PREVENTIVE NUTRITION
NUTRITION ◇ AND ◇ HEALTH
Adrienne Bendich, Series Editor

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Preventive Nutrition

The Comprehensive Guide for Health Professionals

Second Edition

Edited by

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DEDICATION

A. B. dedicates this book to Tyler James Schiff, her first grandchild, with the hope that preventive nutrition becomes an accepted component of medical practice in his lifetime.

R. J. D. thanks his wife, Kaya, and children Ariel, Dan, Mikael, and Leona for their understanding and support.

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FOREWORD

Nutrition has been recognized as a major determinant of health for centuries. Traditionally, nutritional sciences have primarily targeted the prevention of diseases resulting from clinical deficiencies of essential nutrients, such as scurvy and rickets. Contemporary nutritional research has focused on the prevention of major diseases of Western civilization, particularly cardiovascular disease and cancer, as well as promoting maternal and child health and healthy aging. Heart disease and cancer, which were rare in most developing countries several decades ago, are increasing dramatically in these countries, in parallel with economic development and dietary transitions, decreases in infectious diseases, and increasing sedentary lifestyle and obesity. Substantial evidence indicates major chronic diseases such as coronary heart disease, type 2 diabetes, and some cancers are largely preventable by relatively simple diet and lifestyle modifications.

Despite the great potential of nutrition in preventing diseases and improving health, nutrition is not routinely emphasized in the education and training of physicians and other health care professionals. This has resulted in inadequate nutritional knowledge and lack of skills in providing dietary counseling among many health care professionals. Furthermore, in the past decade, the public’s access to nutritional information has been increasing rapidly, particularly through the Internet. There are now hundreds of websites providing a wide range of nutritional information and selling numerous dietary products. Because of the explosion in nutritional information, the public’s demand for nutritional advice has been increasing rapidly and will continue to rise. This requires physicians and other health professionals to acquire additional knowledge and skills in nutrition.

Preventive Nutrition, 2nd edition, edited by Drs. Bendich and Deckelbaum, which has expanded to keep abreast of recent developments in nutritional sciences, is an extremely important and timely resource for physicians and other health professionals to seek valuable information. Preventive Nutrition, 2nd edition, encompasses a broad range of topics, from cancer and cardiovascular disease to infectious disease in children, and from reproductive and prenatal nutrition to global public health nutrition strategies. This book is notable not only for its comprehensiveness, but also its up-to-date scientific information. In the past decade, our understanding of the nutrients and foods most likely to promote health has improved substantially, owing to the advances in basic science and epidemiologic research. For example, the importance of different types of dietary fat, especially trans fatty acids from partially hydrogenated vegetable oil, in influencing risk of coronary heart disease is increasingly recognized. In addition, several lines of evidence indicate a protective role of omega-3 fatty acids in cardiovascular health as well as in perinatal neurological development. Meanwhile, growing evidence supports an important role of plant constituents such as antioxidant vitamins and phytochemicals in the prevention of chronic diseases. Folic acid, which is critical in preventing birth defects, may also have a role in the prevention of cardiovascular disease and cancer. Increasing evidence has also suggested the potential of nutritional factors in reducing various mor-
bid conditions, such as age-related macular degeneration, osteoporosis, diabetes, infections, and adverse pregnancy outcomes. Many cutting-edge developments in nutrition and health are reviewed in detail.

The translation of nutritional research into clinical and public health recommendations has always been a challenge for nutritional researchers and health professionals. This book gives careful consideration to practical dietary recommendations for all relevant nutritional factors. In addition, the impact of dietary transition on chronic diseases in developing countries is thoroughly discussed, as are global nutritional strategies to curb the growing epidemic of overnutrition-related health problems worldwide. The integration of theory and practice will undoubtedly benefit physicians and other health professionals in achieving a deeper understanding of nutritional sciences and providing dietary counseling. Drs. Bendich and Deckelbaum are to be commended for Preventive Nutrition, 2nd edition, which remains the most comprehensive and up-to-date textbook in the field.

