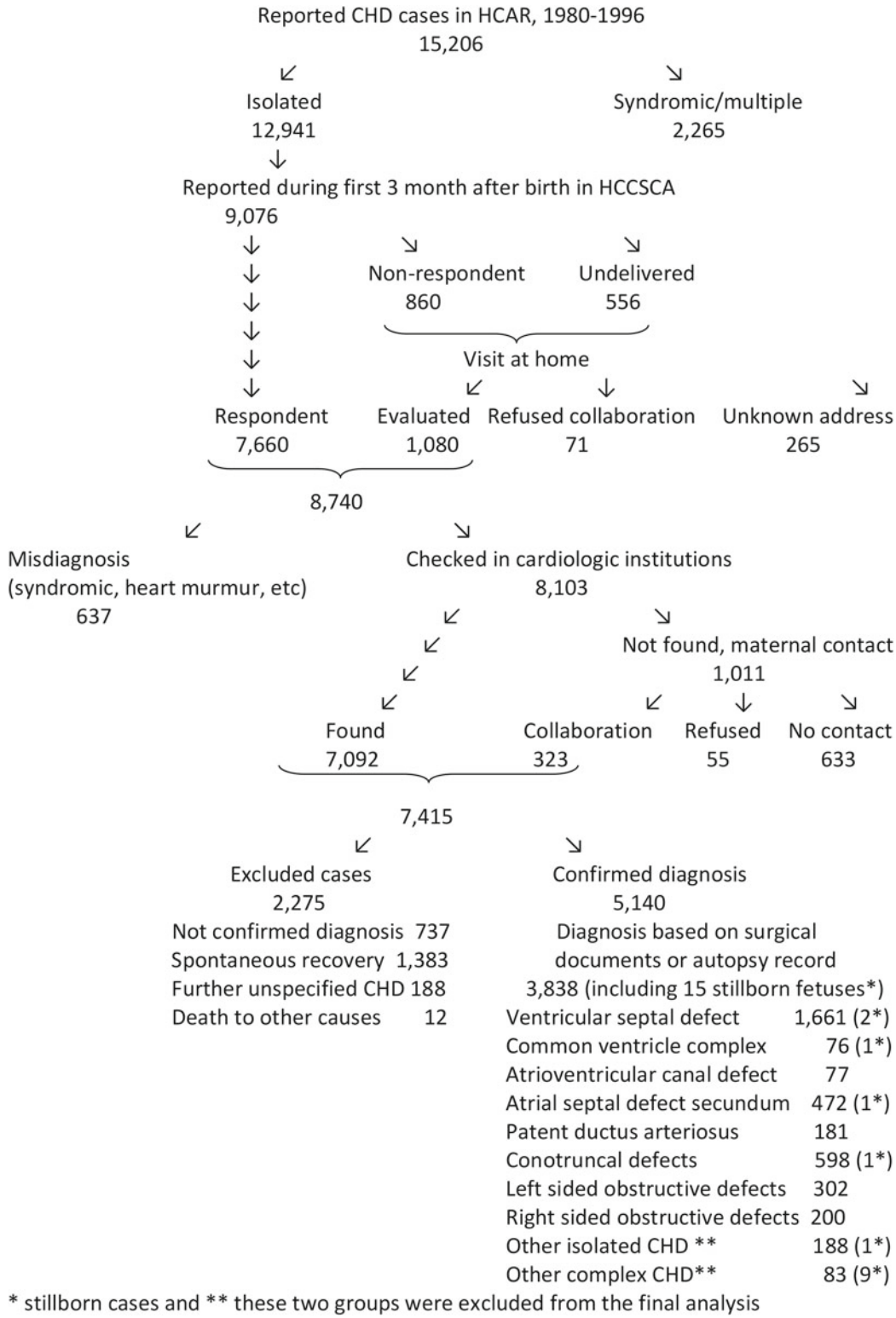

Fig. 32.7 Flow of cases with CHD from the HCAR to the study material of the HCCSCA

Table 32.6 Socio-demographic characteristics of mothers of cases with CHD and matched controls with or without folic acid supplementation

Variables	Mothers of cases				Mothers of matched controls			
	Without		With		Without		With	
	Folic acid use				Folic acid use			
	(*N* = 1808)		(*N* = 1759)		(*N* = 2443)		(*N* = 2952)	
Quantitative	No.	%	No.	%	No.	%	No.	%
Maternal age (years)								
–19	180	10.8	161	9.2	212	8.7	240	8.1
20–29	1231	68.1	1215	69.1	1763	72.2	2193	74.3
30–	397	22	383	21.8	468	19.2	519	17.6
Mean ± SD	25.8 ± 5.4		25.7 ± 5.2		25.5 ± 5.1		25.3 ± 4.7	
Birth order								
1	752	41.6	780	44.3	1105	45.2	1461	49.5
2	634	35.1	641	36.4	909	37.2	1110	37.6
3 or more	422	23.3	338	19.2	429	17.6	381	12.9
Mean ± SD	2.0 ± 1.3		1.9 ± 1.1		1.8 ± 1.1		1.7 ± 0.9	
Categorical	No.	%	No.	%	No.	%	No.	%
Unmarried	112	6.2	92	5.2	105	4.3	107	3.6
Employment status								
Professional	138	7.6	173	9.8	255	10.4	343	11.6
Managerial	355	19.6	385	21.9	597	24.4	809	27.4
Skilled worker	487	26.9	551	31.3	792	32.4	968	32.8
Semiskilled worker	325	18	280	15.9	372	15.2	462	15.7
Unskilled worker	159	8.8	122	6.9	158	6.5	136	4.6
Housewife	224	12.4	184	10.5	187	7.7	149	5
Others	120	6.6	64	3.6	82	3.4	85	2.9

supplementation was considered the most reliable source of verification of folic acid exposure; therefore, the final results were based on medically recorded folic acid use.

In general the onset of folic acid supplementation was related to the first visit to the prenatal care clinics which occurred between the 6th and 11th gestational week. The proportion of preconceptional supplementation of folic acid was rare (less than 3 % before 1992, but about 11 % in 1993–1996). Types of CHD with a critical period after the third gestational month had higher proportion of medically recorded folic acid use, e.g., in the mothers of cases with PDA (23.6 %) than in the mothers of cases with CACD (4.8 %) that has a critical period in the second gestational month. However, most women who used folic acid in the second gestational month started this supplementation in the preconception period.

Only VSDs (Figs. 32.3 and 32.4) showed a reduction after folic acid use in all the three time periods (Table 32.7). However, medically recorded folic acid use during the critical period of formation of the ASD secundum, PDA, and two types of CTD: D-TGA (Fig. 32.5) and TOF (Fig. 32.6) was also associated with a reduction in the birth prevalence of these CHD-entities. If preterm births were excluded at the evaluation of cases with PDA, the previously found reduction disappeared. However, it is worth mentioning that among 12 other CHD-entities, 11 had OR less than 1.0 based on medically recorded folic acid use during their critical period, though these figures showed only a trend without significant reduction. The exception was SVC that included only four cases (and three controls) with medically recorded folic acid use during the critical period.

The number of tablets, i.e., total dose of folic acid, could be obtained from the data set of the HCCSCA and the follow-up study of cases with CHD in 61 % of mothers of cases and in 72 % of mothers of matched controls. The estimated daily average dose of folic acid was calculated to be 5.6 mg.

The dose-dependent effect of folic acid was evaluated in cases with VSD, ASD secundum, CTD, and other groups together (Table 32.8). One tablet (i.e., 3 mg) of medically recorded folic acid during

Table 32.7 Folic acid use in the mothers of cases with CHD and their matched controls (MC), the defined critical period of each CHD type or group is shown in the second column

Groups/entities of CHD	Critical period: month	Number of total Cases No.	Number of total MC No.	Anytime during pregnancy Cases No. %	Anytime during pregnancy MC No. %	Anytime during pregnancy OR 95% CI	Critical period Cases No. %	Critical period MC No. %	Critical period OR 95% CI	Critical period, medically recorded Cases No. %	Critical period, medically recorded MC No. %	Critical period, medically recorded OR 95% CI
Ventricular septal defect (perimembranous and muscular types)	II–III	1661	2534	813 48.9	1378 54.4	**0.83** **0.66–0.97**	389 23.4	723 28.5	**0.76** **0.63–0.91**	188 11.3	457 18.0	**0.57** **0.45–0.73**
Single (common) ventricle complex	II	76	119	45 59.2	76 63.9	0.83 0.34–1.96	6 7.9	12 10.1	0.71 0.23–2.21	4 5.3	3 2.5	1.70 0.26–10.90
Common atrioventricular canal (endocardial cushion defect)	II	77	104	30 39.0	52 50.0	0.78 0.38–1.92	8 10.4	18 17.3	0.68 0.02–2.23	2 2.6	5 4.8	0.49 0.05–4.51
Atrial septal defect, secundum	II–III	472	678	230 48.7	372 54.9	0.90 0.75–1.36	113 23.9	202 29.8	**0.70** **0.41–0.96**	62 13.1	126 18.6	**0.63** **0.40–0.98**
Patent ductus arteriosus	VII–IX	181	285	98 54.1	167 58.6	1.00 0.67–1.50	68 37.6	129 45.3	0.89 0.64–1.52	29 16.0	93 32.6	**0.47** **0.27–0.80**
Conotruncal defects												
Truncus arteriosus	II–III	44	58	19 43.2	29 50.0	0.78 0.17–1.68	9 20.5	15 25.9	0.79 0.13–2.90	4 9.1	7 12.1	0.76 0.07–2.85
D-transposition of great arteries	II–III	307	489	151 49.2	276 56.4	**0.71** **0.52–0.97**	75 24.4	140 28.6	0.80 0.51–1.25	31 10.1	96 19.6	**0.47** **0.26–0.86**
Tetralogy of Fallot	II–III	223	323	112 50.2	167 51.7	0.95 0.66–1.38	59 26.5	91 28.2	0.85 0.34–1.48	28 12.6	60 18.6	**0.59** **0.17–0.94**
Double outlet of right ventricle	II–III	24	32	11 45.8	18 56.3	0.88 0.48–2.89	7 29.2	10 31.3	0.96 0.22–16.41	4 16.7	8 25.0	0.70 0.44–1.94
Together	II–III	598	902	293 49.0	490 54.3	0.86 0.63–1.11	150 25.1	256 28.4	0.81 0.52–1.08	67 11.2	171 19.0	**0.55** **0.29–0.89**

(continued)

Table 32.7 (continued)

Groups/entities of CHD	Critical period: month	Number of total		Anytime during pregnancy			Critical period			Critical period, medically recorded		
		Cases	MC	Cases	MC	OR	Cases	MC	OR	Cases	MC	OR
		No.	No.	No. %	No. %	95% CI	No. %	No. %	95% CI	No. %	No. %	95% CI
Left-sided obstructive defects												
Valvar aortic stenosis	III–VII	56	77	29 51.8	31 40.3	1.38 0.66–4.40	28 50.0	29 37.7	1.26 0.39–13.04	8 14.3	18 23.4	0.62 0.20–1.94
Hypoplastic left heart syndrome	II–IV	76	125	42 55.3	82 65.6	0.78 0.38–1.31	27 35.5	62 49.6	0.85 0.43–1.70	15 19.7	42 33.6	0.64 0.28–1.48
Coarctation of the aorta	III–VII	113	182	57 50.4	101 55.5	0.89 0.55–1.48	54 47.8	98 53.8	0.92 0.54–1.56	24 21.2	66 36.3	0.65 0.23–1.39
Other CAs of aorta	II–VII	57	85	21 36.8	45 52.9	0.58 0.16–1.08	20 35.1	40 47.1	0.67 0.15–1.19	10 17.5	24 28.2	0.52 0.19–1.46
Together	II–VII	302	469	149 49.3	259 55.2	0.83 0.61–1.14	129 42.7	229 48.8	0.94 0.67–1.13	57 18.9	150 32.0	0.63 0.35–1.08
Right-sided obstructive defects												
Valvar pulmonic stenosis	VI–IX	72	121	37 51.4	68 56.2	0.96 0.56–1.99	26 36.1	53 43.8	0.89 0.45–1.77	12 16.7	32 26.4	0.75 0.29–1.50
Tricuspid atresia/stenosis	VI–IX	13	19	10 76.9	10 52.6	1.29 0.30–5.50	6 46.2	7 36.8	1.11 0.09–4.51	2 15.4	5 26.3	0.81 0.11–2.85
Ebstein's anomaly	VI–IX	7	12	3 42.9	6 50.0	0.83 0.09–3.81	2 28.6	4 33.3	0.82 0.01–4.85	0 0.00	4 33.3	0.00 0.00–0.00
Pulmonary atresia/stenosis	III–VII	108	152	51 47.2	74 48.7	1.00 0.58–1.74	47 43.5	69 45.4	1.03 0.58–1.82	19 17.6	43 28.3	0.50 0.24–1.17
Together	III–IX	200	304	101 50.5	158 52.0	1.02 0.70–1.50	81 40.5	133 43.8	1.01 0.68–1.50	33 16.5	84 27.6	0.66 0.34–1.09

OR were adjusted for maternal age, birth order (parity), and socioeconomic status
Bold numbers show significant associations

the critical period of the given CHD type or group was associated with a reduction in the birth prevalence of VSD, conotruncal defect and other CHD groups. Two tablets (6 mg) of folic acid daily reduced the risk of all CHD groups while it was not observed after the use of three tablets (9 mg) but the number of cases and matched controls was limited in this group.

Table 32.8 The dose-dependent effect of folic acid (FA) in the mothers of cases (Case) and matched controls (MC)

		VSD		ASD II		Conotruncal		Other CHD		Total	
Doses of FA		Case	MC	Case	MC	Case	MC	Case	MC	Case	MC
Total *N*		1661	2534	472	678	598	902	836	1281	3567	5395
FA user *N*		813	1378	230	372	293	490	423	712	1759	2952
One tablet											
FA	No.	141	229	40	59	50	83	69	115	300	486
	%	11.5	12.7	11.3	12.4	11.1	12.2	10.8	12.4	11.2	12.5
FA:MR	No.	65	159	21	38	22	52	34	77	142	326
	%	5.3	8.8	5.9	8	4.9	7.7	5.3	8.3	5.3	8.4
FA:CP	No.	70	121	23	31	27	46	46	78	166	276
	%	5.7	6.7	6.6	6.6	6	6.8	6.9	8.4	6.2	7.1
FA: MR/CP	No.	37	85	13	19	11	31	22	55	83	190
	%	3	4.7	3.6	4	2.5	4.5	3.5	6.2	3.1	4.9
	OR	**0.62**		0.9		**0.54**		**0.55**		**0.61**	
	95 % CI	**0.43–0.87**		0.46–1.74		**0.28–0.98**		**0.34–0.86**		**0.48–0.77**	
Two tablets											
FA	No.	410	673	118	180	152	256	233	363	913	1472
	%	33.4	37.3	33.3	37.9	33.9	37.8	36.2	39.2	34.1	37.9
FA:MR	No.	176	437	61	117	70	174	100	231	407	959
	%	14.3	24.2	17.2	24.6	15.6	25.7	15.5	24.9	15.2	24.7
FA:CP	No.	195	355	54	102	76	133	162	261	487	851
	%	15.9	19.7	15.3	21.5	16.9	19.6	25.2	28.2	18.2	21.9
FA: MR/CP	No.	92	216	29	63	32	88	66	165	219	532
	%	7.5	12	8.2	13.3	7.1	13	10.2	17.8	8.2	13.7
	OR	**0.62**		**0.57**		**0.52**		**0.54**		**0.56**	
	95%CI	**0.48–0.75**		**0.37–0.86**		**0.35–0.76**		**0.41–0.70**		**0.49–0.65**	
Three tablets											
FA	No.	49	81	15	22	18	29	25	39	107	171
	%	4	4.5	4.2	4.6	4	4.3	3.9	4.2	4	4.4
FA:MR	No.	25	54	8	18	10	22	12	26	55	120
	%	2	3	2.3	3.8	2.2	3.2	1.9	2.8	2	3.1
FA:CP	No.	22	40	7	9	10	14	15	30	54	93
	%	1.8	2.2	2	1.9	2.3	2.1	2.3	3.2	2	2.4
FA: MR/CP	No.	10	23	5	6	7	9	7	24	29	62
	%	0.8	1.3	1.4	1.3	1.6	1.3	1.1	2.6	1.1	1.6
	OR	0.63		1.12		1.04		0.49		0.7	
	95%CI	0.31–1.20		0.35–3.40		0.39–2.66		0.20–1.07		0.46–1.04	
FA user without known No. of tablets		213	395	57	111	73	122	95	195	438	823

Ventricular septal defects (VSD), atrial septal defect secundum (ASD II)
Only medically recorded (MR) FA use any time during pregnancy (FA:MR)
FA use during the critical period (CP) of the given CHD groups evaluated (FA:CP)
Medically recorded FA use during the critical period of CHD (FA: MR/CP)
OR were adjusted for age, birth order (parity), and socioeconomic status of mothers in the subgroup of FA:MR/CP
Bold numbers show significant associations

Discussion

The objective of our study was to estimate the possible reduction of different severe CHD in newborn infants born to mothers who had taken medically recorded high doses of folic acid during the critical period of the formation of CHD-entities. There was a significant reduction in the risk of VSD (Figs. 32.3 and 32.4), TOF (Fig. 32.5), and D-TGA (Fig. 32.6) with the use of 3 mg/day doses of folic acid. ASD secundum was reduced after the use of 6 mg/day of folic acid. Of the other CHD studied in this analysis, none had a significant reduction after folic acid use, though there was a decreasing trend in their occurrence, except SVC.

As far as we know our population-based case–control study is the first to evaluate all frequent CHD without other noncardiac CA in cases with lethal outcome and/or catheter examination and surgical correction, i.e., with controlled diagnoses. The major finding of the study is that there is a reduced risk of certain types of CHD after the use of medically recorded high doses of folic acid in pregnancy. In this study the most frequently occurring CHD were evaluated, and the major finding is that about 40 % of major CHD may be preventable by the use of higher doses of folic acid supplementation during the critical period of formation of these CHD-entities.

Our findings showed mainly the reduction of VSDs and CTD after the use of higher doses of folic acid alone. The previous Hungarian trials [5–10] and US [38–41] observational studies using periconceptional folic aid-containing multivitamins reported that the strongest risk reduction was found in cases with VSDs and CTD. In addition the use of high [11] and low [44, 45] doses of folic acid during early pregnancy also associated with the reduction of birth prevalence of cases with CHD. After the introduction of folic acid fortification of grains some reduction of certain CHD-entities/groups were observed in Canada [47, 48].

In general, there was no obvious difference in the reduction of CHD after one or two tablets containing 3 mg/tablet/day of folic acid; the exception was ASD secundum. A dose–response relationship is known between the dose of folic acid and the reduction of blood homocysteine level [56]. Thus 3 mg folic acid may reduce the risk of some major CHD-entities. Nevertheless, it would be necessary to determine the lowest effective dose of folic acid alone in the precancerous conditions of CHD in a well-controlled intervention trials before public health recommendations can be made.

There was a recent concern regarding the possible mitosis-stimulating effect of higher doses of folic acid in people with precancerous conditions. However, a recent meta-analysis of randomized trials of 50,000 individuals did not find association between the effect of folic acid supplementation and overall and site-specific cancer incidence [57].

Folic acid antagonist drugs that inhibit dihydrofolate reductase which is required for DNA synthesis increased the risk of CHD in the fetuses of pregnant women [58]. However, the most important argument for the role of folic acid in the pathogenesis of CHD was that the risk of CHD after the use of folic acid antagonists without concomitant use of folic acid-containing multivitamins was 7.7 (95 % CI: 2.8–21.7) while this risk was only 1.5 (95 % CI: 0.6–3.8) after the parallel use of folic acid antagonist drugs and multivitamins [58].

Fetal alcohol spectrum disorder comprises a range of birth defects including CHD. In a recent zebrafish embryo experiments ethanol exposures interrupted divergent cardiac morphogenetic events causing CHD. Folic acid supplementation was effective in preventing a wide spectrum of ethanol-induced heart developmental defects [59]. In avian and mouse vertebrate models CHD was induced by alcohol, lithium or elevation of metabolite homocysteine and these CHDs were prevented with higher doses of folic acid [60]. All three factors affected the important Wnt signaling pathway suppressing Wnt-mediated gene expression in heart fields, resulting in a delay of cardiomyocyte migration, cardiomyogenesis, and CHD [60].

Several studies indicated the association of hyperhomocysteinemia with higher risk of CHD [61]. Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme for the metabolism of folate/folic acid and the detoxication of homocysteine because it directly influences plasma folate levels. The

polymorphism of MTHFR gene is very common. The frequency of homozygous 677TT and heterozygous 677CT genotypes is 11.1 % and 45.2 % in the Hungarian population, respectively [62]. The activity of MTHFR is approximately 30 and 65 % lower in persons with 677CT and 677TT compared with the 677CC genotype and these gene-polymorphisms associate with elevated plasma homocysteine and lower red blood cell folate levels [63]. The majority of CHD may result from disruption of development of the cardiac neural crest because cardiac neural crest is highly responsive to folate and homocysteine [64]. The roles of folate may be one-carbon metabolism in support of mitosis and gene methylation, in addition gene regulation via direct activity of the folate receptor. The role of hyperhomocysteinemia may increase oxidative stress; disruption of gene methylation; homocysteinylation of key proteins; and NMDA receptor binding [64]. In 2013 a meta-analysis of 29 studies showed that both infant and maternal MTHFR 677CT polymorphisms may contribute to the risk of CHD [65]. However, in 2013 another meta-analysis of 7697 cases and 13,125 controls did not find an association of MTHFR C677 polymorphism with higher risk of CHD [66].

Obviously other genetic factors may be also important in the multifactorial origin of CHD. Mothers carrying the MDR1 3435T allele, using medication without folic acid, are at nearly threefold increased risk for CHD in the offspring, but it is decreased in folic acid users [67]. Recent studies have implicated maternal single nucleotide polymorphisms (SNPs) and altered metabolism on folate-related pathways as CHD risk factors. Four SNPs were identified in the methionine adenosyltransferase II alpha (MAT2A) gene that were associated with methionine levels. Three SNPs in tRNA aspartic acid methyltransferase 1 (TRDMT1) gene were associated with total plasma folate levels. Glutamylcysteine (GluCys) levels were associated with multiple SNPs within the glutathione peroxidase 6 (GPX6) and O-6-methylguanine-DNA methyltransferase (MGMT) genes. The regression model revealed interactions between genotypes and case–control status in the association of total plasma folate, total glutathione (GSH), and free GSH, to SNPs within the MGMT1, 5,10-methenyltetrahydrofolate synthetase (MTHFS), and catalase (CAT) genes, respectively [68]. Finally mutation in folate metabolism causes epigenetic instability and transgenerational effects on development [69].

The data in our study strongly suggest that a greater emphasis should be placed on the inclusion of CHD documentation with the same scrutiny as given to NTD currently. In addition, discussions should be undertaken concerning the potential for reduction of CHD in addition to NTD as part of the public health awareness for women of childbearing potential as well as family members planning a pregnancy. Importantly:

1. Cases with CHD represent the most common CA-group, CHD account for a quarter of infant deaths [24, 26], the cost of their surgical and other managements is tremendous [26].
2. Available findings indicate that about 40 % of CHD may be preventable by the use of folic acid or folic acid-containing multivitamins during pregnancy.
3. Obviously the efficacy of primary prevention of NTD by folic acid-containing multivitamins or folic acid alone is better documented currently, but we should consider the absolute numbers of NTD versus CHD. The total birth prevalence of cases with NTD is 2.78 per 1000 in Hungary [70]. The efficacy of folic acid in the prevention of NTD is about 70 %, thus theoretically the population-based rate of NTD is reduced to 0.84 per 1000, and this reduction is equal to 195 cases per 100,000 births. However, the birth prevalence rate of cases with CHD is 10.2 per 1000 in Hungary [22] and it may be possible to reduce to 6.1 per 1000 on the basis of 40 % efficacy of folic acid, thus the preventable absolute number of CHD cases is 408 for 100,000 births. Of course, these estimations depend on the population's baseline prevalence rates of CHD and NTD.

The strength of our study is based upon the large population-based data set of the HCCSCA including cases with CHD and their matched controls in the ethnically homogeneous Hungarian (Caucasian) population. The validity of CHD-diagnoses has been improved due to the follow-up of cases in all cardiologic institutes and the correspondence with mothers. We evaluated CHD-entities as homogeneously as possible; therefore cases with syndromic/multiple CAs and complex CHD cases without specification were excluded; and only cases with severe (lethal and/or catheter examination

and surgically corrected) single and specified complex CHD-entities were included in the study. Folic acid use was assessed from medical records. The exposure time of folic acid was known and evaluated particularly during the critical period of the given CHD-entities/groups. Finally potential confounders were measured.

However, there are some weaknesses in our study: (a) Our cases had serious manifestation of CHD, and do not represent the whole spectrum of CHD. However, if folic acid can prevent severe CHD, we can suppose that it may be effective in less severe cases as well. (b) High doses of folic acid were used in Hungarian obstetrical practice in the 1980s. As we mentioned previously, there was only one kind of folic acid tablets in Hungary containing 3 mg and the recommendation was 1 tablet per day for pregnant women from 1978. However, several obstetricians suggested two or three tablets. The results of MRC Vitamin Study [71] showed the efficacy of 4 mg folic acid in the reduction of recurrent NTD. This publication was well known in Hungary because nearly half of the participants were Hungarian in the MRC Vitamin Study and two tablets, i.e., 6 mg have become a widely used practice. Hungarian obstetricians did not know or did not accept the international recommendation of 0.4 or 0.8 mg folic acid for healthy pregnant women during the 1990s [72]. (c) The study period covered 17 years between 1980 and 1996 because the data of recent years in the HCCSCA after my retirement have not been validated. However, this weakness has some benefits because on the one hand only one type of folic acid tablet was used during this time period. On the other hand we had time to organize long-term follow-up of our CHD cases with the considerable improvement of diagnoses. (d) The standard care of pregnant women in prenatal clinics included recording of folic acid use, nevertheless only 22.6 % and 36.2 % of pregnant women with folic acid supplement had medically recorded folic acid supplementation in their prenatal maternity logbook in the mothers of cases and controls, respectively. However, the above 22.6 % is a relative figure because the proportion of only maternal self-reported folic acid use was 26.7 % by the mother of CHD cases compared to 18.8 % of mothers of matched controls. These data confirm the importance of recall bias in control mothers. On the other hand some obstetricians did not understand the importance of folic acid in the past. It is sad but the available number of pregnant women was enough to evaluate only medically recorded folic acid use. (e) The use of folic acid was known in 96.3 % of cases and 83.0 % of controls at 3.5 and 5.2 months after birth; however, our validation studies [53, 54] showed that this selection bias did not exist if only medically recorded folic acid use was evaluated.

Conclusion and Recommendation

There is only one optimal medical intervention for CA and it is their primary prevention. About 40 % of severe CHD may be preventable by folic acid use alone during the critical periods of these CHD-entities during pregnancy. Therefore it would be worth considering the incorporation of an evaluation of CHD as well as an educational program about the potential for reduction of CHD into the public health NTD preventive program by folic acid [72].

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Chapter 33
Maternal Nutrition and Preterm Delivery

Theresa O. Scholl and Xinhua Chen

Key Points

The rate of preterm delivery in the USA is one of the highest in the developed world. Research on the influence of maternal nutritional status and diet suggests that the following may be associated with this increased risk:

- Maternal overweight/obesity and underweight
- Excessive or inadequate weight gain during pregnancy
- Low energy intake, fasting, and long intervals (>12 h) between meals
- Maternal anemia and iron deficiency anemia early in pregnancy
- Low intake of iron, folate, omega 3 fatty acids, calcium, and zinc
- Poor vitamin D status
- High maternal homocysteine
- Lack of prenatal vitamin/mineral supplementation
- High levels of triglycerides, cholesterol, free fatty acids, and markers of endothelial dysfunction

Keywords Nutrition • Pregnancy • Iron • Folate • Zinc • Lipids • Multivitamins • Preterm delivery

Introduction

The USA has one of the highest rates of preterm birth (<37 weeks gestation) in the developed world. The zenith was reached in 2006 with a rate of 12.6 %, an increase of more that 15–30 % compared to prior years (9.7 % in 1990, 11.0 % in 2005). The rate has decreased since then and by the year 2012 amounted to 11.55 % [1].

Preterm delivery is an important public health issue. Disorders related to short gestation and low birth weight are the leading cause of neonatal mortality (<28 days) and rank second only to congenital defects as the leading cause of death during the first year (infant mortality) [2]. In addition to an increased mortality, infants delivered preterm are at greater immediate risk of life-threatening complications (e.g., respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis). While neonatal intensive care and the use of corticosteroids and surfactants have increased survivorship, in the longer-term such children remain at increased risk of serious disorders (e.g., seizures, cerebral palsy, and mental retardation) and experience persistent learning and behavioral deficits [3–5]. Recent comparisons to family members (siblings, cousins)

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_33

suggest that those born preterm have more social and medical disability: autism, attention deficit hyperactivity disorder, and substance use, and criminal conviction [5]. In later life preterm delivery is associated with heart abnormalities (smaller heart, thicker walls) and increased risks of chronic disease including cardiovascular disease and type 2 diabetes [6, 7].

There is no way to prevent preterm delivery that is universally or consistently effective. The few interventions that at times reduce risk are not applicable to most women or all populations. For example, while treatment with antibiotics eradicates bacterial vaginosis, an important risk factor, antibiotics do not consistently reduce and in some cases will increase preterm delivery rates [8, 9]. While 17 alpha-hydroxyprogesterone caproate has been shown to reduce risk, its efficacy is limited to those women with short cervical length or a prior history of delivering before term [10, 11]. Thus, it is important to prevent preterm delivery and to do that it is necessary to understand its etiology. This review focuses on the influence that maternal nutrition and maternal nutritional status have on that risk.

Risk Factors Associated with Preterm Delivery

One problem hindering the identification of risk factors is the heterogeneous nature of preterm delivery. The most common cause is a spontaneous preterm delivery from preterm labor or preterm premature rupture of the fetal membranes (PROM). In addition, there are numerous complications like preeclampsia, placental abruption, fetal distress, or fetal growth restriction that lead to an indicated preterm birth [12]. Each of the proximate causes may have separate risk factors. Consequently, only a fraction of the factors and exposures that give rise to preterm delivery have been identified. These include a prior history of preterm delivery, by far the strongest risk factor—but also inflammation and infection, a multiple pregnancy, preeclampsia or gestational hypertension, a short cervical length along with social characteristics of the mother—being poor, African American, a cigarette smoker, and at the extremes of maternal age [3]. Of the risk factors that are recognized, several suggest the importance of maternal diet and nutritional status (maternal underweight and overweight, inadequate weight gain during pregnancy, anemia) [3]. Metabolic constraint on mother and fetus—the "Energetics of Gestation and Growth" (EGG hypothesis)—has been proposed as a determining factor. Gestation duration is hypothesized to be a hormonal response to fetal energy demands that exceed the maternal supply of glucose, amino acids, and other nutrients for continued fetal growth and development [13].

Maternal Weight and Weight Gain

Most research on maternal nutritional status focused on the relationship between pregravid weight, total weight gain, and birth weight, and this body of evidence has been extensively reviewed by the Institute of Medicine (IOM) [14]. Studies have been virtually unanimous in showing an inverse relationship between pregravid weight or BMI and weight gain in that heavier women, particularly those who are obese, generally gain less weight when pregnant than women who are lighter. Pregravid weight and weight gain have independent and additive effects on fetal growth. The average magnitude of this effect on the fetus for women with a normal weight-for-height is approximately 16.6–22.6 g birth weight/k total gain [15]. Pregravid weight-for-height is a strong effect modifier; risk of fetal small for gestational age (SGA) is increased and large for gestational age (LGA) decreased with low weight gain in women with low to normal BMI [14, 16]. Among overweight and obese women LGA is increased with excessive gestational weight gain whereas SGA varies only a little [16]. Limiting weight gain diminishes risk of an excessive gain among normal weight women and increases the proportion of normal weight, overweight, and obese women returning to pregravid weight by 6 months postpartum but it also decreases infant birth weight by approximately 100 g [14, 17].

Maternal underweight and overweight/obesity are both associated with an increased risk of preterm delivery [15, 18–20]. The likelihood of delivering preterm is greater when a low weight gain occurs is an underweight woman; in one study this amounted to a threefold increase of delivering moderately preterm (32 to <37 weeks) and nearly a tenfold increase for delivering very preterm (20 to <32 weeks) [20]. Although the finding of an association between low pregravid maternal weight/BMI and preterm delivery is fairly consistent, the extent to which it represents a size bias in the estimation of gestation by ultrasound or the extent to which gestational weight gain and diet during pregnancy can overcome the deficit is not well known.

The pattern of weight gain and rate of gain also appear to be important [21, 22]. In pregnant adolescents from Camden, an early inadequate weight gain increased the risk of SGA births [21]. Preterm delivery was increased with inadequate weight gain late in pregnancy, even when the total pregnancy weight gain never fell below the targets set in clinical standards. Studies of adults also have shown that low rates of weight gain, usually in the latter half of pregnancy, are associated with preterm delivery [20–24].

An excessive rate of weight gain and maternal obesity [14, 18] also increase risk of preterm delivery. In the case of excessive gain it has been speculated that the association is a function of late edema from preeclampsia or pregnancy related hypertension [18, 20]. While obese women also experience increased preterm delivery, this may reflect an excess of indicated deliveries associated with obesity related complications like gestational diabetes, preeclampsia, and hypertension [18, 25, 26].

Carmichael and Abrams [24] reported that 11/13 studies showed a significant association between maternal weight gain and preterm delivery, principally when gestational weight gain was inadequate. Gestational weight gain in later pregnancy was consistently associated with increased rates of preterm delivery while inadequate gain in early pregnancy was not.

Diet and Gestational Weight Gain

One reason that maternal weight gain during pregnancy and gestation duration is linked may be the maternal diet. Although this association appears to be a reasonable one, a link between diet and gestational gain has not often been described.

The first report was made by Thomson [27], who found a correlation of 0.30 between energy intake and weight gain in Scottish primigravidae eating “to appetite.” Among Camden gravidae, a significantly lower energy intake (approximately 150–300 kcal/day less) was associated with an inadequate gestational gain [28]. When asked a series of behavioral questions about changes in food intake during pregnancy consuming “a lot less food” was related to weight gain below IOM guidelines whereas maintaining usual diet was associated with an appropriate gestational weight gain. Conversely, eating “much more food” correlated with an increased risk of an excessive weight gain as did little or no physical activity [29, 30]. All told, several reports have related gestational weight gain to energy intake, the glycemic index, and to consumption of various amounts of a variety of foods [14].

Diet and Preterm Delivery

The relationship between poor diet and inadequate gestational weight gain as well as the observation that a low pregravid weight (or BMI) and low rate of weight gain were each associated with preterm delivery suggests that a poor maternal diet could be a factor. In the African country of Gambia the food supply fluctuates between dry and rainy seasons. The duration of pregnancies conceived in the months when food is scarce and women are at their lowest weights was shorter than pregnancies

conceived when food was more plentiful [31]. During the Dutch Famine of 1944–1945, third trimester exposure to intense famine shortened gestation by about 4 days while exposure during the first trimester was associated with a clear excess of preterm birth. Famine-related amenorrhea, which would have made gestational dating insecure, may have underestimated risk [32].

Fasting during pregnancy may have effects analogous to famine and food shortage. In sheep a short interval of food deprivation around the time of conception—from 2 months before to the first month after conception—increased the rate of preterm birth. The nutritional restriction was brief, maternal weight reduced by only 15 %; restriction was followed by ad libitum feeding for the remainder of gestation [33]. Since the nutritional demands of the fetus early in gestation are modest it was unclear why preterm delivery had been triggered or what triggered it. The authors speculated that the shortage of an essential nutrient(s) was more likely than a lack of calories [34]. However, later in pregnancy a fast of 24–48 h also elicits labor and/or delivery. This occurs across species: ewes [35], mares [36], and primates [37]. In primates, maternal fasting reduces circulating glucose, increases free fatty acid production, raises fetal cortisol, and increases prostaglandin synthesis [37]. Fasting prompts a similar response in humans: abstaining during Yom Kippur (but not Ramadan) increases the likelihood of labor in women who are close to term [38, 39]; women in preterm labor were more likely to have detectable ketone bodies than controls [38, 39]. While this might mean that women eat less early in labor but it is also likely that the women are in "accelerated starvation" and metabolizing fat stores [40]. Periods of extended fasting—13 h or more during the second and third trimesters—are associated with increased levels of corticotropin releasing hormone (CRH) [41]. Higher CRH correlates with shorter gestation duration and an increased preterm delivery risk [42]. Thus, famine and fasting, perhaps in concert with low levels of maternal glucose and other circulating nutrients, potentially influence CRH production. An under-expanded plasma volume in poorly nourished pregnant women may be another underlying cause [43] and could explain why hydration can temporarily arrest preterm labor in some women.

Calories are not consumed independent of food and the diet as a whole contains many hundreds of nutrients. Thus, the type of diet that a woman eats may be related to her risk of preterm delivery. Swedish women eating a prudent diet—defined as one rich in fruits, vegetables, and whole grains—or one that is traditional—emphasizing lean fish and fish products, boiled potatoes, and low fat milk—have lower rates of preterm delivery [44]. Those with diets rich in probiotics (e.g., yogurt), or prebiotics and antimicrobials (e.g., onions and garlic) were also less likely to deliver preterm [45, 46]. Specific nutrients consistently associated with preterm delivery are discussed below.

Iron

Iron is as essential element in the production of hemoglobin for the transport of oxygen to tissues and in the synthesis of enzymes that are required to use oxygen for the production of cellular energy. Supplementation with iron is generally recommended during pregnancy to meet the energy needs of both mother and rapidly growing fetus. Anemia (low hemoglobin levels) and iron deficiency anemia (IDA) sometimes serve as indicators of overall poor maternal nutritional status during pregnancy. When overall dietary intake is inadequate, anemia is one of the most obvious symptoms. Not all anemia is nutritional in origin—some arises from infection or as a consequence of chronic disease.

When detected early in pregnancy, IDA is indeed related to diet—associated with a lower energy and iron intake, an inadequate gestational weight gain over the whole of pregnancy, as well as with a greater than twofold increase in the risk of preterm delivery [47, 48]. Maternal anemia before mid-pregnancy is also associated with an increased risk of preterm birth [49–51]. During the third trimester, anemia may be a good prognostic sign reflecting expansion of the maternal plasma volume. Late anemia often is associated with a decreased rate of preterm birth rather than an increased risk [48, 51].

Scanlon and colleagues used data from Pregnancy Nutritional Surveillance to examine the relationship between maternal anemia and preterm delivery in 173,031 low-income gravidas [51]. Preterm delivery was increased for anemic women and women with low hemoglobin during the first or second trimester. For women with moderate to severe anemia, risk was approximately doubled, for the others risk of preterm delivery was increased between 10 and 40 %. During the third trimester the association reversed—anemia and low hemoglobin were each associated with a decreased risk of preterm birth [51]. The association of maternal anemia in early pregnancy with preterm delivery and other poor outcomes has been confirmed in recent studies mostly from the developing world [50–55]. For example, data from more than 70,000 Korean women obtained from before pregnancy showed that approximately 12 % of the women were anemic [53]. The effect of pregravid anemia depended upon its severity and during gestation severe anemia (hemoglobin <100 g/L) was associated with a 50 % increase in risk of preterm delivery. A meta-analysis of cohort studies showed a 20 % increase in risk of preterm birth with anemia in the first or second trimester [52].

The increased risk of preterm delivery may be specific to iron-deficiency anemia, and not anemia from other causes. Scholl [47] reported data on 755 pregnant women receiving initial antenatal care at 16.7 ± 5.4 weeks gestation in Camden, New Jersey. Serum ferritin (<12 μg/L) was used to characterize IDA. While anemia based upon low hemoglobin was 27.9 % at the initial antenatal visit, prevalence of IDA (anemia with serum ferritin concentrations <12.0 μg/L) was lower, amounting to 3.5 %. After controlling for confounding variables, the risk for preterm delivery increased more than twofold for women with IDA in early pregnancy, while anemia from other causes were not associated with any increased risk for preterm delivery. Consistent data were obtained from Papua, New Guinea. Severe anemia (<80 g/L) in early pregnancy attributed to iron deficiency, risk of low birth weight (<2500 g) was increased approximately sixfold in primiparae. Risk was not increased with anemia diagnosed at delivery [56].

Thus, it seems reasonable to presume that some but not all of what seems to be anemia or IDA is caused by the expansion of the maternal plasma volume. At present this state is poorly differentiated from anemia or iron-deficiency anemia late in pregnancy but is easier to distinguish during the first or second trimester.

The relationship between maternal hemoglobin and pregnancy outcome is said to be U shaped [57, 58] with low hemoglobin reflecting a mix of true and physiologic anemia and high hemoglobin reflecting failure of the plasma volume to expand (Table 33.1). High maternal hemoglobin is consistently associated with adverse outcomes—gestational hypertension, preeclampsia, and diabetes, which are underlying causes for an indicated preterm delivery [59]. Increased levels of the iron storage protein ferritin also are related to preterm delivery but likely via chronic inflammatory changes reflecting maternal infection (clinical chorioamnionitis and infant sepsis) [59–61]. Mechanisms whereby maternal anemia and iron deficiency might influence the outcome of pregnancy include chronic hypoxia that initiates a stress response, increased oxidative stress that damages the maternal–fetal unit and reduced immune function from maternal infection [62].

Despite the long-standing connection between maternal anemia and preterm delivery it is uncertain if adverse pregnancy outcomes can be prevented by supplementing pregnant women with iron. A Cochrane review reported that iron/folic acid supplementation resulted in significantly heavier infants (+58 g) compared to placebo or other regimens with no difference in preterm delivery, SGA, or low birth weight infants [59]. Another meta-analysis [63] reported a 25 % reduction in risk of low birth weight, increased gestation duration (+0.23 weeks), and reduced rate of very preterm delivery <34 weeks (0.98 % vs. 1.8 %) after including a study from rural China [64]. Thus, supplementation may more effectively reduce risk of adverse outcomes when a high proportion of women are iron deficient or anemic. It is also plausible that duration of the supplement is a limiting factor and may need to be started early in pregnancy if not beforehand and continued throughout the reproductive years in order to reduce risk [59, 65].

Table 33.1 Maternal nutrition and preterm delivery, study characteristics

Nutrient	Reference	Study design, size, and setting	Age (year)	SES	Race/ethnicity	Findings
Iron/anemia	Klebanoff et al. [49]	Case–control study of 1706 (725 preterm, 981 term) deliveries from Kaiser Permanente Births Defects Study, Hct levels measured throughout gestation	–	–	14 % Asian 28 % Black 21 % Mexican 37 % White	Hct values fell during early second trimester and began increase again at 31–33 weeks Moderate relationship between second trimester anemia (Hct <10th percentile for ethnicity and gestation) and preterm delivery
	Zhou et al. [50]	Observational study of 829 women from Shanghai. Population homogeneous for race, parity, prenatal care, smoking. Hgb sampled at 4–8 weeks, 16–20 and 28–32 weeks gestation	27.7 ± 4.7 years	–	100 % Chinese	Inverse U-shaped relationship between maternal Hgb at 4–8 weeks and preterm delivery and low birth weight (LBW). Odds of preterm delivery increased 2.5-fold for women with Hgb > 130 g/L or between 90 and 99 g/L. Odds increased 3.5-fold for entry Hgb between 60 and 89 g/L
	Scanlon et al. [51]	Retrospective analysis of data from 173,631 pregnant WIC participants	70 % 20–35 years	Low	55 % White 20 % Black 19 % Hispanic 6 % Other	Risk of preterm delivery increased with low Hgb in first and second trimester. SGA not associated with anemia. Odds of preterm delivery with first trimester severe anemia increased 1.7×
	Yi et al. [53]	Retrospective analysis of 70,895 women with hemoglobin measured before pregnancy	57 % 25–29 years	Mixed	100 % Asian	Risk of preterm depended upon degree of anemia. For severe anemia (<100 g/L) risk increased 1.5-fold. Infant LBW and SGA also increased with anemia
	Garn et al. [57]	Observational study of over 50,000 women followed in the National Collaborative Perinatal Project (NCPP), lowest pregnancy values of hemoglobin (Hgb)	–	Mixed	Whites and Blacks	Maternal Hgb and Hct levels had "U-shaped" relationship to preterm delivery (≤37 weeks), with the risk being higher at Hgb <100 g/L and >120 g/L. and hematocrit (Hct). The increased risk with Hgb >120 indicated a failure of plasma-volume expansion
	Murphy et al. [58]	Observational study of nearly 55,000 women in the Cardiff Births Survey, Hgb levels ascertained at entry to prenatal care	–	Mixed	Welsh	Preterm delivery (<37 completed weeks) showed a "U-shaped" relationship to Hgb levels. At <13 weeks, risk was increased ~50 % with Hgb <104 g/L; at week 13–19 risk not increased; at week 20–24, risk was increased for low (104 g/L) and high (>145 g/L) Hgb > 50 %
Iron/iron-deficiency anemia	Scholl et al. [47, 48]	Observational study of iron-deficiency anemia (IDA, anemia with serum ferritin <12 μg/L) among 779 Camden, NJ women	18.4 ± 3.7 years	78 % low	66 % Black	IDA (12.5 % of all anemia) was associated with lower energy and iron intakes early in pregnancy. The risk or preterm delivery increased >2× with IDA, but was not with anemia from other causes

Iron/ferritin	Scholl [60]	Observational study of serum ferritin (15 and 28 weeks) in 1162 gravidas from the Camden Study	12–29 years	100 % low	33.3 % Hispanic 57.3 % Black 9.4 % White	At week 28 high concentrations of ferritin (>90th percentile) from failure of ferritin to fall with gestation, associated with ninefold increase in very preterm delivery, fourfold increase in preterm delivery and fivefold increase in LBW ($p<0.01$ for each)
	Goldenberg et al. [61]	Observational study of serum ferritin at (19, 26, and 36 weeks) and preterm delivery in 580 gravidas who participated in a randomized study of zinc supplementation	–	100 % low	100 % Black	Ferritin in highest quartile at week 28 associated with twofold increase in odds of preterm delivery (<37 weeks) and infant LBW, threefold increase in odds of very preterm delivery (≤32 weeks) and a fourfold increase in birth weight ≤1500 g
Zinc	Scholl et al. [28]	Observational study of dietary zinc intake in 818 Camden, NJ pregnant women	19.0 ± 4.1 years	Low	60 % Black	Low dietary zinc intake associated with twice greater preterm delivery (<37 weeks) and infant LBW, 3–4 times greater very early preterm delivery (<33 weeks) ($p<0.05$ for each). Risk or preterm delivery with PROM increased 3.5 times with low zinc ($p<0.05$).
	Neggers et al. [67]	Observational study of 476 Alabama women	21.8 ± 4.9 (12–42) years	Low	76.5 % Black	Low plasma zinc at entry to prenatal care (7.0–12.2 μmol/L) increased risk of infant LBW 8× compared with women in the highest quartile (15.9–25.4 μmol/L, $p<0.05$). Plasma zinc was linearly related to gestation duration (0.17 week/μmol/L zinc, $p<0.05$)
	Cherry et al. [71]	Randomized, controlled trial of zinc supplementation among pregnant New Orleans teenagers, assigned to placebo ($N=556$) or 30 mg zinc supplement ($N=581$)	17.6 (13.5–19.6) years	Low	95 % Black	With stratification by maternal weight at delivery, zinc supplementation was related to a lower risk of preterm delivery (38 weeks) among normal weight women (18.3 % vs. 28 %, $p<0.05$)
						Zinc supplemented low weight multiparas had increased gestation duration (+1.2 weeks, $p<0.008$). There was a tendency ($p<0.15$) for weight gain rates to be greater among zinc supplemented women (0.52 kg/week) compared with placebo (0.42 kg/week)
	Goldenberg et al. [72]	Randomized, controlled trial of zinc supplementation among pregnant Alabama women with plasma zinc below median at entry to prenatal care, assigned to multivitamin tablet with additional 25 mg zinc ($N=294$) or placebo ($N=286$)	23.5 ± 5.5 years	Low	100 % Black	Birth weight increased with zinc supplementation by +126 g ($p<0.05$) and gestation duration by 0.5 week ($p=0.06$). Women with pregravid BMI <26 had a birth weight increase +248 g ($p<0.005$) and an increase in gestation of +0.7 week ($p=0.08$)
	Osendarp et al. [73]	Randomized controlled trial of zinc supplementation (30 mg$N=269$) vs. placebo ($N=290$) from 12 to 16 weeks gestation until delivery	23.5 ± 5.0 years	Low	100 % Bangladeshi	No significant effect of zinc on preterm delivery or LBW

(continued)

Table 33.1 (continued)

Nutrient	Reference	Study design, size, and setting	Age (year)	SES	Race/ethnicity	Findings
Folic acid/folate	Shaw [80]	Observational study before vs. after folate food fortification in 5.9 million California births, 1990–2000	–	Mixed	–	Unadjusted analyses showed no change in % preterm or LBW. Adjusted data (age, parity, ethnicity, education, year, fortification period). Showed reductions in risk of preterm (–4 %), LBW (–6 %) VLBW (–9.8 %)
	Scholl et al. [81]	Observational study of folate from diet and supplements and serum folate in Camden, NJ ($N=832$)	18.8 ± 5.0 years	Low	67 % Black	Women with low folate intake (≤240 μg/day) had three times greater preterm delivery and infant LBW ($p<0.05$). Risk of preterm delivery without PROM increased 3 times ($p<0.05$). Odds of preterm delivery increased 1.5 % per unit decrease in serum folate ($p<0.05$)
	Siega-Riz [83]	Observational study of folate from diet and supplements and serum, RBC folate in North Carolina 1995–2000	–	Mixed	40 % Black 54 % White	Women with low folate intake (≤500 μg/day) in second trimester 1.8× more preterm deliveries <37 weeks. Odds ratios for preterm <37 weeks 1.8 with low serum folate (<16.3 ng/mL) and low RBC folate (<626.6 ng/mL)
Folic acid	Bukowski et al. [86]	34,480 low risk pregnant women with preconceptional folate	28–32 years	Mixed	68 % White 22 % Hispanic 10 % Black or other	Preconceptional folate use >1 year decreased risk of preterm delivery 20–28 weeks by 70 % and 50 % between 28 and 32 weeks; risk of delivery >32 weeks not altered
	Li et al. [88]	Prospective cohort study of periconceptional folic acid use	24–25.5 years	Low	100 % Asian	Folic acid use associated with 14 % reduction in preterm delivery. Risk reduction strongest for spontaneous preterm delivery (23 % reduction in risk)
Folic acid/ homocysteine	Vollset et al. [98]	Retrospective cohort analysis of 5883 women linking current homocysteine to past pregnancy outcomes	40–42 years	–	Norwegian	Current homocysteine (highest quartile) associated with past history of adverse pregnancy outcome including preeclampsia, preterm delivery, LBW, stillbirth, neural tube defects and club foot
	Ronnenberg et al. [99]	Case–control study of pre-pregnancy homocysteine, B vitamins (B_6, B_{12}, folate) and pregnancy outcome in 405 women from Anquin, China	24–27 years	Low	100 % Chinese	Risk of preterm delivery (<37 weeks) increased 3.6× for women with elevated homocysteine (≥12.4 μ md/L). Preterm delivery, 50–60 % lower for women with non-deficient levels of B_{12} and B_6. Serum folate not associated with preterm delivery
	Malinow [101]	Observational study of circulating serum homocysteine and serum folate in 35 healthy nulliparous gravidas at delivery	24.2 ± 5.9 years	–	American	Maternal homocysteine correlated inversely ($p<0.05$) with gestation duration ($r=-0.42$) serum folate correlated positively ($p<0.05$) with gestation duration ($r=0.23$). Maternal folate and homocysteine correlated ($r=0.54$) with each other ($p<0.05$). Maternal serum B_{12} was not related to gestation duration ($r=0.08$, $p=0.67$)
	Bergen et al. [103]	Prospective cohort study of 5805 women from the Netherlands	29.8 years	Mid	60 % White	Lowest folate quintile associated with twofold increase in preterm delivery and lower birth weight, lower placental weight and increased pre-eclampsia risk

Calcium	Belizan et al. [108]	Randomized, controlled trial of calcium supplementation among 1194 gravidas in Rosario, Argentina	23.7 ± 5.5 years	Mixed	Argentinian	Percent with preterm delivery (<37 weeks) among 579 calcium-supplemented women 6.3 vs. 6.8 % for 588 women receiving placebo
	Lopez-Jaramillo et al. [113, 114]	Randomized, controlled trial of calcium supplementation among 56 nulliparous clinic patients with overall low calcium intake at risk for pregnancy-induced hypertension in Quito, Ecuador	19.4 ± 1.8 years	Low	Ecuadorian	Gestation duration longer for 22 supplemented women (2 g calcium/day) vs. placebo group ($N = 34$). Gestation 39.2 ± 1.2 week for calcium supplemented, 37.4 ± 2.3 week for placebo ($p < 0.01$)
	Bucher et al. [116]	Medline and Embase searches for randomized trials of calcium and preeclampsia during pregnancy (2459 subjects enrolled in 14 trials) for meta-analysis	–	Mixed	–	The summary odds ratio for preterm delivery was 0.69 (95 % CI 0.48–1.01) and not statistically significant. All the trials which included preterm delivery showed a reduced risk with calcium supplementation during pregnancy only one was statistically significant
	Levine et al. [117]	Randomized trial of calcium supplementation (500 mg/day) or placebo in 4589 healthy nulliparous at 13–21 weeks gestation. Eligible women underwent test of compliance before enrollment in trial	21 ± 4 years	Mixed	35 % White 17 % Hispanic 47 % Black 2 % Other	Calcium supplemented gravidas delivered at 38.9 ± 2.5 weeks vs. 38.9 ± 2.4 weeks for placebo (NS). Preterm delivery (<37 weeks) and very preterm delivery (<34 weeks) occurred in 10.8 % and 4.2 % of supplemented vs. 10.0 % and 3.7 % of controls (NS). LBW (<2500 g) was 8.8 % in supplemented, 9.6 % in controls (NS)
	Crowther et al. [118]	Randomized trial of calcium supplementation (1.8 g/day) or placebo in 456 nulliparous with singleton pregnancies	25 ± 5 years	Mixed	Australian	Treatment with calcium reduced risk of preeclampsia and preterm birth with more than twofold reduction in risk for each
	Villar et al. [119]	Randomized trial of calcium supplements (1.5 g/day) vs. placebo in 8325 nulliparae <20 weeks at entry from populations w low calcium intake (<600 mg/day)	22.6 ± 4.4 years	Mixed	–	Calcium supplemented gravidae <20 years had fewer preterm delivery <37 weeks (OR = 0.82) and very preterm deliveries <34 weeks (OR = 0.64). No effect on preeclampsia but reduced risk of eclampsia (OR = 0.68) and severe gestational hypertension (OR = 0.71), severe preeclamptic complications (OR = 0.76) and severe maternal morbidity/mortality (OR = 0.80) with calcium for women, all ages
	Bodnar et al. [130, 131]	Maternal vitamin D status as 25 hydroxyvitamin D (25(OH)D) in (1) Singleton pregnancies from Project (CPP $N = 767$ cases) and (2) Twin gestations from a multicenter US trial ($N = 211$)	(1) 20–29 years (53 %) (2) 30+ years (52 %)	Low Mid	(1) CPP: 28 % White 72 % Non-White (2) Twins: 62 % White	CPP data (singletons) showed decreasing risk of preterm delivery from the (20–30 %) with the US Collaborative Perinatal increasing vitamin D only in non-white women. In twin gestations low vitamin D (<75 nmol/L) increased preterm risk by 60 %
	Schneuer et al. [135]	Prospective cohort study of 25(OH)D at 10–14 weeks gestation in 5109 women in New South Wales	>30 years (68 %)	Mixed	75 % Australia New Zealand, Europe	Low 25(OH)D in first trimester not associated with preterm delivery, LBW, SGA, or preeclampsia

(continued)

Table 33.1 (continued)

Nutrient	Reference	Study design, size, and setting	Age (year)	SES	Race/ethnicity	Findings
Fish oils (*n*-3 fatty acids)	Olsen et al. [141]	Randomized, controlled trial of supplementation with fish oil, 533 women assigned 2/1/1 to fish oil, olive oil, or no oil supplementation, Aarhus, Denmark	29.4 ± 4.4 years	–	Danish	Mean gestation duration longer ($p < 0.006$) for the 266 women receiving fish oil (283.3 ± 11.1 days) compared with olive oil (279.4 ± 13.1 days) or no oil groups (281.7 ± 11.6 days)
	Harper et al. [144]	Randomized controlled trial of 852 women with prior preterm delivery assigned to Omega 3 fatty acids (1200 mg/EPA and 800 mg/day DHA) or placebo. All received 250 mg 17 hydroxyprogesterone caproate	27.5 years	Mixed	57 % White 34 % Black	Omega 3 fatty acids did not reduce preterm recurrence
	Makrides et al. [145]	Cochrane review of randomized trials of fish oil/prostaglandin precursor supplements during pregnancy (>2700 subjects enrolled in 6 trials) for meta-analysis	–	Mixed	–	No decrease in preterm delivery but very preterm delivery was reduced (0.69, 95 % CI 0.49–0.99) and gestation duration increased by 2.5 days ($p < 0.05$). No reductions in LBW, SGA or other outcomes
Multivitamin/ minerals	Scholl et al. [154]	Observational study of 1430 gravidas from the Camden Study who entered care during trimesters 1 and 2. Prenatal supplement users compared to gravidas entering care in same time frame who did not use supplements	12–29 years	Low	58.9 % Black	Prenatal supplement use associated with two- to fourfold reduction in odds of preterm delivery and very preterm delivery ($p < 0.001$). Significant reductions in odds of very LBW (six- to sevenfold) and infant LBW (twofold) ($p < 0.001$). Prenatal supplement use corroborated by assay of circulating micronutrients at 15 and 28 weeks ($p < 0.05$)
	Catov [155]	Observational study of 1832 gravidae and periconceptional multivitamin use within 6 months of first prenatal visit (9.9 ± 3.9 weeks)	25 ± 5.5 years	–	35 % Black	Periconceptional prenatal supplement use associated with threefold reduction in odds of preterm delivery (<34 weeks) all women and 85 % reduction in SGA (<5th percentile) non-obese women. No change in preterm delivery 34 to <37 weeks or SGA 5th to <10th percentiles
	Fawzi et al. [157]	Randomized study of 1075 HIV-1 infected gravidas from Tanzania randomly assigned to placebo, vitamin A, or multivitamins in a 2 × 2 factorial design	–	Low	Tanzanian	Women receiving multivitamins had significantly decreased ($p < 0.01$) infant LBW by (44 %), very preterm birth (39 %) and fetal growth restriction (43 %) CD4, CD8 and CD3 counts were significantly increased ($p < 0.05$). Vitamin A had no effect on outcomes
	Fawzi et al. [158]	Randomized study of 8468 HIV negative gravidae from Tanzania randomly assigned to multivitamins or placebo all received iron (60 mg) plus folate (0.25 mg)	–	Low	Tanzanian	Women receiving multivitamins had significantly decreased ($p < 0.01$) infant LBW by (20 %) and SGA by 27 %. Gestation duration increased by +0.2 week ($p = 0.02$) but preterm birth unchanged

	Christian et al. [159]	Cluster randomized controlled trial of 426 rural communities in SE Nepal. Communities randomized to five regimes of supplementation vs. Vitamin A control	55 % 20–29 years	Low	Nepalese	None of the supplements reduced risk of preterm delivery. In comparison to Vitamin A control, multiple micronutrients increased birth weight (+64 g) and reduced LBW by 14 %. Folic acid plus iron also increased birth weight (+37 g, NS) and reduced LBW by 16 %
	Ramakrishnan et al. [160]	Randomized trial of multi-vitamin supplements vs. iron	23 ± 5 years	Low	Mexican	Multivitamin and iron supplements did not yield differences in birthweight, gestation duration, LBW, IUGR or ponderal index
	Liu et al. [162]	Randomized trial of 18,775 N Chinese women with mild or no anemia. Folic acid, folic acid + iron, Folic acid + iron + 13 micronutrients from 20 weeks gestation to delivery	23.7 ± 5.0 years	Mixed	100 % Asian	Received folic acid alone (400 mg, control), folic acid + iron (30 mg). Preterm delivery, perinatal mortality and other adverse outcomes not reduced compared to folic acid control
	Zeng et al. [163]	Cluster randomized study of rural NW Chinese women received folic acid alone (control), folic acid + iron or 15 micronutrients (MMN)	25 ± 5.0 years	Mixed	100 % Asian	Women from poorest households given folic acid + iron had reduced preterm delivery by 45 % and early neonatal mortality by 90 % compared to folic acid alone. MMN also reduced risk of LBW and early neonatal death among poor women compared to folic acid alone
Dyslipidemia	Scholl et al. [174]	Prospective study of CRP in Camden women with normal glucose tolerance with (N=520)	22 ± 5.0 years	Low	52 % Hispanic 43 % Black	High CRP related to diet in lean women BMI <25 (increased protein, cholesterol, dietary glycemic index, less carbohydrate). High CRP increased early preterm delivery risk (<34 weeks) 3.8-fold and doubled risk of preeclampsia
	Khoury et al. [175]	Randomized trial (N=290) low risk gravidae, nonsmokers with no prior pregnancy complications assigned to usual (Control) or cholesterol lowering diet (Intervention)	21–38 years	–	100 % White	Diet lowered total and LDL cholesterol, cord lipids NS. Preterm delivery <37 weeks reduced significantly from 7.4 % (controls) vs. 0.7 % (intervention)
	Chen, Scholl [176]	Observational study of fasting free fatty acids at week 30 in Camden (N=523)	22.5 ± 5 years	–	53 % Hispanic 34 % Black	Women with high FFAs (>432 μM) had 3× increased risk for preterm delivery <37 weeks

While clinical trials have shown almost uniformly that iron supplementation results in a reduction in anemia they also have raised the possibility that giving too much iron to non-anemic women, i.e., daily administration of 50–60 mg/day or more, can increase blood viscosity and associated poor outcomes [59, 65]. Supplementation of women with poor iron stores thus may be preferable to routine supplementation. Iron is potentially toxic and could expose women who are not deficient to high levels of oxidative stress with no benefit for mother or fetus [59].

Micronutrients

During pregnancy, low intakes of two micronutrients, zinc and folate, are associated with an increased risk of preterm delivery. Zinc is an element involved either directly as a metalloenzyme in the production of enzymes that include DNA and RNA polymerase or as a catalyst in the synthesis of other enzyme. Folic acid functions as a coenzyme in the transfer of single carbon atoms to intermediates in the synthesis of amino acids and nucleic acids. While many other nutrients in addition to these two would be limited in a marginal maternal diet, inadequate intake of either zinc, folate, or both potentially leads to impaired cell division and alterations in protein synthesis. Such alterations are most notable and have the greatest potential to do harm during times of rapid tissue growth, such as pregnancy.

Zinc

Studies of circulating levels of zinc or dietary zinc intake [28, 66–68] have often suggested that higher intakes or greater circulating concentrations of zinc are associated with improved pregnancy outcomes including preterm birth (Table 33.1). Clinical trials have yielded equivocal results often focusing on entire groups of low-income women where the mean zinc intake is below the RDA for pregnancy, an approach that selects a population, as opposed to individuals, at risk. Two recent meta analyses of clinical trials involving approximately 11,000–15,000 pregnant women each showed a small but significant (14 %) reduction in risk of preterm delivery when zinc supplemented women, most from low income populations, were compared to women on placebo [69, 70]. There was a no accompanying increase in infant birth weight or improvement in other neonatal outcomes, no decrease in maternal complications such as preeclampsia or preterm premature rupture of membranes (PPROM) among the zinc supplemented women.

In two trials effects of zinc were conditional on maternal weight [71, 72] with a lower rate of preterm delivery evidenced in zinc-supplemented women who were not overweight (Table 33.1). Cherry et al. [71] reported the frequency of preterm delivery to be reduced in the zinc treated normal-weight women; treatment of underweight multiparous women also was associated with a gestational age increase of nearly 3 weeks.

The trial conducted by Goldenberg and colleagues [72] recruited women with plasma zinc levels below the median and randomly assigned them to zinc or placebo. When stratified by body mass index, zinc supplementation increased gestation duration of approximately half a week ($p = 0.06$) and increased birth weight along with the duration of gestation. Women with a BMI <26 benefited most with a 248 g increase in infant birth weight and infants with a larger head circumference. Thus, consistent with prior results, effects were increased for women with lower pregravid body mass index (Table 33.1). In contrast, two recent trials from the developing world (Peru, Bangladesh) where one might suppose that zinc deficiency would be prevalent, were negative [73, 74].

Low plasma zinc has a number of potential causes. In addition to its effect on protein synthesis zinc also has an antiseptic action. In theory, a low zinc intake could be associated with preterm delivery by increasing risk of infection during pregnancy leading to fragile fetal membranes or by altering hormones related to labor onset (progesterone and prolactin) [70]. Conversely, a low plasma zinc level could be caused by a secondary deficiency—an acute phase response to a stressor such as maternal infection—rather than a primary deficiency (dietary). It is also likely that low income gravidas from the developing have multiple nutritional deficiencies that reduce the bioavailability of zinc. UNICEF is promoting use of multiple micronutrients during pregnancy which include zinc as opposed to supplementation with zinc alone. Several of the trials included in the meta-analyses discussed above contained studies where women were supplemented with zinc in combination with iron, folate, and other vitamins [69].

Folate

During gestation marginal maternal folate nutriture impairs cellular growth and replication of the fetus and the placenta which could harm placentation and increase the risk of preterm delivery [75]. Pregnant women living under circumstances where preterm delivery is common have been reported to consume diets with a lower density of vitamins and minerals, including folate and to limit consumption of folate-containing dietary supplements [76, 77]. The US food supply was fortified with folic acid in 1998 in order to reduce risk of neural tube defects for women in their reproductive years. Fortification of flour and cereals with folic acid resulted in a 19 % decline in infants with neural tube defects, increased serum and red cell folate, and decreased homocysteine levels [78, 79]. Rates of preterm delivery, very low birth weight, and low birth weight also declined between 4 and 10 % in California before and after folic acid food fortification [80].

In Camden low dietary and circulating folate early in gestation tripled risk of low infant birth weight and preterm delivery and interacted with a deletion allele for a folate metabolizing enzyme dihydrofolate reductase (DHFR) that converts folic acid that is used in supplements and for fortification to the reduced folate forms used by cells. Presence of the DHFR deletion allele increased risk of preterm delivery threefold and when folate intake also was low, increased risk of preterm birth fivefold [81, 82]. Lower folate intake (<500 μg/day) at mid gestation was associated with approximately twofold increased risk of preterm delivery in North Carolina as were low levels of serum and RBC folate [83], and the folate metabolite 5-methyltetrafolate [84]. Others have reported positive correlations between maternal serum and red cell folate with birth weight or gestation duration [85].

Apart from reducing occurrence of congenital defects, the consequences of folic acid supplementation before and during pregnancy are unclear. Some studies have reported a reduction in risk for preterm delivery with pre-pregnancy use of folic acid [86–88]. Supplementation with folic acid (with and without multivitamins) for at least 1 year before pregnancy was associated with a 70 % reduction in risk of preterm delivery before week 28 and a 50 % reduction between 28 and 32 weeks in a large cohort of low risk women [86]. Hungarian primiparae who used folic acid without multivitamins around the time of conception had a rate of preterm delivery significantly below women who did not supplement (7.6 % vs. 11.8 %) [87]. Data from a large population based cohort from south China with showed a reduced risk of preterm birth (5.28 %) among periconceptual folic acid users (without multivitamins) compared to women who were not users (6.1 %); the relationship was strongest for spontaneous preterm delivery [88].

Recent meta-analyses of folic acid supplementation trials [89, 90] found overall effects on mean birth weight corresponding to a 2 % increase for each doubling of the supplement [89]. While neither preterm delivery nor gestation duration was altered only 3 of 31 trials examined either as an outcome [90].

One large randomized study, reported that a high dose of folic acid (5 mg/day) was associated with a 37 % reduction in risk of infant low birth weight [91]. Likewise, while supplementation with folic acid (with/without iron) was without effect on white South African women, the risk of bearing an infant weighing less than 1870 g was reduced fourfold among the Bantu participants, who subsisted primarily on maize porridge [92]. It should also be borne in mind that many of the investigators who did not find lower folate (circulating levels, diet, or supplements) to be related to preterm delivery or gestation duration did not confirm the mother's last menstrual period by ultrasound with resulting misclassification and loss of precision [84].

Folic acid may increase risk as well as give benefit; fortification has been associated with increased rates of colorectal cancer and cognitive decline [93, 94]. Supplementation was recently report to increased risk of preterm delivery when started more than 8 weeks before conception [95] although this may have been due to residual confounding since there was no adverse influence of supplementation starting after 8 weeks. Another consequence may be epigenetic modification of the fetal genome. Maternal supplementation with ≥400 μg folic acid/day was linked to increased methylation of IGF2 DMRs among infants [96] and increased methylation of H19 DMR but not IGF2 DMR in cord blood [97]. The long-term consequences of these epigenetic changes, if any, are not known.

Homocysteine

Hyperhomocysteinemia may result from a deficiency in folate (B9), B6, or B12. Genetic and environmental factors that increase the metabolic requirement for folate and other B vitamins give rise to high homocysteine, a risk factor for cardiovascular disease, and a known cause of vascular damage and endothelial dysfunction [75].

High maternal circulating homocysteine correlates with older age at delivery, higher cholesterol, less multivitamin usage, lifestyle factors (cigarette smoking and high coffee consumption), and a past reproductive history that included preeclampsia and preterm delivery [98]. In China, preconceptional homocysteine was associated with a substantially increased risk of preterm delivery as were low levels of B6 and B12 but not folate [99]. Others reported higher maternal plasma homocysteine measured before or during pregnancy in association with significantly lower infant birth weight [100, 101] and shorter gestation duration [101]. A longitudinal study, with repeated measurement of maternal homocysteine from before conception, reported that lower weight infants (~200 g) were born to mothers with higher homocysteine; whether or not this was attributable to shorter gestation duration is unclear [100]. Lower folate and higher homocysteine were observed in women delivering preterm compared to term controls; those delivering preterm with preeclampsia also had higher homocysteine [102]. Investigators from the Generation R study reported twofold increases in preterm delivery and preeclampsia, lower placental weight and infant birth weight with low folate; high homocysteine was associated with increased risks of preeclampsia and SGA infants [103]. That low folate was more regularly associated with increased placental vascular resistance during mid to late gestation than homocysteine might suggest that high homocysteine is a response to poor folate status [103].

In summary, while observational studies of folate and pregnancy suggest a potential benefit of folic acid supplementation during pregnancy—a decrease in serious complications and an improvement in birth weight and gestation, randomized trials indicate that routine folic acid supplementation is not uniformly beneficial. Some who are at risk from common genetic polymorphisms that alter folate metabolism or because of environmental factors associated with poor diet would seem to benefit the most. Homocysteine may prove to be a more sensitive indicator of risk than diet, serum, or red cell folate. It should also be borne in mind that folic acid fortification of the food supply may have had unknown consequences for the population including the fetus as it grows and develops.

Other Nutrients

Calcium

During pregnancy, there is an increased physiologic need for calcium. A full-term infant accretes about 30 g of calcium, primarily in the third trimester when the fetal skeleton is actively ossifying, and to meet this there is enhanced absorption of calcium from the maternal gut [104]. Diets low in calcium, both in general and especially during pregnancy, have been associated with high blood pressure possibly via increased parathyroid hormone (PTH) secretion [105]. High PTH increases intracellular calcium which heightens smooth muscle reactivity and the likelihood of preterm labor and delivery [106–108]. Calcium supplementation during pregnancy has been shown to lower maternal blood pressure levels [109] and increase uteroplacental blood flow [110].

Maternal growth and pregnancy often coincide and approximately half of all pregnant teenagers continue to grow while pregnant [111]. Bone ultrasound measures of the os calcis during pregnancy showed greater loss for growing teenage gravidas (−5.5 %) than for mature women (−1.9 %) [112]. Thus, in the case of an adolescent pregnancy, calcium may be limited by maternal diet, but simultaneously driven by the need to retain enough calcium to mineralize two skeletons.

Two calcium supplementation trials [113, 114] among high-risk women with very low intakes in Quito, Ecuador, and teenagers in Baltimore [115] showed promising results in decreasing preterm delivery (Table 33.1). On the other hand, a large calcium supplementation trial of over 1000 adult women from Argentina showed the expected decrease in the incidence of pregnancy induced hypertension (PIH), but no effect on preterm delivery [108]. A meta-analysis [116] of 14 randomized controlled trials of calcium supplementation involving several thousand gravidas showed significant reductions in systolic and diastolic blood pressure and preeclampsia with the administration of calcium salts. However, the analysis yielded no effect of calcium intake on preterm delivery or fetal growth restriction (Table 33.1).

A multicenter double-blind placebo controlled trial of more than 5000 low-risk nulliparous women supplemented with 2000 mg/day elemental calcium or placebo before mid-pregnancy was conducted by the NIH [117]. Calcium did not reduce risk of preeclampsia, and had no effect on obstetrical outcomes including preterm delivery. However, the women recruited into this trial had calcium intakes that were atypically high, averaging 1100 mg/day before supplementation. A trial of calcium supplementation in Australian gravidae ($N=456$) with apparently adequate dietary calcium intakes (median 1100–1200 g/day) and other characteristics very similar to those in the US study [117] reported a positive effect [118]. There was a greater than twofold reduction in the risk of preeclampsia and a reduction in the risk of preterm delivery from 10 % (placebo) to 4.4 % (calcium supplemented) with reductions in admissions for threatened preterm labor ($p=0.03$), and preterm premature rupture of membranes ($p=0.08$).

The WHO trial of supplemental calcium (1.5 g calcium/day) or placebo in gravidae with low calcium intake (<600 mg/day) showed no reduction in risk of preeclampsia [119]. However, there was a significant effect of calcium on preterm delivery in women <20 years where odds of preterm delivery were reduced by 22 % (10.6 % vs. 12.8 %) and very preterm delivery (<32 weeks gestation) by 56 % (2.4 % vs. 3.8 %). In women of all ages the reduction was small (6.9 % vs. 7.2 % preterm) and not statistically significant.

Two meta-analyses of calcium supplementation came to different conclusions. One meta-analysis, which included data from more than 15,000 women randomized to calcium or placebo, reported that calcium supplements reduced the risk of preterm delivery by ~30 % (RR = 0.76, 95 % CI 0.60–0.97) and by 50 % ($N=568$) for women at high risk for preeclampsia (RR = 0.45, 95 % CI 0.24–0.83) [120]. While the second analysis, which also included some trials where participants were not randomly allocated or the study placebo controlled, found a small reduction in risk that

was not statistically significant for preterm delivery (<37 weeks) and very preterm delivery (<34) with calcium supplementation [121].

Thus, it seems that the capacity of supplemental calcium to decrease risk may be confined largely to populations where maternal calcium metabolism is stressed. The vitamin D status of the mother may be an additional factor [105, 122]. When administered together, calcium and vitamin D have a larger effect than calcium alone on lowering systolic blood pressure and PTH [104]. When vitamin D is deficient even high calcium intakes may be inadequate to maintain maternal calcium metabolism [104, 122].

Vitamin D

Vitamin D is a pro-hormone that is synthesized mainly by the skin after sun exposure. While present naturally in few food (e.g., fatty fish, fish liver oils) vitamin D is used extensively in food fortification (e.g., fortified milk, breakfast cereals, orange juice) and included in many nutritional supplements [104]. Circulating levels of 25(OH)D are the best indicator of vitamin D status representing the sum of Vitamin D produced cutaneously and ingested from diet and supplements [104].

Vitamin D helps maintain calcium balance by regulating release of PTH to increase calcium absorption. The consequences of a too little calcium intake or poor vitamin D status during pregnancy include elevated PTH, generally regarded as a sign of stress to calcium metabolism [104, 122]. In Camden women when vitamin D was insufficient even a high calcium intake equivalent to the RDA for pregnancy was unable to maintain a normal concentration for PTH or to moderate the proportion with elevated PTH. Risk of abnormally elevated PTH was increased threefold when vitamin D was insufficient regardless of calcium intake and increased twofold among vitamin D sufficient women with very low calcium intakes [122].

Higher PTH leads to an increase in intracellular calcium which increases uterine and vascular smooth muscle reactivity and is associated with labor onset—both term and preterm [123, 124]. Belizan and colleagues hypothesized that calcium supplementation, by reducing PTH and intracellular calcium, might alter rates of preterm delivery in addition to reducing preeclampsia risk [124, 115]. However dietary calcium cannot be absorbed when vitamin D is insufficient [104]. Calcium supplementation did reduce risk of preterm delivery in an Australian study [118] and among younger women in the WHO trial [119]. Although 25(OH)D was not measured and vitamin D not included in the supplement many of participants from both WHO and Australian trials were from areas of the world with significant amounts of sunshine and thus had a low expected prevalence of vitamin D insufficiency.

The sequelae of calcium metabolic stress related to vitamin D insufficiency include preeclampsia [105] and decreased fetal growth [122]. The sequelae of calcium metabolic stress from serious disorders like primary maternal hyperparathyroidism also include preterm delivery [125] and preeclampsia [126]. During pregnancy higher PTH is related to higher maternal systolic and diastolic blood pressures [105]; high blood pressure or a substantial blood pressure rise during pregnancy is associated with an increased risk of preterm delivery [106, 127, 128].

Poor maternal vitamin D status—albeit without information on calcium metabolic stress—has sometimes been reported to influence gestation duration or preterm delivery. Gravidae with very low levels of 25(OH)D (<11.2 ng/mL) had gestations that were 0.7 week (4.9 days) shorter than women with higher circulating levels; birth weight was also reduced but the difference (−157 g) was not statistically significant [129]. Bodnar [130, 131] has twice reported increased risk of preterm delivery <35 weeks with low 25(OH)D (<12 ng/mL) for minority participants (African American and Puerto Rican) from the Collaborative Perinatal Project and from all participants (<30 ng/mL) from a study of twin gestations [131]. The only clinical trial to date, powered to detect differences in circulating

25(OH)D and 1,25-dihydroxyvitamin D_3 with increasing vitamin D supplementation but not clinical outcomes, did suggest a trend in increasing gestation duration ($p=0.1$) amounting to 0.5 week between the highest and lowest levels of supplementation as well as reduced rates of preeclampsia/gestational hypertension [132]. Others have reported null results [133–135], including a large study of Australian gravidae from latitudes receiving significant sunshine. In that study, the small group of vitamin D deficient gravidae had a 2.6-fold (<10 ng/mL) and 1.8-fold (<15 ng/mL) increases in risk for spontaneous preterm birth <34 weeks, neither statistically significant [135]. While it seems plausible that, like calcium, the capacity of vitamin D to decrease risk of delivering preterm may be confined largely to populations where maternal calcium metabolic stress is prevalent this has yet to be demonstrated.

n-*3 Fatty Acids*

Some studies have associated the consumption of supplements that contain fish oil, as well as a diet rich in marine foods, with beneficial effects including longer gestation duration and improved fetal growth. Omega (*n*)-3 and omega (*n*)-6 polyunsaturated fatty acids (PUFA) are essential fatty acids that must be consumed. The most bioactive forms of *n*-3 PUFA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the key *n*-6 PUFA is arachidonic acid (AA). All of the *n*-3 and *n*-6 PUFA in the fetus come from the mother, either in the form of the essential fatty acids or their derivatives EPA and DHA [136]. Thus, moderate consumption of an oily fish like salmon will increase both maternal and fetal levels [137, 138].

Canadian Inuit who eat diets rich in fish oil have longer gestations (+5.4 days) and increased infant birth weights (+77 g) [139]. The trial conducted by the People's League of Health [140] during 1938–1939 suggested that supplementing the usual diet of British women with minerals, vitamins and halibut oil extended gestation. Fewer infants were born before the 40th week of gestation to supplemented women (20 % vs. 24 %) although there was no difference in birth weight. A trial of 533 Danish women who received fish oils, olive oil, or no supplement by 30 weeks gestation showed that fish oils resulted in longer average gestations (+4 days) and infants with higher average weights (+107 g), mostly attributable to increased gestation [141]. The effect of fish oil was strongest with low fish consumption and amounted to +7.4 days longer gestation in this group. A trial in a high risk, low income population that was primarily African American showed that the consumption of eggs fortified with DHA late in second trimester increased gestation duration by 6 days on average [142].

Fish oil or use of *n*-3 PUFA supplements during pregnancy seem to increase the length of gestation, whether such supplements could prevent preterm delivery or other adverse pregnancy outcomes has not been confirmed. A multicenter trial enrolled high-risk women with a history of preterm delivery, fetal growth restriction, or preeclampsia. Women were randomized to either fish oil or olive oil. Fish oil reduced the recurrence risk of preterm delivery approximately twofold but did not alter the recurrence of the other poor outcomes [143]. But another study of women with a prior history of preterm birth showed that adding omega 3 fatty acids to injections of 17 alpha-hydroxyprogesterone caporate did little to reduce recurrence [144]. A Cochrane review ($N=2793$) reported that pregnant women taking fish oil supplements had a small but significant lengthening of gestation (+2.6 days) compared to controls. There was no reduction in risk of preterm delivery (<37 completed weeks) although there was a 45 % reduction in the odds of giving birth <34 completed weeks' gestation [145]. Likewise, low risk women assigned to receive omega 3 fatty acids (600 mg/day) had significant reductions in delivering <34 weeks (4.8 % vs. 0.6 %) but not <37 weeks (8.8 % vs. 7.8 %) [138].

A meta-analysis of several randomized trials suggested that while the effect of *n*-3 PUFAs on gestation duration was present it was weak (+1.5 days). There was little effect of the fish oils when fish consumption was high (−1.6 days) [146]. Likewise while moderate fish consumption (variously defined as >1 or <3 fish meals per week) may decrease risk of preterm delivery, higher consumption

may be of little benefit [147, 148]. Oily fish provide a uniquely rich food source of long-chain PUFA, especially DHA and EPA as well as vitamin D. The benefit of maternal seafood intake and the competing risk of maternal mercury exposure have been evaluated [149]. Methylmercury crosses the placenta. The US Food and Drug Administration (FDA) advised all pregnant women to limit seafood consumption to 340 g/week (0.75 lb), which provides approximately 200 mg of DHA per day, to reduce fetal exposure to trace amounts of neurotoxins from methylmercury.

Multivitamin-Mineral Supplements

In the USA, there is limited regular use of dietary supplements by reproductive-age women; overall about 31 % of women report taking vitamin or mineral supplements, folic acid or a prenatal vitamin every day during the month before becoming pregnant [150]. This varies by ethnicity and age; with white gravidae (35 %) reporting daily use more frequently than Blacks (20 %) or Hispanics (23 %) and women aged 18–24 being less likely to supplement every day (16 %) than those who were older (34.5 % 25–34 years, 41.6 % 35–44 years).

Level of nutrition knowledge has a strong independent effect on regular supplement use during pregnancy [151] as do education and social class making older better educated women of higher SES the most likely to supplement consistently [151–153]. Reasons for inconsistent use included: maternal confidence that diet was good, perceived side effects from the supplement and skepticism that health effects were real [153]. In Camden, few (17 %) low-income minority gravidae reported using supplements before they got pregnant [154]. Periconceptional use was more likely to occur among women with a history of an adverse pregnancy outcome, principally spontaneous abortion in past pregnancies, and with spotting and bleeding during the current pregnancy. Thus, low-income women appear to use supplements when they previously had or currently anticipated a problem with their pregnancies. Other predictors of not using prenatal vitamin/mineral include being unmarried, parous, under the age of 20, with less than a high school education [150, 153, 154].

Camden women who used supplements before or during pregnancy had reduced risks of preterm delivery (twofold lower than controls) and very preterm delivery (fourfold reduction in risk with first trimester use, twofold reduction with second trimester use) with use corroborated by assay of circulating micronutrients [154]. Prospective data from US and Danish women underscore this result. Multivitamin use at least once a week before pregnancy was associated with a threefold reduced risk of preterm delivery <34 completed weeks in a large US cohort [155]. Likewise there was a reduced risk of preterm labor and delivery (<37 weeks) in non-overweight Danish women who regularly used multivitamins around the time of conception [156].

Randomized trials of multivitamins/minerals use are not ethically feasible in Europe or North America but have been carried out in the developing world where few pregnant women can afford to supplement. Fawzi and colleagues [157] examined effects on more than 1000 HIV positive women from Tanzania supplementing then with multivitamins (mostly 2 RDAs), vitamin A or placebo in a double blind-placebo controlled trial. Multivitamin supplementation decreased the risk of very preterm delivery approximately twofold along with infant low birth weight and fetal growth restriction. A second trial [158] undertaken among the HIV negative ($N = 8468$) suggested a 0.2 week increase in gestation duration but no reduction in risk for preterm delivery <37 weeks (16.9 % multivitamin vs. 16.7 % placebo), or <34 weeks (4.9 % multivitamin, 5.6 % placebo). Other clinical trials conducted in Nepal, Mexico, China, and elsewhere found no effect of multivitamins (1 RDA) on preterm delivery. In Nepal, pregnant women received vitamin A alone (control) or vitamin A with the following: folic acid, folic acid + iron, folic acid + iron + zinc, or multiple micronutrients [159]. The multivitamin formulation increased birth weight by 64 g and decreased low birth weight by 16 %; both changes were statistically significant. The folic acid + iron regimen also decreased low birth weight by 16 %;

folic acid without iron had no effect on pregnancy outcome. None of the regimens decreased risk of preterm delivery. In comparison to iron alone, multivitamins did not alter birth weight, decrease risk of low birth weight or of preterm delivery in women from Central Mexico [160]. In Guinea Bissau, a randomized trial showed increased birth weight in women supplemented with 2 RDAs (+88 g) and reduced the odds of a birth below 3000 g by 58 %. Effects on preterm delivery and gestation duration were not reported [161]. Micronutrients provided to more than 18,000 non-anemic Chinese women prevented later pregnancy anemia but had little effect on adverse outcomes [162]. On the other hand, another large study of rural Chinese detected a response to supplementation that was contingent upon wealth; among the poorest women and in comparison to folic acid alone, both multiple micronutrients and iron/folic acid were associated with approximately twofold reductions in risk of preterm delivery and low birth weight [163]. There was no effect of supplements when women were economically better off. Recent meta-analyses have confirmed effects on low birth weight, and SGA compared to folic acid alone or iron/folic acid [164–166] along with gestation duration [167]. Thus, despite their modest number, studies from the developing world are beginning to suggest that micronutrient supplements may be of some importance for pregnant women apart from a reduced risk of congenital defects.

Dyslipidemia and Low Grade Inflammation

Several large epidemiologic studies linking birth records to maternal death certificates showed that women with serious complications of pregnancy (history of preeclampsia, gestational hypertension, or gestational diabetes) were at increased risk of having cerebrovascular events like stroke in later life [168, 169]. It is less clear if women, who deliver preterm without a serious complication, are also at risk [169, 170]. One study which linked birth and death data from Norwegian women found an eightfold increase in cardiovascular death for women delivering preterm with a history of preeclampsia and a threefold increased risk without prior preeclampsia [168]. An indicated preterm delivery, largely because of preeclampsia or fetal growth restriction, may have a stronger association with death from ischemic heart disease than spontaneous preterm delivery [171]. A recent study that examined cardiovascular morbidity in a cohort of women who delivered preterm found that a preterm birth increased risk of a cardiovascular event by approximately 40 % after control for preeclampsia and gestational or pre-gestational diabetes [172]. Thus it is plausible that preterm delivery is an independent risk factor for cardiovascular morbidity and mortality occurring years in the future.

Mechanisms linking preterm birth with maternal cardiovascular risk are not understood but inflammation may be one of the processes. Biomarkers of the inflammatory process and of dyslipidemia (elevations in maternal plasma triglycerides, cholesterol, and C-reactive protein (CRP) [173–175]) have been linked to preterm delivery. Triglycerides must be hydrolyzed to fatty acids to cross the placenta. In Camden women who had elevated fasting free fatty acids had a greater than 3.5-fold increased risk of preterm delivery and spontaneous preterm delivery [176].

Endothelial dysfunction is considered a key element in the development of atherosclerosis and is increased in diabetes, insulin resistance, obesity, and preeclampsia [177–180].

Vascular endothelial cells are activated by inflammatory changes during pregnancy with increased expression of soluble intercellular adhesion molecule-1 (sICAM-1) vascular cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin (sE-selectin) [177, 181]. Elevated levels of sICAM-1 and sVCAM-1 are associated with an increased risk of preterm delivery and spontaneous preterm delivery in Camden gravidae [182]. The associations were detectable as early as 16 week gestation and the effects persisted to the third trimester. Elevated sE-selectin level was not increased unless a preterm or a term delivery was complicated by preeclampsia. These findings thus suggest that endothelial dysfunction is a pathological state that manifests in some women who deliver preterm that may eventually increase their risk of CVD [182].

It is well known that cardiovascular disease is linked to diets rich in saturated fat which raise cholesterol and increase risk of atherosclerosis. Higher concentrations of inflammatory markers—mainly CRP—correlate positively with a Western diet characterized by the increased consumption of red meat and other high cholesterol foods, poor quality carbohydrate and a low intake of fiber [183]. Likewise diets rich in fruits, vegetables, and whole grains correlate negatively with inflammation [183]. Chronic low grade inflammation may be one of the routes by which diet increases cardiovascular risk. During pregnancy, high maternal CRP is associated with an increased risk of preterm delivery as well as with diet [174]. Lean women (BMI <25) from Camden with high CRP had increased intakes of energy adjusted protein and cholesterol and a higher dietary glycemic index as well as a threefold increase in risk of preterm delivery. Thus it is plausible that the maternal diet, by exacerbating the inflammatory response, is an underlying factor that influences preterm delivery as well as later cardiovascular risk.

Conclusions and Recommendation

While the relationship between nutrition and growth, both prenatal and postnatal, is well established, maternal nutrition has emerged as a factor related to preterm delivery.

In the developing world the interaction between nutrition and infection is a well-known cause of childhood malnutrition, morbidity from infectious disease and death. Inflammation is often an acute response aimed at removing an infection and allowing repair of the damaged tissue. A different sort of inflammation occurs in response to tissue damage, trauma, dyslipidemia, cancer and obesity [184]. It differs from the acute inflammatory response in that it is low grade and chronic, with systemic effects linked to an increased risk of future chronic disease. Low grade inflammation is related to many adverse pregnancy outcomes including preterm delivery. While it is possible that inflammation during pregnancy is a consequence of impaired maternal immunity or that the inflammation causes an acute phase response which affects circulating micronutrient levels in the mother [185, 186] it is also plausible that diet is an important contributor to chronic, low grade systemic inflammation [187]. Thus, it is prudent to eat a healthful diet that includes fish and to complement it with iron/folic acid—containing multivitamin/mineral supplements before and during pregnancy. And, since a poor diet rarely occurs in isolation, it is also important to maintain a lifestyle free of cigarettes, alcohol, and drugs.

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Chapter 34
Linking Prenatal Nutrition to Adult Mental Health

David St. Clair and Ezra Susser

Key Points

- Studies on Dutch and Chinese cohorts have found an association between prenatal exposure to famine and risk of developing schizophrenia.
- The mechanisms responsible for this increased risk are unknown at present but could include elevated mutation rate, altered epigenetic regulation, and/or gene–environment interactions.
- As examples of possible contributing nutritional factors, the effects of selenium, zinc, and folate deficiencies upon brain development, mutation rate, and methylation, as well as their interaction with human polymorphisms are discussed.
- The interaction between micronutrients in the body is complex and must be considered in any study examining the association between a given deficiency and risk of schizophrenia.
- Understanding the role of micronutrient deficiencies in the development of schizophrenia may help elucidate risk factors for other mental disorders.

Keywords Fetal malnutrition • Famine • Micronutrients • Schizophrenia

Introduction

The effects on child health and development of exposure to either general malnutrition or to specific micronutrient deficiencies during early life are well documented [1]. For example, prenatal folate deficiency increases the risk of neural tube defects, and low maternal iodine intake can cause fetal iodine deficiency syndrome and cretinism [2–5]. However, the impact of malnutrition during early life is not restricted to infancy and childhood. It is now clear that there are latent effects that may only become evident in adult life. The prenatal antecedents responsible for these latent effects can arise from exposures at any point from conception onward [6, 7].

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_34,

Although low birth weight has been implicated in increased risk of cardiovascular diseases and several adult psychiatric disorders and on initial inspection it might seem a good indicator of prenatal nutritional stress, unfortunately multiple mechanisms (hypoxia, periventricular hemorrhages, genetic, etc.), independent of malnutrition, can cause low birth weight. It should therefore be considered an unreliable proxy indicator of fetal or maternal undernutrition.

Indeed studies based on the Dutch famine, described later, suggest that, to the extent that birth weight does reflect maternal nutrition, it reflects mainly nutritional intake during the last trimester of gestation [8, 9]. There have, in fact, been very few studies with direct measures of both maternal prenatal nutrition and adult health in offspring [10]. The central studies in this field have been follow-ups of the birth cohorts exposed and unexposed to prenatal famine during the Dutch Hunger Winter of 1944–1945. These studies find some evidence of effects of prenatal famine on obesity and insulin resistance, but as yet, the effects are modest [11–14]. Indeed some have argued that the fetal origins hypothesis as formulated by Barker has been overstretched [15].

Despite the evidence noted earlier that prenatal nutrition influences neurodevelopment, even fewer studies have examined the relation of maternal nutrition to adult mental health. Again, the results derive mainly from studies of the Dutch Hunger Winter. The strongest positive result links early gestational exposure to famine with an increased risk of schizophrenia [16–19]. There have also been reports of correlations between exposure to the famine and other psychiatric disorders. Males exposed to the Hunger Winter during the first trimester had a significantly greater likelihood of being treated for addiction as adults [20]. Males exposed during either the first or second trimester were more likely to develop antisocial personality disorder as young adults [21]. Also, second and third trimester exposure increased the odds of being hospitalized for major affective disorders [22, 23]. Using different definitions of prenatal exposure periods, a follow-up of a cohort in midlife found an association between preconceptional exposure and measures of depressive symptoms as well as quality of life; the exposure defined a preconceptional in this study would have been defined as early gestation in the studies of mental illness [24]. No effect of prenatal famine exposure on IQ, as measured at 18 years in males at military induction, or at 59 years in the population as a whole has been found [25, 26]. The link between exposure to famine and schizophrenia has now been replicated in a second series of studies based on the Chinese famine of 1959–1961 [27, 28]. The similarities and differences between the Dutch and Chinese schizophrenia studies were recently discussed [29]. Together they represent the strongest evidence linking prenatal starvation with the later development of adult mental disorders.

Given the strong data implicating prenatal malnutrition with increased risk of adult schizophrenia this chapter will focus on this "latent" effect. By this we mean that the mental disorder is not detectable in childhood, though we do not exclude the possibility of subtle manifestations in childhood. Establishing one clear example of a latent effect on a major adult mental disorder opens the door to considering a range of other potential effects. We discuss some of the mechanisms through which prenatal nutritional deficiencies could influence the risk of developing schizophrenia, and we highlight specific micronutrients whose intake would be expected to fall during famine and which could work through the mechanisms discussed.

Prenatal Famine and Schizophrenia

Schizophrenia is a disorder characterized by psychotic symptoms such as delusions and hallucinations, as well as deficits in other domains, such as motivation and affect. As the disorder is often associated with long-term disability, it ranks among the top ten causes of disability in the WHO classification [30]. Currently, most investigators consider schizophrenia to be a neurodevelopmental disorder, in the sense that it has some early origins in brain development, but the disorder is not diagnosed

until adolescence or adulthood. Typically, the full syndrome required for diagnosis emerges between age 16 and 29 years. In many cases, however, certain signs or symptoms are apparent long before the full syndrome and in other cases the syndrome does not emerge until midlife or even late life [31].

The hypothesis that prenatal nutrition may be related to schizophrenia dates back to the mid-twentieth century [32]. It was not tested, however, until the end of the twentieth century [18]. The first test was based around the historical circumstance of the Dutch Hunger Winter of 1944–1945. This famine was precipitated by a Nazi blockade of the occupied region of Holland in October 1944 and exacerbated by the severe winter which soon followed. The food shortage was most severe in the occupied cities of western Holland; in the rural areas, there was more access to supplementary food.

Three remarkable features of this famine made it possible to study its effects on schizophrenia in adulthood. First, the food rations distributed to the population were documented. Although individuals found ways to supplement the official ration, the caloric content of the ration was strongly correlated with intake. When rations fell, food intake fell. Second, the peak period of famine was of short duration. The famine ended abruptly in May 1945, when the Allied troops liberated western Holland. In the last months of the famine, the ration fell to extremely low levels, and supplementary food was increasingly difficult to find. In addition, the population was nutritionally depleted. Thus, the period of most severe starvation was approximately from February 1945 until liberation in early May. The increased severity in these last months is reflected in data on mortality, fertility, and birth weight. Third, information could be obtained on schizophrenia admissions in adulthood for the individuals born in the famine cities of western Holland before, during, and after the famine. The Dutch national psychiatric registry recorded hospital admissions for specific diagnoses from 1970 onward. Taken together, these features made it possible to define birth cohorts exposed to famine at specific periods of gestation and to test whether the exposure was linked to an increased risk of schizophrenia.

Although the result for schizophrenia emerged through a series of studies [17], we summarize here the main findings in a single figure. In Fig. 34.1, we define the birth cohort of October 16–December 31, 1945 as severely exposed to famine in early gestation. Based on their birth dates, we can infer that the vast majority of this birth cohort was conceived or in early gestation during the peak of the famine. This inference is supported by the excess of neural tube defects and other congenital anomalies of the central nervous system in this birth cohort (Fig. 34.1). It is also supported by the drop in the birth rate, which reached a nadir in September 1945 and remained low until the end of 1945 (Fig. 34.1); the decline in fertility correlated strongly with rations around the time of conception in these Dutch birth cohorts [8]. Figure 34.1 also illustrates that this exposed birth cohort had a sharply increased risk of schizophrenia in adulthood, a result based on the national psychiatric registry data. Finally, the figure illustrates that among males there was an excess of Schizoid Personality Disorder diagnosed at age 18 in the same exposed birth cohort [19, 33]. This result was derived from military induction data: all males born 1944–1946 were subject to a military draft at age 18 years, and the induction examination included a psychiatric assessment. Current diagnostic practice would probably classify the individuals with Schizoid Personality as having a "Schizophrenia Spectrum" personality disorder since familial aggregation studies and other evidence suggest that "Schizophrenia Spectrum" personality disorders are etiologically related to schizophrenia [34].

Although these data offered fairly compelling evidence for a link between prenatal malnutrition and the risk of schizophrenia in offspring, the number of exposed cases was modest, and a single study is rarely sufficient basis for a causal inference. Also, other interpretations were plausible. For instance, during periods of famine people often resort to food substitutes such as tulip bulbs in the Dutch Hunger Winter that might be toxic to the developing brain. Although starvation would have led to the ingestion of these food substitutes, a causal pathway mediated by ingestion of toxic food substitutes would have different implications for the pathophysiology and ultimately for preventive interventions.

It proved difficult, however, to find another historical circumstance in which this result could be tested. Famines are common, but the documentation of psychiatric outcomes in a defined population for

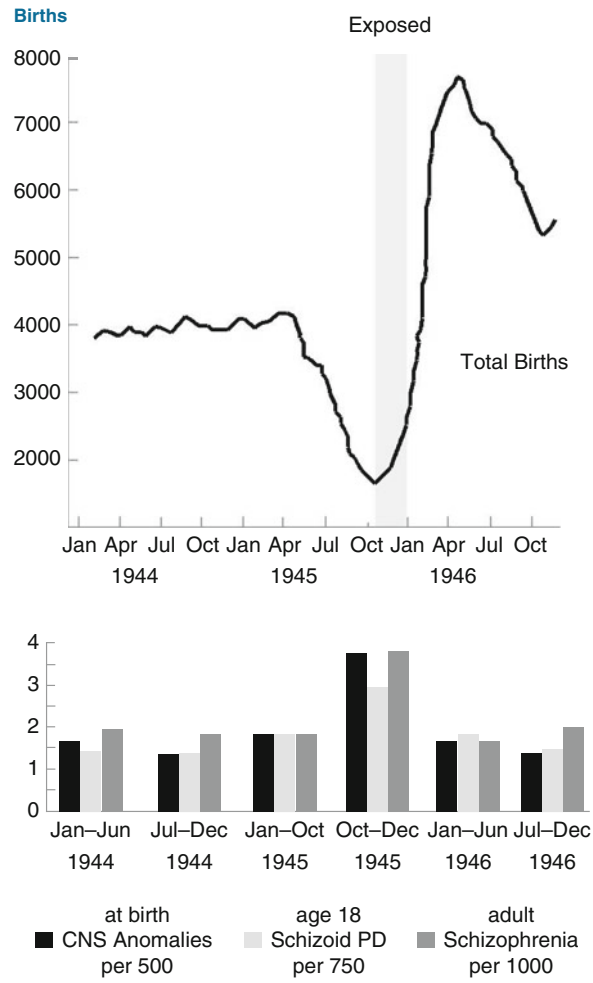

Fig. 34.1 Dutch famine birth cohorts of October 16–December 31, 1945. Reproduced from Susser et al. [133] with permission from the New York Academy of Sciences

decades after a famine is rare indeed. It was nearly a decade before the finding was replicated, and what is more by an independent group [27]. The study was conducted in the Wuhu region of Anhui Province, China and based on the devastating famine which afflicted China following the Great Leap Forward. In Wuhu, the peak of this famine was in 1959 and 1960. The key data available in Wuhu were the number of births in each year and the number of people born in these years who subsequently received outpatient or inpatient treatment for schizophrenia. The authors were able to demarcate a district that was served by the same psychiatric hospital over the main decades of risk for schizophrenia in the relevant birth cohorts. Also, the population of this district was remarkably stable over these decades, in part because of tradition and in part because changes in district of residence were controlled.