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Preventive nutrition can be defined as dietary practices and interventions directed toward the reduction in disease risk and/or improvement in health outcomes. Preventive nutrition is a critical component of preventive medicine approaches that seeks to prevent disease rather than treat the condition after it manifests clinically. Examples of preventive nutrition include current recommendations to reduce fat and saturated fat intakes for cardiovascular disease prevention, the inclusion of iodine in salt for the prevention of iodine deficiency disease, and the inclusion of certain B vitamins, vitamins A and D, iron, and calcium in staple foods, such as grain products, milk, and cereals, for the prevention of essential nutrient-related deficiencies. These preventive nutrition strategies have been underway as part of public health policy for more than a generation and have been shown to be extremely effective.

Within the past 20 years, further nutrient-based research has demonstrated the potential for essential micronutrients to reduce the risk of many common chronic diseases. Thus, the overall goal of the second edition of *Preventive Nutrition* is to assess and describe the most recent nutritional approaches for promoting health and preventing, delaying, or modifying disease processes, with increasing recognition of the role of nutrition in health promotion and disease risk prevention.

This second edition of *Preventive Nutrition* provides updates of most of the original chapters in the first edition by documenting and reviewing recent advances described in the literature over the last few years. The new research results on dietary components that are not considered to have traditional “nutritional value,” but have been shown to have important health consequences, such as fiber, specific long-chain fatty acids, non-pro vitamin A carotenoids, and other phytochemicals, are placed in perspective with regard to the available knowledge to date.

The first objective of our revised volume is to provide practicing health professionals, including physicians, nutritionists, dentists, pharmacists, dietitians, health educators, policy makers, and research investigators, with the newest research indicating that the risk of many of the major diseases affecting middle-aged adults can be prevented, or at least delayed, with simple nutritional approaches. Many health professionals are asked daily about the new studies with vitamins or other nutrients discussed in their local newspaper or on the evening news. As examples, patients want advice from their health care providers about β-carotene, antioxidants, fiber, and the myriad of bioactive phytochemicals, such as those found in garlic and other foods.

*Preventive Nutrition*, 2nd edition, like the first, provides answers based on the totality of evidence, rather than on the findings of any single study. Major disease categories are included, such as the leading two causes of mortality in the United States and elsewhere, cardiovascular disease and cancer, as well as such progressively debilitating conditions as diabetes, cataracts, and osteoporosis. The potential of nutrients to affect immunocompetence, which may be an underlying factor in many of the abovementioned and other conditions, is examined.
The second objective is to examine key research linking nutritional status with the prevention of birth defects and optimization of birth outcomes. Recent evidence that micronutrient status can also improve the potential for the health, vision, and intellectual capacity of children is discussed. The need for physicians and nutritional care providers as well as both potential parents to strongly advocate a new paradigm of long-range planning for pregnancy is underscored. Research clearly shows that the preconceptional period, about three months before conception, through the third month of pregnancy is the time when many serious birth defects occur; thus, the old paradigm that women can wait for prenatal care until weeks or months after conception is no longer valid.

A unique feature of this volume is the section that examines the successes, and consequent public health implications, of national preventive nutrition strategies, not only in the United States and Europe, but also in “Westernizing” nations and developing countries. As the demographics of US and European populations change and become more multicultural, it is increasingly important for health professionals to understand the nutritional backgrounds and diversities of their patients. As important, there may be significant national dietary initiatives that provide roadmaps for effective implementation of preventive nutrition within an overall strategy of health improvement, especially for vulnerable members of the population, such as the poor.

The evaluation of the totality of the evidence will be critical in leading to recommendations that can lower risk of disease, morbidity, and mortality and at the same time reduce the burden of health care costs for all. The economic consequences of preventive nutrition cannot be easily overlooked. Based on the annual costs associated with hospitalizations alone, documented in the 1992 National Hospital Discharge Survey, the estimated savings associated with reducing chronic disease risk has been shown to be substantial. For coronary heart disease, the chronic disease responsible for the largest number of hospitalizations per year in the United States, the total for hospitalization charges, excluding physician fees, was approximately $57.6 billion in 1995 dollars. Using the recent epidemiologic literature examining the reduction in risk of heart disease associated with the highest antioxidant status, it can be estimated that $22 billion per year could be saved in this disease category alone once preventive nutrition measures were fully implemented (Chapter 9, 1st edition).