The Chinese famine was long lasting, and the Wuhu data on birth rates were available for years rather than months or weeks. Following the result for the Dutch study, however, it could be hypothesized that the schizophrenia risk should peak in those birth years in which the birth rate dropped. (The Dutch famine results in Fig. 34.1 indicate that the schizophrenia risk peaked shortly after the nadir in the monthly birth rate.) This is exactly what was observed (Fig. 34.2). Although the measure of exposure was less precise, the numbers were much larger, making these two studies complementary.

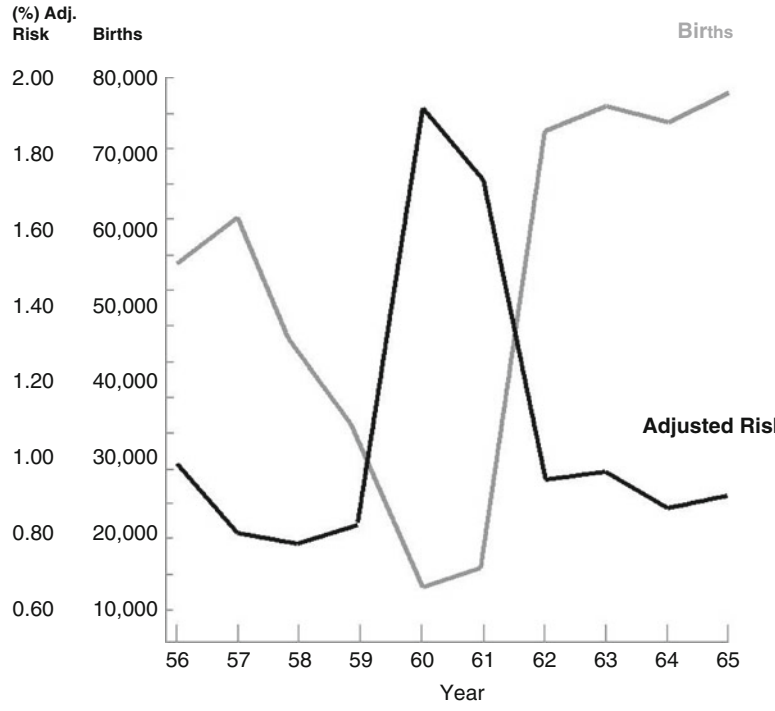

Fig. 34.2 Adjusted risk of schizophrenia versus birth rate for years 1956–1964. Wuhu prefecture, Anhui. Reproduced from Susser et al. [133], with permission from the New York Academy of Sciences

Two studies with concordant results substantially strengthened the hypothesis that prenatal malnutrition had latent effects on adult schizophrenia. A third study was conducted in the region of Liuzhou, in southern China, using essentially the same design as the Wuhu study. Again the results were concordant; that is, the risk of schizophrenia peaked in the annual birth cohorts in which the birth rate dropped [28].

Some special features of this third study added strength to the hypothesis. It was based on a very large number of people, even larger than the Wuhu study. It was conducted in a region of China that differed in customs, ethnic diversity, and historical famine experience from the Wuhu region. Finally, and most important, it permitted us to clearly differentiate the impact in urban and rural areas. This was important because, in contrast to Holland, the famine in China affected mainly the rural areas. Urban residents received rations and generally suffered little or no starvation, while the rural population suffered starvation on a massive scale. Therefore, an increased risk of schizophrenia due to prenatal malnutrition should be evident in the rural but not the urban area. This is what we observed [28].

In sum, the results from the Dutch and/or Chinese famine studies suggest that in the birth cohort conceived or in early gestation at the height of the famine there was a sharp increase in rates of neurodevelopmental disorders at birth, in adolescence, and in adulthood. It appears then that the same exposure can lead to different neurodevelopmental disorders at different points in the life course. A similar overlap applies to the genetics of neurodevelopmental disorders. The same rare pathogenic DNA copy number variants (CNVs) can increase risk of mental retardation at birth, autism in childhood, or have no apparent harmful effects until schizophrenia presents in adulthood [35]. The term pleiotropy is often used to describe these differing clinical and temporal presentations caused by the same genetic lesion. Should we extend the use of this genetic term to the effects of early life environmental exposures? It is important also to be clear that prenatal exposures such as famine, like rare pathogenic CNVs, only increase **risk** of neurodevelopmental disorders. They are neither sufficient nor necessary to cause these diverse phenotypes.

Mechanisms

The two Chinese studies, together with the earlier Dutch study, make a very compelling case that prenatal exposure to famine increases risk of schizophrenia and possibly other forms of major mental illness in later life. The specific risk factors and mechanisms involved are unclear, however. Various biological pathways by which prenatal nutritional adversity may produce increased risk of adult physical disorders and of schizophrenia have been enumerated elsewhere [14, 36–39].

Not all the plausible pathways are related to nutrition. For instance, one intriguing speculation is that the effects of famine are mediated through the hypothalamic pituitary adrenal (HPA) axis. Some investigators have argued that exposure to high levels of glucocorticoids, induced by a stressful prenatal environment, might "program" the HPA axis for life or disturb brain development. Famine might be thought of as a stressor, since fetal undernutrition is associated with greater transplacental transfer of glucocorticoids and reduced HPA axis function The outcome and mechanisms are discussed [40–42]. Mothers who experience the death or major illness of a close relative during the first trimester of pregnancy may be more likely to give birth to children that would later develop schizophrenia; some studies report this association while others do not [43, 44]. It is possible that exposure to nutritional stress in early gestation might have similar effects to those caused by sudden bereavement.

In this chapter, however, we will first describe three distinct but not mutually exclusive pathways by which prenatal micronutrient deficiencies might affect the risk of schizophrenia. Each of these pathways represents a form of interplay between genes and environment. Thus, we examine possible roles for (1) mutation rate, (2) epigenetic regulation, and (3) gene environment interactions. Then we consider three specific micronutrients selenium, zinc, and folate to demonstrate the plausibility of these pathways.

Nutritional deficiency can indirectly affect brain development by interfering with DNA stability. A number of micronutrients play key roles in DNA synthesis, methylation, and repair. Antioxidants protect against oxidative damage, a major cause of DNA damage, and trace metals are integral parts of many of the proteins involved in DNA transcription and repair. For these reasons, the rate of *de novo* mutations which is normally very low may increase under nutritional deprivation, and this could result in genetic changes that give rise to schizophrenia in later life. Advancing paternal age is a major source of new mutations in humans [45], and the relative risk of schizophrenia rises with age of the father [46], an analogous phenomenon may occur in response to prenatal nutritional stress [38].

The influence of nutrition on epigenetic regulation is well documented in humans, rodents, and sheep [47–50]. Epigenetic regulation of genes critical for brain development may also be important in the development of schizophrenia [51, 52]. It is known that disorders of imprinted genes such as Beckwith–Wiedemann and Prader Wili syndrome play important roles in fetal growth and development of the brain and can affect cognition and behavior throughout life.

For this reason, epigenetic changes could increase the risk of schizophrenia, by changing the expression of genes either involved in neuropsychiatric pathology specifically or related to fetal growth in general. Again, because epigenetic changes occur more frequently with advancing age, increased risk of schizophrenia with advancing paternal age is consistent with an epigenetic mechanism, as well as a DNA mutation one [53]. To our knowledge, the only published studies on humans are again from the Dutch Hunger Winter: exposure to famine in early gestation was associated with altered methylation of insulin-like growth factor 2 (IGF2) in midlife [54] and from genome-wide screening a range of prenatal malnutrition associated with differentially methylated regions [55].

Finally, gene–environment interactions may play a role in the development of schizophrenia. For example, the results of a study of Finnish children born to schizophrenic mothers and subsequently adopted suggest that these children's genetic background may make them especially susceptible to their household environment, with a greater risk of exhibiting thought disorders when placed with a family that communicates poorly and a lower risk when placed with a family that communicates well [56]. Might similar patterns with regard to prenatal nutritional environment be important? In some

cases, variation in genes involved in micronutrient-dependent pathways could result in changes to the in utero environment that are conducive to the development of schizophrenia but only in the presence of a deficiency of the relevant nutrient.

In the remainder of this chapter, we will describe what is known about the effects of specific micronutrient deficiencies, selenium, zinc, and folate, in regard to the mechanisms just discussed. Although famine may reduce the intake of many different micronutrients, the functions of these particular micronutrients make them especially promising for future study. Vitamin D and iron deficiency are also implicated in risk of schizophrenia; they are not discussed in this chapter but the evidence of their potential involvement in schizophrenia and clinical implications is reviewed in detail elsewhere [57, 58].

Selenium

It has been proposed previously that selenium deficiency influences the risk of developing schizophrenia [59]. Ecologic studies have linked selenium deficient soil with high rates of schizophrenia. An analysis of U.S. state and county medical hospital records from 1965 found that of 219 environmental variables, low selenium levels in fodder crops had the strongest positive correlation with high schizophrenia prevalence [60]. A subsequent analysis of prevalence data drawn from nine U.S. schizophrenia surveys conducted between 1880 and 1963 reported a significant correlation between low selenium soil and high schizophrenia rates at the state level [61]. We cannot infer from these data that prenatal or postnatal selenium is causally related to schizophrenia. They do, however, strengthen the rationale for studies explicitly focused on the relationship between selenium deficiency during prenatal development and the later development of schizophrenia. By contrast, assays of selenium in adult schizophrenia show variable results and may be due to the metabolic effects of neuroleptic medication [62].

Selenium is thought to play an important role in brain development, but exactly what this role is has yet to be elucidated. When animals are deprived of sufficient levels of selenium, supply to the brain is prioritized [63] although in severe deficiency the activity of important selenium-containing enzymes falls [64]. That the brain receives such high priority suggests that selenium plays a particularly important role in the function of this organ [65, 66]. It has been shown that selenite is an essential component of the medium used to culture central neurons and that selenoprotein P (SePP) promotes the survival of cultured neurons [67]. Selenium has also been shown to protect against neuronal damage and death in response to free radicals [68]. Finally, mRNA levels of brain-derived neurotrophic factor fall in pups born to dams fed a selenium deficient diet, as does the production of seleno enzymes required for the expression of thyroid enzymes essential for normal brain development at certain stages [69].

There is also good evidence that selenium levels influence the methylation of genes, and thus affect epigenetic regulation. Selenium reverses the hypermethylation of genes, such as those coding for tumor suppressors, in human prostate cancer cells, reactivating them by modifying methylation patterns [70].

Further, selenium deficiency apparently has different effects upon methylation pathways across species such as mice and rats [71, 72]. Thus, while the data indicate an important role for selenium in epigenetic regulation, the nature of this role appears to be quite complex.

Finally, gene interactions with selenium deficiency appear to influence the development of a number of human diseases. Polymorphisms in the selenoenzyme GPX-1 that change the enzyme's activity [73] have been found at higher prevalence in individuals with Keshan disease [74]. In addition, Keshan disease is linked to low selenium levels. It appears that the interaction between these genetic polymorphisms and selenium deficiency is multiplicative in determining the risk of the disease. If low levels of selenium contribute to schizophrenia, then environmental interactions with such polymorphisms in the prenatal environment may be important.

Zinc

The hypothesis that prenatal zinc deficiency contributes to schizophrenia is not a new one [75, 76]. Zinc deficiency is important in the neurodevelopment of humans, and insufficient zinc levels early in pregnancy can lead to serious deformities, such as anencephaly. In a randomized controlled trial, pregnant women given zinc supplements upon beginning prenatal care had babies with improved neurobehavioral development, as measured by fetal heart rate and movement patterns [77]. Studies in animal models are consistent with findings in humans and provide a better picture of the mechanisms at work. Embryonic rat neural cells are especially susceptible to zinc deficiency, dying at a higher rate than other types of cells [78]. Not surprisingly, exposure of fetal rats to zinc deficiency results in reduced fetal growth and number of brain cells [79]. There is also evidence that maternal zinc deficiency in mice suppresses the development of neural stem cells, which could lead to future neuroanatomical and behavioral abnormalities [80]. In addition to physical changes found in animal models, a number of behavioral sequelae have been noted which persist into adulthood even after the provision of a normal diet [81–83].

Zinc also plays an important role in guarding against DNA damage by reactive oxygen species. Rats fed a low zinc diet [84] and cells grown in zinc-deficient culture [85] experience increased oxidative stress. In addition to playing an important role in DNA transcription factor function, many of the proteins involved in base and nucleotide excision repair are either zinc finger or zinc-associated proteins. Zinc deficiency alters the expression of genes and the conformation of proteins 84 needed to respond adequately to DNA damage. For this reason, dietary deficiency can lead to single- and double-strand breaks in DNA [86, 87] as well as oxidative modifications to it [85].

Zinc also plays an important role in methylation, which is central to epigenetic regulation. It is a cofactor for DNA methyltransferase [88] and zinc-finger domains are found in methyl-DNA-binding proteins [89]. Proteins featuring zinc-finger domains establish and maintain methylation patterns [90] and are associated with epigenetic reprogramming events [91]. Thus, zinc is essential for proper functioning at many levels in the process of epigenetic regulation, and inadequate amounts of this metal have the potential to alter methylation patterns.

Though gene–environmental zinc interactions may be important, little is known about this subject in humans. Mutations in various genes result in the inability to secrete zinc in milk [92] and in the disorder acrodermatitis enteropathica [93]. Little is known, however, about potential interactions between the genetic changes responsible for these human diseases and normally encountered variation in zinc intake. It is not clear what sort of polymorphisms in zinc homeostasis proteins are present in humans, but if functional genetic differences are present, an interaction with zinc levels that affects brain development is a possibility.

Folate

We have long been intrigued by the notion that folate deficiency might link prenatal famine to schizophrenia [94]. An excess of neural tube defects was found in the same Dutch cohorts in which we found an excess of schizophrenia, and neural tube defects are known to be related to periconceptional folate intake. As noted earlier, a polymorphism in an important enzyme in the folate pathway, MTHFR, has been associated with both neural tube defects [95] and with schizophrenia [96]. Furthermore, there is strong evidence that a folate deficiency could activate the pathways discussed here [51].

It is well established that the folate metabolic pathway is central to DNA synthesis and repair, and we have previously hypothesized that mutagenic effects of folate deficiency might explain the Dutch and Chinese famine results [37]. Both in vitro and in vivo studies suggest that folate deficiency induces

genomic instability [97–99]. Also, reduced levels of folate in seminal fluid have been associated with genomic instability in sperm [100, 101]. This raises the intriguing possibility that the effect might originate in the father's germ cells rather than in utero [102].

The folate pathway is also essential for the methylation of DNA. This is one of the most important means by which epigenetic effects are established. In some of the earliest animal studies that documented epigenetic effects in the prenatal period, folate and other carbon donors such as methionine and betaine were used extensively [46, 103, 104]. It is probably fair to say that, at this time, folate level is the best established source of epigenetic variation in utero. Although folate levels degrade in stored sera, homocysteine represents an excellent proxy given that levels of this amino acid reliably increase when folate levels decline. The relationship between maternal homocysteine and offspring risk of schizophrenia was investigated in the Child Health and Development Study (CHDS) birth cohort in northern California, which had available archived sera drawn during pregnancy in nearly all members of this cohort. Elevated third trimester maternal homocysteine levels were associated with a greater than twofold increase in the risk of schizophrenia [105]. Moreover, recent studies in the Norwegian Mother and Child Cohort suggest that folate supplementation in early pregnancy may be protective language delay and autism, and the CHARGE study in California found a similar result for autism. As noted earlier, genetic causes of neurodevelopmental disorders overlap, and this may also pertain to micronutrients (or other environmental factors) that influence risk [106–108].

Complexities

The relationship between the nutrients described is complex. For example, there is evidence that high levels of folate interfere with absorption of zinc in the gut [109–111]. And because selenium and folate influence one-carbon metabolism differently, results suggest that selenium deprivation ameliorates some of the effects of folate deficiency [112]. Similarly, selenium and iodine levels interact. These elements are both required for the production of thyroid hormones essential for brain development, and in areas with dual selenium and iodine deficiency, adverse neurodevelopmental outcomes such as cretinism appear to be higher [113]. However, high levels of selenium coupled with low levels of iodine actually exacerbate hypothyroidism [114]. Zinc deficiency, in the presence of iodine and selenium deficiencies singly or together, adds even more complexity to this picture [115]. Because micronutrient deficiencies are often found in multiples, and many micronutrients play overlapping but different roles in neurodevelopment, taking into account the interactions between them is a challenging necessity.

Micronutrients, Infections, and Schizophrenia

Many studies have implicated exposure to infection in utero in the development of schizophrenia [116, 117]. Because so many types of prenatal infection have been linked to increased rates of schizophrenia, a hypothesis that alterations in cytokine production in the fetal brain are responsible has been proposed [118]. It has been demonstrated that micronutrient deficiencies, including those discussed here, alter the immune response. This may enhance the risk to the fetus associated with prenatal infection exposure. Gestational zinc deficiency causes immune dysfunction in the mother and also has adverse effects on the developing immune system of the baby [119]. Because zinc is necessary in order for immunoglobulins to cross the placenta, perinatal zinc deficiency may reduce the availability of maternal antibodies to the fetus [120]. Similarly, selenium deficiency has been found to alter cytokine and chemokine expression in response to influenza infection. For example, in an adult mouse

model, there is an increase in proinflammatory cytokines and chemokines and a decrease in anti-inflammatory ones [121]. In humans, marginal or deficient adults who received selenium supplements were able to more rapidly clear administered, attenuated viruses than those who did not receive supplements [122, 123]. Thus, maternal micronutrient deficiencies may result in increased susceptibility to infection and/or increased morbidity in the fetus.

On the other hand, maternal infections can result in the sequestration of important nutrients, such as folate, selenium, and zinc, as part of the acute phase response [124–129]. Even temporary drops in the availability of these nutrients may result in damage to the fetus, if they occur during important developmental windows. In this way, infections may initiate or exacerbate micronutrient deficiencies in pregnant women, with effects on fetal neurodevelopment.

Conclusion

The example of schizophrenia demonstrates that prenatal malnutrition is associated with latent effects on adult mental disorders. The challenge now is to understand the causal pathways that account for these latent effects. Until we do, we cannot be entirely sure that the relationship is causal and cannot use these findings to tailor interventions to prevent schizophrenia or other diseases. This is the focus of our current work, and the mechanisms and micronutrients described in this chapter point the way toward some areas for future study that may prove useful. The large numbers exposed to the Chinese famine including individuals who later developed schizophrenia offer the promise of testing some of these hypotheses. Unfortunately, recruitment in rural China of well-documented schizophrenia cases, together with parents and/or siblings with documented precise birth and famine exposure dates has proved much harder than originally anticipated. Also, in the past 20 years, large randomized trials and quasi-experimental interventions have been conducted with early prenatal nutritional supplements, and in coming years the follow-up of the offspring from these trials will permit testing of other hypothesized pathways. Finally, we have already made use of archived biological specimens from pregnancy/birth cohorts established in the 1950s and 1960s to investigate associations between prenatal micronutrients and schizophrenia [105, 130]. A new wave of pregnancy/birth cohorts recently initiated are much larger and their biological specimens are far richer. By 2030, we can expect cohorts such as the Norwegian Mother and Child Study [131] and the Danish National Birth Cohort to answer some of the questions posed in this chapter about prenatal nutrition and schizophrenia. Finally, if the ethical and political problems associated with access to Dried Down Neonatal Blood Spots can be overcome by a combination of neonatal micronutrient assays, Mendelian randomization and adult phenotypic data should allow adequately powered studies of potential gene environment interactions to answer some of these questions much sooner [52, 132].

So far, most research on prenatal nutritional deficiency and adult mental health has focused on schizophrenia. We have yet to determine, however, whether this exposure also influences the risk of developing other mental disorders over the life course. Understanding the role of a given micronutrient deficiency in one disorder, schizophrenia, may shed light on risk factors for others.

Recommendation

At this point, the study of prenatal nutritional deficiency and its latent effects on adult mental health is in its infancy. Therefore, more study on this subject is needed before specific recommendations can be made. In the meantime, providing pregnant women with access to good nutrition has long been known to improve the general health of infants and may also contribute to their life-long mental health.

Acknowledgements This chapter is an updated and expanded version of a previous article [133] The authors thank Kim Fader for her help and the Robert Wood Johnson Health & Society Scholars Program for its financial support.

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Part VII
Nutrition Transitions Around the World

Chapter 35
Nutritional Habits and Obesity in Latin America: An Analysis of the Region

Nicole Figari, Oscar Castillo, and Jaime Rozowski

Key Points

- The dietary pattern in Latin America has become "westernized," moving away from traditional habits.
- Despite the efforts to prevent it, the prevalence of obesity keeps increasing in most countries.
- Socioeconomic level (SEL) is a major determinant of food intake in the region.
- Most worrisome is the increase in the prevalence of obesity in children which increases the risk of chronic diseases in the adult.
- A few intervention programs with a focus on education and change in lifestyle are showing some effectiveness.

Keywords Latin America • Eating habits • Obesity • Programs

Introduction

The stigma of undernutrition has been prevalent for hundreds of years in the world. However, a series of measures have been able to decrease its prevalence, albeit with great differences depending on the socioeconomic situation of the country. In the last decade, obesity has been creeping in most countries, this being particularly true for Latin America (LA), where some countries have to deal simultaneously with undernourishment and obesity. In most countries, obesity has become one of the major health problems and its prevention a priority. The increase in the prevalence of obesity and a reduction of physical activity have become a major health item at the national and global level. The World Health Organization (WHO) 2014 report on chronic disease prevention highlights nine global targets, of which seven are related to nutrition and physical activity, including a reduction of mortality from chronic diseases, reduction in alcohol intake, physical inactivity, and in sodium consumption [1].

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_35

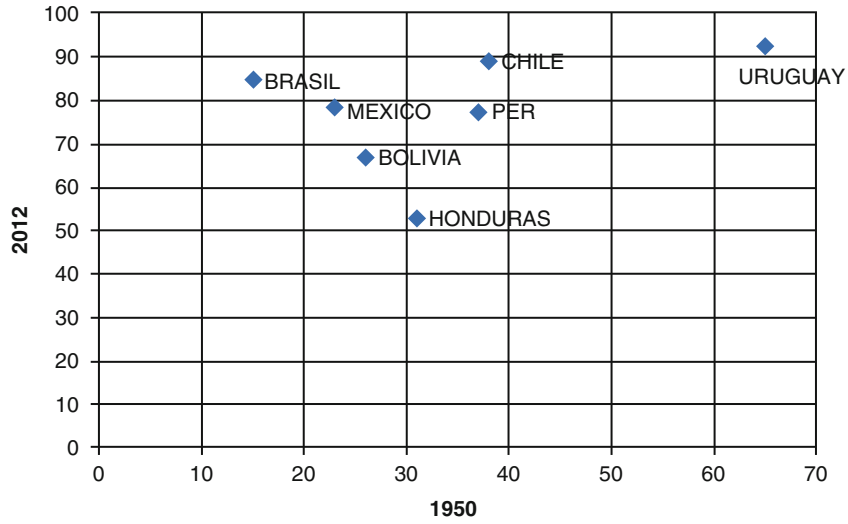

Fig. 35.1 Changes in urbanization from 1950 to 2012 in selected countries from Latin America [5]

Latin American (LA) countries show a significant heterogeneity in their ethnicity, SEL, and health. The region holds 590,000 million inhabitants highly urbanized [2]. It shows great inequalities in SEL, with more than 1/3 of the population living in poverty. All this makes it extremely hard to discuss average values of whatever indicator is used. For instance, although the average infant mortality in LA is 18.7/1000 born alive, the range varies between 4.5/1000 and 50/1000. Likewise, in 2011 life expectancy at birth, though showing an average of 74.2 years, ranged from 66.8 to 79.2 years, more than a 12-year difference [2].

Urbanization has been a major trend in LA during the last decades, reaching currently more than 75 % of the population [3]. Figure 35.1 shows urbanization in selected LA countries in 1950 and 2012. Although some countries still have a substantial rural population, the tendency to move into urban areas is very strong. Undeniably, while urbanization in industrialized countries usually brings along general progress in technology and social development, in developing countries the benefits of urban growth usually show only in the higher socioeconomic levels (SELs). In the lower socioeconomic groups, urbanization generally leads to a relative decrease in income, which, from the nutritional point of view, produces a shift toward consumption of high-calorie foods with a reduced nutrient density, incorporating into their diets foods that are more commonly consumed in industrialized nations. In fact, this trend is observed in the majority of the countries in the region in what could be called the "westernization of eating habits," characterized by an increased consumption of the so-called fast foods coupled with a tendency to decrease physical activity. Although this phenomenon is observed across all SELs, it is more prevalent in poor families.

The incorporation of these new food habits trends has led the LA region as a whole and every country in particular to change morbidity and mortality profiles showing an increase in the prevalence of chronic diseases above that of infectious diseases, the usual killers in the past. Currently, chronic diseases are the main cause of death in most of the LA countries [3].

An example is the case of Chile, where the infant mortality rate in 1955 was approximately 120/1000 live births, mainly as a result of malnutrition and infectious diseases. At that time, cancer and cardiovascular diseases combined accounted for 25.8 % of all deaths in the country. In 2013 the infant mortality rate was 7.7/1000 live births, with cancer and cardiovascular diseases accounting for more than 50 % of all deaths. At the same time, the mortality rates attributed to diarrhea and

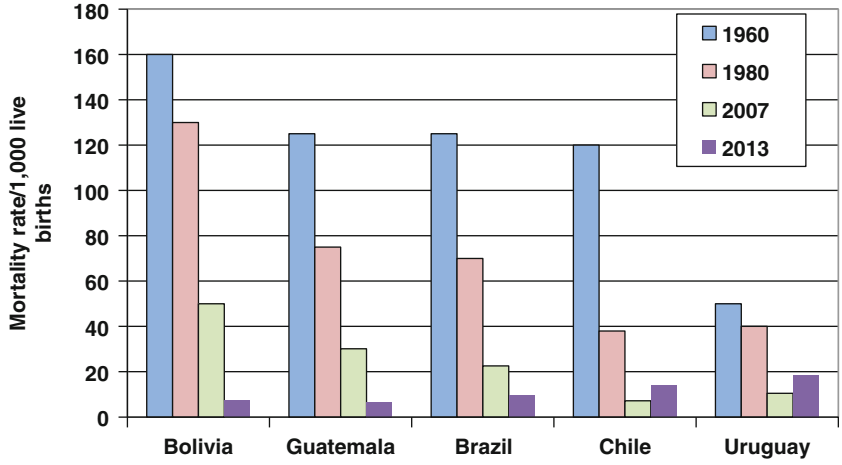

Fig. 35.2 Infant mortality rate (deaths/1000 live births) in selected countries of Latin America 1960–2013 [2]

gastroenteritis of presumed infectious origin decreased in those years from 20/1000 to 0.035/1000 live births [4].

A similar pattern has been seen in other countries of the region. Figure 35.2 shows the infant mortality rates from 1960 to 2012 in five countries that represent a range of SELs in LA [3]. Although all of these countries show a reduction in this indicator, this decline has been variable. The reduced infant mortality and the general improvement of health care are reflected in the proportion of individuals older than 65 years of age, which depicts the increase in this population group since 1960. The increase relates to the improvement of the socioeconomic situation. Brazil has more than double the proportion of that age segment. The aging of the population, when coupled with a decline in early deaths and an increase in life expectancy, necessarily forecasts an increase in the prevalence of the chronic diseases of the aged.

The increase in adult population in most countries in the LA region causes a growing demand on health services, not only to take care of the nutritional needs of the mother and child (malnutrition and micronutrient deficiency), but also to provide for the treatment of nontransmissible chronic diseases (NTCD) and their associated risk factors. Thus, it seems urgent to decrease the nutrition-related risk factors involved in NTCD, such as overweight and high intake of saturated fats; one mechanism is through health promotion via healthy lifestyle programs.

Socioeconomic Changes

A thorough discussion of socioeconomic changes in the region is beyond the scope of this chapter. However, it is important to discuss some aspects that are relevant from the nutritional point of view. The gross national product (GNP) of the different countries in Latin America has shown substantial increases in the last decades. Nevertheless, this increase has not always been accompanied by an improvement in the health situation. For instance, Venezuela, with a GDP/capita of US$ 14,414, has almost double the infant mortality rate of Chile, which has a GDP/capita of US$ 15,732 [5].

The improvement in GNP and in the general well-being of many countries has produced an influx of foreign fast-food enterprises with a wide range of food items at very low cost and, therefore, at the reach of people from all SELs. Also involved in the development of obesity has been the increase in portion sizes, which have become progressively larger in the last decades. Rolls et al. showed that larger food portions led to greater energy intake in volunteers, independently of body mass index (BMI) [6].

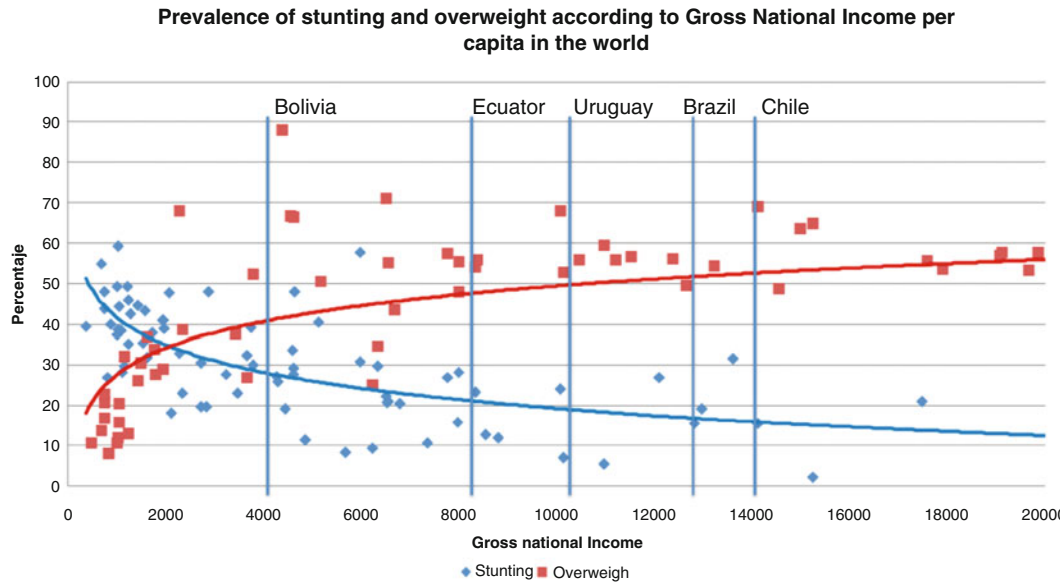

Fig. 35.3 Prevalence of stunting in children and overweight in adults according to their Gross National Income per capita in countries of the world. Selected countries of LA are indicated in the vertical lines. Adapted from The World Bank [5]

There is a clear inverse relationship between income and undernutrition, and a direct relationship with overweight and obesity. This is shown in Fig. 35.3 where the changes in Gross National Income per capita are plotted in relation to the nutrition condition of the population in the world. The vertical lines show the situation in selected countries in LA. As income per capita grows, the prevalence of undernutrition decreases and the prevalence of overweight increases [5].

Food Habits

A thorough analysis of food habits in Latin America is hampered by the lack of national food consumption surveys. Except for a few countries, most information available is from small surveys that are often not representative of the population. In addition, many of these surveys are published in local journals that are not always widely available.

There is a great diversity of national nutritional habits in Latin America. In the Southern cone countries (Chile, Argentina, and Uruguay), wheat represents the main component of the national diets, with the addition of meat and dairy products especially in Argentina and Uruguay. Corn is the main staple in Mexico and most of Central America. The rest of the countries in that area have a rather diverse diet, combining the three main cereals (wheat, corn, and rice). In the Andean countries (Peru, Bolivia, Ecuador), and Paraguay, potatoes represent an important contributor to their diets. In many countries, sugar is an important contributor of calories, representing about 10 % of caloric intake as an average. However, in some groups it can provide as much as 20 % of dietary calories [7].

SEL is a major determinant of eating habits [8, 9]. Food prices, according to FAO have augmented markedly in the last years, as shown in Fig. 35.4. This increase certainly has an influence in the purchasing habits of a sizable proportion of the poor population inducing them to consume cheaper high-calorie foods. For instance, the price of cereal in LA has increased more than 250 % from 1990 to 2014 [10].

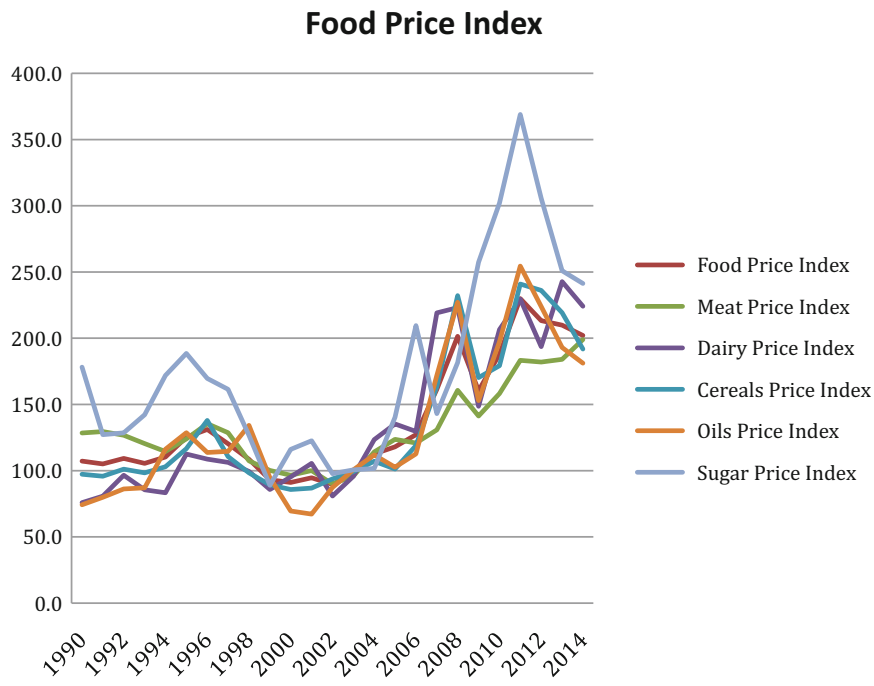

Fig. 35.4 Variations in the world Food Price Index in general and in selected foods [10]

Figure 35.5 shows the availability of energy in several countries in LA. Most countries show an availability that on the average is above recommendations and again is related to SEL. The structure of national diets has shown a tendency to change in most of the countries when income per capita has increased [6]. Calories from fat have increased because of a higher supply of butter, margarine, and different types of oils. There is a clear trend in the region to increase the consumption of fats, with many countries showing an increase in availability in the last 20 years. Nevertheless, only two countries, Argentina and Uruguay, consume a proportion of calories from fats that are considered elevated (>30 %). Cuba is the only country that has shown a substantial decrease in the availability of fat (25 %), while the largest increase (24 %) has been seen in Chile [10]. The increase in fat consumption is observed as a hallmark of the westernization of the diet. This has been confirmed in several countries [7, 8] where the pattern of fat consumption has shown to be correlated with an increase in blood lipids [9, 11]. In terms of the type of fats consumed, there has been a shift from fats of vegetable origin to animal fats, most certainly due to the increased consumption of meat and prepared foods. During the period 1980–2011, we can see a variation in the consumption of foods in Chile. An increase in the consumption of chicken, pork, and bovine meat while a decrease in the intake of vegetables. Calorie intake from complex carbohydrates has shown a tendency to decrease in time while the ones from sugar tend to increase [10]. Protein calories availability tend to remain stable or to increase slowly but with a rapid rise in the intake of proteins from animal origin. The increase in availability is observed mainly in fats and sugar, accompanied by a decrease in availability of healthier foods like legumes. Red meat availability has also increased, showing that as time passes the Chilean diet gets further from a healthy pattern. In addition, increases in BMI parallel increases in income and dietary fat content.

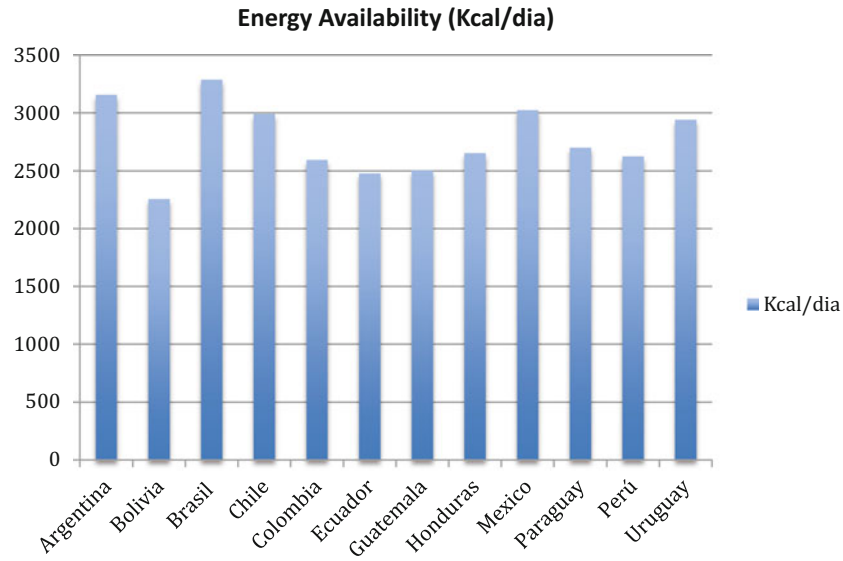

Fig. 35.5 Energy availability in selected countries in LA according to FAO Tables [10]

Carbohydrates

In general, most countries from the region show a decrease in carbohydrate consumption, with the exception of Cuba, and, more importantly, they also show a shift from complex carbohydrates to refined ones, mainly sugar. In general, several studies show that poorer populations consume more carbohydrates than the better-to-do ones [12]. As mentioned earlier, cereals constitute the mainstay of intake in many countries, Mexico showing the largest intake. However, this is changing to a diet that is more refined and has more sugar, fats, animal proteins, and salt.

A pattern that is worrisome is the low consumption of fruits and vegetables. The 2012 national nutrition survey in Mexico showed that only 34 % of children follow the recommendation for these foods [13].

Consumption of salt has increased markedly in the last decade, most probably due to a higher consumption of convenience foods [14]. An increased salt intake has a direct correlation with the appearance of high blood pressure, and many institutions like WHO have recommended a decrease in salt intake which is one of their health targets [1]. Many substitutes of sodium have appeared in the markets that replace 50 % of the sodium for potassium, but their use is still not widespread. Measurements of urinary sodium in the National Health Survey 2009/2010 in Chile showed that on the average the Chilean adult population consumes 9 g of salt/day [15], much above the recommendation of no more than 5 g/day.

Food Consumption According to SEL

As mentioned earlier, it is obvious in all countries that there are differences in consumption according to SEL. A notable example is the case of Chile. The last national Food Consumption Survey (ENCA) that took place in 2012 showed that the intake of red meat, fish, and seafood diminish as SEL decreases [14]. Processed meats and poultry are consumed by higher SEL while the diet of people in low SEL

is abundant in legumes. While only 5 % of the population in the highest income quintile consumed less than the protein requirement (0.75 g/kg body weight), 51 % of people in the poorest quintile consumed less protein than the requirements. Crovetto et al. estimated consumption based on the amount of money spent on food, showing a remarkable increase in the 1997 survey compared with 1988 [16]. Macronutrient intake increased in all income levels in that period of time, the increase being more pronounced in lipids (50 % on the average) than in the other macronutrients. The average increase in calories was more pronounced in poorer groups (quintile I to III). The same was observed for proteins and lipids, which it is consistent with the prevalence of obesity in those groups.

Qualitatively, the principal foods consumed by the rich and the poor in Chile are essentially the same, but the wealthier 60 % of the population consumes a diet quantitatively different from the poorer 40 %. Over time and socioeconomic sectors, the dominant contributors to total calories in the diet have been bread and other cereals. Although bread consumption in Chile has decreased about 25 % in the last two decades, it is currently estimated to be at a level of 90 kg/person/year and even higher in rural areas (120–140 kg/person/year) [14]. In comparison, developed countries consume an average of 50 kg/person. Besides being a traditional staple in the Chilean diet, bread is an inexpensive item widely used by the poorer groups to satiate hunger.

Prevalence of Obesity

Obesity can be described as a nutritional disease with serious consequences. Its prevalence increases with age and though the increase in weight with age has been accepted as inevitable, data show that this weight gain augments the risk of mortality [17]. The treatment of obesity is difficult and usually unsatisfactory, therefore making its prevention very important [18, 19].

Overnutrition has become in the last decades an important public health problem, both in developed and developing countries. WHO has recently estimated that approximately 1.9 billion people in the world show overweight and at least 600 million of them are obese, making this disease a true epidemic [5]. In addition, obesity is a major risk factor for mortality, particularly from cardiovascular disease [18, 20].

Recent decades have witnessed not only a dramatic increase in the prevalence of obesity in developed countries, but also in developing ones, including those in LA. Figure 35.6 shows the prevalence of obesity in adults observed in several selected countries from the region [3].

In Mexico, the National Nutrition Survey of 2012 showed a prevalence of overweight and obesity in adults of 69.4 % in males and 73 % in females, respectively. In adolescents, the prevalence of overweight + obesity was 34.1 % in males and 35.8 % in females. In children, the prevalence was 36.9 % in boys and 32 % in girls [13, 21].

Brazil, the largest country in the region, has shown a steady decrease in malnutrition and an increase in the prevalence of obesity. A national survey was carried out in 1989 using a representative sample of 14,455 households and compared with a previous survey of 55,000 households performed in 1974–1975. An increase in prevalence was observed in both overweight and obesity in the period of time between the two surveys. Women were 2.5 times as likely as men to be obese, but the highest relative increase was seen in men, where prevalence almost doubled in 15 years. In men the prevalence was much higher in urban areas than in rural areas and showed a direct relationship with income. In both sexes, the more developed regions in the country were associated with a higher prevalence of overweight/obesity [22].

While obesity prevalence in Brazil showed an increase in obesity in lower socioeconomic groups, a decrease in the prevalence of obesity in well-to-do women has been observed. As a matter of fact, this seems to be the only favorable change observed in the region at present [22]. In their analysis, the authors showed that in women obesity is associated with income and education (negatively). Men

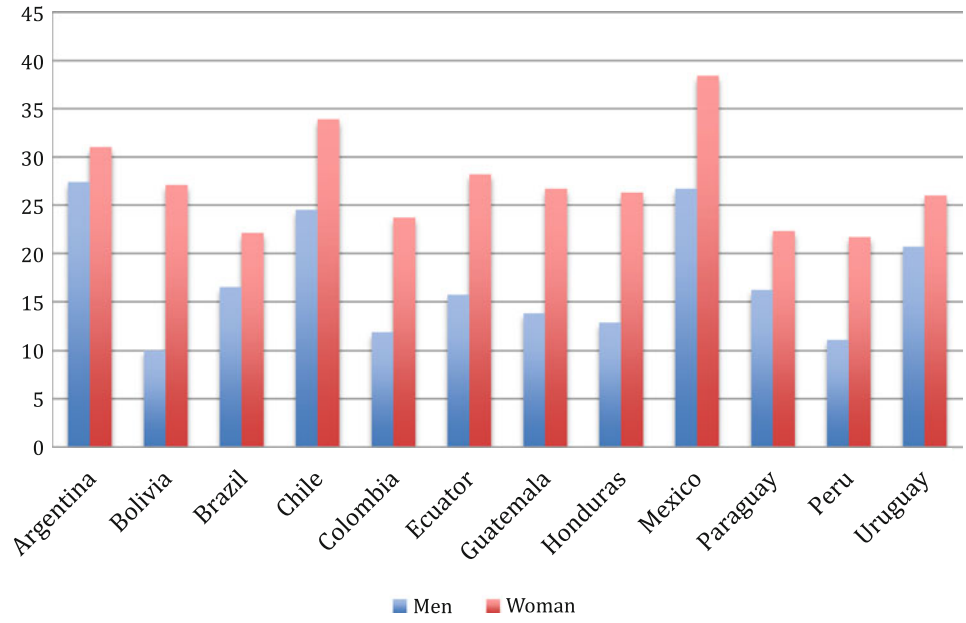

Fig. 35.6 Prevalence of obesity (BMI>30 kg/m^2) in adults from selected countries of Latin America, 2012 [3]

from the same region only showed a positive association with income. In the more developed regions, obesity prevalence was associated negatively only with education in both sexes while in men it was positively associated with income. Similarly, Jacoby et al. in urban Peru showed that in women, education was negatively associated with overweight and obesity [23]. The National Nutrition Survey of 2009 in Brazil also showed a high prevalence of overweight and obesity in adults, reaching 62.6 % for males and 64.9 % for female. In adolescents it was 30.8 % and 30 % [24].

A serious problem that we face now is the remarkable prevalence of obesity in young children. The reasons for this are diverse. Nowadays, children from developed countries and from late developing countries perform much less physical activity and tend to eat or drink high-fat, high carbohydrate foods and sweetened beverages. The last National Nutrition Survey of Mexico also showed the presence of some degree of food insecurity (FI) in 70 % of all households. FI was related to anemia in children (40 %) and 16.5 % in the elderly adults and those households presenting severe food insecurity the prevalence of anemia in children was 40 %. In those with moderate and severe FI, the prevalence of overweight and obesity were 30 % in schoolchildren, 32 % in adolescents, 70.1 % in adults, and 64.2 % in the elderly [25].

A study in Brazil showed that in children 6–9 years old and 10–18 years old, the prevalence of obesity increased, from 1974 to 1997, from 4.9 % to 17.4 % and 3.7 % to 6 %, respectively, and from 4.9 to 18.4 % in urban children [26]. In the last National Survey, these numbers increased [24].

In Chile, the increase of obesity in preschool children has been notoriously rapid, rising from 5.8 % in 1994 to 7.4 % in 2006, and to 10 % in 2013 [27] and obesity in children entering school raised from 6 % in 1987 to 19.4 % in 2006 [17, 27]. De Onis et al. published in 2000 a review of obesity in preschool children in different countries in the world, including Latin America [28]. In LA, the highest prevalence was observed in Argentina, Chile, Peru, and Bolivia while the lowest prevalence was observed in Haiti. In general, there is a correlation of overweight in preschool children and the purchasing power parity (PPP) in countries in LA. The countries with higher PPP tend to have a higher prevalence of overweight in preschool children. When we evaluate the prevalence of overweight and obesity related to socioeconomic status, we find a similar pattern than that in developed countries, showing

poorer people with a higher prevalence of overweight and obesity than well-to-do individuals. This is accompanied by a deficient diet and a lack of physical activity. This was confirmed by a study of Chilean girls aged 8–13 years, which found a lower prevalence of overweight and obesity in the middle–high SEL, presenting a healthier food consumption characterized by a higher intake of dairy products, and a reduced frequency of bread, sweet and salty snacks, and sugary drinks consumption [29].

This data has to be considered seriously since the probability for an obese child to be obese at age 35 years is 80 % [30] and obesity in childhood has serious influence in their physical health and self-esteem [31]. In addition, obesity in adolescence is strong predictors of overweight in adult life and is associated to health problems, regardless of their weight in adult life [32].

What is the cause of such a high obesity in preschool children? Undeniably, there has been an historical decrease in physical activity in children of all ages, which seems to be more prevalent in children from low-income families. Although it is hard to establish the reasons for this decrease, two factors seem to be most relevant. First, the increased amount of time spent in activities that do not require any effort, like TV watching or computer use, in detriment of physical activity. In the Mexican survey, it was found that 51 % of adolescents spent more than 12 h per week watching television. Of these, more than 50 % of them watched TV more than 21 h per week [25]. In Chile, Olivares et al. found that 22.3 % of a sample of 1701 schoolchildren of low socioeconomic status watched TV more than 3 h per day during the week and more than double of them (47 %) during the weekend [33, 34]. Unfortunately, food is the most advertised product category in children's television, with most of the products directed to sweetened products and fast-food restaurants [34].

The other factor responsible for the decrease in physical activity is safety concern of parents regarding their children playing on the street, although attraction for sedentary activities appears to be more important for children. Fortunately, programs are being developed in different countries promoting exercise, and data is showing that they are being successful (see below).

A study has shown that there was a positive significant correlation between consumption of sugar-sweetened drinks and obesity in children [35], although it has been questioned by others [36]. The consumption of fast foods has been often blamed for the increase in the prevalence of obesity in children from LA countries. However, it is hard to blame exclusively the food industry for the increase in obesity. Rather, it is the whole environment (including lack of exercise, lack of nutrition education, and consumption of high-calorie food) that is conductive to this condition.

An added component that we have to consider when we discuss obesity is the effect of early malnutrition and stunting on the appearance of obesity at later years. The studies of Barker strongly suggest that early undernutrition results in increased adiposity in later life [37]. If this is so, we will be faced in the future with the consequences of malnutrition observed in the last decades in Latin America children which will impose an unbearable burden to the health system when they reach adult life.

How Do We Tackle the Obesity Problem?

People that achieve economic progress increase their food-purchasing power, which is corresponded by an increased availability of food by industry. Although the role of food companies in health has long been debated, the reality is that their traditional role is to make a profit for their shareholders. Only public education will be able to change eating trends, and most surely food companies will respond to them. The influence of foreign food styles and imports in Latin American countries has been increasing steadily and certainly will keep this trend.

Fast-food restaurants have shown a striking increase in some Latin American countries, like Chile, Argentina, Brazil, and Uruguay, where most of these traditionally American fast-food restaurants have had explosive growth. As an example, a well-known international fast-food chain increased the number of outlets in Chile from 1 in 1990 to 70 in 2006. This trend is seen all over the region [38].

These types of restaurants are attractive for the population because of the low cost, easy accessibility, and good taste. Unfortunately, their foods are high in calories, saturated fat, and salt. They are particularly attractive for the low-income population because they provide a clean place where they can eat a tasty meal within their financial means. Aware of their success, many middle-income local food service stores have turned into fast-food outlets with mass produced items. As a result, items like hamburgers and French fries are more frequently consumed compared to years ago, when their intake was only sporadic.

In the future, consumption of prepared foods, either from large supermarkets or from fast-food outlets is bound to increase, unless improving nutrition education of the general population counteracts it. It is expected that industry will respond to public demand, if it exists, to develop foods that have a low fat content, are rich in complex carbohydrates, and are low in salt.

We have to consider special care when we propose programs to prevent obesity. In the US, billions of dollars have been spent and nevertheless obesity prevalence keeps increasing [38]. We are fighting not only environmental components such as food availability at a low price, but also biological components, since the signal to eat is stronger than the signal to not to eat, brought about by millions of years of adaptation to a hunting and gathering lifestyle [19].

Do Programs Intended to Prevent Obesity Work?

Several revisions have dealt with the efficacy of obesity prevention programs. A review by Showell showed a low effectiveness in home-based child obesity prevention programs. They conclude that additional research is needed to test interventions in the home setting, particularly those incorporating parenting strategies and addressing environmental influences [39]. Wang et al., in a review about the effectiveness of programs to reduce childhood obesity, conclude that the efficacy of the intervention is moderate. Those programs with most success are those that involve intervention on diet, physical activity both in school and home [41, 42].

In a study of the relationship between socioeconomic status and the prevalence of obesity worldwide, Wang and Lim conclude that the obesity–SES association varies by gender, age, and country [42]. Those groups with more access to energy-dense diets (low SES in industrialized countries and high SES in developing countries) are at increased risk of being obese than their counterparts.