The costs associated with hospitalizations resulting from cancer are also substantial. The average annual hospitalization charges for stomach cancer are about $1 billion; breast cancer costs about $1.8 billion; for head and neck cancers, which are more rare than the two other cancers discussed, the hospitalization costs are still high, although under $1 billion per year. It is estimated that hospitalization costs associated with stomach and breast cancer could each be reduced by one-third; head and neck cancers could be halved based on projections that use published estimates of risk reduction associated with the highest intakes of antioxidant micronutrients.

Cardiovascular disease, cancer, and cataracts are examples of chronic diseases with long durations of onset; thus, long-term preventive nutrition strategies are needed. Therefore, the economic benefits that are projected would not be realized in a short period of time, and it may take years before the economic as well as personal and national health benefits can be seen. There are, however, other adverse health conditions that are more acute in time frame, and the economic consequences could be measured in a shorter time period than required for prevention of chronic diseases. For example, the effects of
preventive nutrition strategies on the hospitalization costs involved in adverse pregnancy outcomes could be documented in a relatively short period of time.

Birth defects are the number one cause of hospitalizations associated with birth-related disorders. Low birth weight accounts for the second largest number of hospitalizations. Birth defects and low birth weight are also the two major causes of infant mortality in the United States. Thus, the potential to reduce both infant morbidity and mortality through nutritional interventions provides a real possibility of verifying the economic and consequent health benefits of relatively short-term dietary changes.

As a specific example, within the past decade, significant research has documented that women who take a folic acid-containing multivitamin daily for at least one month before conception and during their pregnancies have approximately a 50% decrease in neural tube defect (NTD) outcomes (Chapter 15). The expected annual savings associated with lowered NTD-related outcomes is about $70 million. By far the greatest savings would be seen in the reduction of cardiovascular birth defects, which are the greatest cause of birth-related hospitalizations. Based on intervention and epidemiologic studies, it is estimated that the annual savings could reach $800 million. In addition to NTD and cardiovascular birth defects, there are also significant reductions in renal defects, cleft lip/palate, and limb reductions seen in women who use multivitamin supplements before and during pregnancy.

Low-birth-weight infants include those from premature births as well as small for gestational age term infants. In both cases, hospitalization costs are projected to be over $2 billion annually. There are studies that indicate that reduction in iron deficiency anemia, as well as improved zinc and/or folic acid status, can significantly reduce the risk of low-birth-weight pregnancy outcomes (Chapter 17). The estimated hospital-associated savings would be many millions of dollars per year (Table 1).

Economic estimates have not been made for all of the areas covered in the chapters in this book. However, it seems logical that the improvement seen in the immune responses of the elderly who took a multivitamin supplement (Chapter 13) would result in lowered hospitalizations associated with respiratory infections, for instance. Likewise, improved immune status via vitamin A supplementation in children could prevent infection-associated morbidity and mortality (Chapter 14).

It should be realized that many of the nutrient recommendations provided in this volume that appear to be related to one specific health factor or disease in fact “cross over”

<table>
<thead>
<tr>
<th>Disease</th>
<th>Costs/yr, $</th>
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<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>22 billion</td>
</tr>
<tr>
<td>Cancer</td>
<td>1 billion</td>
</tr>
<tr>
<td>Cardiovascular birth defects</td>
<td>800 million</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>500 million</td>
</tr>
<tr>
<td>Neural tube birth defects</td>
<td>70 million</td>
</tr>
<tr>
<td>Cataract</td>
<td>2 million</td>
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</tbody>
</table>

Table 1: Potential Economic Consequences of Preventive Nutrition
and appear valid for many of the health areas discussed (Table 2). Importantly, there are many more commonalities in the recommendations provided for disease risk reduction than there are differences. For example, lowering saturated fat intake and increasing antioxidants, fiber, and calcium intake are suggested for reducing cardiovascular and cancer risks and at the same time may lower the risk of osteoporosis and cataracts. Increased intake of folic acid would likely lead to decreases in NTDs as well as cardiovascular disease. Of critical importance, no single recommendation provided in this volume targeted to a single condition will lead to adverse effects in another health area. Thus, overall, the guidelines suggested in the individual chapters have the potential not only to reduce individual morbidity and health care costs, but also to contribute positively to the national health care debate.

As editors, we are very excited about the contents of the second edition. Generally, each chapter is organized to provide an overview of the field, the author’s own research, and how those findings fit with the overview. Extensive summary tables and figures illustrate the depth of knowledge in the area and recommendations for various patient groups. There is an extensive index. Also included is a list of journals that specialize in publishing clinical studies in preventive nutrition and a bibliography of recent, relevant books and a list of websites of importance to nutrition topics. By addressing the nutrition questions most often raised, and by examining the issues based on disease as well as age, it is hoped that this volume will serve as the critical resource for health professionals interested in enhancing their ability to utilize nutrition to improve health outcomes of individuals, and assist in the planning of national disease prevention programs for enhancing the health status of populations.

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CANCER PREVENTION
1. BACKGROUND

Cancer is the most common fatal disease of childhood in the United States. Between ages 1 and 15, only accidents kill more children. Approximately 14/100,000 children develop cancer each year, or about 7500 children in the United States (1,2). This incidence rate indicates that about 1 in 500 children develop cancer before the age of 15. The common cancers of childhood are not those of later life; leukemia accounts for about one-third of childhood cancers and brain tumors about one-fifth. The other major cancers, in order of frequency, are lymphoma, neuroblastoma, Wilms' tumor, soft tissue sarcoma, osteogenic sarcoma, and retinoblastoma (2). Since the early 1970s, the incidence of childhood cancer appears to be increasing slowly (1), but whether the observed increase reflects better diagnosis or real change is not known. The same time period has also seen a dramatic improvement in the survival of children with cancer with approx 70% of these children now alive 5 yr after diagnosis (2). However, some are left with long-term medical and cognitive problems.

Little is known about the etiology of cancers in children. The medical literature contained few epidemiologic studies of childhood cancer before the 1970s, but the extent of interest and investigation has increased dramatically since then. Many risk factors have been investigated, including genetic abnormalities and parental occupational exposures, in addition to aspects of diet that are the focus of this review.

The relationship between diet and childhood cancer has been studied little. In fact, nearly all the data discussed here come from 12 studies (see Table 1) (3–15). The possibility that a child’s diet or the mother’s diet during pregnancy can raise or diminish the risk of these rare cancers at first seems unlikely. The adult cancers most strongly linked with diet, such as stomach and colon, are believed to have latency periods of several decades. In contrast, cancers in children by definition have latencies of no more than 15 yr and often less than 5 yr. Furthermore, cancers of the digestive tract and of other sites linked to diet in adults rarely occur in children. Therefore, researchers first focused their search for causes on genetic predisposition and exposure to environmental toxins rather than diet.