Several authors have reviewed the effectiveness of intervention programs to prevent obesity [41, 43, 44]. In general, the results show that the effectiveness of these programs is moderate, and the most effective programs involve an active participation of the family in addition to intervention in the school environment.

There are some successful activities in the LA region implemented to promote physical activity and improve nutrition education. One of them is the AGITA Sao Paulo experience in Brazil initiated in 1996 [45–47]. This is an intervention program directed to promote physical activity in a community-wide intervention designed to increase knowledge on the benefits and levels of physical exercise. It involves the community, government and nongovernment organizations, and the school environment. The message is that the majority of the population should accumulate at least 30 min of physical activity during most days of the week. Activities are encouraged in three settings: home, transport, and leisure time. There are more than 160 groups involved, and the Brazilian government decided to make it a nationwide program (Agita Brasil). The impact of the program has reached other countries of the region.

The program has been successful in decreasing sedentary habits by 70 % and has diminished the hospitalization by stroke and diabetes type 2 by 50 % and 57 %, respectively. The program is special in that involves multiple organizations and has been well received by the population [45]. The program message has been received by more than 60 % of the population, and among these, 23.1 % know

the main message. Recall of Agita and knowledge of its purpose were well distributed among different SELs, being known by 67 % of the most educated. The prevalence of people reaching the recommendation was 54.8 % (men 48.7 %, women 61 %); and risk of being sedentary was smaller among those who knew the Agita message (7.1 %) compared with those who did not know (13.1 %). Based upon this Agita Sao Paulo experience, it appears that a multilevel, community-wide intervention to promote physical activity may obtain good results as a prevention strategy.

The Brazilian government has also addressed the prevention of nutrition-related noncommunicable diseases by means of innovative legislative and regulatory actions, mass communications, and capacity building creating a comprehensive approach for addressing poor dietary and activity patterns in Brazil related to obesity. Some of these measures are new nutrition-related initiatives in the labeling area, shifts in the types of food purchased for the school food program, use of mass media to communicate components of the food guidelines, establishment of a smart shopping initiative, and training of teachers and health workers. This has represented an effort that took several years to get underway [46].

In Chile, several programs concentrated in the school and community which have been based on the training of teachers to improve nutrition education in class and optimization of the physical education class have resulted in the decrease in the prevalence of obesity. However, once the intense training is finished, children go back to their original weight.

Conclusion

Obesity in LA presents a significant problem nowadays. Its prevalence keeps going up in practically all countries, many of them having to deal also with the burden of undernutrition. The data cited earlier show that eating habits have undergone a transition toward a diet high in calories, and that programs to diminish obesity in children are deficient. A successful program needs to secure the participation of local government to create safe spaces to allow children to actively play, the school to develop a systematic curriculum to promote physical activity and home, where children are stimulated to exercise as opposed to sit in front of a screen.

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Chapter 36
Sodium Consumption in Southeast Asia: An Updated Review of Intake Levels and Dietary Sources in Six Countries

Maria Sofia Amarra and Geok Lin Khor

Key Points

- Hypertension is a major risk factor for cardiovascular disease in Southeast Asia.
- Traditional salted and fermented foods and condiments are an integral part of Southeast Asian cuisine and contribute to increased sodium intakes.
- Discretionary use of salt and condiments contribute significantly to sodium intakes in Southeast Asia.
- The most accurate method to assess level of sodium intake is 24-h urinary sodium excretion, while multiple 24-h recalls are more accurate than food frequency questionnaires to determine dietary sources of sodium.
- Current nationwide survey data from six Southeast Asian countries provide insufficient evidence regarding levels of sodium intake of different population groups, as well as major dietary sources of sodium.
- Only Singapore and Vietnam have accurate data on sodium intake levels of adults, based on 24-h urinary sodium excretion method.
- Efforts should be undertaken to reduce sodium while increasing potassium intakes in Southeast Asian populations.

Keywords Southeast Asia • Sodium intake • Salt • Hypertension • Diet • Cardiovascular disease • Fermented foods • Traditional salted foods • Asian condiments • Asian sauces

Introduction

Sodium is an essential nutrient and the main cation in extracellular fluid. Its functions include maintenance of hydration and fluid volume, stabilizing the potassium/sodium ratio which determines the membrane potentials of cells and the action potentials underlying transmission of nerve impulses and muscular

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_36

Table 36.1 Prevalence of raised blood pressure among adults, age-standardized mortality rates for cardiovascular disease and diabetes, and estimated deaths from hypertensive heart disease in six Southeast Asian countries [9–11]

Country	Prevalence of raised blood pressure among adults aged ≥25 years (%) 2008		Age-standardized mortality rate (ages 30–70 per 100,000 population) for cardiovascular disease and diabetes (2008)	Estimated deaths from hypertensive heart disease 2012 ('000)
	Male	Female		
Indonesia	32.5	29.3	308	42.2
Malaysia	28.8	24.6	217	1.7
Philippines	28.7	23.6	362	21.0
Singapore	24.3	18.5	115	0.4
Thailand	24.6	20.2	244	7.1
Vietnam	29.1	23.3	232	8.8

contractions [1]. Signs of sodium deficiency include thirst, nausea, weakness, fatigue, and cramps. The most common source of dietary sodium is table salt or sodium chloride, comprising 40 % sodium [2].

Studies have shown that habitual high salt intake is one of the risk factors influencing population-wide blood pressure patterns [3]. Hypertension is a leading risk factor for cardiovascular disease and premature deaths globally, and is especially common in Asian populations [4]. With increasing industrialization and urbanization, as well as increasing levels of overweight and obesity, the total burden of cardiovascular diseases is increasing throughout the Asia-Pacific Region. It has been shown that Southeast Asians develop hypertension at lower levels of BMI compared with other Asian populations, due to ethnic variation in body fat. Using nationally representative data, Tuan et al. [5] found that BMI cutoffs that predicted hypertension in Chinese, Indonesian, and Vietnamese adults were 23–24, 21–22.5, and 20.5–21, respectively. Epidemiological cohort studies [6] have shown that hypertension (particularly systolic blood pressure) is a major risk factor for fatal and nonfatal stroke in Asian populations [4, 7, 8].

Statistics for six Southeast Asian countries (Table 36.1) show that in 2008, the prevalence of raised blood pressure among adults aged ≥25 years ranged from 18.5 to 32.5 %, while age-standardized mortality rates for cardiovascular disease and diabetes ranged from 115 to 362 for ages 30–70 per 100,000 population [9, 10]. Indonesia had the highest prevalence of high blood pressure while the Philippines had the highest mortality rate for cardiovascular disease and diabetes. In 2012, both countries also had higher mortality rates from hypertensive heart disease compared with other countries in the region [11].

No segment of the population is immune from the adverse health effects of excess sodium [12]. A meta-analysis of controlled trials on infants and children [13] showed that 54 % reduction in salt intake among infants (aged newborn to 3 months old) resulted in a significant decrease in systolic blood pressure (−2.47 mmHg; $p<0.01$), while 42 % salt intake reduction among children aged 8–16 years resulted in reduced systolic blood pressure (−1.17 mmHg, $p<0.001$) and reduced diastolic blood pressure (−1.29 mmHg, $p<0.0001$). It is now recognized that blood pressure in childhood tracks into adulthood, and although a positive sodium balance is needed for growth during the first year of life, a low salt diet for older children and adolescents has been shown to have the same cardiovascular protective effects as for adults [14–16].

Traditional Role of Fermented and Salted Foods in Southeast Asian Cuisine

Fermented Foods

For centuries, consumption of salted and fermented foods has been part of the traditional food culture in Southeast Asian countries. Among indigenous groups, the use of salt to preserve food helped overcome the uncertainties of nature and enabled consistent consumption. Fish is a major part of the diet

Table 36.2 Traditional fermented fish products in Southeast Asian countries [17–19]

Fermented fish food product	Local name
Fish sauce	*petis* (Indonesia), *budu* (Malaysia), *patis* (Philippines), *nam-pla* (Thailand), *nuoc-mam* (Vietnam)
Fish paste	*otak-otak* (Indonesia), *kapi* (Thailand), *mam-cho* (Vietnam), *badging, belibel, bagoong isda* (Philippines)
Shrimp paste	*trassi* (Indonesia), *sambal belachan* (Malaysia), *kapi* (Thailand), *bagoong alamang, guinamos* (Philippines), *mam ruoc, mam tom* (Vietnam)
Shrimp sauce	*alamang patis* (Philippines), *nam kapi* (Thailand), *nam tom* (Vietnam)
Other fermented fish and seafood	*belachan* (Malaysia), *angkak* (Philippines), *pla ra* (Thailand)

in Southeast Asia. Dried salted fish and fermented fish products are widely consumed, particularly among low-income groups. For countries in the region, fermented fish food products are consumed in greater amounts and are of greater importance than dried salted fish [17]. Researchers have suggested that fish fermentation arose in areas where rice was cultivated, salt was easily obtained, and fish was available seasonally [18, 19]. In these rice-eating countries, a protein food or a condiment that could season the bland flavor of rice was needed, and fermented fish products met this need [18]. At present, the widest variety of fermented fish products and their principal dietary role occurs in Southeast Asia, which makes the region the most likely center of the origin of these foods [19].

Fermentation of fish products results from the action of enzymes and microorganisms in the fish, and requires large amounts of salt to prevent putrefaction [18]. Hydrolysis of fish proteins occurs, resulting in free amino acids, peptides, and ammonia. This, together with salt to prevent microbial growth, results in a desirable taste and aroma called *umami* [20]. *Umami* is defined by the Japanese as the taste of free glutamic acid [19] which enhances the palatability of food by binding to glutamate receptors in the tongue [21] and facilitates rice consumption [19].

Fermented fish sauces and pastes are prepared from different kinds of fish, shrimp, and shellfish. Salt is added and mixed, ranging from 20 to 40 % for fish sauce, and 15 to 25 % for fish pastes [17]. The liquid portion of fermented fish is called fish sauce, and the solid portion is called fish paste. Both products are widely used as staples, side dishes, condiments for cooking, or as dips and sauces in Southeast Asian cuisine [17, 20]. In the Philippines, fish paste and dried processed fish are important dietary components and their use as a vehicle to address iodine deficiency has been recommended. It is estimated that, at existing levels of consumption, iodized salt in dried fish and in fish paste could provide 30 % and 64 %, respectively, of the Recommended Nutrient Intake (RNI) levels for iodine among pregnant and lactating women, while iodized salt in dried fish alone could provide at least 107 % of the RNI for children aged 1–6 years and 64 % of the RNI for reproductive age women [22].

Table 36.2 shows traditional fermented fish products and the local nomenclature in their respective countries.

Other Asian Sauces

In addition to fermented fish products, a variety of sauces that contain salt are commonly used in Asian cuisine. An example is oyster sauce, traditionally produced by slowly simmering oysters in water until the juices caramelize into a thick, brown, intensely flavorful sauce [23]. Oyster sauce is a staple in Chinese cooking, giving this cuisine which is widely adopted across Southeast Asia, its distinct flavor and sensory characteristics. In Indonesia, sweet soy sauce "*kecap manis*" is found in almost every household [24]. In Thailand, fermented seasonings, such as *nam pla* or fish sauce, is found in almost all Thai dishes in every region of the country [25]. Commonly used Asian sauces are shown in Table 36.3, with their corresponding sodium content.

Table 36.3 Sodium content of commonly used Asian sauces and condiments [27, 92]

Sauce	Mg Na per 100 g serving
Black bean sauce (sauce with fermented black beans, sugar, salt, soy sauce for simmering meats)	3200
Chili sauce (spicy condiment for use as a dipping sauce)	1473
Curry sauce (sauce to simmer foods; made from lemongrass, coconut milk, salt, spices)	794
Fish sauce	4926
Hoisin sauce (marinade sauce from soybeans, rice vinegar, salt, spices)	600
Marinade powder for satay (barbecue)	5569
Oyster sauce (dark brown sauce made from boiled oysters and seasonings; used as dressing, marinade, or to simmer meats and vegetables)	3379
Plum sauce (sauce with plum puree; used as marinade or dipping sauce)	842
Shrimp paste	6000
Soy sauce (dark)	3472
Soy sauce (light)	9489
Tomato ketchup (dipping sauce made from tomato paste and seasonings)	954

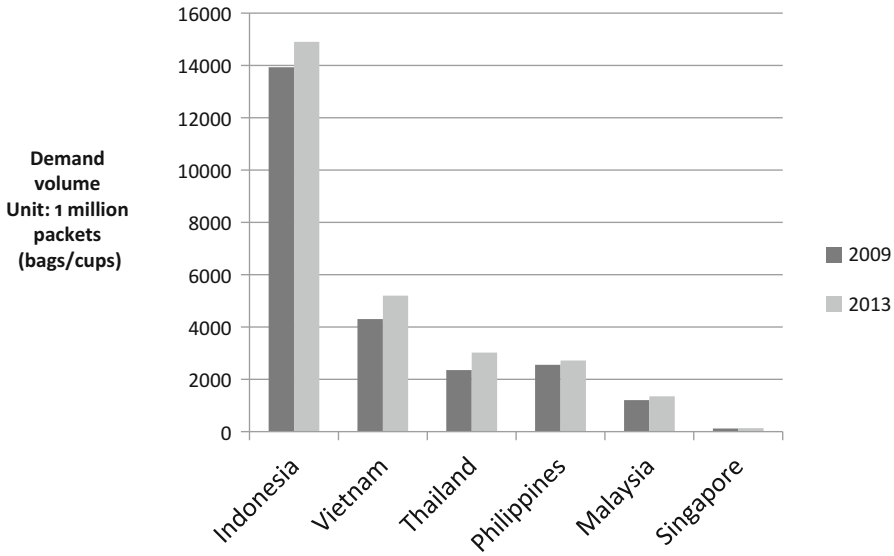

Fig. 36.1 Demand for instant noodles in Southeast Asia [28]

Salted Noodles

Aside from fermented foods, noodles made from wheat flour form an important part of the Southeast Asian diet. Noodles mean long life and good health, and are a traditional component of birthday celebrations in the region. Asian wheat noodles can be classified based on the presence of salt or alkali. White salted noodles contain only common salt (1–3 %), while yellow alkaline noodles contain alkaline salt such as sodium and/or potassium carbonates (1–1.5 %), with or without common salt. Ramen, or instant noodles, contain 0.1–0.6 % sodium and potassium carbonates; instant noodle powder contains other alkalis such as sodium pyrophosphate and sodium metaphosphate [26]. A 100 g serving of instant noodles with seasoning contains 1975 mg sodium [27]. Billions of instant noodle packages are consumed annually in Southeast Asia. Figure 36.1 shows how demand for instant noodles has risen in Southeast Asian countries from 2009 to 2013 [28].

Impact of Urbanization on the Amount of Sodium in the Southeast Asian Diet

The introduction of modern processed western foods as a result of urbanization has further increased the amount of salt and sodium in the food supply of Southeast Asian countries. Sodium-containing compounds are used to improve flavor, reduce the growth of pathogens, and improve the shelf life of processed foods. Compounds used in food preservation include disodium ethylenediaminetetraacetic acid (EDTA), sodium acetate, sodium ascorbate, sodium benzoate, sodium lactate, sodium sulfite, sodium nitrite, and sodium nitrate [12]. Aside from preservation, other roles of sodium-containing compounds include the development of physical properties of foods that are beneficial for processing (e.g., to control the stickiness of some doughs) or developing final product qualities (e.g., improved texture). A variety of sodium-containing compounds also act as emulsifying agents, buffering agents, anticaking agents, leavening agents, stabilizers, neutralizers, thickeners, moisture-retaining agents, and bleaching agents [12].

In Southeast Asia, increased urbanization is not necessarily accompanied by a shift in preference from traditional to western foods, resulting in the nutrition transition seen in developing countries. Lipoeto et al.'s [29] study of food consumption patterns among adults living in urban and rural areas in the Philippines, Malaysia, and Indonesia showed that Filipinos, Malaysians, and Indonesians have retained many aspects of their traditional diets, and that western-style foods and fast foods were considered as snack or recreational foods to be consumed only once in a while. But while traditional food patterns were maintained, more sugar and vegetable oils were consumed and added to traditional recipes. Participants in urban areas consumed more varieties of traditional foods compared to those in rural areas, mainly due to the increased availability of these foods and participants' own increased purchasing power. Hence the authors concluded that the rapid nutrition transition in this region may be due, instead, to increasing food availability and food purchasing power rather than to a shift in preference toward modern western foods. In Thailand, the 2013 Survey on Food Consumption Behavior reported that 70.8 % of respondents did not eat western fast food [30].

In Southeast Asia, both home-cooked foods and foods eaten away from home contribute to increased sodium intake. This is in contrast to Western countries where foods eaten away from home are the major contributors to intake [31]. In Vietnam, out-of-home traditional street foods contributed a higher percentage to daily sodium intake among urban adolescents compared with rural adolescents (33.1 ± 2.1 % vs. 12.1 ± 2.1 %, respectively). However, rural adolescents had higher total sodium intakes compared with urban adolescents (1643.2 ± 124.8 mg Na vs. 1500.7 ± 124.8 mg Na, respectively) [32]. In Singapore, data from the Singapore Chinese Health Study showed that respondents reporting frequent intake of Western-style fast food items (≥2 times/week) also consumed more *dim sum*, noodles, and sugar-sweetened beverages, and greater amounts of sodium (864 mg Na/1000 kcal) compared with those who did not eat fast foods (651.7 mg Na/1000 kcal) [33].

Recommendations for Sodium Intake

WHO and the United Nations have called for global reductions in salt intake and salt content of foods, as a cost-effective way to prevent disease and reduce health care costs [34, 35]. WHO recommends an intake <2 g (or 2000 mg)/day sodium (<5 g/day salt or sodium chloride) to reduce blood pressure and risk of CVD, stroke, and coronary heart disease in adults. The same recommendation applies to children, with the maximum level of intake of 2 g/day adjusted downward based on the energy requirements of children relative to those of adults [36].

The U.S. Institute of Medicine (IOM) defines adequate intake (AI) level as "the recommended average daily intake level based on observed or experimentally determined approximations of

nutrient intake by a group of apparently healthy people that are assumed to be adequate (used when the recommended dietary allowance (RDA) cannot be determined).” The following AI levels for sodium are recommended [37]: 1–3 years (1.0 g Na/day), 4–8 years (1.2 g Na/day), 9–50 years (1.5 g Na/day), 51–70 years (1.3 g Na/day), >70 years (1.2 g Na/day).

Other Recommendations

The 2010 U.S. Dietary Guidelines recommends a daily sodium intake <2300 mg/day, and 1500 mg for individuals aged 51 years and older, African Americans, and those with hypertension, diabetes, or chronic kidney disease [38]. The American Heart Association (AHA) currently recommends a sodium intake <1500 mg/day for the entire U.S. population [39].

Methods of Determining Sodium Intake

Biochemical Assessment Methods

The measurement of 24-h urinary sodium excretion is considered the “gold standard” method of obtaining data on sodium intakes in population surveys [40, 41] and reflects about 85–90 % of ingested sodium. Two or more collections are preferred to estimate within-person variability and decrease misclassification. The method accounts for electrolyte loss via the kidney and excludes other routes of elimination, thereby underestimating true intake by 10–15 % [42].

To reduce participant burden, overnight and spot (casual) urine collections have been suggested as alternatives to 24-h urine collections. Evidence suggests that the use of overnight urine samples may result in biased estimates of sodium excretion [42]. Wang et al. [43] found that, among adults aged 18–39 years, overnight urine samples had the lowest sodium concentrations compared with urine samples collected during other times of the day.

Spot urine collection means taking a single sample at a certain time of day. The use of spot urine samples is discouraged [41] because of the limitations and uncertainty inherent in the method. If the method is used, a “calibration” should be carried out, based on the expected 24-h volume of urine or the 24-h total excretion of creatinine, by applying recommended equations. These equations yield “correction factors” that should be calculated in a subsample of individuals from the same population subjected to the same environmental conditions and studied in a 24-h period. Equations and their corresponding correction factors are specific to certain populations and cannot be reliably extrapolated from one site/population to another, making these “correction factors” difficult to determine [41]. Until more studies are carried out to assess simpler but reliable methods of urine collection for the purpose of estimating daily excretion of sodium, 24-h urine collections are recommended [41].

Food Consumption and Household Survey Methods

Sources of information on dietary sodium intake include household expenditure surveys and nutrition (i.e., food consumption) surveys [12, 44]. National household expenditure surveys (HES) evaluate food and nonfood consumption of a country’s population [45]. Food data collected in HESs reflect the quantity of food “acquired” by a household, including their food purchases, foods consumed from their own farms or gardens, and foods received in kind. Estimated quantities, expressed in metric units,

serve as the basis for calculating indicators of food security such as diet quantity and diet quality [46]. HESs do not measure wastage and spoilage and therefore do not reflect food actually consumed.

National nutrition surveys represent the best way to assess food actually consumed in a population. However, many developing countries including those in Southeast Asia do not have the resources to mount nationwide surveys [47]. In the absence of large surveys, small studies done on specific groups serve as the main sources of information on individual intakes [47].

The most commonly used methods in nutrition surveys are 24-h recalls and food frequency questionnaires. Weighed food records are the most precise method but are tedious and more often used in small studies. For large surveys, multiple 24-h recalls provide more precise information than food frequency questionnaires (FFQ). A single 24-h recall is sufficient to describe the average intake of a population, while multiple days of recalls are needed to model estimates of the population's usual intake distributions and their relationships with other factors [48, 49]. Day et al. [50] examined the performance of a semiquantitative FFQ and 7-day food diary against urinary measures of sodium. Their results showed that, while both methods underestimated sodium intakes, the 7-day diary provided a better estimate of average intake compared with the FFQ. Low correlations between urinary and dietary assessment measures were observed, that is, 0.36 for the 7-day food diary and 0.13 for the FFQ.

Levels of Sodium Intake and Dietary Sources in Southeast Asia

A search of online and published literature was conducted to examine the best available evidence regarding levels and sources of sodium intake in six Southeast Asian countries: Indonesia, Malaysia, Philippines, Singapore, Thailand, and Vietnam. The objectives were: (1) to describe levels of sodium consumption, as shown in available national surveys and individual studies, and (2) to identify sources of sodium including traditional foods and condiments and their levels of use.

Materials and Methods

Materials for the review consisted of government and news reports, reports from international organizations, conference and seminar proceedings, and scientific studies.

Search Strategy

For nationally representative data, a Google search of government and international organization websites was conducted using the following terms: national nutrition survey, food consumption survey, household expenditure survey, food security, Indonesia, Malaysia, Thailand, Philippines, Singapore, Vietnam. Meeting and news reports, conference proceedings were hand searched. For individual studies, a PubMed search was conducted using the terms: sodium, salt, diet, nutrient intake, seasoning, condiment, hypertension, blood pressure, micronutrients, cardiovascular disease, stroke, urinary sodium, Indonesia, Malaysia, Thailand, Philippines, Singapore, Vietnam. Unpublished theses and dissertations were hand searched.

Inclusion Criteria

Materials were selected for inclusion based on the following criteria:

(1) Nationally representative surveys (household expenditure and food consumption surveys) that were the latest available online as of November 2014; (2) Individual studies that: (a) were conducted among all age groups; (b) examined dietary intakes of salt, sodium, and salty foods; (c) were among populations in Indonesia, Malaysia, Philippines, Singapore, Thailand, and Vietnam; and (d) covered the period 2000 up to November 2014.

Exclusion Criteria

Excluded were: (1) studies based on dietary simulations or total diet studies; (2) studies among pregnant women, institutionalized persons, case studies.

Data Synthesis

For each country, information relating to salt and sodium intake measurements was extracted. Table salt (sodium chloride) is approximately 40 % sodium. To provide standard information, all estimates of salt intake were converted as mass of sodium per day (mg/day) where 1 g sodium chloride = 17.1 mmol sodium or 393.4 mg sodium. To convert mmol of sodium to mg, the mmol value was multiplied by 23 (the molecular weight of sodium) [37].

For studies reporting on the consumption of condiments and traditional salted and fermented foods, the amounts consumed were extracted and daily consumption was estimated by converting weekly or monthly portions into daily portions. The sodium content of the respective food items was then obtained from existing food composition databases—that is, the ASEAN Food Composition Database [27], the USDA Nutrient Database [51], Malaysian food composition table [52]—and applied to amounts consumed in order to estimate sodium intakes.

A total of 31 papers (10 nationally representative surveys/reports, 21 individual studies) were obtained. Figure 36.2 shows the flow chart for inclusion of studies included in the review. Characteristics

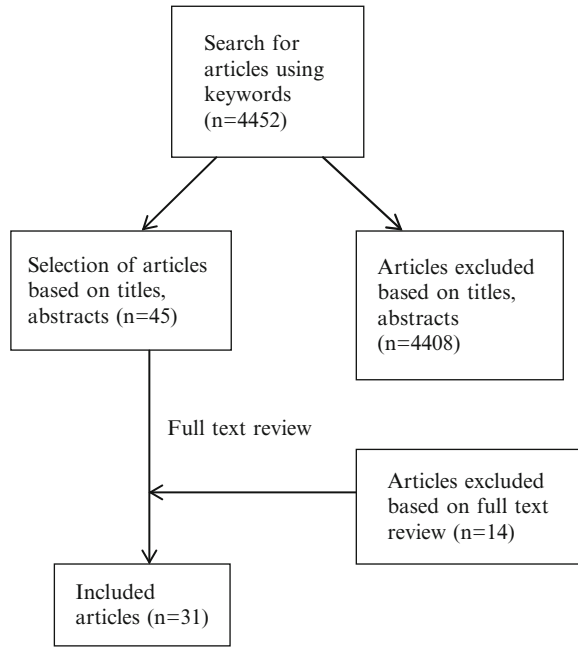

Fig. 36.2 Schematic diagram for selection of studies and reports for inclusion

Table 36.4 Characteristics of nationally representative surveys and studies included in the review

Country	Source of information	Sample size; age and sex examined	Method of measuring sodium intake	Level of sodium intake identified	Dietary sources of sodium identified
All	Powles et al. (2014) on behalf of the Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCODE) [53]	Published and unpublished country surveys of 24-h urinary sodium and dietary sodium	24-h urinary sodium excretion; dietary estimates converted to urine equivalents	Yes	No
Indonesia	National Socio-Economic Survey (SUSENAS) 2011 and 2012 [59, 60]	75,000 households	Per capita consumption and expenditure (salt and condiments)	No	Partly
Malaysia	Malaysian Adult Nutrition Survey (MANS) 2002–2003 [54]	7349 adults; aged 18–59 years; 51 % men, 41 % women	One day 24-h recall	Yes	No
Philippines	2008 National Nutrition Survey [58]	4880 households	One day household food weighing (per capita consumption of discretionary salt, i.e., salt added during cooking or at the table)	Partly	Partly
Thailand	Fourth National Health Examination Survey 2009 [56]	Population aged 19–59 years	One day 24-h recall	Yes	No
	Thailand Household Socio-Economic Survey 2011 [65]	52,000 households	Questionnaire on quantities consumed from a list of 193 food items	No	Partly
	Thailand Health Profile 2008–2010 [93]	Survey conducted in eight provinces in all four regions	Questionnaire on food consumption behavior	No	Partly
Singapore	National Nutrition Survey 2010 [55]	1661 adults aged 18–69 years	24-h urinary sodium excretion; questionnaire on food practices	Yes	No
Vietnam	2009 Vietnam STEPwise Approach to Surveillance (STEPS) Survey 2014 [57]	14,706 adults aged 25–65 years from 8 provinces – Males = 7850 – Females = 6804	Fasting, mid-morning spot urine sample previously validated against 24-h urine sample	Yes	No
	Vietnam Living Standards Survey 2012 [66]	>30,000 households	Per capita consumption and expenditure (fish sauce)	No	Partly

Table 36.5 Characteristics of individual studies included in the review

Country	Author, year	Sampling method	Sample size; age and sex examined	Method of measuring sodium intake	Level of sodium intake identified	Dietary sources of sodium identified
Indonesia	Andarwulan et al. (2011) [24]	Not specified	222 households; Bogor (rural) = 110, Jakarta (urban) = 112	Questionnaire on use (frequency and amounts) of different condiments and seasonings; per capita intake reported	No	Partly
	Gunung (2008) [95]	Systematic sampling	8 families consisting of 33 subjects; 20 males, 13 females	Dietary assessment method not specified	Yes	No
	Kamso et al. (2007) [61]	Purposive sampling	556 elderly aged 55–80 years; 161 males, 395 females	One day 24-h recall	Yes	No
	Mustafa et al. (2006) [62]	Purposive sampling	15 boys and their mothers; boys aged 8.7 ± 0.6 years, mothers aged 33.2 ± 4 years	24-h urinary sodium excretion for total sodium intake; Lithium-labeled salt for discretionary salt intake	Yes	Partly
Malaysia	Karupaiah et al. (2013) [96]	Purposive sampling	128 adult women aged 21 to >60 years – 19–29 years (n = 19) – 30–50 (n = 85) – 51–59 (n = 19) – 60–65 (n = 5)	3 day food records	Yes	No
	Choong et al. (2012) [67]	Not specified	300 university students aged 20.9 ± 1.67 years	Food frequency questionnaire regarding – Consumption of selected salty foods – Adding salt/soy sauce to food while eating – Dipping food in soy sauce mixed with chilies/garlic/sambal belacan	No	Partly
	Gan et al. (2011) [97]	Random sampling	584 university students aged 20.6 ± 1.4 years	Two day 24-h recalls	Yes	No
Philippines	Lee (2009) [68]	Cluster sampling	1776 female participants in the Cebu Longitudinal Health and Nutrition Study, aged 35–68 years	Two day 24-h recalls	Yes	Yes

Thailand	Leelajaratkoon et al. (2010) [64]	Purposive sampling	225 preschoolers from a well child clinic in Bangkok; aged 1–5 years	Two day 24-h recalls; food frequency questionnaire for high sodium foods	Yes	Yes
	Ramathibodi Hospital Cardiovascular and Metabolic Centre (2010) [98]	Not specified	2500 employees of the Electricity Generating Authority of Thailand	Not specified	Yes	No
	Pavadhgul et al. (2009) [69]	Purposive sampling	170 undergraduate students aged 17–20 years	Semiquantitative food frequency questionnaire	Yes	Yes
	Ministry of Public Health, 2008—poll conducted among Bangkok residents in 2006 [94]	Not specified	Not specified	Questionnaire on food practices	No	Partly
	Klunklin and Channoonmuang (2006) [63]	Not specified	170 preschoolers from rural villages in northeastern Thailand; aged 2–6 years	5-day food records	Yes	No
Vietnam	Do (2014) [72]	Random sampling	121 men and women aged 25–64 years from a rural commune of northern Vietnam	One day food weighing	Yes	Yes
	Thu Hien et al. (2013) [75]	Multistage cluster sampling	1528 adults living in rural and urban areas; aged ≥20 years	MSG weighing for 3 consecutive days	No	Partly
	Laillou et al. (2012) [70]	Random sampling	1503 women participating in the 2009 Food Consumption Survey; aged 15–60 years	One day 24-h recall combined with controlled food weighing	Partly	Partly
	Laillou et al. (2012) [71]	Random sampling	415 children from 19 provinces; aged 6–60 months	One day 24-h recall combined with controlled food weighing	Partly	Partly
	Lachat et al. (2009) [32]	Cluster sampling	1172 adolescents living in urban and rural areas; aged 16.4 ± 0.03 years	One day 24-h recall	Yes	Partly
	Thuy et al. (2005) [73]	Random sampling	576 women from 2 communes in Nam Dinh province; aged 16–49 years	One day 24-h recall	Partly	Partly
	Duong et al. (2003) [74]	Purposive sampling	357 adults in Ho Chi Minh City; aged 19–85 years	Survey on health practices	No	Partly
	Hanh et al. (2001) [99]	Random sampling	217 elderly from an urban and rural area in Ho Chi Minh City aged 60–69 years	24-h urinary sodium excretion	Yes	No

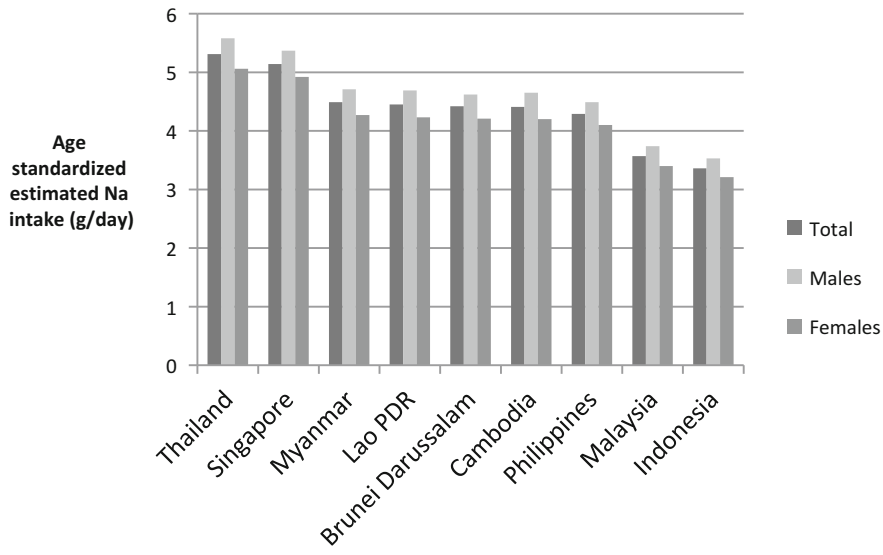

Fig. 36.3 Age-standardized estimated Na intake (gm/day) for ages 20+ years in 2010 [53]

of nationally representative surveys and studies included in the review are shown in Table 36.4; those of individual studies are shown in Table 36.5.

Results

Findings from the Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCODE)

Powles et al. [53] estimated global and national sodium intakes among adults from 187 countries in 1990 and 2010, using published and unpublished surveys of 24 h urinary sodium and dietary sodium. Dietary estimates were converted to urine equivalents, and Bayesian hierarchical modeling was used to estimate mean sodium intake by sex, 5 years age groups, and associated uncertainty for persons aged 20+ years. Results showed that in 2010, estimated sodium intake of adults in Southeast Asian countries exceeded 3 g/day (Fig. 36.3). The highest levels of intake were found in Thailand and the lowest in Indonesia, with males in all countries having higher intakes than females.

Findings from Nationally Representative Surveys and Individual Studies

Levels of Sodium Intake

Table 36.6 summarizes the levels of sodium intake in each country based on findings from national surveys and individual studies, categorized according to age groups.

Nationally Representative Surveys. Sodium intake in national surveys focused only on the adult population. Malaysia [54], Singapore [55], Thailand [56], and Vietnam [57] examined sodium intake levels

Table 36.6 Levels of sodium intake in six Southeast Asian countries

Country	Reference	Mean sodium ± SD (mg/day)			
		Mean adult intake	Males	Females	Other results
A. Nationally representative surveys					
Malaysia	Malaysian Adult Nutrition Survey 2002–2003 [54]	2575 ± 23	2819 ± 34.4	2322 ± 29.2	Age group Na intake
					– 18–19 years = 2494 ± 116.4
					– 20–29 years = 2645 ± 40.1
					– 30–39 years = 2671 ± 39.3
					– 40–49 years = 2500 ± 42.4
					– 50–59 years = 2414 ± 59
					Ethnic group intake
					– Malay = 2507 ± 30
					– Chinese = 2916 ± 49
					– Indian = 1965 ± 61.9
					– Orang Asli PM = 945 ± 334.8
					– Sabah Bumiputera = 2929 ± 107.8
					– Sarawak Bumiputera = 2707 ± 96.1
					– Others Bumiputera = 2518 ± 179.8
					Urban = 2601 ± 31.5
					Rural = 2538 ± 33.1
					Intake by educational attainment
					– Primary = 2429 ± 46.2
					– Lower secondary = 2588 ± 46.7
					– Higher secondary = 2663 ± 39.2
					– Matriculation/Form 6 = 2605 ± 101.7
					– College/university = 2734 ± 70.1
					– Least educated = 1973 ± 98.4
Thailand	4th National Health Examination Survey 2009 [56]	3300	–	–	–
Vietnam	2009 Vietnam STEPwise approach to Surveillance (STEPS) survey [57]	3934	3776.64 ± 51.14	4052.02 ± 70.81	–

(continued)

Table 36.6 (continued)

Country	Reference	Mean sodium ± SD (mg/day)			
		Mean adult intake	Males	Females	Other results
Singapore	National Nutrition Survey 2010 [55]	3270.6	3772	2831.3	Ethnic group intake
					– Indian = 3415.5
					– Chinese = 3293.6
					– Malay = 2990
B. Individual studies					
		Mean sodium ± SD (mg/day)			
		Mean intake (all)	Males	Females	Other results
1. Adults and adult equivalent					
Indonesia	Gunung (2008) [95]	Per capita intake = 2612.18 ± 751.4	–	–	–
	Kamso et al. (2007) [61]	–	198 ± 18.4	161 ± 12.5	–
	Mustafa et al. (2006) [62]	–	–	2720.9 ± 1338.6	
Malaysia	Karupaiah et al. (2013) [96]	–	–	19–29 years = 2833 ± 431	–
				30–50 years = 2724 ± 517	
				51–59 years = 2709 ± 349	
				60–65 years = 2683 ± 435	
	Gan et al. (2011) [97]	–	2971 ± 1273	2322 ± 958	–
Philippines	Lee (2009) [68]	–	–	2900	49.9 % of respondents consumed >2300 mg Na/day
					14.9 % of respondents consumed at least 4600 mg Na/day
Thailand	Ramathibodi Hospital, Cardiovascular and Metabolic Centre (2010) [98]	–	–	–	Intake range = 3934–4720
	Pavadhgul et al. (2009) [69]	–	5225 ± 2290	4498 ± 2091	–

Vietnam	Do (2014) [72]	4338.4 ± 1322	4526 ± 1357.6	4165.7 ± 1320.3	97.5 % of subjects intakes above 2000 mg Na/day
	Hanh et al. (2001) [99]	2725	2759	2694	mg Na/24 h in urban and rural residents
					– Urban
					Males = 2334 ± 1178
					Females = 2764 ± 1221
					– Rural
					Males = 3184 ± 1662
					Females = 2624 ± 1327
					mg Na/1000 kcal in urban and rural residents
					– Urban
					Males = 1347 ± 547
					Females = 2220 ± 1202
					– Rural
					Males = 2377 ± 1437
					Females = 3180 ± 2350
2. Adolescents					
Vietnam	Lachat et al. (2009) [32]	1572 ± 86.3	–	–	mg Na intake of rural and urban adolescents
					– Rural = 1643.2 ± 124.8
					– Urban = 1500.7 ± 124.8
3. Children					
Indonesia	Mustafa et al. (2006) [62]		1752.6 ± 742.9	–	–
Thailand	Leelajaratkoon et al. (2010) [64]	1–3 years = 1301.5 ± 409.7	–	–	–
		4–6 years = 1448 ± 518.5			
	Klunklin and Channoonmuang (2006) [63]	2–3 years = 541 ± 107	–	–	–
		4–6 years = 582 ± 150			

-No data

as part of their national surveys. Data from Malaysia and Thailand were based on single 24-h food recalls, while data from Singapore and Vietnam were based on 24-h urinary sodium excretion and spot urinary sodium excretion, respectively. Regardless of the method used, findings showed high levels of sodium intake in all countries that exceeded the WHO recommendation of <2000 mg Na/day, with the highest levels in Vietnam (3934 mg Na/day) and lowest in Malaysia (2575 mg Na/day). Philippines [58] and Indonesia [59, 60] had no information regarding the total sodium intake of their respective populations.

Individual Studies. In all countries, individual studies among adult subjects showed high levels of sodium intake exceeding the WHO recommendation, consistent with results obtained from nationally representative surveys. One exception is a study among the elderly in Indonesia [61], which showed low levels of sodium intake, but did not include discretionary salt intake.

Very few studies exist on sodium intakes of younger age groups and the results vary. In Indonesia, one study [62] showed a high mean intake exceeding 1500 mg Na/day. In Thailand, one study among preschool children in rural villages showed low sodium intake levels [63] while another study in a well-child clinic in Bangkok showed high levels of intake [64]. In Vietnam, one study among adolescents showed high levels of sodium intake among those living in rural areas but not in urban areas [32]. The lower sodium intake of urban Vietnamese adolescents appeared to be associated with their lower energy-dense diets.

Sources of Sodium

Table 36.7 summarizes the sources of sodium based on national surveys and individual studies, including traditional salted foods and condiments and their levels of use in each country.

Nationally Representative Surveys. Household expenditure surveys from Indonesia [59, 60], Thailand [65], and Vietnam [66], and a survey on food consumption behavior in Thailand [30] suggested that use of condiments such as table salt, soy sauce, and fish sauce contributed significant amounts of dietary sodium, in addition to the sodium obtained from traditional salted products (e.g., fish paste, dried fish) and modern processed foods.

In the Philippines, discretionary salt intake alone (measured by household food weighing) contributed more than 1500 mg Na/capita/day [58]. The exception is Singapore, where only 19 % of respondents in the latest survey reported adding salt or sauces to their food at the table [55], indicating that discretionary use of salt or condiments may not account for their high sodium intakes.

Individual Studies. Individual studies showed that adding salt or seasoning at the table (e.g., dipping food in soy sauce) and during cooking is a common practice across Southeast Asia. Popular condiments are soy sauce in Indonesia, fish sauce in Vietnam and Thailand, soy sauce and sambal in Malaysia, table salt in Philippines.

In Indonesia, one study [24] showed that, compared with other condiments, sweet soy sauce contributed most to individual sodium intakes in both urban and rural areas. A study that used lithium-labeled salt to estimate discretionary salt intake [62] among mothers and their children found that discretionary salt contributed 50 ± 17 % (i.e., 2.9 ± 5.9 g salt/day) and 48 ± 17 % (i.e., 2.5 ± 1.2 g salt/day) of the total salt ingested by mothers and their children, respectively. This translates into approximately 1141 and 984 mg Na/day for mothers and children, respectively. While discretionary salt intake of mothers was not different from those of their children, children had significantly higher intake of salt per kg body weight than that of mothers (0.27 vs. 0.12 g/kg body weight, respectively, $p < 0.001$).

In Malaysia, a study among university students [67] found that French fries and instant noodles (with soup) were the most preferred and frequently (at least once a week) consumed salty food by male and female students, respectively. Majority of subjects reported usually adding salt/soy sauce to food and dipping food in soy sauce mixed with chilies/garlic and/or *sambal belacan*, with more subjects dipping in sauce rather than adding.

In the Philippines, Lee's [68] study on 1776 women aged 35–68 years showed that salty condiments added during cooking or at the table accounted for 76.3 % of total sodium intake. The most significant source of sodium was table salt, contributing 53.3 % for women who consumed <4600 mg Na/day and 66.5 % for women who consumed higher amounts of sodium.

In Thailand, one study [69] found that the main source of sodium in a group of university students aged 17–20 years was one-plate meals (defined as foods cooked by the seller when the customer orders it, such as noodles and fried rice) sold in the campus cafeteria. These meals provided an average sodium intake of 2852.3 ± 1421.8 mg for males and 2042.3 ± 1214.2 mg for females, comprising 54.6 % and 45.5 % of the total daily sodium intakes of males and females, respectively. The most popular one-plate meals were rice noodles with meat balls and soup, and rice with fried meat and holy basil leaves. Eighty percent of students added seasoning to their meals, which contributed 539.8 ± 498.8 and 473.3 ± 514.5 mg Na/day for males and females, respectively. Favorite seasonings were fish sauce, tomato ketchup, soy sauce, and chili ketchup. A study among Thai preschoolers [64] found that frequently consumed foods with high sodium content were fish sauce/soy sauce, bread and bakery products, fried mackerel, fried rice, fried prawn/chicken/fish balls, and fried seaweed.

Table 36.7 Sources of sodium in six Southeast Asian countries

		Sources of sodium			
A. Nationally representative surveys					
Indonesia	National Socio-Economic Survey (SUSENAS) 2011 and 2012 [59, 60]	Food sources	Estimated per capita intake (mg Na/day)		
			2011		2012
		Table salt	488.35		461.22
		Instant noodles	198.49		144.13
		Soya sauce	134.3		115.32
		Fish paste/sauce	19.62		17.72
		Sambal/chili sauce/tomato sauce	4.58		3.43
		Fermented soybean paste	0.57		0.57
		Monosodium glutamate (MSG)	0.06		0.07
Philippines	2008 National Nutrition Survey [58]	mg Na from discretionary salt = 1573.6 mg/capita/day			
Thailand	Thailand Household Socio-Economic Survey 2011 [65]		Estimated per capita intake (mg Na/day)		
		Food sources	Urban	Rural	Total
		Fish sauce	359.6	502.45	862.05
		Fermented fish (preserved)	106.54	294.55	401.09
		Curry paste	60.58	75.72	136.3
		Other fish/seafood (preserved)	34.6	50.17	84.77
		Other meats (preserved)	14.96	14.96	29.92
		MSG	0.1	0.16	0.25
	Thailand Health Profile 2008–2010 [93]	Food consumption behavior and % Thais with such behavior – Eat salty foods such as salted beef, salted fish, salted eggs, pickled mustard, pickled garlic = 91.8 % – Add fish sauce or soy sauce to food prior to eating it = 82.5 %			
Vietnam	Vietnam Living Standards Survey 2012 [66]	Daily per capita Na intake from fish sauce = 493 mg Na/capita/day (10 mL fish sauce/day)			

(continued)

Table 36.7 (continued)

		Sources of sodium			
B. Individual studies					
1. Adults and adult equivalent					
Indonesia	Andarwulan et al. (2011) [24]	Condiment/seasoning	Estimated per capita intake (mg Na/day)		Other findings
			Rural	Urban	Total
		Sweet soy sauce	64.28	140.07	204.35
		Premix seasoning: liquid, cubes, powder	21.23	38.77	60.01
		Chili sauce	11.64	19.67	31.31
		Ready to use seasoning	–	24.00	24.00
		Oyster and fish sauce	5.70	15.19	20.89
		Teriyaki and others	–	20.33	20.33
		Seasoned flour	0.45	13.15	13.60
		Fermented fish/shrimp paste	5.58	6.86	12.44
		Salty soybean sauce	–	4.38	4.38
		Fermented soybean paste	3.73	0.29	4.02
		Dip and sauce	–	3.23	3.23
		Tomato sauce	0.19	1.19	1.38
		MSG	0.13	0.09	0.22
		Spread	–	0.19	0.19
		Mayonnaise and mustard	0.07	–	0.07
					Condiments most commonly used by 71 % of households (urban and rural) – Sweet soy sauce – MSG – Premix seasoning Condiments commonly used by 58 % or less in urban households and 53 % or less in rural households – Chili sauce – Fermented shrimp/fish paste – Tomato sauce – Fermented soybean paste – Ready to use seasoning – Seasoned flour
	Mustafa et al. (2006) [62]	mg Na from discretionary salt intake = 1140.8 mg Na/adult woman/day (approx. 41 % of total Na intake/day)			
Malaysia	Choong et al. (2012) [67]	1. Frequently consumed salty foods (in descending order)			
		Males	Females		
		– French fries	– Instant noodles		
		– Hotdog/sausage	– Hotdog/sausage		
		– Instant noodles	– French fries		
		– Fried nuggets/balls	– Fried nuggets/balls		
		– Potato chips	– Spaghetti with canned tomato sauce		
		– Chicken ham	– Potato chips		
		– Spaghetti with canned tomato sauce	– Chicken ham		
		– Salted biscuit	– Salted biscuits		
		– Pizza	– Asam laksa		
		– Canned soup	– Pizza		
		– Salted nuts	– Salted vegetables		
		– Asam laksa	– Canned soup		
		– Belacan fried rice	– Salted nuts		
		– Salted fish	– Belacan fried rice		
		– Salted vegetables	– Salted fish		
		2. Majority of subjects practiced usual and occasional adding and dipping of food in soy sauce with chilies/garlic/belacan			
		3. Compared with adding, more subjects preferred dipping food in soy sauce mixed with chilies/garlic/sambal belacan			

(continued)

		Sources of sodium		
Philippines	Lee (2009) [68]	Condiments added during cooking or at the table accounted for 76.3 % of total sodium intake; Percentage contribution of foods and condiments to total sodium intake – Intrinsic sodium from foods in the diet = 23.7 % – Salt = 58.1 % – Soy sauce = 13.9 % – MSG = 2.4 % – Fish sauce = 1.4 % – Other flavorings = 0.6 %		
Thailand	Ministry of Public Health (2008) [94]	Food items that had to be regularly consumed and % of respondents consuming – Pickled fruits = 35.1 % – MSG = 34.8 %		
	Pavadhgul et al. (2009) [69]	Food groups and their estimated % contribution to mean Na intake/day % of total mean Na intake/day		
		One-plate meals	50.2	
		Meat products	12.6	
		Flour, nut, seed products	10.7	
		Seasoning added during eating	10.4	
		Snacks and desserts	8.1	
		Fast foods	5.0	
		Beverages	3.4	
		mg Na from seasoning added during eating – Males = 540 ± 499 – Females = 473 ± 514		
Vietnam	Do (2014) [72]	Contribution of foods to total Na intake (%) – Condiments added during cooking or at the table—81 % – Processed foods—11.6 % – Natural foods—7.4 % Contribution of condiments to total Na intake (%) – Mixed seasoning (*bot canh*)—35.1 % – Fish sauce—31.6 % – MSG—7.4 % – Table salt—6.1 % Mean Na intake from processed foods (mg Na/person/day) – Instant noodle = 470.1 – Salted vegetable = 54.7 – Bread = 8.1 – Other processed food = 77.1		
	Thu Hien et al. (2013) [75]	– 81 % of respondents used MSG – Average Na intake from MSG = 0.264 ± 0.216 mg Na/person/day (2.2 ± 1.8 g MSG/day)		
	Laillou et al. (2012) [70]		Mg Na/capita/day	
		Socioeconomic level	Flavoring powders	Fish sauce
		1	363 ± 30.3	714 ± 162.6
		2	348 ± 30.3	414 ± 44.3
		3	371 ± 37.8	498 ± 49.3
		4	341 ± 15.1	557 ± 108.4
		5	409 ± 22.7	507 ± 24.6
		All	366	538
	Thuy et al. (2005) [73]	Mean Na intake from fish sauce = 887 mg Na/person/day (18 mL/d fish sauce)		
	Duong et al. (2003) [74]	98 % of respondents cooked with salt and 75 % added salt when eating		

(continued)

Table 36.7 (continued)

		Sources of sodium
2. Adolescents		
Vietnam	Lachat et al. (2009) [32]	Percentage contribution of out-of-home foods to daily Na intake – Rural = 12.1 ± 2.1 % – Urban = 33.1 ± 2.1 % – All = 22.6 ± 4.2 %
3. Children		
Indonesia	Mustafa et al. (2006) [62]	mg Na from discretionary salt intake = 983.5 (approx. 56 % of total Na intake/day)
Thailand	Leelajaratkoon et al. (2010) [64]	High sodium foods eaten frequently eaten during the week 1. Seasoning (added during eating) ○ Fish sauce/soy sauce ○ Seasoning sauce 2. Flour and seeds products ○ Bread and bakery products ○ Instant rice porridge ○ Instant wheat noodles 3. Meat and meat products ○ Fried mackerel ○ Sausage 4. One-plate meals ○ Fried rice ○ Rice streams with chicken soup ○ Rice noodle with meat/meat ball 5. Hawker food ○ Fried prawn/chicken/fish balls 6. Snacks ○ Fried seaweed ○ Potatoes/potatoes flour chips ○ Fish minced strips
Vietnam	Laillou et al. (2012) [71]	Median Na intake (mg/capita/day) from fish and soy sauces by age group (months) – 6–11.9 months = 123 mg Na/capita/day – 12–23.9 months = 197 – 24–35.9 months = 172 – 36–59.9 months = 246 – All ages = 197

In Vietnam, Laillou et al.'s [70] study of the intake of condiments among Vietnamese women of reproductive age (19–50 years) using data from the 2009 Food Consumption Survey (FCS) showed that flavoring (seasoning) powders were consumed daily by almost the entire population whereas fish and soy sauces were consumed by two-thirds. Estimated mean consumption of sodium from flavoring powders and fish sauce was approximately 366 and 538 mg/day, respectively. Fish sauce was consumed in greater amounts compared with flavoring powders. Using the same dataset, the investigators [71] examined dietary intakes of Vietnamese children aged 6–60 months in order to identify potential vehicles for fortification. Fish and soy sauces were consumed by 50.8 % of children, with median consumption of 4 g sauce/day, equivalent to approximately 197 mg Na/child/day.