The cancers of childhood, mainly leukemia, brain tumor, lymphoma, and sarcoma, occur relatively rarely in adulthood, and the etiologic hypotheses about these cancers...
<table>
<thead>
<tr>
<th>Authors and reference</th>
<th>Sample size</th>
<th>Age</th>
<th>Cancer type</th>
<th>Paternal cured meat consumption</th>
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<td>209 Cases 209 Controls</td>
<td>0–14</td>
<td>Tumors of brain and cranial meninges</td>
<td></td>
</tr>
<tr>
<td>Howe et al., 1989 (4)</td>
<td>74 Cases 138 Controls</td>
<td>0–9</td>
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<tr>
<td>Kuijten et al., 1990 (5)</td>
<td>163 Cases 163 Controls</td>
<td>0–14</td>
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<td>Bunin et al., 1993 (6)</td>
<td>166 Cases 166 Controls</td>
<td>0–6</td>
<td>Medulloblastoma/primitive neuroectodermal tumor</td>
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<td>155 Cases 155 Controls</td>
<td>0–6</td>
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<td>McCredie et al., 1994 (8,9)</td>
<td>82 Cases 164 Controls</td>
<td>0–14</td>
<td>Tumor of brain and cranial nerves</td>
<td></td>
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<td>McCredie et al., 1994 (8,9)</td>
<td>All cured meats, OR = 2.5(^e)</td>
<td>Fruit, OR = 0.7</td>
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<td></td>
<td>Hot dogs, OR = 1.99</td>
<td>Vegetables, OR = 0.4</td>
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<td>Sarasua and Savitz, 1994 (10)(^f)</td>
<td>Hot dogs, OR = 2.3(^e)</td>
<td>Fruit, OR = 1.5</td>
<td>Vitamin supplement, OR = 0.7(^e)</td>
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<td>Other individual cured meats, ORs = 0.4 and 1.0</td>
<td>Carrots, leeks, green peppers, ORs = 0.3–0.4(^e)</td>
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<td>Cordier et al., 1994 (11)</td>
<td>Individual cured meats, ORs = 0.5–0.8</td>
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<td>Preston-Martin et al., 1996 (12)</td>
<td>All cured meats, OR = 2.1(^e)</td>
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<td>Multivitamin, OR = 0.5–0.7 (depending on duration of use)</td>
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<td>Hot dogs, OR = 1.4(^e)</td>
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<td>Bacon, OR = 1.6(^e)</td>
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<td>Other individual cured meats, ORs = 1.0–1.9(^e)</td>
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<td>Peters et al., 1994 (13)</td>
<td>Hot dogs, OR = 2.4</td>
<td>Oranges, OR = 0.4</td>
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<td>Other individual cured meats, ORs = 1.0–1.3</td>
<td>Grapefruit, OR = 0.6</td>
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<td>Oranges or orange juice, OR = 0.8</td>
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<td>Grapefruit or grapefruit juice, OR = 1.1</td>
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<td>Apple juice, OR = 0.9</td>
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<td>Sarasua and Savitz, 1994 (10)(^f)</td>
<td>Hot dogs, OR = 0.9</td>
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<td>Vitamin supplement, OR = 0.5(^e)</td>
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<td>Other individual cured meats, ORs = 1.0 and 1.5</td>
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<td>Shu et al., 1988 (14)</td>
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<th>Authors and reference</th>
<th>Infant’s/child’s cured meat consumption</th>
<th>Infant’s/child’s fruit consumption</th>
<th>Infant’s/child’s vitamin supplement use</th>
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<td>Preston-Martin et al., 1982 (3)</td>
<td>All cured meats, OR = 2.3¢</td>
<td>Fruit juice, OR = 0.2¢</td>
<td>Vitamin supplement, no association</td>
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<td>Howe et al., 1989 (4)</td>
<td>All cured meats, OR = 1.1</td>
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<td>Vitamin C supplement, OR = 0.9</td>
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<td>No fruit as infant, OR = 4.3¢</td>
<td>Multivitamin as infant, OR = 0.7</td>
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<td>Fruit as infant, OR = 0.4</td>
<td>Orange juice as infant, OR = 1.8</td>
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<td>Sarasua and Savitz, 1994 (10)²</td>
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<td>Vitamin syrup as infant, OR = 0.5</td>
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<td>Apple juice as infant, OR = 0.3</td>
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<td>Individual cured meats in absence of vitamin supplements, ORs = 3.2–6.8¢</td>
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<td>Study</td>
<td>Foods</td>
<td>Odds Ratio(s)</td>
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<tr>
<td>Peters et al., 1994 (13)</td>
<td>Hot dogs, OR = 5.8(^c)</td>
<td>Oranges or orange juice, OR = 1.1</td>
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<td>Grapefruit or grapefruit juice, OR = 1.0</td>
<td></td>
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<tr>
<td>Sarasua and Savitz, 1994 (10)(^d)</td>
<td>Hot dogs, OR = 1.3</td>
<td>Vitamin supplement, OR = 0.6</td>
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<td></td>
<td>Other individual cured meats, ORs = 1.1 and 1.2</td>
<td>Individual cured meats in absence of vitamin supplements, ORs = 2.9(^c)</td>
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<tr>
<td>Shu et al., 1988 (14)</td>
<td></td>
<td>Cod-liver oil, OR = 0.3</td>
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\(^{a}\) For all foods, odds ratio presented is that for highest level of consumption.

\(^{b}\) OR: odds ratio.

\(^{c}\) Statistically significant.

\(^{d}\) Listed as two studies because separate analyses presented for brain tumors and acute lymphocytic leukemia (ALL).

\(^{e}\) Calculated from data presented in reference.
have generally not been dietary. Therefore, the literature on adult cancers adds only a limited amount to the discussion, but is considered where relevant.