A recent study [72] among rural adults showed that condiments added during cooking or at the table contributed 81 % of sodium intake. Condiments that provided the greatest amount of sodium were mixed seasoning (*bot canh*) and fish sauce. Another study among rural women [73] showed mean intakes of 887 mg Na/day from fish sauce. Duong et al.'s [74] survey among 357 adults aged 19–85 years in Ho Chi Minh City to determine risks associated with hypertension found that majority (98 %) cooked with salt and 75 % added salt when eating. Using MSG weighing for 3 days, Thu Hien et al. [75] found that average MSG intake was 2.2 ± 1.8 g/person/day (equivalent to 0.26 mg Na/person/day) among 1528 adults aged ≥20 years living in rural and urban areas.

A study among Vietnamese adolescents [32] showed that foods eaten away from home (usually traditional street foods) contributed 22.6 ± 4.2 % of daily sodium intakes, with higher levels contributed in urban compared with rural adolescents (33 % vs. 12 % of total Na intake/day, respectively). Away from home meals that were frequently consumed included bread with liver pâté and vegetables, bread with salted and shredded meat, and noodle soup with beef.

Discussion

Assessment of Evidence Regarding Levels and Sources of Sodium Intake in Southeast Asia

Levels of Intake

Insufficient evidence exists in the region regarding levels of sodium intake. Among the six countries, only Singapore and Vietnam have information on sodium intakes based on urinary sodium excretion of a nationally representative sample of adults. Malaysia and Thailand have nationally representative sodium intake data for adults, but based only on dietary assessment methods (i.e., 24-h recall). The Philippines has nationally representative household data (i.e., per capita intake) for discretionary salt but lacks data on total sodium intake. Indonesia does not have nationally representative data regarding the level of sodium intake of its population. None of the countries have nationally representative data on sodium intakes of younger age groups. Based on 24-h urinary sodium excretion measures, sodium intakes of adults in Singapore and Vietnam (3271 and 3934 mg Na/day, respectively) exceeded the WHO recommendation of <2000 mg Na/day.

Main Sources of Sodium

Insufficient evidence exists regarding the main contributors to dietary sodium in Southeast Asia. A complete profile of the dietary sources of salt can be obtained by identifying the following [41]: (a) foods that people eat and the amounts and frequency of consumption, (b) sodium content of the most commonly consumed foods, (c) the amount of salt added at the table and in cooking, and (d) intake of high sodium foods that are culturally or regionally specific. Ideally, this information is obtained through nationally representative food consumption surveys, using 24-h recalls or validated food frequency questionnaires [41, 76]. Based on these criteria, the present review shows that none of the countries have identified the main dietary sources of sodium at the population level.

While some information can be gleaned from national household expenditure surveys and individual studies, the low accuracy of the data (from household expenditure surveys) and small nonrepresentative samples (from individual studies) preclude identifying specific foods that can be targeted for salt reduction in each country. The exception may be table salt and culturally specific condiments and seasonings, as the available studies showed that discretionary use of these food items contributed a large proportion to individuals' total sodium intake. Added salt or condiments contributed approximately 76 % of total sodium intake in the Philippines [68], 41–56 % in Indonesia [62], and 81 % in Vietnam [72]. Other sources of sodium identified in individual studies were traditional dishes (e.g., one-plate meals) [69], foods sold by vendors [32], instant noodles [67, 72], and western processed and fast foods [67].

Other types of studies indicate that modern processed foods should also be considered potential targets for salt reduction in Southeast Asia. Using market sales information obtained from an international database, Baker and Friel [77] identified processed food categories that were the most significant "product vectors" for salt. Per capita sales volumes of various food product categories and their linked ingredients were analyzed in Asian countries with varying income levels (including Philippines, Vietnam, Indonesia, Malaysia, Thailand, Singapore). The most significant product vectors for salt in these countries were baked goods, biscuits, sweet/savory snacks, dried processed food, and noodles.

Current Salt Reduction Initiatives in the Region

A 2013 WHO meeting in Thailand [78] reviewed the situation on salt and health in the Southeast Asia Region, and reported that detection, treatment, and control rates of hypertension in some countries were low and suboptimal, following the "rule of halves" (i.e., less than half with hypertension are detected, less than half of those detected receive treatment, and less than half of those receiving treatment have blood pressure adequately controlled). The meeting emphasized the need to implement population-wide salt reduction programs especially in countries where hypertension control rates were less than 10 %.

Recommended interventions to reduce salt intake consists of three main strategies [42]: product reformulation (i.e., reducing the salt content of commercialized foods and meals), consumer education (i.e., raising awareness on the harmful effects of excessive salt consumption and educating consumers with regard to reading food labels and choosing healthier options), and environmental change (i.e., building an environment where choosing the healthiest foods is the easiest and most affordable option, e.g., through pricing strategies and development of clear labeling systems). A review by Batcagan-Abueg et al. [79] showed that consumer education (through the use of dietary guidelines) was a common strategy used in all six countries. In addition to national dietary guidelines, voluntary product reformulation was utilized in Malaysia and Singapore, while environmental change (through the use of product labeling) was implemented in Indonesia, Singapore, and Thailand.

Current Issues Regarding Cutoff Levels for Sodium Intake

There is an ongoing debate regarding optimal levels of sodium intake. Low sodium intakes are thought to adversely affect blood pressure levels by stimulating the renin–angiotensin–aldosterone system (RAAS). Below 1000 mg Na/day, renin rises exponentially [80]. Plasma renin activity has been proposed as a predictor of cardiovascular risk and has been related to cardiovascular risk factors (e.g., hypertension, left ventricular hypertrophy, lipid levels) and with insulin resistance [80]. A meta-analysis of 23 cohort studies and 2 follow-up studies of randomized controlled trials [81] found that both low (<2645 mg Na) and high sodium (>4945 mg Na) intakes were associated with increased all-cause mortality, consistent with a U-shaped association. The authors suggested an optimal range of intake between 2645 and 4945 mg Na/day, within which variation in sodium intake is not associated with variation in mortality, and is consistent with the current dietary intake of most of the world's population. These findings are supported by those of the Prospective Urban Rural Epidemiology (PURE) study, a prospective study involving 102,216 participants aged 35–70 years from 667 communities in 18 low-, middle-, and high-income countries on 5 continents. The study showed that an estimated sodium intake (based on spot urinary Na excretion) between 3 and 6 g Na per day was associated with lower risk of death and cardiovascular events, as compared with a higher or lower estimated level of intake [82].

However, Cobb et al. [83] assessed the quality of cohort studies examining the relationship between sodium intake and subsequent cardiovascular disease. Methodological issues across cohort studies were observed. These included systematic error in the sodium assessment method and the potential for reverse causality (i.e., the likelihood that the illness is responsible for the low level of sodium intake rather than the low of level of sodium intake leading to illness). The authors emphasized that until high quality rigorous studies are done, the body of high quality evidence linking sodium intake to blood pressure should remain the basis for setting recommended levels of sodium intake for the general population. In support of current recommendations, the Trials of Hypertension Prevention (TOHP) involving 2275 participants not included in a sodium reduction intervention during 10 or 15 years of posttrial follow-up found a linear association of sodium with cardiovascular events [84]. Using multiple 24-h urine specimens among prehypertensive individuals, the study found a 17 % increase in risk of cardiovascular events per 1000 mg/day increase in sodium ($p=0.05$), and reduction in risk that continued from 3600 to 2300 and 1500 mg Na/day. The authors concluded that these

findings are consistent with current recommendations to reduce sodium intake to 1500–2300 mg per day for majority of the population in order to maintain good health.

These differing findings highlight the need for more well-designed studies and standardized methodological approaches to measure sodium intake in population groups [80]. Improved methodological approaches include standardizing the use of 24-h urine collections and validating sodium intake estimates with data on urine volume, urine creatinine, and body weight [80]. In a recent policy statement, the World Hypertension League expressed the need for rigorous research study design and conduct to determine whether reducing dietary sodium is harmful or beneficial [85]. A working group was created for the setting of standards for research examining sodium intake and health. The standards setting process would be aided by systematic reviews of the evidence and overseen by respected international and national health and scientific organizations, with results expected by end of 2014.

Role of Potassium and Other Dietary Factors in Development of Hypertension

It has been shown that optimal blood pressure reduction occurs when sodium reduction is combined with a healthier food-based dietary pattern. This is due to the synergy of composite effects and interactions of multiple factors, including carbohydrate quality, fiber content, specific fatty acids and proteins, food structure, and bioavailability of inherent micronutrients and phytochemicals [86]. Dietary patterns with beneficial effects on blood pressure and CVD risk factors include the DASH (Dietary Approaches to Stop Hypertension) pattern and the traditional Mediterranean pattern [86].

Among food components, the role of potassium in reducing blood pressure has been highlighted. WHO [87] suggested a potassium intake of at least 3510 mg/day (90 mmol/day) for adults to reduce blood pressure and risk of cardiovascular disease, stroke, and coronary heart disease. Increased potassium intake was also recommended for children to control blood pressure, with the recommended amount adjusted downward based on the energy requirements of children relative to those of adults.

It was emphasized that the recommendation for potassium intake should complement the WHO guideline on sodium [87]. If both guidelines are achieved, the molar ratio of sodium to potassium would be approximately one to one. To maintain this molar ratio at higher levels of sodium consumption, the recommended level of intake of ≥90 mmol/day potassium should be increased [87]. Studies have shown that urinary Na^+/K^+ has a strong positive correlation with blood pressure in multiethnic and normotensive populations [88, 89], making it important for individuals to achieve a dietary Na/K ratio ≤1.

Other nutrients have also been associated with blood pressure. Using an environment-wide association approach to analyze data from the INTERMAP (International Collaborative Study on Macro-/Micronutrients and Blood Pressure) study, Tzoulaki et al. [89] found inverse associations between systolic blood pressure and dietary intakes of B vitamins (folacin, riboflavin, thiamin), nonheme iron, phosphorus, and magnesium, while a direct association was found between alcohol intake and blood pressure.

Use of Sodium-Reduced Potassium-Enriched Salt and Condiments

In Southeast Asian populations with traditionally high levels of salt use, replacing table salt with potassium-enriched condiments may be a viable alternative for reduction of blood pressure levels. In a randomized, double-blind placebo controlled trial among 173 Vietnamese adults aged 45–64 years with untreated elevated blood pressure, Do [72] showed reduction in blood pressure from baseline among subjects receiving sodium-reduced potassium-enriched condiments (i.e., table salt and a seasoning mix "*bot canh*"). Use of the condiments for 8 weeks resulted in a mean change of −2.6 mmHg for systolic BP and −1.6 mmHg for diastolic BP in the intervention group compared to the control group. The urinary Na/K ratio decreased from 4.4 to 3.6 in the intervention group, but increased from 4.5 to 5.0 in the control group.

An earlier study among 1981 elderly Taiwanese men found that subjects given potassium-enriched salt in place of table salt lived 0.3–0.90 years longer and spent significantly less in inpatient care for CVD compared with the control group, after controlling for age and previous hospitalization expenditures [90]. In Japan, a parallel controlled trial among 41 elderly subjects with mild essential hypertension showed that those given a reduced-sodium mineral salt containing potassium and magnesium had significant reductions in systolic and diastolic BP compared to baseline (i.e., from 134.7 ± 17.2/77.2 ± 9.7 mmHg at baseline to 127.3 ± 12.0/73.5 ± 8.9 mmHg at the end of 5 weeks intervention), with no change among subjects receiving regular salt [91].

Conclusion

In Southeast Asia, hypertension is a major risk factor for cardiovascular disease. Due to the use of traditional fermented and salted food products, sodium intakes in the region are predictably higher compared to western countries. The review of six Southeast Asian countries showed that only two countries (Singapore and Vietnam) had sufficient information regarding the level of sodium intake of their respective adult populations, and that all six countries had insufficient information regarding the main sources of sodium. In spite of the paucity of data, the available studies indicate that sodium intakes of adults in these countries are most likely in excess of the WHO recommendation of <2000 mg Na/day, and that table salt and traditional sauces and seasonings added to food during cooking and at the table are major sources of dietary sodium. None of the countries had nationally representative information regarding sodium intakes of younger age groups.

For accurate assessment of sodium intake levels, countries should use urinary sodium excretion measures (preferably 24 h urinary sodium excretion) to indicate the magnitude of the problem within the population. In order to identify dietary sources of sodium, national food consumption surveys (preferably using 24-h recalls) should be conducted and sodium content of the foods consumed by individuals estimated, including discretionary use of salt/condiments in cooking and at the table. Surveys assessing sodium intake levels and sources should be repeated at regular intervals to monitor changes in consumption and to provide data for evaluating the effects of salt reduction initiatives. Given the high prevalence of hypertension in the region, countries should aim to reduce sodium and increase potassium intakes of the population.

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Chapter 37
The Role of Food Security in Preventing the Rise of the Nutritional Double Burden in Low-Income Countries

Andrew D. Jones

Key Points

- The prevalence of nutrition-related chronic disease is rising rapidly in low-income countries (LICs) alongside persistent problems of undernutrition.
- Food insecurity is an important contributing factor to this double burden of malnutrition in LICs via several distinct pathways related to food availability, food access, and food utilization.
- Nutrition-sensitive food security programs have made important contributions to improving nutrition outcomes in LICs and hold great potential to simultaneously reduce divergent forms of malnutrition by addressing common underlying determinants.
- Food security programs and policies based on an evidence-based understanding of the pathways to improved nutrition, and that simultaneously address sustainable livelihood and environmental goals are needed across contexts to attain equitable global food and nutrition security.

Keywords Food security • Double burden of malnutrition • Nutrition transition • Food access • Low-income countries • Malnutrition • Undernutrition • Obesity • Urbanization • Demographic transition

Introduction: The Double Burden of Malnutrition in Low-Income Countries

Undernutrition remains one of the most pressing public health challenges facing low-income countries (LICs). In 2011, 165 million children under the age of five worldwide suffered from linear growth stunting [1], a consequence of chronic undernourishment that not only increases the risk of death from infectious diseases [2], but is also predictive of poorer cognitive and educational outcomes in later childhood [3], as well as an enhanced risk of adult chronic disease [4]. At the same time, billions of individuals across the globe suffer from micronutrient deficiencies of which iron, zinc, and vitamin A are the most prominent [5]. These deficiencies contribute in part to the growth deficits observed with child stunting, but are also responsible for an enormous burden of morbidity and mortality across different life stages. Iron deficiency, for example, affects more than one billion people, especially young children and women of childbearing age [6]. Infants with anemia caused by iron

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_37,

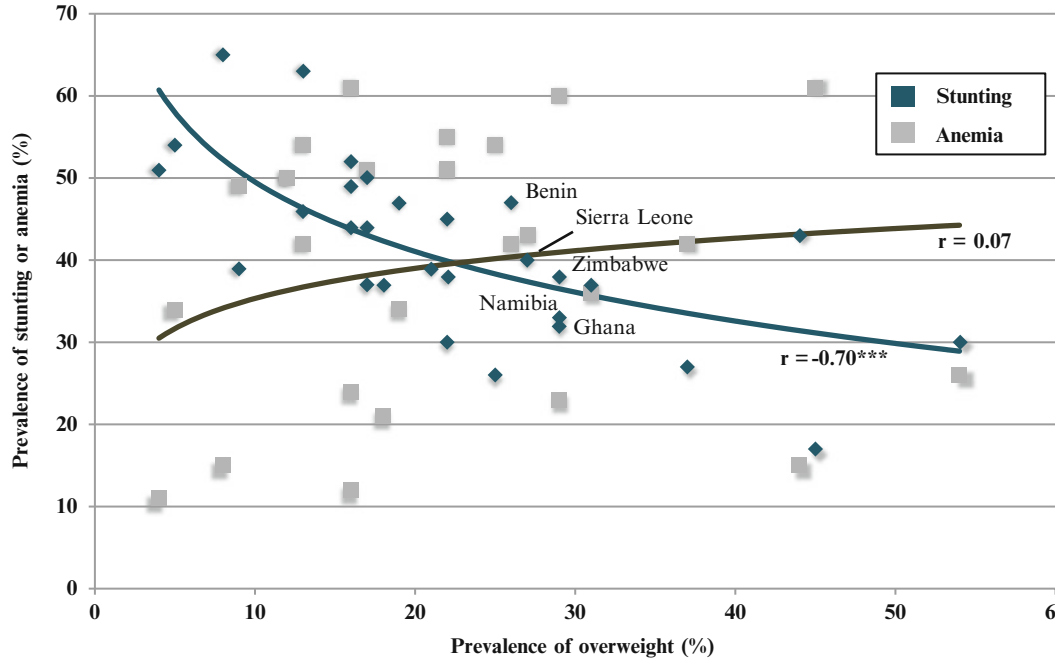

Fig. 37.1 Associations between the prevalence of overweight among women of childbearing age (15–49 years) and both stunting among preschool-aged children (12–59 months) and anemia among women of childbearing age in 30 countries of sub-Saharan Africa. Data are from Demographic and Health Surveys (DHS) from 30 countries in sub-Saharan Africa for which the most recent standard DHS survey was administered using Phase V or Phase VI core questionnaires (2006–2012). We defined overweight or obesity using standard body mass index (BMI) cut-offs ($\geq$25 kg m^{-2}) [70], anemia as a hemoglobin (Hb) concentration <120 g/L [71], and child stunting as height-for-age Z-score (HAZ) <2 SD below the mean according to the World Health Organization (WHO) Child Growth Standards [72]. Pearson correlations (r) indicating the magnitude of the association between overweight and stunting, and overweight and anemia, respectively, are shown. *P<0.05, **P<0.01, ***P<0.001

deficiency may have poorer neurocognitive, motor, and social–emotional development [7], while iron-deficient pregnant women may give birth to children with restricted intrauterine growth [8].

These seemingly intractable problems of undernourishment in LICs are increasingly occurring alongside rapidly changing food and physical activity environments characterized by increased access to vegetable oils, refined and processed foods, sugar-sweetened beverages, and more sedentary lifestyles. This so-called nutrition transition is associated with an increase in the prevalence of overweight and cardiometabolic disease in low- and middle-income countries (LMICs) [9]. For example, data from the Global Burden of Disease study demonstrate that the contribution of ischemic heart disease to disability-adjusted life years (DALY) in developing countries rose by 60 % between 1990 and 2010, making it the number three contributor to overall DALYs in these countries behind diarrheal disease and lower respiratory infections [10]. Though most research examining the convergence of undernutrition and overweight has focused on middle-income countries (e.g., Brazil, China, Mexico, and South Africa) [11–13], even low-income countries such as those in sub-Saharan Africa (SSA) are experiencing the nutrition transition and its associated changes in vulnerability to malnutrition. In examining 30 low-income countries from SSA for which Demographic and Health Survey data were available, one-fifth or more of women of childbearing age were overweight while nearly half of preschool-aged children remained stunted (Fig. 37.1). Importantly, child stunting was negatively correlated with adult overweight, an association commonly observed in developing countries more broadly [14]. However, there were many countries for which 30 % or more of children were

stunted while one-quarter or more of women were overweight (e.g., Benin, Sierra Leone, Ghana, Zimbabwe, Namibia). At the same time, the prevalence of anemia among women of childbearing age, closely linked to iron deficiency, showed no strong relationship with overweight indicating the persistence of this public health problem across all levels of overweight.

This chapter examines one potentially important contributing factor to this emerging double burden of malnutrition in LICs, namely, food insecurity. Much of the literature to date on the intersection of food insecurity and nutrition has emphasized the adverse consequences of inadequate food production on undernutrition in rural regions of developing countries. However, food insecurity encompasses more than just adequate food availability and may play an important role in shaping both undernutrition and chronic disease risk across populations from rural to urban. This chapter examines the conceptual pathways between food insecurity and malnutrition, and assesses the potential for nutrition-sensitive food security programs and policies to impact nutrition outcomes in LICs in the context of rapidly transitioning food and activity environments.

Conceptual Linkages Between Food Insecurity and Malnutrition

At the 1996 World Food Summit in Rome, Italy, the following definition of food security was adopted: "Food security, at the individual, household, national, regional and global levels [is achieved] when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" [15]. This definition encompasses four commonly recognized pillars of food security, namely, availability, access, utilization, and stability [16]. Availability refers to the physical availability of food at different scales. Food balance sheet data from the Food and Agriculture Organization (FAO), for example, report nationally aggregated data on food supply and utilization including the amount of food produced and imported as well as the amount exported, fed to livestock, and used for other purposes [17]. Food access includes several components including physical accessibility (e.g., the extent to which markets are accessible based on overall distance, access to transportation, and the quality of road infrastructure), economic accessibility (e.g., the extent to which households or individuals have sufficient economic resources, or "entitlements" to purchase or grow food [18]), food safety and quality (e.g., the extent to which foods are safe from contamination by pesticide residues, microbial agents, and toxins such as aflatoxin, as well as the nutritional quality of foods), and sociocultural acceptability (e.g., the extent to which households must rely on culturally inappropriate foods or socially unacceptable methods to access food) [19]. Food preferences or acquisition behaviors are often included in definitions of food access as well, as households may have limited knowledge of what foods constitute a healthy diet or may have limited capacity to prepare certain kinds of foods. Some scholars have argued, however, that it is important to differentiate food acquisition behaviors from food access given that a household may have the economic resources to access sufficient, safe, and nutritious food, but may choose to purchase more unhealthy options [20]. Food utilization, the third pillar, encompasses the allocation of food to individuals within households, as well as the bioavailability of nutrients in diets that may be determined in part by the source of the food (i.e., of plant or animal origin) and the health status of the individual. Finally, stability is the temporal dimension of food security and refers to the extent to which food insecurity is experienced seasonally, chronically, or transiently.

Household food security is an integral part of most conceptual frameworks that describe the determinants of malnutrition. One of the most well recognized frameworks, developed by UNICEF nearly 25 years ago, presents household food security as one of three underlying determinants of child undernutrition [21]. "Food" (i.e., household food security), "health" (i.e., access to health services, improved water and sanitation, and hygienic physical environments), and "care" (i.e., access by child caregivers to education, time, social support, and other resources to adequately feed and provide psychosocial

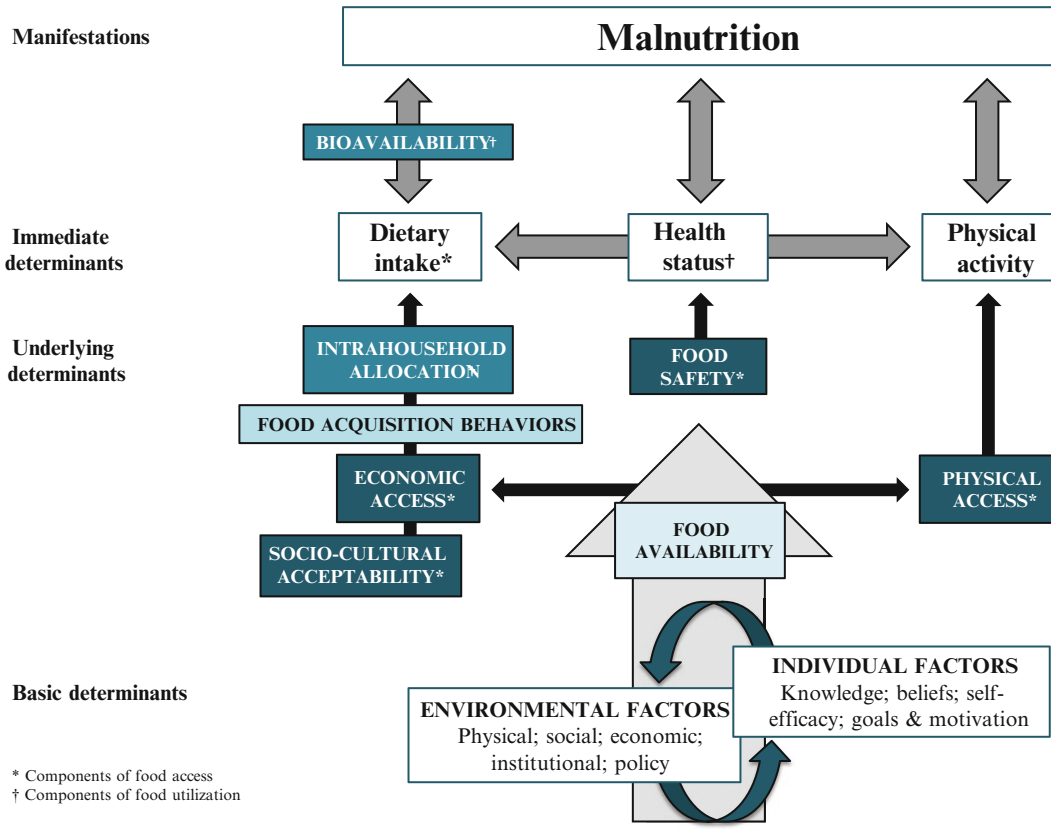

Fig. 37.2 A conceptual framework of the pathways from food security to malnutrition. Adapted from [21]

care for infants and children [22]) are the terms commonly used to refer to these underlying determinants. Household food security is conceived as directly impacting dietary intake, which in turn influences the healthy growth and development of children. However, the multiple dimensions of food security that have been identified and increasingly accepted over the past three decades (as articulated in the definition earlier) suggest that food security may operate to influence child undernutrition via pathways that are not currently recognized in this framework. Figure 37.2 presents an adapted version of the UNICEF conceptual framework highlighting the principal pathways through which the different components of household food security may affect undernutrition as well as overweight and associated chronic disease.

The social–ecological model of behavior change suggests that environmental factors at varying scales are essential for understanding health-related behaviors and outcomes [23]. Figure 37.2 indicates five different categories ofenvironmental factors that are basic determinants of household-level food security. These include: (1) physical environments (e.g., food environments that include access to food vendors, markets, and retail stores, and exposure to food advertising), (2) natural environments (e.g., local and regional climate, soils, water, and other natural resources), (3) social environments (e.g., family, community and peer support networks, social dynamics of the immediate home environment, access to educational opportunities), (4) economic environments (e.g., macro-economic context, employment opportunities, market prices and protections), and (5) institutional and policy environments (e.g., trade policies, regulatory environments, political and legislative protections for land tenure, women's rights, provision of extension services, investments in research and technology,

financial institutions, and access to credit). Individual-level factors such as knowledge, education, and beliefs, an individual's self-efficacy, as well as goals and motivation all dynamically interact with environmental factors to determine individual- and household-level actions and outcomes that affect food availability.

Household food access is the component of food security that is perhaps most commonly implied when referring to the underlying determinant of "food" in the UNICEF framework. In Fig. 37.2, household food access is disaggregated into its constituent components. Physical access may in part influence physical activity levels. In rural regions of LICs, communities are often located long distances from markets with limited access to personal or public transportation. Thus, traveling several hours on foot over difficult terrain to reach markets (and expending large amounts of energy) is not uncommon. Physical and economic access to food are clearly linked. Wealthier households may be able to afford personal transportation or to hire transportation to reach markets more easily or to reach markets that provide a larger diversity of food. Furthermore, large investments of time spent traveling to markets to buy, sell, or trade directly affects potential time allocation to income-earning opportunities and may adversely impact income. Though not shown in Fig. 37.2, physical access may directly influence the dietary intake of individuals, especially young children, if the capacity of caregivers to dedicate time to child feeding is adversely affected by time dedicated to traveling. Similarly, traveling or engaging in market activities may expose caregiver and child to environments that are not amenable to proper breastfeeding or complementary feeding [24].

Greater access to wealth (i.e., economic access) may allow households to purchase more food or a greater diversity of food. Alternatively, greater wealth might allow subsistence farmers to invest in agricultural inputs (e.g., fertilizer, irrigation systems, improved seeds, technology, storage facilities) that may facilitate greater yields and thus increased food access. As noted earlier, however, the impacts of these pathways on individual dietary intakes are mediated by the acquisition behaviors of households. Furthermore, the quantity or quality of foods fed to different individuals within households, especially women, children, or siblings of different sexes, may differ [25]. Intra-household food allocation then (included in the food utilization component of food security) may also mediate the relationship between household food access and individual dietary intake. Food safety, another important component of food access, has direct implications for health (e.g., aflatoxin exposure may lead to hepatic cirrhosis or delayed development, and microbial contamination of food with *E. coli* O157:H7 can lead to acute gastrointestinal illness [26]). Finally, though diet quality is often conceived of as distinct from food security, the quality of diets is in fact explicitly included in the definition of food security. Figure 37.2 indicates "dietary intake" as a component of food access. This includes not only the caloric sufficiency of diets, but also the extent to which diets meet requirements for micronutrients; do not exceed calorie requirements; contain sufficient quantities of dietary fiber and other beneficial food constituents; and minimize intakes of refined sugars, trans fats, saturated fats, salt, and highly processed foods. The influence of dietary intake on malnutrition is modified both by the bioavailability of nutrients in foods (e.g., iron and vitamin A from animal-source foods are more highly bioavailable than from plant sources) as well as the health status of individuals [27]—both of which influence food utilization. Importantly, "malnutrition" in this conceptual framework refers to both undernutrition and overweight or obesity. Malnutrition is commonly indicated by measurements of body composition; though blood biomarkers of nutrient status or other clinical indicators of chronic disease risk associated with malnutrition (e.g., glucose or triglyceride profiles, blood pressure) may also be relevant for assessing malnutrition.

The relationships indicated in Fig. 37.2 may be modified by sociodemographic factorssuch as age, life stage, household composition, genetic factors, and the control of resources and decision making within households. Control of resources may be especially important for translating household food security into positive nutrition outcomes. Studies from diverse world regions indicate that income controlled by women has a significantly greater positive effect on child nutrition and household food security than income controlled by men, perhaps in part because women typically spend a higher

proportion of their income on food and health care for children than men [28, 29]. The stability component of food security is also not directly indicated in Fig. 37.2 but is certainly an important determinant of the overall influence of food security on nutrition outcomes. Food insecurity experienced transiently because of changes in household composition or economic shocks will likely have differential impacts in the short- and long-term on the nutritional status of household members as compared to chronic food insecurity or food insecurity experienced seasonally among agricultural families in the months leading up to harvest [30]. Certainly, the temporal nature of food insecurity may change for families as well with certain economic shocks catalyzing transient, acute food insecurity that leads to long-term economic hardship and chronic food insecurity.

The previous analysis suggests that food security may influence malnutrition across the spectrum from undernutrition to overweight and obesity via multiple pathways. The following section builds from this understanding of the conceptual linkages between food security and malnutrition to explore current programmatic approaches to addressing undernutrition and overweight in LICs, and examines the evidence for the nutritional impacts of programs that aim to improve food security.

Current Approaches to Improving Nutrition Through Food Security Programs in LICs

Programmatic paradigms for addressing malnutrition in LICs have evolved over the past 60 years from efforts focused almost exclusively on increasing global protein intakes to a more recent emphasis on "hidden hunger" or micronutrient malnutrition [31]. Direct, technical solutions and clinical approaches have been perhaps the most common strategies adopted and evaluated. These so-called nutrition-specific interventions include, for example: supplementation of women of reproductive age and pregnant women with folic acid, iron, calcium, and/or multiple micronutrients; delayed cord clamping and supplementation with vitamin A among neonates; provision of micronutrients, home-based micronutrient powders, and/or lipid-based nutrient supplements to infants and children; promotion of breastfeeding; and management of severe acute malnutrition. If scaled up to 90 % coverage, these interventions could reduce under-five child mortality by nearly 15 % and reduce stunting by 20 % [32]. Clearly, these interventions have the potential to make substantial contributions to improving the nutritional status of vulnerable populations in LICs. However, 90 % coverage of many of these interventions is unlikely to be attained in the near future because of weak institutions, inadequate physical infrastructure, and overly burdened health systems in LICs. Even assuming attainment of this high level of coverage, a substantial proportion of the global morbidity and mortality burden would remain unresolved. Furthermore, the rapid rise in noncommunicable disease (e.g., cancer, cardiovascular disease, and diabetes) in LICs associated with overweight and unhealthy diets [33], alongside persistent challenges of undernutrition and infectious disease, means that addressing malnutrition will increasingly require approaches that simultaneously confront this "double burden" of undernutrition and overweight and obesity. More holistic approaches that target the underlying determinants of malnutrition may be most appropriate for meeting this challenge. Programs focused on enhancing food security through social safety net programs, and agriculture and food systems, are examples of so-called nutrition-sensitive approaches that seek to address to the underlying determinants of malnutrition across the spectrum.

Social safety net programs distribute cash or in-kind transfers to eligible, low-income populations. There are examples of such programs in many LMICs (e.g., India's Public Distribution System, Ethiopia's Productive Safety Net Programme, or Brazil's Zero Hunger Program). The scale and reach of these programs and the size of transfers varies widely across countries and regions, though supplementing the income of vulnerable households and linking transfers to health and nutrition services or education (i.e., conditional cash transfer (CCT) programs) are common features [34]. Mexico's

Oportunidades programis one of the most well known, and most rigorously evaluated CCT programs, and, similar to several other such programs in Latin America, has demonstrated positive impacts on food insecurity, dietary diversity, as well as the uptake of preventive health services, and women's control of household resources [35–37]. The evidence supporting the nutritional impacts of these programs, however, is mixed with most programs showing little to no effect on anthropometric outcomes or micronutrient status with the exception of those groups with the most potential to benefit (i.e., the youngest or poorest children) [34].

Programs that seek to improve household food security and/or nutrition outcomes through interventions in agriculture and food systems are another example of nutrition-sensitive approaches. Most of the evidence to date on the impacts of these programs on food and nutrition security is from programs focused on small-scale horticulture and homestead animal rearing. Helen Keller International was among the first organizations to implement this approach at a large scale [38]. Its Enhanced Homestead Food Production program, aimed at improving production and consumption of nutrient-rich fruits, vegetables, and animal-source foods through homestead gardens and the rearing of small animals, now operates in ten countries throughout sub-Saharan Africa and Asia [39]. These programs and other similar programs have demonstrated consistent improvements in the production and consumption of nutrient-rich foods, and when measured, women's control of decision making and household resources [40]. However, few programs have demonstrated improvements in child anthropometry or micronutrient status, and most have employed study designs that have not allowed for causal inferences and/or have lacked statistical power to detect differences in outcomes [41].

The biofortification of staple crops with a higher content of micronutrients has been another nutrition-sensitive agriculture approach for improving food and nutrition security [42]. The HarvestPlus programhas led this effort by using traditional crop-breeding approaches to increase the iron, zinc, and β-carotene content, respectively, of select staple crops from different world regions including cassava, sweet potato, rice, wheat, maize, beans, and pearl millet. These programs have only been implemented at a small scale to date with most of the efficacy and effectiveness trials evaluating the nutritional impact of regular consumption of these biofortified foods to be carried out in the next 5 years [34]. Available evidence from trials of the orange-fleshed sweet potato, as well as iron-fortified rice, and pearl millet, however, indicates that these crops have the potential to improve both consumption of targeted micronutrients as well the micronutrient status of vulnerable groups [43–45].

Social safety net programs and programs aimed at improving the consumption of specific micronutrients through crop breeding or cultivation of nutrient-rich foods have the potential to not only increase household access to nutrient-rich foods but may also play an important role in increasing household incomes and helping to shift the locus of control of resources and decision-making within households. However, there are many limitations to these approaches that suggest additional approaches to improving nutrition through food security are also needed. First, these programs have not yet demonstrated substantial or consistent impacts on nutrition outcomes. For homestead food production programs, this lack of evidence may stem in part from the limitations of the study designs employed to date. However, the multifactorial determinants of child stunting among preschool-aged children may mean that any single approach will be constrained in its ability to reduce stunting without adequate attention to other determinants (e.g., access to improved water and sanitation). Second, many of these programs still operate on a small scale, and for homestead gardening or animal-rearing programs at least, may not be amenable to scaling up in a homogenous manner across contexts. Indeed, local climatic conditions, water availability, soil structures, and other agronomic concerns, in addition to sociocultural factors, will dictate in part the extent to which certain crops or management practices will able to be introduced in a given region. This concern need not limit the expansion of these programs; however, it may limit the pace of such an expansion given the need to locally adapt these approaches. Third, fewer farmers in LICs are producing food primarily for their own consumption. Low-income, small- and medium-holder farmers are increasingly raising crops and animals for sale in local, regional, and export markets, are diversifying their livelihoods to include non-farm

income, or are leaving agriculture altogether [46]. Nutrition-sensitive agriculture programs focused on subsistence production then may have little potential to improve diets and nutrition outcomes for families that are increasingly selling what they produce and relying on market-purchased foods. Fourth, interventions to improve food safety (a central component of food access) in LICs have not been widely implemented or rigorously evaluated. Aflatoxin contamination of staple crops such as maize and groundnut is of particular concern, especially in sub-Saharan Africa, given the potential adverse health and nutrition consequences not only from acute exposures, but also from low-level chronic intakes [47]. Animal agriculture may also contribute to microbial contamination of food or water, or exposure to fecal bacteria near homesteads that may directly contribute to environmental enteric dysfunction and anemia, and in turn, child growth faltering [48]. Finally, nutrition-sensitive programs aimed at improving nutrition through food security in LICs have almost exclusively focused on reducing undernutrition without explicit attention to the rapidly increasing problems of overweight and associated chronic disease in these countries. Some hypothesize that early life undernutrition and adult chronic disease may in fact be linked (i.e., nutrient restriction in utero may "program" individuals to expect scarcity, and they may therefore eat more, be less physically active, and store fat more efficiently) [49]. Therefore, efforts to accelerate growth postnatally following a period of intrauterine growth restriction may lead to an increased risk of glucose intolerance, insulin resistance, type 2 diabetes, or obesity in adulthood [50]. Efforts to improve undernutrition among vulnerable children may in some cases also have more immediate unintended health effects on adults within the same households. In the *Oportunidades* program in Mexico, for example, cumulative cash transfers to households were associated with higher BMI, diastolic blood pressure, as well as prevalence of overweight and obesity among adults [51]. The rapid advance of the nutrition transition in LMICs means that approaches to reducing malnutrition need to adapt just as quickly to confront the changing nature of the public health challenges associated with this transition. Interventions will increasingly need to address both undernutrition and obesity, or at least ensure that efforts to reduce growth deficits or micronutrient deficiencies, for example, do not increase the risk of overweight and associated chronic disease, and vice versa. Nutrition-sensitive approaches aimed at enhancing food security hold great potential to simultaneously reduce divergent forms of malnutrition by addressing common underlying determinants.

In the next section, this chapter ends with a set of recommendations to help guide the design of future nutrition-sensitive interventions in LICs in order to maximize the nutritional benefits of efforts to improve food security in the context of the nutrition transition.

Strengthening Nutrition-Sensitive Food Security Interventions in LICs to Address the Double Burden of Malnutrition

The emerging double burden of malnutrition in LICs requires new and different approaches to addressing the unique challenges associated with the convergence of both undernutrition and nutrition-related chronic disease in these countries. The nature and health consequences of this changing nutrition landscape in LICs are likely to be different than those observed in middle- and high-income countries over the past several decades for at least two important reasons: (1) changes in food and physical activity environments associated with population growth and urbanization in LICs are occurring at a much faster pace than observed previously, and (2) the capacity of institutions and infrastructure in these countries to adapt to these changes is extremely limited [52]. LICs then will have to confront this double burden with fewer initial resources, despite the fact that the health consequences of changes in diets and patterns of physical activity in LICs are likely to be more severe than in high-income countries precisely because there are fewer resources (e.g., weaker health systems and

inadequate social safety net programs) to address the problem [33]. Therefore, it is important to identify strategies in LICs that will be able to deliver improved nutrition and health outcomes in the context of resource constraints and increasing external constraints (e.g., population growth and climate change). Below are five recommendations to strengthen the design and implementation of nutrition-sensitive policies and programs that aim to improve nutrition through food security in LICs, emphasizing the importance of mitigating undernutrition as well as overweight and chronic disease.

Seek Opportunities to Employ Nutrition-Sensitive Approaches That Leverage the Multiple Conceptual Pathways from Food to Nutrition Security

Increasing food availability or increasing household income alone is often necessary, but not sufficient to improve nutrition outcomes through food security policies and programs [53]. Food access has multiple components (see Fig. 37.1), and policy or programmatic actions that are able to jointly influence several of these components may be more likely to generate positive nutritional impacts. For example, introducing new knowledge, management systems, facilities, and/or technologies to improve postharvest storage practices could reduce the risk of mycotoxin contamination of harvested crops while expanding opportunities for farmers to increase their income by selling a higher quality product and/or delaying the sale of their harvest to obtain a higher market price. Combining these activities with behavior change efforts focused on increasing acquisition and preparation of nutrient-rich, minimally processed foods could amplify the potential positive nutritional impacts of increased incomes by affecting diet quality as well. Ensuring that food security programs and policies do no harm to nutrition outcomes is equally important [54]. For example, efforts to improve access to fresh fruits and vegetables through homestead gardens, or to incorporate new, nutrient-rich species into cropping systems (e.g., intercropping legumes into maize monoculture systems) may contribute to improved food access and more diverse diets, but could also contribute to increased workloads, especially for women. Time dedicated to agricultural labor could adversely affect women's own nutritional status because of increased energy expenditures [55], but could also detract from time dedicated to child caregiving activities that could have negative consequences for child diets [24, 56]. As another example, households that shift out of subsistence food production in favor of commercialized agriculture may see improved incomes, but may also become more reliant on market-purchased foods. Depending on their physical and economic access to healthy foods, food preferences, and capacity to prepare foods (i.e., both available time, and knowledge and skills related to food preparation), the quality of household diets may be adversely affected. Nutrition-sensitive food security programs and policies then would benefit from cogent assessments of potential impact pathways that would allow program planners and policy makers the ability to leverage multiple pathways from food security to nutrition while planning to mitigate against potential negative consequences for both undernutrition and overweight.

Emphasize Agricultural Value Chains and Consumer Behavior in Nutrition-Sensitive Food Security Programs

Subsistence production of nutrient-rich foods can make important contributions to the adequacy of diets, especially for low-income households [57]. However, as farming households increasingly raise crops and animals for markets rather than for own consumption, and rely on market-purchased foods for larger proportions of their diets, understanding and shaping consumer behavior, specifically, the choices individuals make with respect to food and beverage purchases, will be of paramount importance to improving the quality of diets for alleviation of both undernutrition and chronic disease risk.

Furthermore, the agriculture sector globally is increasingly supplying raw ingredients for the food processing industry rather than producing food for direct consumption [58]. This trend is linked to the rise in ultra-processed foods that are associated with overweight and obesity [59]. However, the increasing reach of primary processing technology may also provide important opportunities for increasing off-season access to perishable fruits and vegetables, and animal-source foods, as well as increasing employment opportunities in the postharvest value chain for low-income households. Leveraging the potential nutritional benefits of these changing trends will be important for nutrition-sensitive agriculture programs and policies as the fate of agricultural goods postharvest becomes increasingly important for diets and nutrition outcomes.

Seek Triple Wins for Nutrition and Health, Livelihoods, and Environmental Sustainability

Food security and the sustainability of human livelihoods are intimately linked to the health and stability of natural ecosystems. Warming surface temperatures, and more frequent and extreme droughts and flood events associated with anthropogenic greenhouse gas emissions will increasingly disrupt these natural systems with potential adverse consequences for anthropogenic health, food access, and nutrition [60]. At the same time, scarcity of critical natural resources such as fresh water and soil organic matter is placing pressure on low-income populations in particular who depend on these resources for livelihood and well-being [61]. Therefore, efforts to address food and nutrition security need to seek triple wins that support sustainable livelihood strategies, provide for the nutrition and health of communities, while at the same time prioritize goals of conserving resources and enhancing the sustainability of natural ecosystems. Agroecological intensification is one such approach that relies on evidence-based agroecological principles and enhanced access to information to increase agricultural productivity, reduce reliance on fossil fuel inputs, and enhance ecological resilience [62]. Reducing food loss and waste offers another potential triple win that would reduce the need for agricultural intensification, allow for new employment opportunities if achieved through investments in rural infrastructure, and could potentially increase access to nutrient-rich foods if perishable horticultural crops and/or animal-source products, in particular, reached vulnerable groups rather than being lost or wasted.

Design Programs and Policies for the Unique Food and Nutrition Security Vulnerabilities of Low-Income Populations in Urban and Peri-urban Regions

Urbanization is a dominant driver of the nutrition transition in LICs. Nearly all of the population growth that will occur in the next 30 years will take place in urban regions of LICs [63]. This urbanization, however, will not take place primarily in mega-cities, but rather in smaller cities and on the periphery of large metropolises where infrastructure, institutions, and public services are absent or underdeveloped [64, 65]. Peri-urban residents, many of whom are recent migrants from rural areas, may lack access to improved water and sanitation, adequate healthcare services, and consistent employment [66]. At the same time, peri-urban residents may be less likely to produce their own food and may be more reliant on inexpensive processed foods and street foods—attractive to low-income households because they can be purchased in small quantities, and require little time investment for preparation [67, 68]. However, these foods may present food safety risks, may be nutrient poor, and could contribute adversely to the risk of chronic disease. All of these factors may contribute to a unique convergence of risk of both undernutrition and overweight among peri-urban residents.

Measure the Diversity of Nutritional Risks Associated with Food Insecurity

Improving nutrition outcomes has been an implicit goal of many food security programs and policies in recent decades, yet few such programs have explicitly measured nutrition. Indeed, the assumption that efforts to improve food availability and access will lead to improvements in nutrition without explicit attention to these outcomes has not been borne out by evidence [69]. Assessing dietary intake (e.g., dietary diversity, energy intake, the macro- and micronutrient content of diets, as well as other dietary compounds), anthropometry, micronutrient status, clinical indicators of chronic disease risk, and/or other nutrition-related biomarkers are essential for understanding the nutritional impacts and impact pathways of food security programs. Measuring nutrition is especially important given the changing context of nutritional risk associated with the nutrition transition. The rapid changes in food environments and interconnected socioenvironmental risk factors associated with both undernutrition and overweight mean that predicting the nutritional impacts of programs and policies is increasingly complex. Therefore, program theory will be increasingly imperative for designing effective programs and policies, yet measuring multiple outcomes, including those that could be potentially indirectly (and negatively) affected by program activities, will also be critical for monitoring the overall nutrition and health impacts of programs and policies.

Conclusions

This chapter has provided an overview of the emerging double burden of undernutrition and nutrition-related chronic disease in LICs, the conceptual linkages between food insecurity and this double burden, and the potential for nutrition-sensitive food security programs and policies to shape nutrition outcomes in the context of the nutrition transition. The enormous burden that nutrition-related disease presents to the public health and the sustainability of economies in LICs is one of the greatest challenges facing policy makers, researchers, and practitioners across sectors in the coming decades. The constraints to environmental sustainability and human development posed by population growth, urbanization, and climate change are equally pressing concerns and are intimately linked to global prospects for attaining food and nutrition security for all. Investments in multisectoral efforts focused on creating more sustainable and nutrition-sensitive food systems will provide a strong foundation for achieving equitable global food and nutrition security.

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Chapter 38
Role of the Food and Supplement Industries in Human Health

Michael I. McBurney and Eric D. Ciappio

Key Points

1. Population growth and urbanization trends will drive dependence upon robust agri-food production and distribution systems to ensure nutrition and food security.
2. Food enrichment and fortification have eradicated nutrition deficiency diseases in many regions of the world. However, consumers seem to be transitioning from in-home use of fortified staples to using more partially or fully prepared foods and this has important health consequences.
3. Innovations in crop, animal, food science, and consumer demand lead to continuous change in the nutritional composition of foods. Consequential changes in the composition of foods and patterns in food choices, coupled with imprecise consumer reporting on food intake and physical activity, make it difficult to accurately assess the impact of diet on health.
4. New technologies need to be adopted to assess nutritional status. By associating assessments of nutritional status with health outcomes, for example, serum 25-hydroxyvitamin D3 concentration and risk of falls and fractures, nutritional guidance can be personalized and individuals at risk of deficiency or excessive nutrient intake can be educated and/or helped.
5. Food groups are becoming irrelevant because the agri-food industry has the competency to add nutrients to almost any food. By embracing best agricultural practices, innovative technologies across all categories, and harmonized regulations globally, future generations will have the greatest diversity of products to choose from to meet their nutrient needs.

Keywords Vitamin • Fortification • Supplements • Adequacy • Nutrition status

Introduction

Nourishment is essential for life. Because of advances in agricultural and food systems, unprecedented gains in human health globally have been achieved in the last century. In just the past 50 years, life expectancy has increased [1] and so has the world population. Fortunately, food production has outpaced population growth [2]. Unfortunately, more than two billion people suffer from one or more nutrient deficiencies [3]. With food demand expected to increase 50 % by 2030 [4], the pressure to

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_38

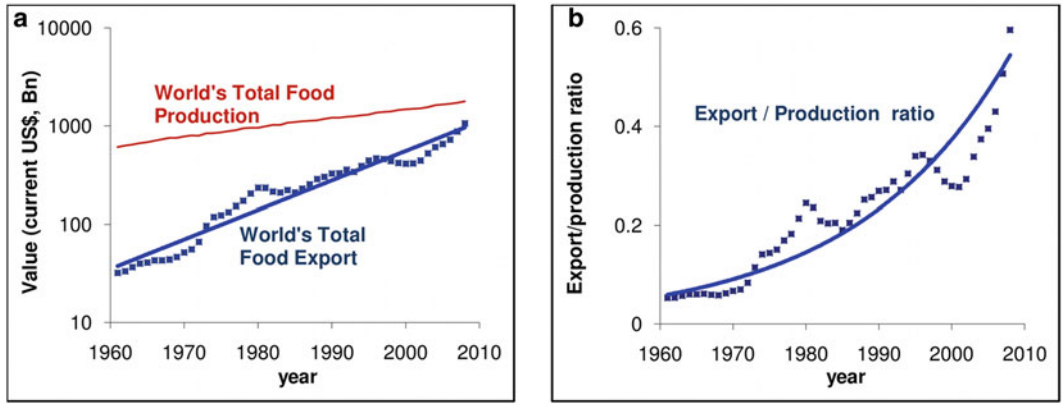

Fig. 38.1 The world's food trade grows faster than the food production

increase the production of foods, especially those containing essential nutrients, will intensify. One of the greatest contributors to world hunger is a lack of access to food. The fact that global food transport is increasing at a faster rate than food production (Fig. 38.1) will help improve food distribution globally to alleviate food insecurity. Even though improper food handling by consumers is often the largest contributor to waste along the food chain [5], foods need to be transported efficiently and stored properly to reduce spoilage. The complexity of moving food around the world and across borders is hampered by national interests in food safety and security [6]. Governments, nongovernment agencies, and industries need to collaborate to expand food production and improve the integrity of food distribution systems as this is the best means to ensure people have access to essential nutrients (*nutrition security*) in sufficient and reliable amounts (*food security*) to maintain health. In addition to expanding existing agri-food systems, feeding and nourishing more than seven billion people will require the further development and application of innovative technologies.

Food Security

Food security is a local phenomenon. Seasons affect agricultural production. War, insurrection, and environmental disasters (flood, fire, wind, hail, etc) can also affect the redistribution and availability of food. As the world population continues to grow and become increasingly more urbanized, people are more dependent upon a global food distribution system. There simply will not be enough food produced within close proximity of urban dwellers to meet their demand. Food security will become increasingly dependent upon robust agri-food production and distribution systems.

Malnutrition occurs with an inadequate intake of nutrients essential for cellular function, for example, vitamins, minerals, essential amino and long-chain poly-unsaturated fatty acids (LCPUFA), and/or an excess of energy macronutrients required to maintain a healthy body weight. Around the globe, it is common to see evidence of inadequate micronutrient intake, that is, stunting in children under 5 years or anemia in women of childbearing years, and excess food energy intake relative to energy expenditure, that is, overweight and obesity, coexisting together [7]. A sustainable food production system must be capable of producing enough food (protein, carbohydrate, and fat) to feed the world's population. In addition to food macronutrients (energy, protein, fat, and carbohydrates) needed to support life, individuals require nutrients, for example, vitamins, minerals, some amino acids, and LCPUFA [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] essential for normal cellular function. Food security depends upon redistributing food so that everyone, regardless of where they live, has access to food.

Nutrition Security

Nutrition security is more than access to enough food calories to overcome hunger. Nutrition security requires adequate intakes of essential amino acids, LCPUFA, vitamins, and minerals for health. Otherwise, *hidden hunger*—the availability of adequate calories but insufficient amounts of vitamins, minerals, and EPA and DHA to support cognitive development and health of individuals—occurs within apparently healthy and overweight populations [7, 8]. Hidden hunger leaves individuals deficient in essential nutrients. Stunting is an obvious indicator of undernutrition. However, a lack of essential micronutrients can negatively impact cognition, function, survival, health, and economic potential of individuals and nations.

The agri-food industry has successfully increased total annual production of food (by weight), food calories, protein, carbohydrate, and fat. One reason is because farmers are choosing to plant, grow, and harvest highly productive cereal and oil crops [9]. Between 1998 and 2008, average yield increased 0.9, 1.0, 1.3, and 1.9 % for wheat, rice, soybean, and maize, respectively [10]. Production efficiencies are also observed in animal husbandry. Over the past 50 years, beef production has doubled and milk yield has been increased [11]. Unfortunately, the use of some technologies, for example, bovine somatotropin to enhance dairy cow productivity and decrease environmental impact, has not been socially acceptable. Twenty-five years ago, poultry producers fed 3.22 kg of feed to raise a 1.40 kg broiler whereas a 2.44 kg bird is produced today with 3.66 kg feed in the same 35 days [12]. China, the world's largest producer of hogs, is trying to meet increased demand for a staple food, pork. Since 1980, China has increased hog production, primarily by technologic adaptations improving feed conversion and scale efficiency [13].

Selection of highly productive systems and species could reduce species diversity and promote dietary convergence. There is evidence that national food supplies may become less heterogeneous as farmers choose more productive crops [9]. The agri-food industry is frequently blamed for the increasing prevalence of overweight and obesity. Clearly, the agri-food industry has increased the per capita availability of food. However, a systematic analysis of 187 nations finds consumption of healthier foods and nutrients increased between 1990 and 2010; unfortunately, there was a greater increase in unhealthy eating patterns [14]. During the past 25 years and based on dietary recommendations, the food industry removed saturated fats from many foods. Saturated fats were replaced with partially hydrogenated oils containing trans-fatty acids which turned out to have unexpected health consequences [15] and were then removed [16].

Because the human diet typically consists of a variety of foods (unlike total mixed rations fed domesticated animals or the enteral or parenteral diets fed in clinical settings), it is very difficult to determine the impact of adding/removing a food group (e.g., total meat) or calorie source (e.g., saturated fat). Invariably, the removal of one component from a food (or a diet) changes the relative proportion of other entities within the remainder, making it very difficult to elucidate nutrient–disease relationships [17].

As an analogy, let's modify the recipe for a hot milk cake (Table 38.1). The removal of nine tablespoons (135 g) of butter from the original recipe with ten tablespoons of butter does not affect the amount of sugar added to the "reduced-butter" recipe (400 g). The proportion of the recipe which is sugar increases from 32 to 36 %. This gives the perception that sugar may have been added to the recipe, possibly to improve sweetness. The reality is that the change in sugar percentage is the result of removing butter.

Nutrition labeling can also contribute to misconceptions about the intentions of the baker (food manufacturer). Let's assume the reference size customarily consumed (RACC) established by regulations for a hot milk cake is 1249 g. With a 1149 g "reduced-butter" cake, the baker (food manufacturer) needs to scale up the "reduced-butter" cake to meet the 1249 g RACC serving size. The nutrition facts panel on the scaled-up "reduced-butter" cake, that is, the "serving-adjusted" column, gives the appearance that sugar was added (448 g vs. 400 g to the original recipe). This is not the case. The

Table 38.1 Effects of changing a hot milk cake recipe on ingredient composition

Hot milk cake recipe	Original		Reduced butter		Serving-adjusted	
Ingredients	g	%	g	%	g	%
4 eggs, large	200	16	200	18	224	18
2 cups sugar	400	32	400	36	448	36
1 teaspoon vanilla extract	28	2	28	3	31	3
2.25 cups all-purpose flour	308	25	308	28	345	28
2.25 teaspoons baking powder	10	1	10	1	11	1
1.25 cups 2 % milk	153	12	153	14	172	14
10 tablespoons butter	150	12	15	1	15	1
Total	1249	100	1114	100	1247	100

proportion of the recipe which is sugar and protein increases predictably in the "reduced-butter" and "serving-adjusted" cakes. Therefore, a health outcome hypothesized to be attributable to reducing butter intake could be a result of increasing the relative proportion of carbohydrate and/or protein in the diet. Dietary macronutrient manipulations are complicated. It is difficult to attribute health benefits to the addition or removal of macronutrients from a food or a diet.

Despite the acrimonious debate about foods contributing to overweight and obesity, the nutritional value of plant or animal products produced by organic methods is similar to those produced conventionally [18]. The biggest nutritional difference found in most foods incorporating organic or conventionally grown staples is if they are enriched/fortified or not [2, 19, 20]. Restricting intake of any macronutrient—fat, protein, or carbohydrate—can lead to weight loss [21–23]. For example, it appears to be the amount (Glycemic Load), not carbohydrate type (Glycemic Index), that contributes to chronic disease risk [24, 25]. Because it is difficult to assign cause to any macronutrient, often the best recommendation to reduce noncommunicable disease risk is modest weight loss [21, 26, 27].

The current emphasis on energy balance implies that health is only affected by the amount and type of calories consumed. Adequate vitamin and mineral intakes are essential for health. Unfortunately, the impact of macronutrient manipulation/substitution on vitamin and mineral intakes (nutritional quality) and noncommunicable disease risk (other than deficiency diseases) is relatively unexplored. Given the existence of hidden hunger, what proportion of the health and mortality risk associated with overweight and obesity is attributable to micronutrient insufficiency?

Past, Present, and Future Impact of Fortification

In the early 1900s, vitamins were discovered (Table 38.2). With the development of commercial production, food enrichment and fortification of foods became possible. For the first time in history, the food industry could enrich and fortify foods to help prevent deficiency diseases. Millions of lives were saved by adding essential vitamins to staple foods—dairy products, flour, salt and in some cases, sugar. The first example in the United States, beginning in the 1920s, was salt fortification with iodine in an effort to control the widespread prevalence of goiter. Shortly thereafter, the isolation and characterization of the B-vitamin, niacin, and its utility in preventing the disease pellagra resulted in significant decreases in pellagra mortality, particularly in the southern United States. As a result of widespread fortification practices, pellagra fell from the eighth leading cause of death in the Southern United States—with an annual mortality of nearly 7000 people per year in the US alone—to being nearly eradicated worldwide [28]. Most recently, fortification of grain products with folic acid in an attempt to reduce the incidence of neural tube defects began in the late 1990s. The result has been nothing short of a public health triumph, with the incidence of neural tube defect decreasing by up to

Table 38.2 History of vitamins

Vitamin	Structure discovered	Appearance	Total synthesis	Industrial production since
Vitamin A (Retinol)	1909	Fish liver oil	1933	1950
Vitamin B1 (Thiamin)	1912	Cereal germs	1936	1938
Vitamin C (Ascorbic acid)	1912	Many fruits	1934	1935
Vitamin D3 (Cholecalciferol)	1918	Fish liver oil	1927	1971
Vitamin B2 (Riboflavin)	1920	Milk, eggs	1935	1942
Vitamin E (ά-Tocopherol)	1922	Wheat germ oil	1938	1941
Vitamin B12 (Cobalamin)	1926	Liver	1948	Resale
Vitamin K1 (Phylloquinone)	1929	Vegetables, fruits	1943	1953
Vitamin B5 (Pantothenic acid)	1931	Liver, kidney	1942	1942
Vitamin B7 (D-Biotin)	1931	Liver, kidney	1942	1948
Vitamin B6 (Pyridoxol)	1934	Meat, liver	1938	1943
Vitamin B3 (Niacin)	1934	Meat, liver	1941	Resale
Vitamin B9 (Folic acid)	1941	Yeast, liver	1946	1948
B-Carotene	1930	Fruits, vegetables	1950	1954
Lycopene	1936	Tomatoes, fruits	1955	1997
Zeaxanthin	1936	Corn, fruits	1975	1999
Apocarotenal	1937	Fruits	1958	1961
Astaxanthin	1951	Crabs, fish	1973	1989
Canthaxanthin	1956	Chanterelle	1956	1964
Apoester	1958	Not natural	1958	1961
Stable Astaxanthin	2002	As Astaxanthin	2002	2008

35 % in just the first 15 years following the introduction of voluntary, and then mandatory, fortification [29]. Some have further speculated that folic acid fortification is a contributing factor to observed declines in other chronic diseases, such as certain pediatric cancers [30] and stroke [31]. Consumption of iron-fortified foodsimproves hemoglobin, serum ferritin, and reduces risk of anemia and iron deficiency [32]. Seasonings are often fortified, for example, fish and soy sauces [33], salt [34], and sugar [35]. Without question, as a public health practice, fortification has been tremendously successful in controlling the prevalence of nutritional deficiency diseases.

Today, despite the advanced understanding on the health effects of vitamins derived in over 100 years of research, national surveys show that inadequate intakes of nutrients are still quite common. If one accounted for the impact of consuming solely nutrients from foods that have not been enriched or fortified, that is, naturally occurring sources of nutrients, inadequate intakes (defined as intake below the Estimated Average Requirement) of vitamin A, folate, thiamin, and calcium would affect the majority (>50 %) of the population, while inadequate takes of vitamin D and vitamin E would be nearly ubiquitous with over 90 % of Americans consuming less than recommended [19]. Due to the high prevalence of certain nutritional inadequacies and the public health impact associated with them, the 2010 Dietary Guidelines Advisory Committee identified several "Nutrients of Public Health Concern" [36]. These included calcium, vitamin D, potassium, and dietary fiber for all adults and children. In 2015, the Dietary Guidelines Advisory Committee identified eight shortfall nutrients: vitamin A, vitamin D, vitamin E, vitamin C, folate, calcium, magnesium, fiber, and potassium [37]. For women of childbearing age, this list was expanded to include iron and folic acid, and vitamin B12 for adults over 50 years of age.

The prevalence of inadequate micronutrient intake is a concern globally. In developed countries, nutrients are often not consumed in recommended amounts from the diet of an overwhelming number of people [2, 19, 38, 39]. In the absence of enrichment and fortification of foods, large proportions of

Table 38.3 Top 15 sources of calories among US children and adolescents 2–18 years, NHANES 2005–2006

Rank	Food category
1	Grain-based desserts[a]
2	Pizza
3	Soda/energy/sports drinks[b]
4	Yeast breads[c]
5	Chicken and chicken mixed dishes[d]
6	Pasta and pasta dishes[e]
7	Reduced fat milk
8	Dairy desserts[f]
9	Potato/corn/other chips
10	Ready-to-eat cereals
11	Tortillas, burritos, tacos[g]
12	Whole milk
13	Candy
14	Fruit drinks[h]
15	Burgers

Source: Adapted from Dietary Guidelines for Americans, 2010. Table 2.2
[a]Includes cake, cookies, pie, cobbler, sweet rolls, pastries, donuts
[b]Sodas, energy drinks, sports drinks and sweetened bottle water including vitamin water
[c]Includes white bread or rolls, mixed-grain flower, flavored bread, whole-wheat bread and bagels
[d]Includes fried or baked chicken parts and chicken strips/patties, chicken stir-fries, chicken casseroles, chicken sandwiches, chicken salads, stewed chicken, and other chicken mixed dishes
[e]Includes macaroni and cheese, spaghetti, other pasta with or without sauces, filled pasta (e.g., lasagna and ravioli), and noodles
[f]Includes ice cream, frozen yogurt, sherbet, milk shakes, and pudding
[g]Includes nachos, quesadillas, and other Mexican mixed dishes
[h]Includes nachos, quesadillas, and other Mexican mixed dishes. Includes fruit-flavored drinks, fruit juice drinks, and fruit punch
[36]Adapted from Dietary Guidelines for Americans, 2010. Table 2.2

the world population would have poorer micronutrient intakes. In European nations, intakes of several vitamins are below recommendations with the most extreme being vitamin D [40].

While whole grains, fruit, vegetables, and legumes are the cornerstone of a healthy diet, most people do not consume recommended quantities. Data from nationally representative surveys show that the most commonly eaten categories of foods are calorie rich and micronutrient poor. Case in point, the top contributor to caloric intake in the United States is the category of grain-based desserts which includes cakes, cookies, and pie (Table 38.3). According to biological measures of nutritional status, significant proportions of the US population are nutritionally "at risk" with respect to vitamins and minerals [41]. In developing nations, fruit, vegetables, whole grains, and dairy products are often not available in sufficient quantities to meet population demands. The consequence is that 84 % of 122 nations report two of the following measures of malnutrition: (1) under 5-year stunting, (2) anemia in women of reproductive age, and (3) adult overweight [7].

Nations regulate the foods that can be fortified as well as the amount of vitamin and mineral which can be added per serving through mandatory fortification efforts and/or voluntary fortification programs [37]. In the modern age, fortification continues to be a major contributor to micronutrient intake. Inadequate intakes of vitamins A, C, D, E, thiamin, folate, calcium, magnesium, and iron would be more prevalent in the US without enrichment and fortification [19]. In children and adolescents, fortification reduces the prevalence of inadequate micronutrient intake, particularly for vitamins A, D, B6, and C [20]. So-called processed foods, often serve as important food sources of nutrients. The top 4 food sources for several vitamins and minerals for children and adolescents 2–18 years of age are all

Table 38.4 Top food sources and source (intrinsic to the food and added to the food) of select vitamins and minerals to diet of children 2–18 years, NHANES 2003–2006

Vitamin source	Rank and % contribution							
Added and intrinsic	First	%	Second	%	Third	%	Fourth	%
Vitamin A	Milk	19.6	Ready-to-eat-cereals	13.7	Cheese	6.0	Carrots, sweet potato, winter squash	5.8
Vitamin D	Milk	51.0	Milk drinks	9.8	Ready-to-eat-cereals	8.0	Fruit juice	3.3
Vitamin C	Fruit juice	32.4	Fruit drinks–ades	23.7	Fruit	11.4	Ready-to-eat-cereals	3.9
Folate	Ready-to-eat-cereals	22.0	Yeast bread, rolls	13.3	Pizza, turnovers	9.7	Pasta dishes	5.0
Iron	Ready-to-eat-cereals	19.2	yeast bread, rolls	11.0	Pizza, turnovers	8.8	Cake, cookie, quick bread, pastry, pie	5.0
Added only								
Vitamin A	Ready-to-eat-cereals	37.6	Milk	29.1	Bars/toaster pastries	0.1	Milk drinks	5.8
Vitamin D	Milk	67.6	Milk drinks	12.2	Ready-to-eat-cereals	11.6	–	
Vitamin C	Fruit drinks–ades	72.1	Ready-to-eat-cereals	19.6	Fruit juice	3.0	Other nonalcoholic beverages	2.3
Folate	Ready-to-eat-cereals	36.1	Yeast bread, rolls	16.3	Pizza, turnovers	10.9	Pasta dishes	6.6
Iron	Ready-to-eat-cereals	44.0	Yeast bread, rolls	14.8	Pizza, turnovers	11.0	Cake, cookie, quick bread, pastry, pie	5.2

Source: Adapted from Berner et al. (2014)

processed foods (Table 38.4). In all cases, the addition of micronutrients increases their contribution to nutrient intake. Milk, fruit juice, ready-to-eat-cereals, and bars/toaster pastries are common breakfast foods which explain the contribution of eating breakfast to nutrient intake [42–44]. Processed foods are the source of 65 % of folate/folic acid, 64 % of iron, 55 % of dietary fiber, 48 % of calcium, and 43 % of potassium consumed by Americans [2]. Without question, fortification has historically been, and continues to be, an effective method to improve micronutrient intakes.

The successes of fortification are being tested by consumers intrigued with natural, organic, and locally grown products. While there are a variety of reasons for seeking organic products, for example, perceived quality, political/ethical reasons, health concerns, family considerations [18, 45], the regular consumption of foods which are not enriched or fortified increases the likelihood of failing to meet dietary recommendations [19, 20]. Not all foods within a category provide the same amount of micronutrients (Table 38.5). When comparing three cereal bars, one doesn't contribute any vitamins or minerals, a second is a good source (10 % of the Daily Value, DV) for 2, and the third is a good or excellent source (20 % DV) for 17 vitamins and minerals. In the ready-to-eat-cereal examples, the organic breakfast cereal isn't a good source of any vitamins and minerals whereas a brand often vilified for its sugar content is a good or excellent source of eight vitamins and minerals. Federal regulations guide serving sizes in these categories so the products are compared presuming a 100 RACC serving size (Table 38.6). There are many nutritional differences between the bars. Within the ready-to-eat cereal selection, differences in the amounts of total fat and total carbohydrate per serving are physiologically inconsequential. However, the contributions of these three ready-to-eat-cereals on vitamin and mineral intake are very different when compared on a standardized serving size. Organic products are not necessarily more nutritious [18], especially compared to enriched/fortified products.

The practice of food fortification is safe. The risk of consuming more than the Tolerable Upper Intake Limit (UL), an average daily intake level unlikely to pose any adverse health risk to almost

Table 38.5 Nutritional content of select cereal bars and ready-to-eat-cereals

	Kind Healthy Grains® vanilla blueberry	Special K® bars chocolatey pretzel	ZonePerfect® chocolate peanut butter	Barbara's® shredded wheat	General Mills® fiber one	Cascadian Farm® organic fruitful O's	Kellogg's® froot loops
Serving size	35 g	22	50	40	30	28	29
Calories	140	90	210	140	60	100	110
Fat calories	35	20	60	10	10	10	10
Total fat	4	2	7	1	1	1	1
Saturated fat	0	1.5	4	0	0	0	0.5
Trans fat	0	0	0	0	0	0	0
Cholesterol	0	0	<5	0	0	0	0
Sodium	75	110	270	160	110	130	150
Total carb	24	16	15	31	25	23	25
Dietary fiber	2.5	3	3	5	14	10	3
Sugars	7	6	15	0	0	8	10
Protein	2	<1	14	5	2	2	1
Good source	*0*	*2*	*2*	*4*	*2*	*0*	*3*
Excellent source	*0*	*0*	*17*	*0*	*8*	*0*	*8*
Vitamin A		0	25	0	0	0	10
Vitamin C		0	50	0	10	0	25
Vitamin E			25				
Calcium	2	0	20	2	10	0	0
Iron		2	10	6	25	2	25
Vitamin D				0	0		10
Thiamin		6	25	6	25		25
Riboflavin		6	50	2	25		25
Niacin		10	45	10	25		25
Vitamin B6		10	50	4	25		25
Folic acid		2	20		25		25
Vitamin B12			25		25		25
Phosphorus			20	15	6		
Magnesium			10	15	4		
Zinc	4		25	8	25		10
Copper				10			
Biotin			25				
Pantothenic acid			25				
Selenium			30				
Chromium			25				
Molybdenum			35				

Information sourced on March 3, 2015 from:
http://www.kindsnacks.com/store/types/healthy-grains-bars/vanilla-blueberry.html
http://www.specialk.com/en_us/products/cereal-bars/chocolatey-pretzel.html
http://zoneperfect.com/products/zoneperfect#chocolate-peanut-butter
http://barbaras.com/products/shredded-wheat/shredded-wheat/
http://www.generalmills.com/en/Brands/Cereals/wheaties/brand-product-list
http://www.cascadianfarm.com/products/cereals/cereal/fruitful-o-s
http://www.kelloggs.com/en_US/kelloggs-froot-loops-cereal.html

Table 38.6 Nutritional content of select cereal bars and ready-to-eat-cereals adjusted to a common 100 g serving size

	Kind Healthy Grains® vanilla blueberry	Special K® bars chocolatey pretzel	ZonePerfect® chocolate peanut butter	Barbara's® shredded wheat	General Mills® fiber one	Cascadian Farm® organic fruitful O's	Kellogg's® froot loops
Per 100 g							
Calories	400	409	420	350	200	357	379
Fat calories	100	91	120	25	33	36	34
Total fat	11	9	14	3	3	4	3
Saturated fat	0	7	8	0	0	0	2
Trans fat	0	0	0	0	0	0	0
Cholesterol	0	0	10	0	0	0	0
Sodium	214	500	540	400	367	464	517
Total carb	69	73	30	78	83	82	86
Dietary fiber	7	14	6	13	47	36	10
Sugars	20	27	30	0	0	29	34
Protein	6	4	28	13	7	7	3
Good source	1	2	0	3	1	0	0
Excellent source	0	2	19	5	11	0	11
Vitamin A	0	0	50	0	0	0	34
Vitamin C	0	0	100	0	33	0	86
Vitamin E	0	0	50	0	0	0	0
Calcium	6	0	40	5	33	0	0
Iron	0	6	20	15	83	7	86
Vitamin D	0	0	0	0	0	0	34
Thiamin	0	17	50	15	83	0	86
Riboflavin	0	17	100	5	83	0	86
Niacin	0	29	90	25	83	0	86
Vitamin B6	0	29	100	10	83	0	86
Folic acid	0	6	40	0	83	0	86
Vitamin B12	0	0	50	0	83	0	86
Phosphorus	0	0	40	38	20	0	0
Magnesium	0	0	20	38	13	0	0
Zinc	11	0	50	20	83	0	34
Copper	0	0	0	25	0	0	0
Biotin	0	0	50	0	0	0	0
Pantothenic acid	0	0	50	0	0	0	0
Selenium	0	0	60	0	0	0	0
Chromium	0	0	50	0	0	0	0
Molybdenum	0	0	70	0	0	0	0

Information sourced on March 3, 2015 from:
http://www.kindsnacks.com/store/types/healthy-grains-bars/vanilla-blueberry.html
http://www.specialk.com/en_us/products/cereal-bars/chocolatey-pretzel.html
http://zoneperfect.com/products/zoneperfect#chocolate-peanut-butter
http://barbaras.com/products/shredded-wheat/shredded-wheat/
http://www.generalmills.com/en/Brands/Cereals/wheaties/brand-product-list
http://www.cascadianfarm.com/products/cereals/cereal/fruitful-o-s
http://www.kelloggs.com/en_US/kelloggs-froot-loops-cereal.html

everyone (97.5 % of the population), is very low even when the contributions from dietary supplements are considered [20, 46]. With the exception of zinc in very young children, individual subgroup analysis does not find nutrient intakes exceeding the upper limit (UL) from food sources. For this reason, many countries rely upon food fortification to address nutritional deficiencies of iron, vitamin A, folic acid, vitamin D, and iodine [8, 32, 33, 35].

Vitamin D is a case study demonstrating the need for continued fortification efforts. Because so few foods are naturally rich in vitamin D, vitamin D fortification of milk and margarine helped prevent rickets [47]. Without vitamin D fortification, cow's milk will not maintain serum $25(OH)D_3$ concentrations in children through winter months [48]. Rickets, caused by severe vitamin D deficiency, tends to occur most often in children whose mothers had poor vitamin D status during pregnancy and then were breast-fed for prolonged periods with little exposure to sun [49]. Rickets is reemerging. Hospitalization rates in Great Britain for rickets are the highest in five decades [50] which may partially reflect a decline in milk intake [51]. Until countries change regulations to increase the number of foods which can be fortified with vitamin D and/or permit higher levels of fortification [52], vitamin D insufficiency is likely to persist unless individuals choose dietary supplementation. It is worth noting that the 2015 Dietary Guidelines Advisory Committee report emphasized that none of the USDA Food Patterns result in consumption of the current Recommended Dietary Allowance (RDA) for vitamin D. On the topic, the committee concluded that "*Additional fortification or supplementation strategies would be needed to reach RDA levels of vitamin D intake consistently, especially in individuals with low intakes of fish/seafood or fortified dairy foods, or other fortified foods (e.g., breakfast cereals) and beverages*" [37].

Another approach to improve the nutritional content of staple crops is biofortification. Selective breeding and genetic modification can improve micronutrient content [53]. Biofortification is a feasible and efficacious way to increase micronutrient intake and status [54]. Sweet potatoes with higher β-carotene concentrations are an effective means to improve vitamin A status [55]. In countries where 75 % of children under 5 years have iron deficiency anemia, increasing the iron content of rice grains through transgenic manipulations has the potential to save millions of lives [56].

Enriched and fortified foods are nutritionally important [2]. Yet there are also life stages where micronutrient demands are increased such that it is difficult to meet nutrient requirements from foods alone. In these cases, recommendations include dietary supplementation to obtain sufficient folic acid and iron during pregnancy, iron during lactation, and vitamin B12 for those 50 years and older [36]. Almost 2/3 of Americans report using dietary supplements. Multivitamin and mineral supplements are nutritionally important [57]. Dietary supplement users tend to have healthier habits, better micronutrient intakes, and better diets than nonusers [58]. In both children and adolescents [59] as well as adults [60], dietary supplement users have a significantly lower prevalence of inadequate vitamin intake. Fifty-one percent of American adults report using multivitamin and mineral supplements and these are a safe, practical means to increase micronutrient status while not exceeding the Tolerable Upper Limit (UL) [57]. More than 90 % of children and adolescents who report using dietary supplements use a multivitamin/mineral or multivitamin product [59, 61]. Dietary supplements, particularly multivitamin/mineral products, remain an effective method to improve nutrient intakes and reduce the prevalence of micronutrient insufficiency.

Public Health and Public Perception

An important challenge to public health strategy is the shift in urbanization. When people live in rural areas, they may be able to grow crops and raise domestic animals. In an analysis of the impact of staple food price increases in eight developing countries, access to land has a positive impact on food security [62]. Households that do not produce food and whose diet depends upon a staple are more

vulnerable to staple food price increases. In other words, poor urban/nonfarm households with a high share of food expenditures are the most nutritionally vulnerable. This is the trend. In 1950, 30 % of the world's population lived in urban areas, today's proportion is 54 % and it is projected to be 66 % in 2050 [36]. Not only will the world population continue to grow but the shift from rural to urban will create new challenges for a global food system. Fewer people will produce food or live in close proximity to others who grow crops or raise production livestock. Most people will live in communities where the number of people needing food will be greater than the local capacity to produce food. People will be increasingly dependent upon foods which need to be transported considerable distance. As socioeconomic indicators increase, people also increase their consumption of prepared meals at home and away from home [63]. Worldwide, consumers are shifting away from preparing foods from staples within the home to eating prepared foods [64, 65]. The historical dependence on fortified staples (flour, salt, milk) which are integrated into meals/foods consumed in the home is disappearing. Consumer behaviors are shifting to ready-to-eat and nearly ready-to-eat foods which are more often consumed away from home. Nutrition fortification policies may need to be adapted because of this trend away from home food preparation with staples.

Nutrition labeling is intended to help consumers make informed packaged food [66–68] and restaurant choices [69, 70]. Nutrient–disease health claims, based on expert consensus, help inform consumers about the health benefits or risks associated with eating certain nutrients. When significant scientific agreement may not be evident, health claims may be "qualified" [71]. Some professional associations certify food products at a cost to the manufacturer when they meet certain nutritional requirements [72]. The intent is to help consumers choose healthy versus less healthy foods. Others subscribe to systems which rank foods [73]. To some extent, these endorsements contribute to belief systems that some foods are nutritionally superior. With a global population of seven billion people and growing, a myriad of approaches and technologies will be needed to eradicate nutrition and food insecurity.

Conclusions and Future Directions

People will continue making food choices based on personal values, food preferences, and cost. There is no reason to constrain consumer food choices to the untested belief systems of nutrition zealots. People choose the foods they eat for many reasons—cost, taste, convenience, cultural, and personal reasons. Nutrition labels provide valuable information when choosing food products.

However, using self-reported dietary intake surveys to research nutrition-health outcomes and define policy is difficult and fraught with error [17]. Underreporting is positively correlated with BMI and likely motivated by social desirability [74]. Interventions that emphasize the importance of healthy behaviors can increase reporting error as investigators "teach" participants socially acceptable responses [75, 76]. When experts acknowledge that energy intake cannot be accurately estimated by coupling self-reported dietary intake with food composition databases [76], it is time to accept that these methods cannot elucidate the effect of micronutrients on health.

Dietary guidance needs to be based on objective measures of nutritional status, for example, serum 25-hydroxyvitamin D concentrations, and associating these with health outcomes, for example, risk of falls and fractures [77]. Only by measuring concentrations of micronutrients in appropriate biological samples can nutrient-health outcomes be established. Based on objective nutrient-health outcomes, a criterion of adequacy can be established, for example, serum 25-hydroxyvitamin D concentration >50 nmol/l for bone health [78]. By measuring individual status, health professionals can provide meaningful nutritional advice to achieve the criterion of adequacy. When individuals (or populations) are found to be nutritionally insufficient, that is, below the criterion of adequacy, individuals can be advised to change dietary patterns or to use dietary supplements. Nutritional

recommendations can be personalized and respectful of cultural (kosher) and personal values (vegetarian, organic).

The food and supplement industry has the capacity to provide nutritious solutions and to distribute food to all regions of the world. Rather than obsessing with food compositional databases in a constantly changing marketplace and trying to couple this data with inaccurate dietary recall information, the time has come to adopt accurate, rapid, cost-effective mobile analytic tools to measure and monitor nutritional status of individuals and populations [79, 80]. By identifying individuals at greatest risk of nutrient insufficiency or excess, extreme behaviors can be addressed or products can be removed from the market. Creating regional maps, like those for obesity [81] and vitamin D [82], can help locate communities of concern. Ultimately, maps could be established by age groups and sex. When the resolution is high, that is, small footprint and many observations, enough to understand the nutritional status of local residents, we will have an opportunity for retailers, local government and nongovernment agencies, and food and supplement manufacturers to work in unison to eradicate hidden hunger in an efficient and targeted manner. By working within local communities and across nations and continents, people not meeting criterions of adequacy, that is, anemia in women of childbearing age or vitamin D inadequacy in winter, programs can be put in place to educate vulnerable groups about their risk and nutrition solutions.

With more than seven billion people needing food and their daily requirement of vitamins and minerals, it is time to shift our preoccupation with the source of nutrients, that is, natural, organic, local, global, processed, packaged, prepared foods, and so on, to achieving criterion of adequacy needed to maintain health. Nutrient recommendations, that is, EARs and RDAs, are statistical approximations of the intake required to meet criteria of adequacy. Based on limited biological data and nutrition epidemiology, nutrition experts advise on dietary policy [37].

Nutrients classically associated with certain food groups are becoming irrelevant [83]. Almost any food can be transformed into an excellent source of nutrients and/or made devoid of others because of technology innovations in production and processing methods. Increasingly, the food and supplement industries can provide consumers with products meeting their specific criteria: organic, gluten-free, low-carbohydrate, trans-fat free, and so on. Animal products aren't the only source of protein. Foods other than dairy products provide calcium. Orange juice can be an excellent source of fat-soluble vitamin D. Rice can be an excellent source of iron. By embracing best agricultural practices and new technologies across all categories, future generations will have the greatest diversity of products to choose from to meet their nutrient needs. Innovation must be encouraged and embraced across all sectors, integrated pest management, genetic attributes, processing technology, and distribution systems.

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Chapter 39
Supplementation: Its Evolving Role in Prevention

Bruce P. Daggy and Francis C. Lau

Key Points

- The supplement industry is global and growing.
- Regulation of the supplement industry is becoming more harmonized across geographies in ways that address good manufacturing practices and permit health claims.
- Suboptimal nutrition addressable by supplementation is commonplace.
- In developing countries, certain supplementation is beneficial and extremely cost-effective.
- Controversy about supplementation is in part a result of methodological challenges, particularly in studies conducted in well-nourished populations.

Keywords Nutritional supplementation • Dietary supplements • Bioactives • Regulatory framework • Traditional medicine

Introduction

Modern Western thinking expresses reliance on nutritional intervention as well as behavioral modalities such as meditation or acupuncture as "alternative medicine," with our twentieth-century construct of pharmaceutical and surgical interventions cast as "mainstream medicine." This nomenclature lacks historical perspective, as traditional, largely plant-based medicine can be found in cultures around the globe, such as the Ayurvedic, Traditional Chinese Medicine, or the European phytochemistry traditions such as are represented today by the German Commission E monographs. Indeed, the modern pharmaceutical industry has heavily relied on natural products for medicinal chemistry leads and sometimes for pharmaceutical actives themselves. All nutrition-based approaches can be considered alternative medicine in the US, as the curriculum of most medical schools is tragically deficient in nutrition education [1].

In the US at the dawn of the twentieth century, traditional medicine had devolved into the morass of patent medicines, at the same time that the growth of knowledge in the fields of chemistry (e.g., elucidation of the structure of organic molecules) and biology (e.g., comprehension and acceptance of germ theory) resulted in the rise of a new medical paradigm. Thomas Edison described this well in his predictions in 1902 [2]:

> Nineteen hundred and three will bring great advances in surgery, in the study of bacteria, in the knowledge of the cause and prevention of disease. Medicine is played out. Every new discovery of bacteria shows us all the more convincingly that we have been wrong and that the million tons of stuff we have taken was all useless.
>
> The doctor of the future will give no medicine, but will instruct his patient in the care of the human frame, in diet and in the cause and prevention of disease.

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_39

> They may even discover the germ of old age. I don't predict it, but it might be by the sacrifice of animal life human life could be prolonged.
>
> Surgery, diet, antiseptics—these three are the vital things of the future in preserving the health of humanity. There were never so many able, active minds at work on the problems of diseases as now, and all their discoveries are tending to the simple truth—that you can't improve on nature.

Edison's predictions were largely borne out, with the exception of his prediction for nutrition. Nutrition was sidelined by legislation declaring that products claiming to treat or prevent disease were, *ipso facto*, drugs. While this had the salutary effect of removing from the market many products making outrageous claims, it also removed a commercial incentive to research the beneficial effects of nutrients, and no doubt it also contributed to the lack of attention paid to nutrition in medical school curricula. In the past quarter century, legislation in the US and elsewhere, as discussed later, has attempted to strike a balance that allows claims for food products, including fortified foods and dietary supplements.

The majority of US adults describe themselves as dietary supplement users [3], and this is also true of US healthcare professionals [4–6]. That said, the merit of nutritional supplementation continues to be questioned as either unhelpful [7–9] or even deleterious [10, 11]. Furthermore, there are frequent criticisms of supplements as un- or underregulated [12, 13].

In this chapter, we will review the regulation of dietary supplements in the US, Canada, China, Japan, and EU and then consider the evidence that supplementation has a role to play in human health.

Regulation of Dietary Supplements

The global supplement market is a 100 billion dollar industry with a projected growth of 4 % annually up to 2018. The US commands the top market value, followed by China, Japan, South Korea, Italy, Australia, Germany, Taiwan, and Canada. Whereas, Singapore, Hong Kong, and Norway are the top spenders by household consumption [14].

As the market grows, so does the focus on its regulatory framework. It is quite surprising that consumers still assume that the dietary supplement industry is unregulated despite significant efforts made by local authorities to regulate the marketing of safe supplement products [15]. This section briefly describes the dietary supplement regulatory framework in major countries and regions including the US, Canada, China, Japan, and EU.

United States

The dietary supplement industry has consistently contributed to the US economy with an impressive growth of 7.5 % to finish 2013 at $34.9 billion [16]. Remarkably during the economic downturn, there was a 5.9 % growth during 2006–2007 period [17]. Even though more than two-thirds of US adults regularly take dietary supplements, the majority of consumers regard the supplement industry as unregulated, a myth still exists today after the passage of the Dietary Supplement Health and Education Act of 1994 (DSHEA) more than 20 years ago.

DSHEA

DSHEA amended the Federal Food, Drug, and Cosmetic Act (FFDCA) established in 1938 by setting standards for dietary supplements. DSHEA differentiated aspects of dietary supplement regulation from regulation of conventional foods with respect to new dietary supplement ingredient, good manufacturing practices (GMP), labeling, and health claims for dietary supplements. The fact of the matter

Table 39.1 Summary of federal mandates on dietary supplements

Year	Milestones of dietary supplement regulation [15, 19, 20]
1906	Pure Food and Drug Act
1938	Federal Food, Drug, and Cosmetic Act (FFDCA) and Wheeler-Lea Act
1958	Food Additives Amendment and publication of the first list of GRAS[a] substances
1962	Consumer Bill of Rights was introduced
1967	Fair Packaging and Labeling Act (FPLA)
1969	White House Conference on Food, Nutrition, and Health: review of GRAS list
1970	Environmental Protection Agency was established to oversee pesticide tolerances
1973	Consumer Product Safety Commission was created
1976	Vitamins and Minerals Amendments or the Proxmire Amendments
1982	Red Book was published
1989	Recall of OTC dietary supplements containing ≥100 mg of L-Tryptophan
1990	Nutrition Labeling and Education Act (NLEA) and Anabolic Steroid Act of 1990
1992	Dietary Supplement Act of 1992
1994	Dietary Supplement Health and Education Act of 1994 (DSHEA)
1996	Food Quality Protection Act
1997	Food and Drug Administration Modernization Act (FDAMA)
1999	ClinicalTrials.gov was founded
2000	Rule on Dietary Supplements regarding the labeling structure/function claim
2002	Public Health Security and Bioterrorism Preparedness and Response Act of 2002
2004	Food Allergy Labeling and Consumer Protection Act and Anabolic Steroid Control Act of 2004
2004	FDA banned dietary supplements containing ephedrine alkaloids
2006	Dietary Supplement and Nonprescription Drug Consumer Protection Act
2007	Food and Drug Administration Amendments Act
2011	FDA Food Safety and Modernization Act (FSMA)

[a]*GRAS* generally recognized as safe

is that the FDA regulates both dietary supplements and dietary ingredients under DSHEA, a set of regulations that is different from those governing conventional foods and drug products [18]. Table 39.1 summarizes key milestones of dietary supplement regulation.

The term dietary supplement is defined by DSHEA as a product taken orally that contains one or more dietary ingredients to supplement the diet. Dietary ingredients may include vitamins, minerals, herbs or other botanicals, amino acids, and substances to supplement the diet by increasing the total dietary intake. A new dietary ingredient is a dietary ingredient that was not sold in the US in a dietary supplement before October 15, 1994 (pre-DSHEA) [18]. According to DSHEA, the manufacturers or distributors are required to notify the FDA at least 75 days prior to marketing any new dietary ingredients (NDIs) or dietary supplements containing NDIs.

Dietary supplements may contain a GRAS substance, which is defined as any substance that is intentionally added to food and is generally recognized to be safe under the conditions of its intended use by qualified experts based on scientific evidence. The use of GRAS substances is not subject to any premarket review or approval by the FDA. Although a GRAS affirmation process was proposed in 1970, the agency has not yet established a final rule on this proposal. However, the FDA does implement a GRAS notification program which is a voluntary procedure. The agency's response to the notice is not an approval and the GRAS substances can be marketed before receiving a response from the FDA [21].

Table 39.2 FDA regulated food/nutrient label claims

Category	Description	Reference
Nutrient content claims	Level of a nutrient in the food	21 CFR 101.13
Health claims	Relationship of any substance to a disease or health-related condition based on significant scientific agreement (SSA)	21 CFR 101.14
Qualified health claims	Health claims that failed to meet SSA and therefore must be accompanied by a disclaimer or otherwise qualified	21 CFR 101.70
Structure/function claim	Describes the role of a nutrient or dietary ingredient in maintaining the normal structure/function; accompanied by the *disclaimer*	21 CFR101.93

Claims

The FDA also regulates food label claims on foods including dietary supplements produced domestically and those from foreign countries under the FFDCA, NLEA, and FPLA laws [22]. There are four categories of claims as summarized in Table 39.2.

The FDA food labeling guidance defines the terms *free, low, reduced/less* when used to claim the nutrient contents of calories, total fat, saturated fat, cholesterol, sodium, and sugars. Additional requirements for nutrient content claims are also outlined in the guidance [22]. Only those claims specifically defined in the regulations may be used on a label [23].

Health claim is defined as any claim on a label that expressly or by implication describes the relationship between a substance and a disease or health-related condition. Health claims can only refer to the reduction of the risk of developing a disease or health-related condition, and cannot claim the diagnosis, cure, mitigation, or treatment of a disease. A substance is eligible for a health claim only if it is associated with a disease or health-related condition for which the general US population or an identified subpopulation is at risk. The FDA evaluates a health claim based on the totality of scientific evidence in the public domain and if there is significant scientific agreement (SSA) supporting the evidence, then and only then the FDA will promulgate regulations authorizing the health claim. Therefore, health claims must be reviewed and evaluated by the FDA prior to use. To date, there are 12 authorized SSA-based health claims for dietary supplements as specified under federal regulations 21 CFR 101.72-83 and 5 FDAMA-based health claims—claims authorized based on an authoritative statement by federal scientific bodies for conventional foods only [24].

When the scientific evidence for claim substantiation does not meet the SSA requirement as deemed by the FDA, qualified health claims (QHCs) on dietary supplements can be made by manufacturers about supplement–disease relationships only if the claims are qualified with appropriate qualifying language as provided by the FDA so that the claims do not mislead the consumers. The petitioner of QHC will receive a letter of enforcement discretion from the FDA specifying the nature of the QHC for which the agency intends to consider the exercise of its enforcement discretion. The issuance of a letter of enforcement discretion indicates that the FDA intends no to object to the use of the QHC, provided that the QHC is stated exactly as specified in the letter [25]. The letters of enforcement discretion for QHCs are published on the FDA website (Table 39.3).

DSHEA added a section to the FFDCA which allows a dietary supplement to bear permitted structure/function claims about the effect of a dietary ingredient or supplement on affecting or maintaining normal body structure or function, or general well-being. Three requirements must be met before the claims can be made. First, the law states that the claims must be substantiated and are truthful and not misleading. Second, the FDA must be notified within 30 days of first marketing the product making the claims. Third, the claims must be accompanied by a mandatory disclaimer statement as provided by the law [26].

Table 39.3 Qualified health claims subject to letters of enforcement discretion as of December, 2014[a]

Atopic dermatitis
• 100 % whey protein partially hydrolyzed infant formula and reduced risk of atopic dermatitis
Cancer risk
• Selenium and a reduced risk of site-specific cancers
• Antioxidant vitamins C and E and reduction in the risk of site-specific cancers
• Tomatoes and prostate, ovarian, gastric, and pancreatic cancers
• Calcium and colon/rectal cancer and calcium and colon/rectal polyps
• Green tea and risk of breast cancer and prostate cancer
Cardiovascular disease
• Folic acid, vitamin B6, and vitamin B12 and vascular disease
• Nuts and coronary heart disease
• Walnuts and coronary heart disease
• Omega-3 fatty acids and reduced risk of coronary heart disease
• Corn oil and corn oil-containing products and a reduced risk of heart disease
• Unsaturated fatty acids from canola oil and reduced risk of coronary heart disease
• Monounsaturated fatty acids from olive oil and coronary heart disease
Cognitive function
• Phosphatidylserine and cognitive function and dementia
Diabetes
• Psyllium husk and a reduced risk of type 2 diabetes
• Whole grains and a reduced risk of diabetes mellitus type 2
• Chromium picolinate and a reduced risk of insulin resistance, type 2 diabetes
Hypertension
• Calcium and hypertension, pregnancy-induced hypertension, and preeclampsia
Neural tube defects
• Folic acid and neural tube defects

[a]http://www.fda.gov/Food/IngredientsPackagingLabeling/LabelingNutrition/ucm072756.htm (Accessed 18 Dec 2014)

Canada

Dietary supplements are known as natural health products (NHPs) in Canada and are defined and subject to the Natural Health Products Regulations (NHPR) which became effective on January 1, 2004. Prior to 2004, NHPs were regulated as either a drug or food under Canadian Food and Drugs Act (CFDA). NHPs are defined as health products including vitamin and mineral supplements, herbal preparations, traditional and homeopathic medicines, probiotics, and enzymes—similar to US definition of dietary ingredients.

The Natural Health Products Directorate (NHPD), responsible for the regulation of NHPs sold in Canada, was established in 1999 as a division of the Health Products and Food Branch of Health Canada. The Directorate was established to ensure that consumers have access to NHPs that are safe, efficacious, and of high quality. To be legally sold in Canada, all NHPs must have a product license and site licenses for the Canadian sites that manufacture, package, label, and import these products. Since 2004, NHPD has authorized over 70,000 NHPs for sale and issued over 2000 site licenses [27].

In December 2012, the NHPD published the "Pathway for Licensing Natural Health Products Making Modern Health Claims" guidance documents to modernize the review process of NHPs, including standards for health claims, the use of risk information, and combination of NHPs.

Under this new approach to nonprescription drugs (NPDs), products with the greatest level of certainty are subject to the shortest review time [27]. The risk-based review system for product license application consists of a three-class system as summarized in Table 39.4.

Table 39.4 The three-class review system for product license application

Class I: High level of certainty—lowest level of premarket review
• Products that have been licensed repeatedly and can be referenced against precleared information (PCI) or monographs
• Products such as vitamins, minerals, and certain herbal products
• Review time is 10 days
Class II: Medium level of certainty—medium level of premarket review
• Products with claim(s)/ingredient(s) supported by a combination of NHPD monographs
• Applicants are required to attest that their product meets the individual monograph parameters
• Review time is 30 days
Class III: Low level of certainty—higher level of premarket review
• Products with previously unlicensed claims, or never before seen ingredients or combinations, or with significant safety concerns
• Applicants are required to submit safety and efficacy evidence for novel ingredients
• Review time is 180 days

Table 39.5 Health claims for Natural Health Products[a]

Claims by health condition
• Serious disease/condition claims—for products indicating treatment, prevention or cure of diseases/conditions that require supervision by a health care practitioner, or are debilitating or potentially life threatening without effective treatment. Treatment is vital to mitigate the health impact
• Major disease/condition claims—for products indicating treatment, prevention, or cure of diseases/conditions that are not naturally resolved within a timely manner or have potentially undesirable effects that may worsen or persist if proper treatment or care is not pursued in a timely manner
• Minor disease/condition claims—for products indicating treatment, prevention, risk reduction, or cure of diseases/conditions or symptoms that are expected to naturally resolve within a timely manner or for which lower than expected performance of the product should not pose a major risk to the person taking it under the recommended conditions of use
Claim by health effect
• Diagnostic claims
• Treatment claims
• Cure claims
• Risk reduction claims
• Prevention claims
• General health maintenance, support, and promotion claims
• Antioxidant claims
General health claims
• For product with low therapeutic impact
• Claims are indexed against the level of evidence provided to support the safe use of the products

[a]http://www.hc-sc.gc.ca/dhp-mps/prodnatur/legislation/docs/modern-eng.php. (Accessed 18 Dec 2014)

Health claim is defined as "a statement that indicates the intended effect of a product when used in accordance with its recommended conditions of use [28]." There are three types of health claims which are summarized in Table 39.5.

The framework of federal regulation of food and health products has undergone a significant process of modernization in Canada. As such, in June of 2014 the NHPD changed its name to the Natural and Non-prescription Health Products Directorate (NNHPD) as a result of the transfer of NPDs and

Table 39.6 Currently approved health claims by CFDA

Claims	Required tests
1. Antioxidation; **2**. Alleviate lead excretion; **3**. Assist in blood lipids reduction; **4**. Assist in blood pressure reduction; **5**. Assist in blood sugar reduction; **6**. Assist in memory improvement; **7**. Assist in protection of gastric mucosa; **8**. Facilitate digestion; **9**. Facilitate feces excretion; **10**. Facilitate milk secretion; **11**. Improve child growth; **12**. Improve nutritional anemia; **13**. Moisten and clear the throat; **14**. Regulate gastrointestinal tract flora; **15**. Reduce weight	Animal and human
16. Alleviate eye fatigue; **17**. Eliminate acne; **18**. Eliminate skin chloasma; **19**. Improve skin oil content; **20**. Improve skin water content	Human
21. Alleviate physical fatigue; **22**. Assist in protection against chemical injury of liver; **23**. Assist in protection against radiation hazard; **24**. Enhance anoxia endurance; **25**. Enhance immunity; **26**. Improve sleep; **27**. Increase bone density	Animal

disinfectants to NHPD. This change enables the development of a system for consumer health products that include NHPs, NPDs, disinfectants, and cosmetics.

Canadian regulators and scientists continue to work with global regulatory partners to create and adopt standards and processes in order to achieve global harmonization.

China

Dietary supplements or health foods were regulated by the Ministry of Health of the People's Republic of China (MOH) prior to government restructuring in 2003. In 2003, the State Drug Administration (SDA) which regulated drug manufacturing, trade, and registration was modernized based on the US FDA to become the State Food and Drug Administration (SFDA). SFDA provided a centralized framework for the regulation of foods, health foods, and drugs [29]. In July 2005, the SFDA revised the Administration Regulation for Health Food issued by the MOH in 1996 and promulgated the Guidelines for Health Food Product Registration [29]. As defined by the Guidelines, health (functional) food is a food that has special health functions, is suitable to be consumed by special groups of people, and is capable of regulating the functions of the human body but is not used to treat diseases [29]. Both nutritional supplements and functional foods fall into the health food category. Health foods are allowed to be marketed with health claims. Currently, there are 27 categories of health claims approved by the Food Safety Law passed in 2009 (Table 39.6). For both imported and domestic health foods to be sold in China, companies are required to undertake a stringent process of product registration with the SFDA. The registration is referred to as a "blue hat" or "blue cap" registration because the logo resembles a blue hat.

The regulatory framework for health foods in China is ever changing. As of March 2013, the SFDA was restructured to become the China Food and Drug Administration (CFDA), a ministry-level agency reporting directly to the State Council, the highest administrative body in China. This change was prompted by a series of food safety scandals such as the melamine-tainted milk and infant formula. The responsibility of CFDA on food regulation more closely resembles that of the US FDA.

Another imminent change is the reduction of the approved health claims from 27 to 18 with emphasis on substantiations through human clinical trials. This may impact the current "blue hat" applications. Therefore, it is important to keep abreast of the latest information on new guidelines of health food regulations which is available at the CFDA website: http://eng.sfda.gov.cn/.

Japan

Functional food is defined by the Ministry of Education, Science and Culture in 1984 as a food having three functions: nutritional function, sensory function, and tertiary or physiological function [30]. The Japanese food regulatory framework classifies foods into three categories: (1) Food with Health Claims

(FHC), (2) Food for Special Dietary Uses (FOSDU), and (3) General Foods or so-called Health Foods. General Foods or so-call Health Foods are not allowed to make any claims. FOSDU are foods that are permitted to display special dietary uses for one of the five FOSDU categories (Table 39.7).

Currently, FHC includes two categories: Food with Nutrient Function Claims (FNFC) and Food with Specified Health Uses (FOSHU). FNFC system was established in 2001 to regulate foods that are labeled with nutrient function claims as specified by the Ministry of Health, Labor and Welfare (MHLW) of Japan. The MHLW has established standards and specifications for indication of nutritional functions for 12 vitamins and 5 minerals so far. FOSHU system was set up by the MHLW in 1991 as a regulatory framework to approve product claims regarding the effects of foods on human body. FOSHU was originally enacted under the Nutrition Improvement Law, currently the Health Promotion Law governing FOSDU [30]. Therefore, FOSHU overlaps one of the FOSDU categories (Table 39.7).

FOSHU is defined by the MHLW as a food containing functional ingredient with sufficient evidence to substantiate a health claim and is officially approved by the government to claim its physiological effects on the human body. FOSHU is intended to be used for the maintenance and/or promotion of health or the control of health conditions such as blood pressure and blood cholesterol.

FOSHU approval involves safety evaluation by the Council on Pharmaceutical Affairs and Food Sanitation and efficacy assessment by the Food Safety Commission, and final approval of the MHLW. Requirements for FOSHU approval are summarized in Table 39.8.

After evaluating the effectiveness of the FOSHU system, the Office of Health Policy on Newly Developed Foods decided to classify FOSHU into four categories in 2005 (Table 39.9). This classification system is based on the strength of evidence for the proposed relation between a product and a

Table 39.7 Categories of food for special dietary uses

Category	Description
1	Medical foods for the ill
2	Formulas for pregnant or lactating women
3	Infant formulas
4	Foods for the elderly with difficulty in masticating or swallowing
5	Foods for specified health uses (FOSHU)

Table 39.8 Requirements for FOSHU Approval

- Effectiveness on the human body is clearly proven
- Absence of any safety issues (animal toxicity tests, confirmation of effects in the cases of excess intake, etc.)
- Use of nutritionally appropriate ingredients (e.g., no excessive use of salt, etc.)
- Guarantee of compatibility with product specifications by the time of consumption
- Established quality control methods, such as specifications of products and ingredients, processes, and methods of analysis

Table 39.9 Classification of FOSHU, 2005 amendment

Category	Description
FOSHU	Foods contain functional ingredient with sufficient scientific evidence to support a health claim
Qualified FOSHU	Foods with health function which are not substantiated by scientific evidence that meets the level of FOSHU, or foods with certain effectiveness but without established mechanism of action for the function
Standardized FOSHU	Foods meet the standards and specifications established for foods with sufficient FOSHU approvals and accumulation of scientific evidence
Disease-risk reduction FOSHU	Reduction of disease risk claim is permitted when reduction of disease risk is clinically and nutritionally established in an ingredient, currently for foods containing calcium or folic acid only

disease or health-related condition [30]. The new classification allows for disease-risk reduction claim which is in harmony with the Codex Alimentarius (Table 39.9).

The introduction of the additional three FOSHU categories was intended to facilitate the FOSHU approval process. However, there were only about 1000 approved product-specific FOSHU claims since 1991. The FOSHU approval process is proven to be lengthy and cost prohibitive.

In order to harmonize with international regulatory framework, a new health claim law is being drafted by the MHLW and is slated to take effect in April of 2015. This new law will work alongside the FOSHU system. Although it is unclear what the new law will look like, according to industry insiders, this new law will bring significant changes to the health food industry in Japan. Currently, the so-called health foods that are not FHC, that is, FOSHU, FOSDU, or FNFC, are not allowed to make any health claims. With the new law this ban on functional claims for health foods may change. It is suggested that the new law will resemble the US structure/function claim DSHEA system. In addition, the new law may allow medical professionals to make recommendations on food supplements to patients. The industry is anxiously awaiting the debut of this new law.

European Union

The European Parliament and the Council of the European Union adopted the Regulation on Nutrition and Health Claims (Regulation 1924/2006) in December of 2006. For the first time, a harmonized regulatory framework for nutrition and health claims on foods, including disease-risk reduction claims, was established across the member states and countries of the EU [31]. This premarketing approval legislation ensures that any claims made on foods that are sold in the EU are clear, accurate, and based on evidence accepted by the whole scientific community thereby enabling the consumers to make informed and meaningful choices [31]. As such, the European Commission has established a Register of Nutrition and Health Claims on foods and the list, including permitted, rejected, and pending health claims, is made available to public.

As defined by the European Commission, a health claim "is any statement about a relationship between food and health." Products bearing health claims must be authorized by the Commission. There are three types of health claims (Table 39.10).

The European Food Safety Authority (EFSA) is responsible for evaluating the scientific evidence supporting health claims. EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA), consisting of highly qualified risk assessment experts from a number of European nationalities, evaluates and prioritizes the submitted health claims. As of 2011, there were approximately 44,000 health claims provided by different member states and countries of the EU to the Commission. These claims were consolidated into a list of 4600 claims and sent to EFSA for evaluation. Information on authorization

Table 39.10 Types of health claims

Claims	Descriptions/examples
Function claims	• Growth, development, and functions of the body
	• Psychological and behavioral functions
	• Slimming or weight control
	• Claims based on newly developed scientific evidence and/or proprietary data
Children's development and health	• Effect of vitamin D on the normal growth and development of bone in children
Disease-risk reduction	• Reducing a risk factor in the development of a disease such as
	• Plant stanol esters have been shown to reduce blood cholesterol. Blood cholesterol is a risk factor in the development of coronary heart disease

procedure for health claims can be found on the European Commission's Health and Consumers website: http://ec.europa.eu/food/food/labellingnutrition/claims/health_claims_en.htm.

The Regulation on Nutrition and Health Claims (Regulation 1924/2006) has limited the ability of botanical products to make health claims. The difference in regulation of botanical ingredients under the legislation on health claims and the law on Traditional Herbal Medicinal Products (THMP) has raised a potential problem. Since health claims can only be used when approved by EFSA based on scientific data for safety and the results from gold standard randomized, controlled human trials (RCT) for efficacy. The beneficial effects of botanicals have been accumulated over time through use and experience. However, traditional use or history of use, which is recognized by the THMP legislation, is not considered as evidence for human efficacy claims. This creates a situation where health claims requirements for a botanical food product are stricter than that for a botanical medicinal product. In fact, based on EFSA's assessment of the scientific evidence conducted in 2009, it became apparent that applications for botanical health claims would most likely result in negative opinions. Currently, the assessment of botanical health claims by EFSA is put on hold by the European Commission.

Global Harmonization

In order to provide informed consumer choice and safety and to facilitate world trade of dietary supplements, efforts have been made to harmonize international guidelines for certain dietary supplements by regulatory authorities such as the Codex Alimentarius Commission created by the Food and Agriculture Organization and the World Health Organization of the United Nations, the European Commission, the US FDA, and the World Trade Organization. Working in concert with these international regulatory bodies to achieve global harmonization are industry organizations such as the International Alliance of Dietary Food Supplement, the Global Harmonization Initiative working groups, and the Association of Southeast Asian Nations. It remains to be seen whether these efforts will result in a harmonized framework for food/dietary supplements regulation across the globe.

Does Supplementation Have a Role to Play in Human Health?

First, we will show data that indicate that supplementation is beneficial in both the developing and developed world. Then we examine the studies involving other bioactive compounds commonly delivered through supplementation.

Is Supplementation Beneficial?

A model to understand the conflicting advice concerning supplementation is shown in Fig. 39.1. In the developing world, it is very easy to demonstrate, in what are generally poorly nourished populations, the benefits of supplementation or fortification—on stunting, vitamin A deficiency blindness, anemia-induced loss of mental acuity, poor immune function, higher infant mortality, poor birth outcomes, and more. In the developed world, it is easy to show that supplementation can improve nutritional status, but reports question whether that is beneficial, especially since at times (e.g., beta-carotene in smokers) it looks *worse* than doing nothing. But when researchers attempt to ask the definitive questions, they are hampered by multiple methodological problems, including cost. This is especially true

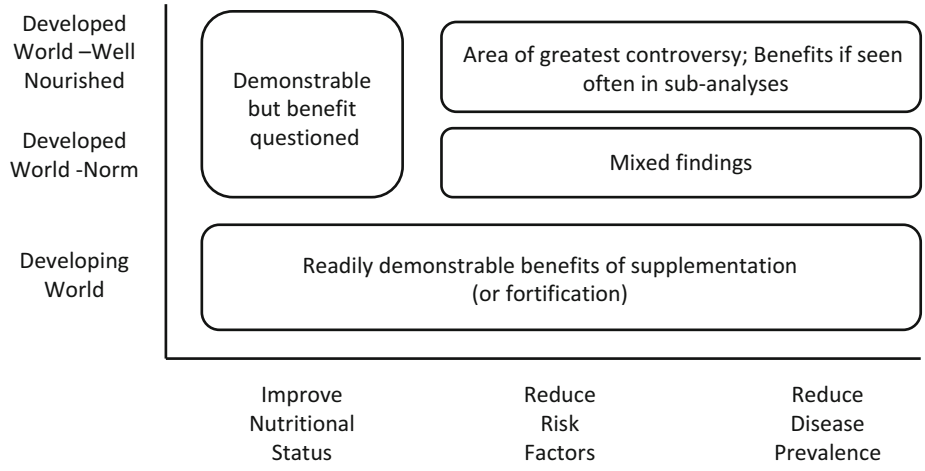

Fig. 39.1 Reporting of supplementation research: general pattern of the dialogue

if the study population consists of better educated, wealthier subjects who are likely to be better nourished and have better access to healthcare than the general population. Intervention trials in the developed world designed with a disease-based primary endpoint often fail to show an overall treatment effect, but one or more subgroup analyses may point to a benefit. Researchers, reporters, and policy makers are therefore faced with a choice: to view these studies and say there is no benefit, that the primary end point of the study showed a nonsignificant difference; or to conclude that the studies do in fact suggest that very safe interventions can benefit a significant fraction of the population. The following section will consider these points in greater detail.

Some of the most clear-cut and compelling arguments for supplementation can be found in developing countries. Micronutrient malnutrition is widespread and is a significant contributor to infant mortality; vitamin A deficiencies alone are estimated to result in 650,000 deaths per year in children under five [32]. Iron deficiency is also widespread in pregnant women, with irreversible impact on cognitive development [33]. In total, maternal and child undernutrition is estimated to result in 3.5 million deaths per year [32]. A meta-analysis of vitamin A supplementation trials estimated a 24 % reduction in deaths in children age 6–59 months [34]. Estimates for the annual cost to supplement the roughly 163 million preschool children who are vitamin A deficient range from $50 million to $391 million [35]. This analysis estimates a benefit-to-cost ratio of 12.5, not factoring in any decreased mortality benefit. In the same report, supplementation and iodine and iron fortification are identified as the inexpensive components of a program to address both stunting and child mortality [35].

While addressing gaps in the delivery of essential nutrients has been the primary focus of interventions in the developing world, in the developed world supplementation covers a wide range of both essential nutrients and nonessential bioactive compounds. All of the defined essential nutrients are available to be consumed via supplementation. These include the vitamins and trace minerals, the 18 carbon omega-3 and omega-6 fatty acids, and the essential amino acids (as free amino acids or via a protein supplement). Dietary fiber, technically not an essential nutrient but included on the Nutrition Facts Panel, is also often consumed in supplemental form, or as an over-the-counter medicine.

Various authoritative bodies have defined levels of intake of the essential nutrients that represent generally adequate intakes in defined populations. In the US, the Dietary Reference Intakes establish adequate intake levels for a core set of micro- and macro-nutrients (93). Adequate intakes are expressed as the Recommended Dietary Allowance (RDA) or, if there is insufficient evidence to

establish an RDA, an Adequate Intake (AI) level. In addition, a Tolerable Upper Intake level (UL) is defined. While the DRI process is now intended to consider mitigation of the risk of chronic disease, the typically wide gulf between the RDA or AI and the UL begs the question as to whether intakes above the RDI/AI can be beneficial. The RDA is an estimate of what daily intake is required to meet the basic (not optimal) needs of most healthy individuals within a particular group. Given the rather high rates of obesity, type 2 and gestational diabetes, hypertension, dyslipidemia, nonalcoholic fatty liver disease, sarcopenia, and so on, and in general population, and the high incidence of chronic disease in the older population [36], it is reasonable to question whether meeting the RDA gives a false sense of security. But first, are the RDAs and AIs being met by the general population with the existing food supply?

The RDAs Are Often Not Being Met

US national survey data have documented inadequate nutrient intake for a number of nutrients covered by the DRI process. The current Dietary Guidelines for Americans (updated every 5 years by the USDA) lists of a number of nutrients of concern: potassium, dietary fiber, calcium, vitamin D; and for certain populations, iron, folate, and B12 [37]. The Guidelines express a bias toward improvement in diet over supplementation as the solution to nutrient gaps. While the Guidelines note that "few American consume potassium in amounts equal to or greater than the AI," they advise "Americans should select a variety of food sources of potassium to meet recommended intake rather than relying on supplements." The Guidelines also express a preference for additional food sources of fiber and calcium. For vitamin D and iron, the Guidelines are more receptive to the use of dietary supplements, and for folate (folic acid) and B12, the Guidelines acknowledge that supplementation can be useful.

It should be noted that without fortification of foods with some of these nutrients, the case for supplementation would be more compelling; food fortification and supplementation jointly play significant roles in closing nutrient gaps as defined by the RDAs. An analysis of NHANES 2003–2006 datafound that supplementation helps close nutrient gaps for a significant proportion of the US population [38]. Similar benefits have been reported in other such analyses [39–41] although whether closing nutrient gaps with supplements in the US population produces differences in health endpoints remains controversial.

Among the essential nutrients not listed as nutrients of concern by the 2010 Institute of Medicine (IOM) statement, deficient intakes can be widespread. For example, an analysis of US national survey data by Moshfegh et al. [42] found intakes from diet alone below the EAR for vitamin E (93 % of all Americans) and vitamin C (34 % for nonsmokers, and most smokers). About two-thirds of the US adult population is below the EAR in magnesium intake, and 16 % of girls and premenopausal women were estimated to have iron intake below the EAR [42]. Looking more broadly across a range of micronutrients, an analysis of 3-day food records of over 21,500 people in the 1977–1978 USDA National Food Consumption Survey found that no individual achieved the RDA for all ten micronutrients evaluated [43]. A recent analysis performed by Agarwal and coauthors (2015) indicated that a significant proportion of the adult population had inadequate intakes of vitamins A, C, D, and E and minerals calcium and magnesium [41]. For example, more than 90 % of adults consumed less than the EAR for vitamins D and E [41]. Taken together, these data suggest that inadequate intakes of micronutrients are prevalent in the US

In the face of consistent findings of suboptimal nutritional intakes, Ames et al. [44] asked "A significant fraction of Americans have micronutrient intakes below the Estimated Average Requirement. Why establish values such as the Estimated Average Requirement and not take simple steps to eliminate deficiencies?"

Is There Evidence of Benefits from Supplementation Above and Beyond the RDAs, and in Well-Nourished Populations?

While the evidence is clear that many individuals are not consistently achieving all the RDAs, the frequent failures of RCTs and meta-analyses to demonstrate a benefit of supplementation are the basis of many criticisms of the practice of nutritional supplementation. The RCT approach is ideally suited for pharmaceutical research, in which a true placebo is possible and a single, often swiftly determined outcome is sought; Heaney has outlined the challenges of applying the RCT approach to the study of nutrients [45]. Ethical considerationswith respect to the control group are problematic enough; but nutrients, unlike the ideal drug, are also not tightly targeted in terms of their target tissues or physiological effects; as Heaney makes clear, the question of outcome measure(s) is (are) also complex [45]. At times, even the minimal intervention given to the control group has been sufficient to prevent disease progression [46].

Bruce Ames has argued "the prevention of more subtle metabolic damage may not be addressed by current RDAs. When one input in the metabolic network is inadequate, repercussions are felt on a large number of systems and can lead to degenerative disease. This may, for example, result in an increase in DNA damage (and cancer), neuron decay (and cognitive dysfunction) or mitochondrial decay (and accelerated aging and degenerative diseases)… A tune-up of micronutrient metabolism should give a marked increase in health at little cost [47]." Heaney has articulated a similar argument, focusing on physiological responses instead of disease avoidance as the measure of adequate intake [48].

The RDAs are based on an avoidance of disease assessment, not optimal health. And yet, 54 % of US supplement users take supplements for overall health and wellness, while only 17 % take supplements to help reduce the risk of serious illness [3]. In this context, do the RDAs provide a false sense of security, to those apparently few individuals who routinely achieve all the RDAs? Per Ames argument earlier, is there evidence supporting health benefits of nutrient intakes above the RDA?

Vitamin D is a good case study for this discussion. The Institute of Medicine in 2010 reevaluated the DRI for vitamin D, and increased the RDA from 400 to 600 IU/day. A 50 % increase in a long-established RDA is itself a cause for reflection; however, also in 2010, the Endocrine Society published its own analysis, recommending 1500–2000 IU/day [49]. In 2014, the American Geriatrics Society published a recommendation based on the prevention of falls and fractures, calling for a total daily input from all sources of 4000 IU, including at least 1000 IU from D supplementation along with calcium [50]. We therefore have three expert bodies, all considering the same data and all focused on disease/injury, with three strikingly different conclusions. Heaney provides an additional perspective based on physiological responses, arriving at an all-input value of 4000–6000 IU/day [48]. So, for vitamin D there are two intake recommendations at or above the UL set by the IOM. What is a clinician (or a consumer) to make of this situation? Heaney argues that the IOM's lower limit of normal D status, that is, 20 ng/mL, is insufficient for a woman to transfer D into her breast milk [48]; the American Geriatrics Society argues that this limit is too low to prevent falls that have serious health consequences [50]. It will be interesting to see how the IOM position evolves in the future.

Other nutrients evaluated in RCTs have also demonstrated benefitswhen supplemented to total intakes above the RDA. For example, a large trial with adults reported benefit from a high dose (8 g) of vitamin C at the onset of cold symptoms [51]. Long-chain omega-3 fatty acids are another good case in point. The American Heart Association recommends eating fish (particularly fatty fish) at least two servings for a total of 7 oz per week [52]. Whether for reasons of availability, taste preference, cost, concerns about contaminants, or other factors, consumption is about half what is recommended [53]. Supplementation with fish oil in fact has become so widespread that even the pharmaceutical industry decided there was an opportunity [54].

Supplementation with Bioactive Compounds

Diets rich in fruits and vegetables are associated with lower risk of cardiovascular disease, cardiovascular risk factors, and risk of certain cancers [55]. Mechanistic studies point to a number of bioactive compounds found in fruits and vegetables, including carotenoids, flavanoids, isothiocyanates, organosulfides, and nitrates [56–62], in addition to vitamins, minerals, and fiber. The Office of Disease Prevention and Health Promotion defines bioactives as "Constituents in foods or dietary supplements, other than those needed to meet basic human nutritional needs, which are responsible for changes in health status" [63]. There are thousands of bioactive compounds, many of which are not fully characterized. The vast majority of phytochemicals found in the human food supply have not been evaluated via a DRI process; while these chemicals may not be essential to life, these phytochemicals are important to human health. While there have been calls to expand the DRI process to cover bioactive compounds, the challenges are considerable [63, 64]. The absence of a DRI process has not prevented supplement companies from making many bioactive compounds available in supplement form, and citing clinical evidence for structure/function claims.

Whether the focus is on essential nutrients or bioactive compounds, studies conducted in nutritionally adequate Western subjects have resulted in considerable debate. In the RCT for osteoporosis within the Women's Health Initiative, over 36,000 postmenopausal women were randomized to receive 1000 mg of calcium plus 400 IU of vitamin D per day or a matching placebo, and were followed for an average of 7 years [65]. No doubt influenced by reporting of previous studies, the control group had a mean daily calcium intake of 1154 mg, with 39.2 % of the control group consuming more than 1200 mg calcium per day [66]. While overall the study found no significant improvement in hip fractures, a modest improvement in bone mineral density did reach statistical significance, and a subgroup analysis found significantly reduced hip fracture risk in women who were judged at least 80 % compliant with the study medication. Although a subgroup analysis based on baseline calcium and D intake found no significant differences, use of personal calcium supplements increased over the course of the study, and calcium intake increased more in those whose intake was low at baseline.

The Physicians Health Study II was a randomized, double-blind, placebo-controlled clinical trial in over 14,000 male US physicians age 50 or older, and included a cohort of 1312 subjects with a history of cancer at enrollment [67]. The intervention was a daily multivitamin, and the primary outcome measure was total cancer, excluding nonmelanoma skin cancer, during a median follow-up of 11.2 years. The study population was not reflective of the US adult population; for example, few subjects were current smokers (3.6 %), aspirin use at baseline was 77.4 %, and reported fruit and vegetable intake exceeded four servings per day. The multivitamin group compared with placebo had a reduced risk of cancer (hazard ratio [HR], 0.92; 95 % CI, 0.86–0.998; $P=0.04$), and this was also true for the cohort with prior cancer (HR, 0.73; 95 % CI, 0.56–0.96; $P=0.02$). The authors reported that these findings were not materially altered by excluding the first 2 or 5 years of follow-up or by adjusting for compliance. The multivitamin supplements were also well tolerated, with rates of adverse experiences generally comparable across treatments [67]. And yet, an accompanying editorial argued against recommending multivitamin use for chemoprotection, based in part on disbelief that a multivitamin could plausibly have such an effect in "a well-nourished population" [68]. An earlier study in a large and less well-nourished Chinese population, the Linxian General Population Nutrition Intervention Trial, tested a combination of beta-carotene, vitamin E, and selenium for 6 years, finding significant improvements in total mortality, cancer mortality, and gastric cancer mortality [69]; the total and cancer mortality benefits were still observed after 10 years of posttrial follow-up [70].

The Age Related Eye Disease Study (AREDS) and the subsequent AREDS2 are nation-wide human clinical trials sponsored by the National Eye Institute to examine the role of nutritional

interventions on age-related macular degeneration (AMD) and cataracts [71–74]. The AREDS study was designed to evaluate the effect of high doses of vitamin C, vitamin E, beta-carotene, and zinc (AREDS formulation) on the progression of AMD and cataract. The multicenter study involved 11 clinical centers nationwide with 4757 participants, 55–80 years of age, and lasted for more than 6 years. Results indicated that high levels of antioxidants and zinc significantly reduced the risk of advanced AMD and its associated vision loss. These same nutrients had no significant effect on the development or progression of cataract [75, 76]. AREDS2 is a 5-year multicenter study designed to evaluate whether the original AREDS formulation could be improved by adding omega-3 fatty acids, adding xanthophylls (lutein and zeaxanthin) and omega-3 (DHA and EPA), removing beta-carotene, or reducing zinc [74]. AREDS2 involved 4203 generally well-nourished participants, ages 50–85 years, who were at risk for advanced AMD. The study was conducted at 82 clinical sites across the country [74]. Results showed that adding omega-3 fatty acids to original AREDS formulation did not improve the effect of treatment for AMD. The plant-derived antioxidants lutein and zeaxanthin also had no overall effect on AMD when added to the AREDS formulation. Removing beta-carotene from or lowering zinc in the AREDS formulation did not reduce the formulation's protective effect against developing advanced AMD. However, when beta-carotene was removed from the original formulation, the addition of lutein and zeaxanthin was shown to reduce the risk of developing advanced AMD by 18 % over a 5-year period. The AREDS2 formulation had no effect on cataract although a subgroup of participants with low dietary lutein and zeaxanthin gained some protection [72, 73, 77].

Interestingly, there are preliminary findings suggesting that bioactive resveratrol may help to protect against AMD [78]. This case report indicated that people on AREDS2 formulation but still suffering from progressing AMD benefited from taking resveratrol supplementation [78]. Indeed a series of case reports including 34 patients with diagnosed dry AMD indicated that a resveratrol/polyphenol-rich supplement taken together with AREDS2 formulation dramatically improved vision and macular structure of people with dry AMD [79].

Long-term, chronic use of multiple dietary supplements is extremely difficult to study. A cross-sectional epidemiology study by Block et al. [80] compared long-term (20+ years) users ($n=278$) of multiple nutritional supplements who were well educated and had higher than average income to two matched control groups from the NHANES databases: nonsupplement users ($n=602$) or users only of a multivitamin ($n=178$). Supplements reported to be consumed daily by more than half of the subjects in the multiple supplement group included a multivitamin–mineral supplement, B-complex, vitamin C, carotenoids, vitamin E, calcium with vitamin D, omega-3 fatty acids, flavonoids, lecithin, alfalfa, CoQ10 with resveratrol, glucosamine, and an herbal immune supplement; in this group, median supplement use was 18 per day. Blood samples were drawn to measure nutritional status and disease risk factors, and detailed medical histories were collected. By multiregression analysis adjusted for age, gender, income, education, and BMI, the multisupplement group exhibited favorable nutrient status for red blood cell folate, retinol, vitamin C, alpha tocopherol, alpha- and beta-carotene, and ferritin in women. No subject in the multisupplement group had low or high vitamin D status (data not available in NHANES). In terms of risk factors, the multisupplement group was at lower risk in terms of homocysteine, C-reactive protein, total- and LDL-cholesterol, triglycerides, (low) HDL, and total-to-HDL ratio. None of the multisupplement subjects had C-reactive protein above 3 mg/dL. This group also had significantly lower odds ratios vs. the no supplement controls for elevated blood pressure (OR, 0.61, 95 % CI, 0.41–0.92), diabetes (0.27; 0.12–0.59), and self-assessed poor health status (0.26; 0.17–0.41). Their odds ratios were trending lower but not statistically significant for known coronary heart disease (0.48), past heart attack (0.51), and angina (0.41). A more recent sampling from this multisupplement cohort compared telomere lengths in this group to those in an age- and gender-matched group of healthy, nonsmoking adults living in the San Francisco Bay area [81]. Subjects ranged in age from about 30 to 80. Telomeres on average were longer in the multisupplement group compared with healthy controls [80].

Supplementation in the Developing World: China

As illustrated in Fig. 39.1, the need for supplementation is readily demonstrable in developing countries such as China. The World Bank does not consider China as a developing country—rather it is now "middle income" country. For example, a recent meta-analysis indicates that hyperhomocysteinemia is prevalent in China, particularly in northern populations, the inlanders, males, and the elderly [82]. Hyperhomocysteinemia, a pathological condition characterized by elevated blood levels of homocysteine, has been associated with increased risk of neural tube defects, cardiovascular diseases, type II diabetes, and certain cancers [82]. However, Yang et al. did not directly assess the association of hyperhomocysteinemia with these conditions [82]. This meta-analysis evaluated 36 studies covering 60,754 subjects from 19 provinces and municipalities in China and showed that the prevalence of hyperhomocysteinemia was over 27.5 % [82].

Hyperhomocysteinemia is clearly preventable. For instance, the prevalence of hyperhomocysteinemia in North America was reduced from 18.7 to 9.8 % after folic acid fortification [83]. A greater reduction was observed in Australia where folic acid fortification decreased the prevalence of hyperhomocysteinemia from 29 to 10 % [84]. Folic acid supplementation not only prevented hyperhomocysteinemia but also reduced its associated diseases such as neural tube defects [85]. Daily periconceptional supplementation of 400 μg of folic acid was shown to prevent 79 % and 41 % of neural tube defects in northern and southern regions of China, respectively [85].

Vitamin B-12 status is another contributing factor to neural tube defects and/or hyperhomocysteinemia [86–89 only one of these relates to NTDs]. A cross-sectional study examined the levels of plasma vitamin B-12 in 2407 apparently healthy Chinese men and women, 35–64 years old, living in the South (Shanghai) and the North (Beijing) of China [90]. The results indicated that plasma vitamin B-12 concentrations were lower among the northerners than the southerners. Specifically, approximately 11 % of the southerners and 39 % of the northerners were found to be vitamin B-12 deficient. Within each region, men were found to have significantly lower plasma vitamin B-12 concentrations and higher prevalence of vitamin B-12 deficiency than women ($P < 0.001$ for all the differences). Subanalyses showed that gender, age, season (spring and fall), and area (urban and rural) had little impact on the observed geographic differences. Instead, diet seemed to contribute largely to the differences. Reduced intakes of animal-based food, especially fish and dairy products, in the northerners were significantly associated with vitamin B-12 deficiency [90]. In the north, 59 % of the participants were deficient in either folate or vitamin B-12, and 17 % had deficiency in both. Higher prevalence of vitamin B deficiency was found in women of reproductive age (35–44 years old) living in the north (37 %) when compared to those living in the south (8 %) which was correlated with a higher incidence of neural tube defects, 5–6/1000 births in the south versus about 1/1000 births in the north [90].

The findings showed that vitamin B-12 deficiency is common in Chinese adults. Taken together, the studies suggest that fortification with both folic acid and vitamin B-12, rather than folic acid alone, may be more effective in reducing the risk of neural tube defects and hyperhomocysteinemia in the Chinese population, particularly in north of China.

Even though vitamin supplementation plays a fundamental role in deficiency-related diseases, herbal/traditional combination health food (or dietary supplement) market is much larger than vitamin market, accounting for 67 % of the total sales (RMB83.0 billion) of vitamins and dietary supplements in 2013 [91]. This is due to the common belief among average Chinese consumers that dietary supplements are efficacious and safe for long-term usage.

Conclusions

Nutritional supplements are available to consumers around the world. While regulations vary from country to country, there has been a growing movement to mandate and enforce GMP and to permit structure/function or other health claims. In an era in which nutritional deficiencies are common and in which healthcare payers are seeking means to control costs via expansion of prevention strategies, it seems prudent to facilitate means of delivering nutrition and health messaging. The challenge is how to do that without allowing "snake oil" products to reemerge in significant numbers. Fortunately today the safety of dietary supplements relative to pharmaceuticals or even to foods is very good [15]. One problem for the supplement industry is actually the practice by some disreputable manufacturers of adulteration of dietary supplements [92]. In addition to whatever regulatory frameworks are put in place, it will be essential for the supplement industry to self-regulate and challenge bad practices whenever found.

Whether one blames the food production and distribution system, individual choice, or both, we live in a world in which a great number of people suffer malnutrition, and a far larger number experience suboptimal nutrition. It makes sense to consider optimizing nutrient delivery from food, food fortification, and supplementation as three complementary means of closing the gaps. It is clear that supplementation can deliver profound clinical benefits in the developing world and do so in a cost-effective manner. While the disease-related benefits are clearly more controversial in the context of a better nourished population, the risks are low and costs minimal, and even modest population-level benefits could result in sizable cost savings to the healthcare system.

The supplement industry also needs encouragement to continue supporting nutrition research; governments have a role to play here as well. One could ask, is there an inherent bias in the medical literature against nutritional approaches, when even RCTs for nutrients that find statistically significant and clinically important benefits are discounted in editorial comment? If there are two possible research outcomes—a significant difference or not—but only one possible conclusion, to recommend no action, what is the point of conducting a RCT of a nutritional intervention?

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Chapter 40
The Role of Preventive Nutrition in Clinical Practice

Atheer Yacoub and Wahida Karmally

Key Points

- Prevention of chronic diseases such as heart disease, diabetes, cancer, and obesity requires early nutrition intervention.
- Lifestyle changes that incorporate physical activity and adherence to the Dietary Guidelines for Americans can help improve quality of life and lower risk of chronic diseases.
- Nutrition counseling using motivational interviewing skills, tailored to meet individual needs, can help overcome barriers to healthy eating.
- Diet quality can be assessed with food diaries, 24 h recalls, Food Frequency Questionnaires (FFQs), and Healthy Eating Index (HEI).
- Diets which emphasize fruits, vegetables, and whole grains such as the DASH diet and the Mediterranean diet may have a beneficial effect on heart disease, diabetes, and obesity.
- Maintaining food safety precautions such as thoroughly washing hands, cutting boards, and separating raw and cooked foods can prevent foodborne illnesses and death.
- Self-monitoring through smartphone apps and online diet analysis tools can be instrumental in improving dietary and physical activity habits in improving dietary and physical activity habits.

Keywords Yacoub • Karmally • Preventive nutrition • Nutrition counseling

Introduction

> "The function of protecting and developing health must rank even above that of restoring it when it is impaired".
>
> *Hippocrates 400 BC.*

The fundamental challenge is to improve the quality of life by preventing disease and disability. Nutrition can play a key role in mitigating or preventing some of the leading causes of death in the United States such as heart disease, stroke, cancer, diabetes, and obesity. In 2010, chronic diseases were responsible for seven out of the ten causes of death, of which 48 % were due to heart disease and cancer. Almost half of US adults have at least one major risk factor for heart disease or stroke which includes uncontrolled high blood pressure, disorders of lipid metabolism, or smoking, and 90 % of

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A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2_40

Americans consume too much sodium in their diets. More than one-third of US adults were obese in 2009–2010, which increases their risk of diabetes heart disease, hypertension, stroke, nonalcoholic fatty liver disease, and some cancers. Uncontrolled diabetes can lead to kidney failure, amputations, and blindness [1].

Compared with 16 high-income or peer countries, the United States is not as healthy when it comes to obesity, diabetes, heart disease, chronic lung disease, and disability, according to the Institute of Medicine. There are many disparities between Americans who are affected by chronic diseases, due to several factors including lower education or income, ethnic backgrounds, and social disadvantages based on geographical location [2]. In 2010, the highest ranked risk factors in high-income North Americans were due to tobacco smoking, including second-hand smoke, high body mass index (BMI), alcohol consumption, high blood pressure, and high fasting blood glucose. Most of these risk factors can be managed or prevented through community and clinical interventions [3]. Nutrition and exercise counseling practices in primary care remain suboptimal, even for persons at high risk for cardiovascular disease (CVD) [4].

Chronic diseases cause both a physical impact on individuals and an economic burden on healthcare spending. In 2010, the combined cost of heart disease and stroke was an estimated $315.4 billion, cancer care was $157 billion, and in 2012 the cost of diagnosed diabetes was estimated at $245 billion. This included $176 billion in direct medical costs and $69 billion in decreased work productivity due to diabetes-related illness. In 2008, medical costs for obesity were estimated to be $147 billion. Excessive alcohol consumption, in 2006, was responsible for about $223.5 billion for healthcare expenses as well as decreased work productivity, and crimes associated with excessive drinking. Smoking, another major contributor to chronic diseases, was estimated at over $289 billion a year during 2009–2012 [1].

Malnutrition in these chronic diseases increases the economic burden in the United States, as measured by community-based disease-associated malnutrition (DAM), with respect to healthcare costs, morbidity, and mortality. In 2010, direct medical costs per patient (with and without malnutrition) were as follows: $10,679 for stroke, $3098 for breast cancer, $21,513 for colorectal cancer, $4822 for coronary heart disease (CHD), $3028 for chronic obstructive pulmonary disease (COPD), $21,769 for dementia, $3102 for musculoskeletal disorders, and $4318 for depression. The prevalence of malnutrition for each disease are as follows: 4.99 % for stroke, 4.3 % for breast cancer, 6.73 % for colorectal cancer, 5.36 % for CHD, 11.04 % for COPD, 7.93 % for dementia, 6.75 % for musculoskeletal disorders, and 10.44 % for depression. Of these eight diseases, the four with the greatest overall economic burden of DAM, and also with the highest prevalence, are depression, COPD, CHD, and dementia [5].

Health needs to be improved in economically responsible ways by focusing on preventive strategies that yield the most benefit for the investment. In this chapter, we will:

1. Outline evidence-based dietary choices affecting wellness.
2. Discuss strategies to help sustained lifestyle patterns.
3. Describe practical strategies clinicians can use for nutrition assessment and counseling.

Dietary Habits of Americans

Unhealthy eating patterns, lack of exercise, drinking alcohol in excess, and smoking are major contributors to chronic diseases. More than a third of US adolescents and adults reported eating fruit less than once a day in 2011. Vegetable intake was reported as less than once a day for about 38 % of adolescents and 23 % of adults [1].

“People whose diets are rich in plant foods such as fruits and vegetables have a lower risk of getting cancers of the mouth, pharynx, larynx, esophagus, stomach, lung, and there is some suggested evidence for a lower risk of cancer of the colon, pancreas, and prostate [6].”

To help prevent these cancers, daily consumption of 5–13 servings of fruits and vegetables are recommended. These include 2–5 servings of fruits and 2–8 servings of vegetables, with special emphasis on dark-green and orange vegetables and legumes. Consistent evidence suggests at least a moderate inverse relationship between vegetable and fruit consumption with myocardial infarction (MI) and stroke, with significantly larger, positive effects noted above five servings of vegetables and fruits per day [6]. The National Cancer Institute (NCI) Dietary Guidelines [7] are:

- Reduce fat intake to 30 % of calories or less.
- Increase fiber to 20–30 g/day with an upper limit of 35 g.
- Include a variety of fruits and vegetables in the daily diet.
- Avoid obesity.
- Consume alcoholic beverages in moderation, if at all.

Four nutrients that are particularly low and cause of concern for the US population are potassium, dietary fiber, calcium, and vitamin D. Following recommendations from the Dietary Guidelines for Americans 2010 can help meet shortfall nutrients and reduce the risk of chronic diseases [8]. The Dietary Guidelines for Americans are published every 5 years with the joint effort of the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (HHS).

The 2015 Dietary Guidelines Advisory Committee established three work groups to shape the report. Work groups are as follows:

1. Environmental Determinants of Food, Diet, and Health.
2. Dietary Patterns of Quality and Optimization through Lifestyle Behavior Change.
3. Foods, Beverages, and Nutrients and their Impact on Healthy Outcomes.

The five primary topic areas to be addressed by the 2015 Dietary Guidelines Advisory Committee are food environment, physical activity environment, agriculture/aquaculture sustainability, food systems, and food safety.

Consuming adequate intakes of fruits and vegetables, whole grains, non-/low-fat milk products can help meet dietary recommendations of these nutrients [8], as indicated by dietary data from the National Health and Nutrition Education Survey (NHANES) from 2003–2004 and 2005–2006 for American adults, adolescents, and children 2 years or older.

The average calorie intake for Americans 2 years and older was 2176 kcal/day, with cake, cookies, quick bread, pastry, and pie comprising the top percentage (7.2 %). Next, are yeast breads and rolls (7.1 %), followed by soft drinks (5.4 %), beef (4.7 %), crackers, popcorn, pretzels, chips (4.7 %), cheese (4.6 %), milk (4.5 %), candy, sugars, sugary foods (4.5 %), poultry (4.3 %), and alcoholic beverages (3.7 %). These foods combined make up more than 50 % of caloric intake. The average daily intake of added sugars was 83.9 g/day [9]. The American Heart Association (AHA) recommends limiting daily added sugar intake to no more than about five teaspoons per day (or 80 cal), for an average adult woman on 1800 kcal/day and no more than nine teaspoons per day (or 144 cal) for an average adult man on 2200 kcal/day [10]. According to a systematic review to establish the World Health Organization Guidelines, there is evidence of moderate quality showing that dental caries is lower when free-sugars intake is <10 % of energy intake. A significant relationship was found in limiting energy intake to <5 %; however, the evidence was judged to be of low quality [11].

The average intake of saturated fat was 27.7 g/day and made up 11.4 % of total calorie intake. The top 10 foods which contributed to saturated fat intake include cheese, beef, milk, other fats and oils, frankfurters, sausages, luncheon meats, cake, cookies, quick bread, pastry, pie, margarine and butter, milk desserts, poultry, crackers, popcorn, pretzels, and chips. These foods accounted for 73.6 % of SFA intake, 65.1 % of monounsaturated fat, and 52.1 % of polyunsaturated fat [9].

While some foods making up the top 10 cal sources, such as beef, poultry, milk, cheese, and baked goods, contributed to some nutrients of concern, soft drinks, soda, candy, sugars, sugary foods, and alcoholic beverages did not provide any nutritional value. The top three sources of saturated fat (cheese, beef, and milk) provided more than 40 % vitamin B_{12}, almost half of vitamin D and calcium [9].

Dietary Recommendations to Lower Risk for CVD

CVD is the leading cause of morbidity and mortality in Americans. A cardioprotective lifestyle is important in the prevention and reduction of CVD. Eating a diet high in saturated fat, trans fat, cholesterol, and increased calorie consumption and sedentary lifestyle can increase the risk of CVD. Reducing cardiovascular risk factors such as overweight and obesity, hypertension, high plasma glucose, and abnormal lipids can help prevent cardiovascular events [12, 13]. AHA guidelines for Americans to reduce disease risk, promote diets high in fruits and vegetables, whole grains, low-fat dairy products, poultry, fish, and nuts, and limit red meat, sugary goods, and beverages. AHA advises to reduce total fat intake to no more than 25–35 % of total calories and to choose healthy sources of fats such as fish, nuts, and plant oils. Saturated fat should also be limited to no more than 7 % of total daily calories and trans fat to no more than 1 % of daily calories. Adults with high LDL cholesterol would benefit by further limiting saturated fat intake to no more than 5–6 % of total calories. Cholesterol should be limited to no more than 200 mg per day [14, 15]. Foods that contribute to saturated fat and dietary cholesterol include egg yolks, high fat cheeses, and dairy and processed meats (Table 40.1). Trans fat, which is found in hydrogenated oils, often in baked goods and fried foods, should be consumed as little as possible. If alcohol is consumed, and permitted by a physician, it should be limited to one drink per day for women and two drinks per day for men [8].

Dietary Sodium and Potassium and Hypertension Risk

About 90 % of Americans consume excess sodium and the majority consumes inadequate potassium. The combination of high sodium intake and low potassium intake are modifiable risk factors for high blood pressure. Hypertension also increases the risk of CVD and stroke. The ratio of sodium to

Table 40.1 Dietary sources of saturated fat and cholesterol [16, 17]

Serving size	Dietary source	Saturated fat (g)	Cholesterol (mg)	Serving size	Dietary source	Saturated fat (g)	Cholesterol (mg)
1.0 tbsp	Butter, salted	7.3	31	1.0 tbsp	Olive oil	1.9	0
3.0 oz.	Beef, ground, 80 % lean meat/20 % fat, cooked	5.2	77	3.0 oz.	Beef, ground, 97 % lean meat/3 % fat, cooked	1.9	75
1.0 cup	Milk, whole, 3.25 % milk fat	4.6	24	1.0 cup	Milk, non-fat (fat free or skim)	0.13	5
0.5 cup	Ice cream, vanilla, full-fat	4.5	29	0.5 cup	Frozen yogurt, fat-free vanilla	0.1	2
1.0 oz	Cheese, mozzarella, whole milk	4.4	25	1.0 oz	Cheese, mozzarella, part skim	2.9	18
3.0 oz	Chicken breast, fried, with skin	4.2	72	3.0 oz	Chicken breast, grilled, without skin	1.1	72
3.0 oz	Shrimp	0.2	179	3.0 oz	Salmon	1.1	60

potassium and the interaction of these nutrients appear to have a stronger effect than sodium or potassium intake alone; however, the ideal ratio has not been determined [18].

The average person should limit sodium to less than 2300 milligrams (mg) per day. Individuals who are 51 years and older, African Americans of any age, and individuals with hypertension, diabetes, or chronic kidney disease should further limit intake to 1500 mg per day [8, 19].

Reducing sodium intake should be included as part of an overall balanced and healthy diet with physical activity. Additionally, avoiding processed foods and choosing lower sodium foods while increasing potassium intake will have positive health outcomes [20] (Table 40.2).

Even moderate decreases in sodium intake can result in great health benefits; however, it remains a challenge with processed foods accounting for 75–80 % of salt intake in the US diet. The analysis of the effects of a population-wide reduction in dietary salt of 3 g/day, compared with current salt consumption, projected to reduce new cases of CHD by 60,000–120,000, with 54,000–99,000 fewer new and recurrent myocardial infarctions (MI), 32,000–66,000 fewer new strokes, and 44,000–92,000 fewer deaths from any cause yearly. Even a reduction of 1 g/day may significantly decrease annual cardiovascular events and deaths, with 20,000–40,000 fewer cases of CHD, 18,000–35,000 fewer new and recurrent MIs, 11,000–23,000 fewer new strokes, and 15,000–32,000 fewer deaths from any cause [21].

The Institute of Medicine recommends 4700 mg/day of potassium for Americans aged ≥14 years; however, current intake is closer to ~2600 mg/day. Potassium-rich foods that can help decrease blood pressure include oranges, tomatoes, dark green vegetables such as spinach and kale, sweet potatoes, low-fat and non-fat yogurt, and dried or no-salt-added/low sodium canned beans. Following the DASH diet (Table 40.3) can help decrease sodium intake while also increasing potassium intake [22].

Table 40.2 Dietary sources of sodium [16, 17]

Serving size	Dietary source	Sodium (mg)
1.0 cup	Chicken and vegetable soup, ready-to-serve	584
0.5 cup	Tomato sauce	581
1.0 piece (134 g)	Frozen entree, lasagna with meat and sauce	465
1.0 oz	Lunch meat, pork	365
1.0 small spear	Pickles, dill	283
1.0 slice	White bread	142

Table 40.3 The DASH diet [22]

Food group	Servings per day	Serving size
Grains and grain products—whole grains are recommended for most servings	6–8	1 slice bread
		½ cup cooked, rice, pasta, or cereal
Fruits	4–5	1 medium fruit, ¼ cup dried fruit, ½ cup fruit juice
Vegetables	4–5	1 cup raw leafy vegetables, ½ cup raw or cooked vegetables
Non-fat or low-fat dairy foods	2–3	1 cup milk or yogurt
		1½ oz cheese
Lean meats, fish, poultry	6 or less	1 oz cooked meats, poultry, fish
Nuts, seeds, legumes (unsalted)	4–5 per week	1/3 cup nuts, 2 Tbsp peanut butter, 2 Tbsp seeds, ½ cup cooked legumes
Sweets and foods with added sugars	5 or less per week	1 Tbsp sugar, 1 Tbsp jelly or jam, 12 cup sorbet, gelatin, 1 cup lemonade
Fats and oils	2–3	1 tsp soft margarine (trans fat free), 1 tsp vegetable oil, 1 Tbsp mayonnaise, 2 Tbsp salad dressing

Fiber in the Cardioprotective Diet

Foods providing 25–30 g of fiber per day, with special emphasis on soluble fiber sources (7–13 g) should be included as part of a cardioprotective diet. Foods rich in soluble fiber are fruits (prunes, apples, and strawberries), vegetables (okra and Brussels sprouts), and whole grains, such as oats, barley, and legumes [23] (Table 40.4) Risk factors associated with CHD (blood pressure, lipoprotein subclasses and particle sizes, insulin resistance, and post-prandial glucose) and CVD (fatal and non-fatal MI and stroke) are decreased as dietary fiber intake increases. Diets high in total and soluble fiber, as part of a cardioprotective diet, can further reduce triglycerides by 2–3 % and LDL up to 7 % [24].

Dietary patterns that emphasize fruits and vegetables intake, high in unsaturated fats from plant sources, and low in saturated fats, are associated with lower risk of CHD [25]. The Mediterranean region is consistent with this dietary pattern [26]. The Mediterranean diet (Table 40.5) is associated with heart health due to its content of olive oil, nuts, fruits, vegetables, and whole grains, with moderate consumption of fish, poultry, lean meat, and eggs, and low intake of saturated fat from dairy, red meat, and processed foods. The diet is also low in sweets, and wine is consumed in moderation. There is some evidence to show that following a Mediterranean diet can help reduce the risk factors for metabolic syndrome, defined as having three or more cardiovascular risk factors such as abdominal obesity, hyperglycemia/insulin resistance, hypertriglyceridemia, low high-density lipoprotein (HDL), cholesterol, and high blood pressure [13].

Table 40.4 Dietary sources of fiber [16, 17]

Serving size	Dietary source	Total dietary fiber (g)	Soluble fiber (g)
1.0 cup	Squash, winter, cooked	6.6	3.8
1.0 cup	Oatmeal, instant, cooked	4.1	2.1
1.0 cup	Rice, brown, cooked	4.0	0.5
0.5 cup	Barley, cooked	4.0	1.0
1.0 slice	Bread, whole-wheat	1.9	0.4
1 medium	Apple	4.0	1.0
1 medium	Banana	3.0	1.0
0.5 cup	Blackberries	4.0	1.0
0.5 cup	Broccoli, cooked	2.6	0.3
0.5 cup	Brussels sprouts, cooked	2.9	0.4
1.0 cup	Carrots, raw	3.6	0.6
0.25 cup	Prunes	3.0	1.5
0.5 cup	Black beans	7.5	0.3
0.5 cup	Kidney beans	5.7	1.3
0.5 cup	Lima beans	4.5	0.7
0.5 cup	Lentils	5.8	0.4

Table 40.5 The Mediterranean diet [13]

Fruits, vegetables, breads, cereals, potatoes, beans, nuts, and seeds as foundation of diet
Olive oil as main fat source
Low to moderate intake of dairy products, fish, and poultry
Very little red meat consumption
Eggs consumed 0–4 times per week
Wine consumed in low to moderate amounts

Table 40.6 Sources of omega-3 fatty acids: plants [16, 17]

Food	Calories	ALA (g)	EPA (g)	DHA (g)
Flax seed, 14 g (~2 Tbsp)	75	3.194	0	0
Chia seed, 14 g (~2 Tbsp)	69	2.527	0	0
Walnuts, 1 oz (14 halves)	185	2.574	0	0
Soybeans, ½ cup cooked	127	0.319	0	0
Canola oil, 1 tsp	40	0.411	0	0
Purslane, ½ cup raw	3	0.011	0	0
Purslane, ½ cup cooked	10	0.032	0	0
Kale, ½ cup cooked	18	0.067	0	0
Spinach, ½ cup cooked	21	0.083	0	0

The randomized control trials and cohort studies did not uniformly define the Mediterranean diet. However, the studies identified several common features of the Mediterranean diets: higher in fruits (particularly fresh), vegetables (emphasizing root and green varieties), whole grain cereals, breads, rice, or pasta, and fatty fish (rich in omega-3 fatty acids); lower in red meat (and emphasizing lean meats); substituting lower fat or fat-free dairy products for higher fat dairy foods, and using oils (olive or canola), nuts (walnuts, almonds, pistachios, or hazelnuts), or margarines blended with rapeseed or flaxseed oils in lieu of butter and other fats. The Mediterranean dietary patterns in these studies tended to be moderate in total fat (25–32 % of total calories), lower in saturated fat (9–10 % of total calories), high in fiber (27–37 g/day), and increased in polyunsaturated fatty acids (PUFAs), particularly omega-3s [13]. The Lyon Heart Trial, a randomized secondary prevention trial, found that following a Mediterranean dietary pattern, high in alpha-linolenic acid (ALA) resulted in fewer coronary events, compared to a Western, control diet [26].

In a randomized, multi-centered trial in Spain, The PREDIMED trial (Prevención con Dieta Mediterránea), Estruch et al. 2013 studied the effect of a Mediterranean diet as a primary prevention for CVD. A total of 7447 patients, ages 55–80 years, with high cardiovascular risk were randomized to one of three diets not restricted in calories: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, and a control group given low-fat diet recommendations. Subjects without CVD were eligible to participate, but had to have either type 2 diabetes, or at least three of the following risk factors: smoking, hypertension, elevated low-density lipoprotein cholesterol levels, low high-density lipoprotein cholesterol levels, overweight or obesity, or a family history of premature CHD. Subjects who followed a Mediterranean diet, either supplemented with extra-virgin olive oil or nuts, showed a significant reduction in cardiovascular risk factors [27].

When counseling on a cardioprotective diet, there are simple changes in the diet that can significantly improve its quality, such as substituting high fat dairy for non-/low-fat, limiting intake of egg yolks to no more than 2–3 per week, limiting intake of red meat and eating lean meat, poultry without skin, or fish instead. Patients/clients should also be advised on food preparation methods such as baking or grilling, preferably without added fat, or using small amounts of unsaturated oils. Fat intake can also be limited by taking skin off poultry or visible fat off of meats. Reducing intake of processed and prepackaged foods, including canned food, and frozen meals, will also help decrease intake of sodium [22].

Table 40.7 Sources of omega-3 fatty acids: seafood [16, 17]

3 oz Cooked	Calories	ALA (g)	EPA (g)	DHA (g)
Flounder and Sole	73	0.018	0.143	0.112
Halibut, Atlantic and Pacific	94	0.011	0.068	0.132
Herring, Atlantic	173	0.112	0.773	0.939
Herring, Atlantic, pickled	223	0	0.717	0.464
Herring, Pacific	212	0.062	1.056	0.751
King Mackerel	114	n/a	0.148	0.193
Salmon, Atlantic, farmed	175	0.096	0.586	1.238
Salmon, Pacific, Sockeye	144	0.073	0.228	0.445
Trout, mixed species	162	0.169	0.220	0.575
Tuna, white, canned in water, drained	109	0.060	0.198	0.535
Tuna, white, canned in oil, drained	158	0.173	0.056	0.151
Oyster, Eastern, farmed	67	0.054	0.195	0.179
Sardine, Atlantic, canned in oil, drained	177	0.424	0.402	0.433
Sardine, Pacific, canned in tomato sauce	157	0.200	0.453	0.735
Scallop, Bay and Sea	94	0.005	0.061	0.088
Tilapia	109	0.038	0.004	0.110

Omega-3 Fatty Acids and CVD

In addition to limiting total fat, saturated fat, trans fat, and cholesterol, increasing intake of omega-3 fatty acids may also be beneficial in reducing CVD risk. ALA is an essential fatty acid found in plant foods such as walnuts, flax, chia, hemp seeds, and vegetable oils such as soybean and canola (Table 40.6). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are longer chain omega-3 fatty acids found primarily in fatty fish such as salmon, sardines, trout, tuna, and herring (Table 40.7). Data from NHANES 2009–2010 show that average daily intake of ALA was 1.77 g and 1.38 g for males and females, respectively. The average daily intake of EPA was 40 mg and 30 mg for men and women, respectively. The Adequate Intake (AI) for ALA is 1.6 and 1.1 g/day for men and women, respectively. The position paper published by the Academy of Nutrition and Dietetics in 2014, reviewed a meta-analysis from 2011, which showed that fish or fish oil may play a positive role in reducing the risk of CVD by lowering inflammation, improving endothelial function, normalizing heart rate, and reducing platelet aggregation. Fish oil supplements are "Generally Regarded as Safe" in quantities up to 3 g/day. The American Heart Association recommends consuming two servings (3.5 oz cooked) of fatty fish per week for prevention of CVD [28].

The 2010 Dietary Guidelines Advisory Committee (DGAC) conducted a literature review to examine the role of seafood consumption and CVD risk in individuals without CVD. The review included 28 studies, and an earlier review from 2004 to 2007, from the Academy of Nutrition and Dietetics. The DGAC concluded "moderate evidence shows that consumption of two servings of seafood per week (4 oz. per serving), which provide an average of 250 mg per day of long-chain *n*-3 fatty acids, is associated with reduced cardiac mortality from CHD or sudden death in persons with CVD [29]."

In addition to eating fatty fish and seafood, the AHA recommends consuming foods with ALA, such as tofu, soybeans, canola, walnut, and flaxseed as well as their oils. While epidemiological studies have shown that consuming omega-3 fatty acids from marine and plant sources can lower the risk of heart disease, there are not enough studies to show causality between ALA and heart disease. While it is better to consume omega-3 fatty acids from foods, certain individuals at risk for heart disease, may not be able to achieve desirable intake solely through diet, and supplements may be an alternative. Supplements may also be beneficial for lowering triglycerides [30].

For patients with a history of CHD, the AHA recommends 1 g of EPA + DHA per day, from fatty fish; if supplementation is needed, it's important to consult a physician.

For patients who would benefit from lowering triglycerides, the recommended dosage of capsules is 2–4 g of EPA + DHA per day, under a physician's care. The AHA warns that anyone taking more than 3 g of omega-3 fatty acid supplements should consult a physician, as there is an increased risk of bleeding for some people. While eating fatty fish is encouraged, the AHA also warns against levels of mercury and other toxins that are present in certain types of fish, usually larger predatory fish such as shark, swordfish, king mackerel, or tilefish. Eating a variety of fish can help reduce the risk of these environmental contaminants [30].

A study was conducted in Japan in a population who ate fish to determine the effect of EPA on major coronary events in hypercholesterolemic patients. Patients were divided into two subgroups: one with coronary artery disease (secondary prevention) and one without (primary intervention) and randomly assigned to receive EPA with statin or statin alone. All patients went through a washout period of 4–8 weeks, where they stayed off of any antihyperlipidemic drugs. All patients received dietary guidance. EPA treatment reduced frequency of major coronary events. In the primary prevention subgroup, EPA resulted in a nonsignificant 18 % reduction in major coronary events. In the secondary prevention subgroup, EPA treatment was associated with a significant 19 % reduction in major coronary events. The risk of angina and non-fatal coronary events were also significantly reduced in the EPA group. There was no significant difference in sudden cardiac death and coronary death between groups [31].

Dietary Recommendations for ObesityPrevention

Obesity is a disease and a risk factor for other diseases such as CVD, diabetes, and metabolic syndrome. According to data between 2011 and 2012 by the National Health and Nutrition Examination Survey (NHANES), over one-third (34.9 %) of adults and 16.9 % of children are obese. However, the prevalence remains stable compared to 2003–2004 data. Obesity was defined as having a BMI at or above the 95th percentile for children and adolescents aged 2–19 years. Adults, aged 20 years or older with a BMI of 30 or above are categorized as obese. There are three grades of obesity, grade 1 (BMI 30–34), grade 2 (BMI 35–39), and grade 3 (BMI $\geq$ 40) [32].

Obesity prevention can occur as early as childhood [33]. If a child is overweight or obese at age 2 years, this is likely to carry into childhood, which increases morbidity and mortality, and risk for nonalcoholic fatty liver disease (NAFLD), type 2 diabetes, and hyperlipidemia. NAFLD is an increasing problem in obese children and is characterized by fat accumulation in hepatocytes. Insulin resistance may also contribute to pediatric NAFLD. Nearly half of children diagnosed with type 2 diabetes are suspected to have a fatty liver indicated by an elevated liver enzyme, alanine transaminase (ALT). Due to the strong association between NAFLD and obesity, insulin resistance, hypertension, and dyslipidemia, it may also increase the risk of atherosclerosis and CVD [34].

The two main interventions for childhood obesity prevention include nutrition and physical activity, which can be implemented as early as pregnancy. The first 2 years of life are especially critical in prevention, as the structure of the hypothalamus responsible for regulating energy intake, expenditure, and body size occurs in the first 1000 days of life. Factors that may reduce the risk of obesity in later life include breastfeeding, portion control, and diet composition [35].

Overweight in pregnancy may contribute to larger gestational weight for the baby. Over the last four decades, average weight gain during pregnancy increased from 10 to 15 kg. In the United States, over the last few decades, there has been an increase between 5 and 9 % of babies born large for gestational age [36]. In addition to maintaining a healthy weight and balanced diet during pregnancy, sufficient intake of folic acid is necessary to prevent neural tube defects. The U.S. Preventive Task

Force recommends that women who or capable of being pregnant or plan to become pregnant take a daily supplement of 0.4–0.8 mg of folic acid [37].

Hormonal and metabolic changes during aging can lead to increased fat mass and decreased muscle mass, or sarcopenia. Low muscle mass can increase the risk of falls, disability, poor quality of life, and mortality [38]. A study of 2629 subjects ≥65 and 998 young adults, age 20–40 years old, were recruited for Sarcopenia and Translational Aging Research in Taiwan (START). Body composition was measured by bio-impedance analysis and physical performance, including upper and lower extremity function. The study showed that subjects with sarcopenia or obesity had poor physical performance, and having both sarcopenia and obesity had a further decline on physical performance and functional impairment [39].

Lifestyle and genetics both contribute to obesity. Some diet-related risk factors include consumption of energy-dense foods such as fast food, fried foods, and fruit juices, as well as an inactive lifestyle. Indicators for individuals who need treatment for overweight and obesity include a BMI ≥ 30 kg/m^2 or a BMI of 25–30 kg/m^2 with one of the following conditions: hypertension, type 2 diabetes, abdominal obesity, high psychosocial stress, or other diseases which are negatively impacted by obesity. Pregnant women or individuals with wasting diseases should not lose weight [40]. For members of the general population, the American Diabetes Association (ADA) recommends testing for diabetes when BMI reaches 25 kg/m^2 or higher . For Asian Americans, ADA is now recommending that screening be done at 23 kg/m^2 or higher [41].

Goals for treatment of obesity should be tailored to each person, however, general guidelines include weight loss of >5 % if BMI is between 25 and 35 and weight loss of 10 % if BMI > 35. These treatment goals can help in the reduction of obesity-related diseases and improvement of obesity-related risk factors, lowering the risk of premature death, prevention of inability to work, reduction of psychosocial disorders, and improvement of quality of life. Achieving these goals is largely dependent on improving dietary habits. The approach to weight loss will vary between individuals. Personalized nutrition counseling as well as group therapy is beneficial [40].

Overweight and obese individuals should be advised that sustained weight loss of 3–5 % can greatly improve health by lowering triglycerides, blood glucose, HbA1C, and the risk of developing type 2 diabetes. This can be achieved by restricting calorie intake to either 1200–1500 kcal/day for women or 1500–1800 kcal/day for men; however, calorie levels may vary depending on body weight. Weight loss can also be achieved by an energy deficit of 500 kcal/day to 750 kcal/day. Participation in a comprehensive lifestyle program for ≥6 months that integrates a physical activity component will also be beneficial. Very low calorie diets (VLCDs) (<800 kcal/day) should only be done under close medical supervision with trained clinicians, as rapid weight loss can cause adverse health effects [42]. Minor side effects include fatigue, constipation, nausea, and diarrhea. Obese individuals on VLCDs are at increased risk for gallstones [43]. Bariatric surgery may be an option for individuals with a BMI ≥ 40 or BMI ≥ 35 with obesity-related comorbidities who have been unsuccessful at losing weight despite being motivated to do so with behavioral intervention. Qualified individuals can be referred to a bariatric surgeon for a consultation [42].

Dietary Recommendations for Prevention of Prediabetes and Type 2 Diabetes

Overweight and obesity increase the risk of diabetes and therefore, weight loss can improve glycemic control. The Academy of Nutrition and Dietetics found that about half of the studies with weight loss interventions in individuals with type 2 diabetes showed improvements in HbA1C at 1 year, and the other studies did not. The two interventions which yielded the largest weight loss at 1 year were the Mediterranean-style eating pattern in newly diagnosed individuals and the Look AHEAD (Action for Health in Diabetes) trial [44].

The Look AHEAD study investigated the effect of an intensive lifestyle intervention in overweight and obese adults with type 2 diabetes and its effect on reducing the risk of cardiovascular morbidity and mortality. The lifestyle intervention included diet, physical activity, and behavior modification, and a weight loss goal of at least 7 %, compared to a control group receiving diabetes support and education. The intensive lifestyle intervention resulted in greater reductions in glycated hemoglobin and weight loss (8.6 % versus 0.7 % in the control group at 1 year and 6 % versus 3.5 % at end of 9.6 years). The trial was stopped early after 9.6 years as there were no significant improvements in cardiovascular risk factors between the intervention and control group [45].

The American Diabetes Association (ADA) reviewed several weight loss trials in overweight and obese individuals with type 2 diabetes and found that average weight loss ranged from 1.9 to 4.9 kg at 1 year; however, improvements in HbA1c, lipids, and blood pressure were not consistent. They also found individuals with diabetes may experience more difficulty with weight loss. Improvements in glycemic control with weight loss may be most beneficial in prediabetes or early onset diabetes. Nutrition therapy should focus on reduced energy intake, lifestyle changes, and regular physical activity [46].

While reducing overall energy intake is key in achieving glycemic control, monitoring intake of carbohydrates is also important. It's more healthful to include complex carbohydrates with fiber from vegetables, fruits, whole grains, and legumes, rather than food with simple sugars. Consuming recommended amount of fiber (25 g/day for women and 30 g/day for men) is also important for individuals with type 2 diabetes. High fiber diets of >50 g/day have shown improved glycemic control in individuals with diabetes [44].

Adopting a Mediterranean-style eating pattern and replacing saturated fats with unsaturated fats may also improve glycemic control and/or serum lipids in individuals with diabetes. While consuming foods high in omega-3 fatty acids are recommended, there is not enough evidence to support the use of omega-3 fatty acid supplements in preventing or treating CVD in this population [44]. The goals of nutrition therapy for individuals at risk for type 2 diabetes should focus on maintaining an overall healthy eating pattern, eating moderate amounts of carbohydrate, moderate weight loss of 7 % of body weight, and engaging in physical activity (150 min/week). There is not enough evidence to support low-glycemic load diets in reducing the risk for diabetes or overweight and obesity. No optimal macronutrient distribution has been determined for weight loss. Both low-fat and low-carbohydrate diets can help with weight loss; however, the difference after 1 year is not significant. There are no nutrition recommendations to prevent type 1 diabetes. Medical nutrition therapy for individuals with prediabetes or diabetes should be tailored to meet their individual needs [46].

Dietary Recommendations for Bone Health and Oral Health

Bone health is a major concern in the United States [47]. The Surgeon General's report on bone health and osteoporosis recommend consuming adequate calcium and vitamin D (Table 40.8). Maintaining a healthful body weight and being physically active are recommended [48]. In addition to meeting calcium intake of 1000–1200 mg/day (depending on age and gender), and vitamin D intake of 800–1000 IU per day [49], a varied, nutrient-dense diet which includes adequate protein, fruits, and vegetables, will aid in the prevention of bone-related diseases. There is also some evidence to show that potassium, magnesium, trace minerals, and vitamins B, K, and C may also play a positive role in bone health [50]. Maintaining adequate levels of phosphorus is also essential, as 85 % of this mineral is found in bone and teeth [51]. Fluoride also promotes healthy bones and teeth, through mineralization, and 99 % of it is found in hard tissues. The Academy of Nutrition and Dietetics encourage the use of fluoride for all age groups for oral health, bone health, and overall health [52].

Scientific and epidemiologic data suggest a lifelong synergy between nutrition and oral health status in health and disease. The links between oral health and nutrition are many. Diet and nutrition may affect the development and progression of diseases of the oral cavity which, in turn can affect nutritional status. Nutritional deficiencies may cause dental problems in that they affect bony tissue synthesis and modify the resistance of the gingival tissues to plaque microorganisms [53].

Table 40.8 Institute of medicine dietary reference intake for calcium and vitamin D [49]

Calcium				Vitamin D		
Life stage group	Estimated average requirement (mg/day)	Recommended dietary allowance (mg/day)	Upper level intake (mg/day)	Estimated average requirement (IU/day)	Recommended dietary allowance (IU/day)	Upper level intake (IU/day)
Infants 0–6 months	[a]	[a]	1000	[b]	[b]	1000
Infants 6–12 months	[a]	[a]	1500	[b]	[b]	1500
1–3 years old	500	700	2500	400	600	2500
4–8 years old	800	1000	2500	400	600	3000
9–13 years old	1100	1300	3000	400	600	4000
14–18 years old	1100	1300	3000	400	600	
19–30 years old	800	1000	2500	400	600	4000
31–50 years old	800	1000	2500	400	600	4000
51–70-year-old males	800	1000	2000	400	600	4000
51–70-year-old females	1000	1200	2000	400	600	4000
>70 years old	1000	1200	2000	400	800	4000
14–18 years old, pregnant/lactating	1100	1300	3000	400	600	4000
19–50 years old, pregnant/lactating	800	1000	2500	400	600	4000

[a]For infants, adequate intake is 200 mg/day for 0–6 months of age and 260 mg/day for 6–12 months of age
[b]For infants, adequate intake is 400 IU/day for 0–6 months of age and 400 IU/day for 6–12 months of age

Throughout life, nutrition and oral health are interdependent and influence individual's overall health status in numerous ways. Good health begins in the mouth for a very simple reason. The mouth is the beginning of the GI tract. It is an important factor in the ability to chew and thus to digest nutrients.

Nutrition plays two different roles in oral health: protective and preventive. The protective role is in promoting healthy development and maintenance of the mouth's tissues and their natural protective mechanisms. The role of nutrition is also to prevent oral disease through the influence of the foods' properties on plaque development and saliva flow. Consuming a variety of foods including vegetables and low-fat dairy to optimize nutrient consumption is of significance in oral health.

With evolving science, specific foods no longer are singled out as major risk factors for dental caries. The direct relationship between diet and dental caries is clearly established. Major components of a preventive dental regimen include nutrition counseling, fluoride therapy, use of sealants, and control of cariogenic bacteria.

The primary factors to consider in determining the cariogenic, cariostatic, and anticariogenic properties of the diet are the food form (liquid, solid, and sticky and long lasting), frequency of consumption of sugar, and other fermentable carbohydrates, nutrient composition, sequence of food intake, and combination of foods.

Periodontal disease increases risk for nutritional deficiencies because the infection can alter tissue capacity to utilize nutrients needed for healing and repair. Optimizing nutritional status and eating patterns combined with removal of the stimuli of inflammatory periodontal lesions are important in diminishing the severity of periodontal lesions.

Risk for oral problems increase with many disease states and medications/treatments used. The diseases include diabetes mellitus, HIV infection, oral and pharyngeal carcinoma. Periodontal disease is the sixth risk factor for diabetes mellitus.

Medical nutrition therapy can reduce the risk of oral infections and improve the outcome of treatment of patients with oral manifestations of acute and chronic diseases.

Healthy Eating Index-2010

The Healthy Eating Index-2010 (HEI-2010) is a measure of diet quality, which has been updated since 2005, to reflect the 2010 Dietary Guidelines for Americans and USDA Food Patterns. The validity and reliability of HEI-2010 was measured against menus of known nutritional quality which includes 2010 USDA Food Patterns, the DASH Eating Plan, Harvard's Healthy Eating Pyramid, and the AHA recommendations. It includes 12 components: (1) total fruit, (2) whole fruit, (3) total vegetables, (4) greens and beans, (5) whole grains, (6) dairy, (7) total protein foods, (8) seafood and plant proteins, (9) fatty acids, (10) refined grains, (11) sodium, and (12) empty calories. The higher the score, the better is the diet quality. Guenther et al. (2014) revealed that the HEI-2010 men's diet quality was poorer than women's, younger adults' diet quality was poorer than older adults, and smokers' diet quality was poorer than nonsmokers [54].

Nutrition Counseling

When counseling a patient/client on implementing preventive nutrition strategies, the Nutrition Care Process is essential in providing individualized and tailored evidence-based recommendations [55].

By using Prochaska's Stages of Change model, also called Transtheoretical Model, the first step the clinician can take is to assess the patient/client's stage of readiness [56]. It's also important to assess the patient's food and nutrition knowledge as well as their typical eating habits, while taking note of any allergies, intolerances, or cultural/religious restrictions that may affect their eating patterns.

Motivational Interviewing is a technique to actively engage with a patient/client to involve them in the decision-making process. It utilizes the Transtheoretical Model to determine which of the five stages the client is in, which includes precontemplation (no intention of change), contemplation (thinking about change), preparation (intention to change within the foreseeable future), action (actively making changes), and maintenance (consistently engaging in a behavior for at least 6 months) [56, 57].

It is important for the counselor to communicate using reflective listening skills, while fostering an open and nonjudgmental environment to move the patient/client from one stage to the next [58].

The patient/client's eating patterns can be assessed by asking open-ended questions such as:

- How would you describe your current eating habits?
- What concerns do you have regarding your health?
- How does physical activity fit into your life?
- What measures have you tried previously to improve your health?
- Why do you want to eat healthfully?

This will help assess what the patient/client's baseline diet is like and asking these questions can help facilitate dialogue and move forward the change process. A 24 h recall, repeated at different stages, or a three non-consecutive day food diary, will provide a better understanding of a patient/client's overall diet. An FFQ may also be administered to assess long-term dietary intake; however, due to limitations in not measuring all aspects of diet, including estimation of portion sizes, an FFQ is best used in combination with 24 h recalls and food diaries [59].

Nutrition Care Process: Diagnosis

Diagnosis is the next step in the Nutrition Care Process for narrowing down the nutrition-related problem so that it may be resolved through nutrition intervention. Identifying the *problem*, *etiology*, and *signs/symptoms* (PES) is a concise way to isolate the main issue and assess where intervention is needed. For example, if an overweight client tells you they are eating fast food every day for dinner because they don't have time to cook, a sample PES statement may be "excessive energy intake related to fast food consumption five times a week, as evidenced by food diary and BMI of 29." The PES statement should ideally identify the main problem that is most nutritionally relevant as well as the barrier that can be addressed with nutrition counseling. This will give focus to the nutrition counseling session. There may be more than one problem contributing to a condition which can be captured by more than one PES statement; however, the clinician should follow the patient/client's lead to which problem they are willing to work on first. The more specific the PES statement, the easier it is to find a nutrition intervention. In the previous example, simply stating that the patient is overweight is not the problem to be addressed, but rather a sign/symptom of the problem. By asking open-ended questions and reviewing the patient/client's dietary intake, the etiology contributing to the patient/client's weight can be identified.

Nutrition Care Process: Intervention

While there are several contributing factors to overweight and obesity, merely repeating scientific evidence and guidelines with the patient/client without identifying their unique situation, is unlikely to produce any meaningful results. Nutrition counseling should be tailored to each individual patient to target their specific barriers and motivation to overcome those barriers. An intervention incorporating behavior change strategies is more likely to be effective and meet the patient/client's individual needs.

After identifying the barrier, the next step is to identify how ready the patient/client is to make a change by identifying their stage of change and ideally moving them forward to the next stage. Patients/clients may be ambivalent about making a change and motivational interviewing can be helpful in resolving uncertainty by actively listening, reflecting, asking open-ended questions, and maintaining a nonjudgmental and supportive environment [60].

It is important to allow the client to identify their own barriers to making changes to further guide them to come up with their own solutions. As the patient/client has a better idea of what is feasible for them, they are more likely to follow through with the changes they suggest. Additional educational information and solutions can also be provided if they can empower the patient/client and make them feel like they have autonomy over their eating and lifestyle habits. This can be done by asking the patient/client what they think about the solution and how helpful it is in overcoming their barrier.

When the patient/client is ready and has moved into the action phase of the transtheoretical model, setting clear and measureable goals is an important next step to the nutrition care process and motivational interviewing. By working together with the patient/client, realistic, small, achievable goals can be set. Starting with smaller goals and working up to bigger goals can help the patient/client gain confidence and feel empowered. Goals should include both short-term and long-term objectives. Setting specific and measurable goals is necessary to monitor and evaluate progress [60].

Using the previous example, if the patient/client reports eating fast food five times a week because they don't have time to cook, examples of small, measurable, short-term goals include:

- Decreasing frequency of eating out to once a week.
- Eating meals at home three times a week.
- Replacing fast food items with healthier options.
- Losing 1–2 lbs per week

Long-term goals may include:

- Eating fast food once a month.
- Cooking meals at home five times a week.
- Losing weight to achieve healthy BMI between 18.5 and 24.9

Nutrition Care Process: Monitoring and Evaluating

After intervention and goal setting, monitoring and evaluating are the next steps in the nutrition care process to measure progress. This can be done in more than one way by comparing current eating patterns to previous assessments, using the four different categories outlined in the Nutrition Care Process.

- Food/Nutrition-Related History. This includes food and nutrient intake, food and nutrient administration, medication, complementary medication use, knowledge/beliefs, food supplies availability, physical activity, and nutrition quality of life. If the problem or nutrition diagnosis is related to a patient's dietary intake, comparing 24 h recalls or 3-day food diaries throughout various visits and to dietary guidelines will help monitor progress and nutrient-related outcomes [55].
- Anthropometric Measurements include height, weight, and BMI. In the previous example, the patient/client's BMI was part of the sign/symptom related to their diagnosis, so height and weight measurements should be taken at each follow-up visit [55].
- Biochemical data, medical tests, and procedures including laboratory data and tests might be measured for monitoring and evaluating, depending on diagnosis and conditions [55]. If a patient/client with high total cholesterol and LDL cholesterol is trying to lower saturated fat and cholesterol intake, relevant laboratory and biochemical data related to their lipid profile should be reviewed during follow-up visits and included in the assessment.
- Nutrition-Focused Physical Findings refers to physical appearance, muscle and fat wasting, swallow function, appetite, and affect [55]. These assessments may not apply to all nutrition diagnoses or health conditions and therefore, only relevant data should be monitored.

Self-Monitoring Apps for Weight Management

Individuals can also monitor and track their nutrition and weight loss goals through smartphone apps designed to track food, exercise, weight, and nutrients listed on the Nutrition Facts label. Depending on the app, some also track fitness goals, provide healthy recipes, and customize individual calorie and physical activity plans. Top-rated apps by registered dietitians include Calorie Counter, Calorie Counter & Diet Tracker by MyFitness Pal, Calorie Counter: Diets & Activities, Calorie Tracker by Livestrong.com, and Sparkpeople Food and Fitness Tracker [61]. Fooducate is another app that is recommended by many healthcare professionals to track food intake and physical activity. It is unique in that it also evaluates the quality of food by assigning a grade from A to D. Foods can be scanned by using the barcode feature. In addition to personalizing health goals based on age, gender, weight, height, and activity level, Fooducate takes into consideration special health conditions and can help avoid genetically modified organisms (GMOs), processed foods, and certain allergens [62]. Fitbit is a popular physical activity tracker that has several different wearable wristbands that can be synced with smartphones or the Internet. The smartphone app also has a food and activity log to track intake. The Fitbit Zip was validated in a study with a healthy and physically active free-living population and was measured against two reference devices, an ActiGraph GT3X accelerometer and a Yamax CW700 pedometer. While Fitbit was found to record significantly more steps than an Actigraph

accelerometer, it could possibly be due to differences in instrument sensitivity or how the device is worn. There were no systematic differences between devices, and most participants favored using the Fitbit tool to track their physical activity [63].

USDA SuperTracker and MyPlate Method

The USDA's SuperTracker is another online self-monitoring dietary and physical activity assessment tool which promotes positive behavior change to help meet 2010 Dietary Guidelines. The online tool can analyze food and nutrient intake, track physical activity and weight, while also personalizing food intake based on age, gender, height, weight, and physical activity level. There is also a daily food plan specifically for expectant mothers at each stage of their pregnancy and during breastfeeding, as well as a separate meal plan for preschoolers. This user-friendly platform compares food intake to nutrient goals and USDA Food Patterns. The food database for SuperTracker is adapted from Agricultural Research Service's Food and Nutrient Database for Dietary Studies (FNDDS) and MyPyramid Equivalents Database (MPED) [64].

Following the MyPlate method, developed by the USDA is another helpful tool that teaches individuals how to build a healthy plate and consume a balanced meal. The MyPlate method emphasizes fruits and vegetables, whole grains, lean protein, and low-fat dairy. Half the plate should consist of a variety of fruits and non-starchy vegetables, especially dark-green such as broccoli, kale, spinach, red and orange vegetables, and beans and peas. A quarter of the plate is for grains of which, at least half should be whole, for example, 100 % whole-grain bread, barley, brown rice, and quinoa. The other quarter of the plate should include a different variety of lean protein-rich foods which includes seafood, nuts and beans, lean meat, poultry, and eggs. A healthful drink can be non-fat or low-fat milk [65].

Foodborne Illness Prevention

Maintaining proper food safety procedures and hygiene can help prevent foodborne illnesses. Each year, 1 in 6 Americans get sick, 128,000 are hospitalized, and 3000 die of foodborne diseases. The prevention of even one fatal case of *E. coli* would save about $7 million [66]. Salmonella infection causes more hospitalizations and deaths than any other germ in food, and 1 million people each year get sick from food contaminated with salmonella. The direct medical cost per year for salmonella infection is $365 million [67]. There are 31 known foodborne pathogens. The unspecified agents which include microbes and chemicals are unproven to cause illness [66]. Some food safety precautions that everyone can take to reduce the risk of foodborne illnesses are:

- Wash hands, cutting boards, utensils, and countertops with soap and water.
- Prevent cross-contamination by keeping raw meats separate from uncooked food such as salads.
- Separate raw meat, poultry, and seafood from ready-to-eat foods.
- Ensure all food is heated properly by using a food thermometer to check internal temperature. Whole meats should be heated to a minimum internal temperature of 145, 160 °F for ground meats, and 165 °F for poultry.
- Keep refrigerator temperature below 40 °F and refrigerate perishable foods.
- Monitor freezer temperature to ensure that it is at or below 0 °F [67].

Table 40.9 Physical Activity Guidelines [68]

Children and adolescents	• Participate in 60 min or more of physical activity per day, with the majority of the activity being moderate or vigorous-intensity
	• Aim for vigorous-intensity aerobic exercise at least 3 days a week
	• As part of the 60 min or more per day, muscle-strengthening activity and bone-strengthening activity should be included at least 3 days a week
Adults	• Participate in at least 150 min a week of moderate-intensity, or 75 min a week of vigorous-intensity physical activity, or a combination of moderate and vigorous intensity of aerobic exercise. It's preferable that activity is spread throughout the week
	• For even more significant health benefits, adults should increase their aerobic physical activity to 300 min or more of moderate intensity, or 150 min a week of vigorous physical activity, or a combination of both
	• Muscle-strengthening activity, at moderate or high intensity, involving all major muscle groups, should be included 2 days a week, to maximize health benefits
Older adults	• Older adults with chronic conditions preventing them from participating in 150 min of moderate-intensity aerobic exercise a week should be as physically active as possible, within the limitations of their conditions. They should understand and be aware of the safety of regular physical activity with relation to their health conditions
	• Older adults who are at risk for falling would benefit from exercises targeted to maintain or improve balance
Women during pregnancy and the postpartum period	• Aim for 150 min of moderate-intensity aerobic activity a week, with exercise spread throughout the week
	• Pregnant women who already engage in regular vigorous-intensity aerobic activity or who are very active can continue this activity into pregnancy and the postpartum period; however, they should discuss with their healthcare provider whether their activity level needs to be adjusted and how
Adults with disabilities	• Adults with disabilities, who are able to, should aim for at least 150 min a week of moderate-intensity or 75 min a week of vigorous-intensity aerobic activity, or a combination. It's preferable that activity is done in 10 min increments, and spread throughout the week
	• If their health condition allows, they should engage in muscle-strengthening activities of moderate or high intensity involving all major muscle groups on 2 or more days a week
	• Adults who are unable to meet guidelines should avoid inactivity and engage in regular physical activity according to their physical ability, and they should also consult a healthcare professional regarding exercises that are safe for them
For safe physical activity	• Be aware of risks involved in physical activity but understand that physical activity is safe for most people
	• Some activities are safer than others and each individual should choose an appropriate type of activity for their fitness level, health goal, and physical ability
	• Gradually increase physical activity over time to meet guidelines or health goals
	• Use appropriate gear and equipment, follow rules, and be aware of what physical activity is appropriate and where and when activity should safely take place
	• Individuals with chronic conditions should consult their healthcare provider about which types of physical activity they may safely engage in

Physical Activity

Physical activity should be included as part of any weight management regimen and for general disease prevention. Similar to the *Dietary Guidelines for Americans*, the U.S. Department of Health and Human services (HHS) issues the *Physical Activity Guidelines for Americans* because regular physical activity can result in long-term health benefits. The health benefits of physical activity vary from type of activity and guidelines are divided according to age group and special populations [68] (Table 40.9).

Conclusions

A holistic and comprehensive nutrition approach which incorporates evidence-based dietary guidelines and recommendations coupled with a physical activity regimen may prevent some of the leading causes of death in the United States. Additionally, proper food handling can prevent illness and death from foodborne diseases. Maintaining a healthy weight is especially important to disease prevention as overweight and obesity increase the risk of CVD, type 2 diabetes, hypertension, stroke, nonalcoholic fatty liver disease, and some cancers [34]. Early prevention is critical. Overweight in pregnancy can lead to large gestational weight. Therefore, proper nutrition should be implemented early in pregnancy [35, 36].

Dietary Guidelines Advisory Committee, National Cancer Institute, American Heart Association, and the Mediterranean diet provide guidelines for healthy eating patterns for the general population. Consuming a varied and balanced diet, with emphasis on adequate intake of fruits and vegetables, lean protein, including fish high in omega-3 fatty acids, unsaturated fats such as nuts, non-/low-fat dairy, and whole grains can help prevent nutrient shortfalls, and lower risk for chronic disease [50].

When making evidence-based recommendations at an individual level, nutrition counseling, using the Nutrition Care Process, is likely to produce more meaningful changes. This tailored and client-driven approach addresses individual barriers, goals, and actively engages patients/clients to take an active role in improving their health. Monitoring and evaluating progress can be achieved by comparing baseline data with each subsequent follow-up visit [55]. Dietary patterns can be assessed with food diaries, 24 h recalls, FFQs, and HEI [54]. Self-monitoring should also be encouraged, which is made easier with the help of several smartphone apps and online tools that track food intake and physical activity, and are also customizable to meet individual health goals.

References

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Appendix A: Recommended Books and Websites

1. Glutamine in Clinical Nutrition, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2014; $249.00
2. Nutrition and Bone Health, Second Edition, edited by Michael F. Holick and Jeri W. Nieves, 2014; $249.00
3. Branched Chain Amino Acids in Clinical Nutrition, Volume 2, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2014; $159.00
4. Branched Chain Amino Acids in Clinical Nutrition, Volume 1, edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2014; $159.00
5. Handbook of Clinical Nutrition and Aging, Third Edition, edited by Connie Watkins Bales, Julie L. Locher, and Edward Saltzman, 2014; $299.00
6. Integrative Weight Management: A Guide for Clinicians, edited by Gerard E. Mullin, Lawrence J. Cheskin, and Laura E. Matarese, 2014; $189.00
7. Adipose Tissue and Adipokines in Health and Disease, Second Edition, edited by Giamila Fantuzzi and Carol Braunschweig, 2014; $189.00
8. Nutrition and Oral Medicine, Second Edition, edited by Dr. Riva Touger-Decker, Dr. Connie C. Mobley, and Dr. Joel B. Epstein, 2014; $139.00
9. Fructose, High Fructose Corn Syrup, Sucrose and Health, edited by Dr. James M. Rippe, 2014; $149.00
10. Nutrition in Kidney Disease, Second Edition, edited by Dr. Laura D. Byham-Gray, Dr. Jerrilynn D. Burrowes, and Dr. Glenn M. Chertow, 2014; $159.00
11. Handbook of Food Fortification and Health, volume I edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013; $209.00
12. Handbook of Food Fortification and Health, volume II edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013; $239.00
13. Nutrition in Pediatric Pulmonary Disease, edited by Dr. Robert Dumont and Dr. Youngran Chung, 2013; $109.00
14. Diet Quality: An Evidence-Based Approach, volume II, edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013; $209.00
15. Diet Quality: An Evidence-Based Approach, volume I, edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013; $209.00
16. The Handbook of Clinical Nutrition and Stroke, edited by Dr. Mandy L. Corrigan, Arlene A. Escuro, MS, and Dr. Donald F. Kirby, 2013; $159.00
17. Nutrition in Infancy, volume II, edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013; $189.00
18. Nutrition in Infancy, volume I, edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013; $189.00

A. Bendich, R.J. Deckelbaum (eds.), *Preventive Nutrition*,
Nutrition and Health, DOI 10.1007/978-3-319-22431-2

19. Nutrition and Diet in Menopause, edited by Caroline J. Hollins, Ronald Ross Watson, and Victor R. Preedy, 2013; $239.00
20. Carotenoids and Human Health, edited by Sherry A. Tanumihardjo, 2013; $189.00
21. Bioactive Dietary Factors and Plant Extracts in Dermatology, edited by Dr. Ronald Ross Watson and Dr. Sherma Zibadi, 2013; $189.00
22. Omega 6/3 Fatty Acids, edited by Dr. Fabien De Meester, Dr. Ronald Ross Watson, and Dr. Sherma Zibadi, 2013; $159.00
23. Magnesium and Health, edited by Dr. Ronald Ross Watson and Dr. Victor R. Preedy, 2012
24. Alcohol, Nutrition and Health Consequences, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
25. Nutritional Health, Strategies for Disease Prevention, Third Edition, edited by Norman J. Temple, Ted Wilson, and David R. Jacobs, Jr., 2012
26. Chocolate in Health and Nutrition, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
27. Iron Physiology and Pathophysiology in Humans, edited by Dr. Gregory J. Anderson and Dr. Gordon D. McLaren, 2012
28. Nitrite and Nitrate in Human Health and Disease, edited by Dr. Nathan S. Bryan and Dr. Joseph Loscalzo, 2011
29. Management of Pediatric Obesity and Diabetes, edited by Dr. Robert J. Ferry, 2011
30. Nutrition Guide for Physicians, edited by Dr. Ted Wilson, Dr. George A. Bray, Dr. Norman J. Temple, and Dr. Marie Boyle Struble, 2010
31. Nutrients, Dietary Supplements and Nutriceuticals, edited by Dr. Ronald Ross Watson, Dr. Joe K. Gerald, and Dr. Victor R. Preedy, 2010
32. Dietary Components and Immune Function edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi, and Dr. Victor R. Preedy, 2010
33. Bioactive Compounds and Cancer, edited by Dr. John A. Milner and Dr. Donato F. Romagnolo, 2010
34. Vitamin D, edited by Dr. Michael F. Holick, 2010
35. Fluids and Electrolytes in Pediatrics, edited by Dr. Leonard G. Feld and Dr. Frederick J. Kaskel
36. Modern Dietary Fat Intakes in Disease Promotion, edited by Dr. Fabien De Meester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson, 2010
37. Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fourth Edition, edited by Dr. Adrianne Bendich and Dr. Richard J. Deckelbaum, 2010
38. Nutrition and Metabolism, edited by Dr. Christos S. Mantzoros, 2009
39. Iron Deficiency and Overload: From Basic Biology to Clinical Practice, edited by Shlomo Yehuda and David Mostofsky, 2009
40. Handbook of Drug-Nutrient Interactions, Second Edition, edited by Dr. Joseph Boullata and Dr. Vincent Armenti, 2009
41. Handbook of Clinical Nutrition and Aging, Second Edition, edited by Dr. Connie Bales and Dr. Christine Ritchie, 2009
42. Probiotics in Pediatric Medicine, edited by Sonia Michail and Philip M. Sherman, 2009
43. Handbook of Nutrition and Pregnancy, edited by Carol J. Lammi-Keefe, Sarah Collins Couch, and Elliot H. Philipson, 2008
44. Nutrition and Health in Developing Countries, Second Edition, edited by Richard D. Semba and Martin W. Bloem, 2008
45. Nutrition and Rheumatic Disease, edited by Laura A. Coleman, 2008
46. Nutrition in Kidney Disease, edited by Laura D. Byham-Gray, Jerrilynn D. Burrowes, and Glenn M. Chertow, 2008
47. Handbook of Nutrition and Ophthalmology, edited by Richard D. Semba, 2007

48. Adipose Tissue and Adipokines in Health and Disease, edited by Giamila Fantuzzi and Theodore Mazzone, 2007
49. Nutritional Health: Strategies for Disease Prevention, Second Edition, edited by Norman J. Temple, Ted Wilson, and David R. Jacobs, Jr., 2006
50. Nutrients, Stress, and Medical Disorders, edited by Shlomo Yehuda and David I. Mostofsky, 2006
51. Calcium in Human Health, edited by Connie M. Weaver and Robert P. Heaney, 2006
52. Preventive Nutrition: The Comprehensive Guide for Health Professionals, Third Edition, edited by Adrianne Bendich and Richard J. Deckelbaum, 2005
53. The Management of Eating Disorders and Obesity, Second Edition, edited by David J. Goldstein, 2005
54. Nutrition and Oral Medicine, edited by Riva Touger-Decker, David A. Sirois, and Connie C. Mobley, 2005
55. IGF and Nutrition in Health and Disease, edited by M. Sue Houston, Jeffrey M. P. Holly, and Eva L. Feldman, 2005
56. Epilepsy and the Ketogenic Diet, edited by Carl E. Stafstrom and Jong M. Rho, 2004
57. Handbook of Drug-Nutrient Interactions, edited by Joseph I. Boullata and Vincent T. Armenti, 2004
58. Nutrition and Bone Health, edited by Michael F. Holick and Bess Dawson-Hughes, 2004
59. Diet and Human Immune Function, edited by David A. Hughes, L. Gail Darlington, and Adrianne Bendich, 2004
60. Beverages in Nutrition and Health, edited by Ted Wilson and Norman J. Temple, 2004
61. Handbook of Clinical Nutrition and Aging, edited by Connie Watkins Bales and Christine Seel Ritchie, 2004
62. Fatty Acids: Physiological and Behavioral Functions, edited by David I. Mostofsky, Shlomo Yehuda, and Norman Salem, Jr., 2001
63. Nutrition and Health in Developing Countries, edited by Richard D. Semba and Martin W. Bloem, 2001
64. Preventive Nutrition: The Comprehensive Guide for Health Professionals, Second Edition, edited by Adrianne Bendich and Richard J. Deckelbaum, 2001
65. Nutritional Health: Strategies for Disease Prevention, edited by Ted Wilson and Norman J. Temple, 2001
66. Clinical Nutrition of the Essential Trace Elements and Minerals: The Guide for Health Professionals, edited by John D. Bogden and Leslie M. Klevay, 2000
67. Primary and Secondary Preventive Nutrition, edited by Adrianne Bendich and Richard J. Deckelbaum, 2000
68. The Management of Eating Disorders and Obesity, edited by David J. Goldstein, 1999
69. Vitamin D: Physiology, Molecular Biology, and Clinical Applications, edited by Michael F. Holick, 1999
70. Preventive Nutrition: The Comprehensive Guide for Health Professionals, edited by Adrianne Bendich and Richard J. Deckelbaum, 1997

Appendix B: Other Relevant Volumes

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Appendix C: Websites of Interest

http://nutrition.org

The American Society for Nutrition (ASN) is a nonprofit organization dedicated to bringing together the world's top researchers, clinical nutritionists, and industry to advance our knowledge and application of nutrition for the sake of humans and animals. Our focus ranges from the most critical details of research and application to the broadest applications in society, in the USA, and around the world.

http://nutrition.gov

The site provides easy, online access to government information on food and human nutrition for consumers. This site is a service of the National Agricultural Library, USDA.

http://cdc.gov

CDC is globally recognized for conducting research and investigations and for its action-oriented approach. CDC applies research and findings to improve people's daily lives and responds to health emergencies—something that distinguishes CDC from its peer agencies. CDC works with states and other partners to provide a system of health surveillance to monitor and prevent disease outbreaks (including bioterrorism), implement disease prevention strategies, and maintain national health statistics. CDC also guards against international disease transmission, with personnel stationed in more than 25 foreign countries. CDC is now focusing on achieving the four overarching Health Protection Goals to become a more performance-based agency focusing on healthy people, healthy places, preparedness, and global health. CDC is one of the 13 major operating components of the Department of Health and Human Services (HHS).

http://ods.od.nih.gov

The NIH Office of Dietary Supplements, established in 1995, provides educational materials and tools and research opportunities for health professionals and consumers including information about dietary supplement ingredients and safety.

http://www.springer.com/series/7659

Nutrition and Health Book Series information on the Springer website provides information about all volumes published in the series, book reviews, and book ordering instructions.

http://www.hsph.harvard.edu/nutritionsource/

The Nutrition Source provides evidence-based diet and nutrition information for clinicians, health professionals, the media, and the public.

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http://globalnutritionreport.org/2014/11/13/global-nutrition-report-2014/

The Global Nutrition Report (GNR) provides a global profile and country profiles on nutrition for each of the United Nations' 193 member states and includes specific progress for each country. It will be a centerpiece of the Second International Conference on Nutrition (ICN2) in Rome on 19–21 November, organized by the UN Food and Agriculture Organization and the World Health Organization. The entire report is downloadable.

http://www.ifst.org/

IFST (Institute of Food Science and Technology) is based in the UK, with members throughout the world, with the purpose of serving the public interest in the application of science and technology for food safety and nutrition as well as furthering the profession of food science and technology. Eligibility for membership can be found at the IFST home page; an index and a search engine are available.

http://www.nysaes.cornell.edu/cifs/start.html

The Cornell Institute of Food Science at Cornell University home page provides information on graduate and undergraduate courses as well as research and extension programs. Links to related sites and newsgroups can be found.

http://www.blonz.com

Created by Ed Blonz, Ph.D., "The Blonz Guide" focuses on the fields of nutrition, foods, food science, and health supplying links and search engines to find quality sources, news, publication, and entertainment sites.

http://www.hnrc.tufts.edu/

The Jean Mayer US Department of Agriculture (USDA) Human Nutrition Research Center on Aging (HNRC) at Tufts University. This research center is one of six mission-oriented centers aimed at studying the relationship between human nutrition and health, operated by Tufts University under the USDA. Research programs; seminar and conference information; publications; nutrition, aging, medical, and science resources; and related links are available.

http://www.fao.org/

The Food and Agriculture Organization (FAO) is the largest autonomous agency within the United Nations, founded "with a mandate to raise levels of nutrition and standards of living, to improve agricultural productivity, and to better the condition of rural population," emphasizing sustainable agriculture and rural development.

http://www.eatright.org/

The Academy of Nutrition and Dietetics is the largest group of food and nutrition professionals in the USA; members are primarily registered dietitians (RDs) and dietetic technicians, registered (DTRs). Programs and services include promoting nutrition information for the public; sponsoring national events, media and marketing programs, and publications; and lobbying for federal legislation. Also available through the website are member services, nutrition resources, news, classifieds, and government affairs. Assistance in finding a dietitian, marketplace news, and links to related sites can also be found.

http://www.foodsciencecentral.com

The International Food Information Service (IFIS) is a leading information, product, and service provider for professionals in food science, food technology, and nutrition. IFIS publishing offers a

wide range of scientific databases, including FSTA—Food Science and Technology Abstracts. IFIS GmbH offers research, educational training, and seminars.

http://www.ift.org/

The Institute of Food Technologists (IFT) is a membership organization advancing the science and technology of food through the sharing of information; publications include *Food Technology* and *Journal of Food Science*; events include the Annual Meeting and Food Expo. Members may choose to join a specialized division of expertise (there are 23 divisions); IFT student associations and committees are also available for membership.

http://www.osteo.org/

The National Institutes of Health Osteoporosis and Related Bone Diseases ~ National Resource Center (NIH ORBD-NRC) mission is to "provide patients, health professionals, and the public with an important link to resources and information on metabolic bone diseases, including osteoporosis, Paget's disease of the bone, osteogenesis imperfecta, and hyperparathyroidism. The Center is operated by the National Osteoporosis Foundation, in collaboration with The Paget Foundation and the Osteogenesis Imperfecta Foundation."

http://www.ag.uiuc.edu/~food-lab/nat/

The Nutrition Analysis Tool (NAT) is a free web-based program designed to be used by anyone to analyze the nutrient content of food intake. Links to an "Energy Calculator" and "Soy Food Finder" are also available. NAT is funded by C-FAR at the University of Illinois.

http://vm.cfsan.fda.gov/

The Center for Food Safety and Applied Nutrition (CFSAN) is one of five product-oriented centers implementing the FDA's mission to regulate domestic and imported food as well as cosmetics. An overview of CFSAN activities can be found along with useful sources for researching various topics such as food biotechnology and seafood safety. Special interest areas, for example, advice for consumers, women's health, and links to other agencies, are also available.

http://www.bcm.tmc.edu/cnrc/

The Children's Nutrition Research Center (CNRC) at Baylor College of Medicine is one of six USDA/ARS human nutrition research centers in the nation, assisting healthcare professionals and policy advisors to make appropriate dietary recommendations. CNRC focuses on the nutrition needs of children, from conception through adolescence, and of pregnant and nursing women. Consumer news, seminars, events, and media information are some of the sections available from this home page.

http://www.usda.gov

The US Department of Agriculture (USDA) provides a broad scope of service to the nation's farmers and ranchers. In addition, the USDA ensures open markets for agricultural products, food safety, environmental protection, conservation of forests and rural land, and the research of human nutrition. Affiliated agencies, services, and programs are accessible through this website.

http://www.nalusda.gov/

The National Agriculture Library (NAL), a primary resource for agriculture information, is one of four national libraries in the USA and a component of the Agriculture Research Service of the US Department of Agriculture. Access to NAL's institutions and resources are available through this site.

http://www.fns.usda.gov/fns/

The Food and Nutrition Service (FNS) administers the US Department of Agriculture's (USDA) 15 food assistance programs for children and needy families with the mission to reduce hunger and food insecurity. Details of nutrition assistance programs and related links can be found.

http://www.agnic.org/

The Agriculture Network Information Center (AgNIC), established through the alliance of the National Agriculture Library (NAL) and other organizations, provides public access to agriculture-related resources.

http://www.who.int/nut/welcome.htm

The World Health Organization (WHO) has regarded nutrition to be of fundamental importance for overall health and sustainable development. The global priority of nutritional issues, activities, mandates, resources, and research are presented in detail.

http://www.clinicaltrials.gov/ct

ClinicalTrials.gov is a registry of federally and privately supported clinical trials conducted in the USA and around the world. ClinicalTrials.gov gives you information about a trial's purpose, who may participate, locations, and phone numbers for more details. This information should be used in conjunction with advice from healthcare professionals.

http://www.faseb.org

A multi-society, interdisciplinary, scientific community that sponsors meeting featuring plenary and award lectures, symposia, oral and poster sessions, career services, and exhibits of scientific equipment, supplies, and publications.

Appendix D
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