Diet might act to alter cancer risk in children by mechanisms similar to and different from those proposed for adult cancers. Antioxidants such as vitamin C and β-carotene may protect against various cancers by their ability to neutralize free radicals and thus prevent oxidative damage to DNA (16). Folic acid deficiency may encourage malignant transformation of normal cells by altering gene expression and weakening chromosomal structure (17). Exposure to N-nitroso compounds may initiate cancer through direct acting or metabolically activated carcinogens in this class of substances. Antioxidants and folic acid may act in fetuses and children through the same mechanisms as they are hypothesized to act in adults. Carcinogens may also act through the same mechanisms at all ages, but fetuses may be more susceptible to carcinogens, as suggested by animal studies of some N-nitroso compounds. Some substances might actually have the opposite effect in fetuses as in adults, as proposed for topoisomerase II inhibitors (see Subheading 4). Mechanisms unique to the embryo or fetus may also exist. An excess or deficiency of a dietary component could result in malformation of an organ or subtle cellular changes that increase the organ’s susceptibility to cancer. This type of altered development has been proposed as a mechanism leading to cancer in young women after prenatal DES exposure (18–20).

2. N-NITROSO COMPOUNDS

Most of the data on childhood cancer and diet come from studies of exposure to N-nitroso compounds (NOC) and risk of brain tumors. The overall category of N-nitroso compounds can be broken down into subgroups that include nitrosamines, nitrosamides, and nitrosoureas. N-nitroso compounds occur in our environment, as do substances that can combine to form these compounds. Nitrite, nitrogen oxides, and other nitrosating agents can react with nitrogen containing compounds such as amines, amides, and ureas to form N-nitroso compounds. Particularly relevant to the discussion of the relationship between diet and cancer are preformed N-nitroso compounds, nitrite, and nitrate, which can be reduced to nitrite in saliva.

Many N-nitroso compounds are potent mutagens and animal carcinogens. N-nitroso compounds have been found to be carcinogenic in a variety of tissues and organs in 40 animal species. Some N-nitroso compounds, when administered to pregnant animals, induce tumors in the offspring. Of particular relevance to childhood cancer is the fact that some nitrosoureas are potent nervous system carcinogens when given transplacentally (21).

Not only are N-nitroso compounds potent carcinogens, but also exposure to these compounds is widespread. Humans are exposed to N-nitroso compounds directly and to precursor compounds that can combine to form NOCs in the body. N-nitroso compounds have been detected in many common products including cigarette smoke, rubber, cured meats, cosmetics, alcoholic beverages, medications, pesticides, automobile interiors, water, air, and in some industrial settings (21–25). Almost all the data on the sources of N-nitroso compounds are on the occurrence of nitrosamines. Less is known about the distribution of other N-nitroso compounds, including the nitrosoureas that are transplacental nervous system carcinogens in animals.

Although N-nitroso compounds occur in the environment, most human exposure is thought to occur via endogenous synthesis. There is evidence that N-nitroso compounds
can be synthesized in the stomach and elsewhere in the body (21). Cured meats, baked goods, and cereal contribute most of the nitrite (a NOC precursor) in the diet (22,24). For nitrates, which can be converted to nitrites, vegetables are the main dietary source (22,24).

The endogenous formation of N-nitroso compounds induces tumors in animals. When animals are fed N-nitroso precursors, for example, nitrite and an amine, the expected compound, in this case a nitrosamine (21). There are substances that inhibit the formation of N-nitroso compounds from precursors in vivo, including vitamin C, vitamin E, selenium, and glutathione (21). In animals, these substances inhibit the formation of N-nitroso compounds from precursors and reduce the proportion of animals that develop tumors (25). In some studies, very large doses of vitamin C prevented 100% of tumors (25). In addition to inhibitors, accelerators of the nitrosation reaction are also known and include metal ions, thiocyanate, and certain carbonyl compounds (21).

Based on the animal data, particularly those concerning transplacental carcinogenesis, Preston-Martin et al. hypothesized that exposure to N-nitroso compounds during gestation increases the risk of brain tumors in children (3). Children whose mothers frequently ate foods containing N-nitroso compounds or nitrite were hypothesized to be at increased risk. The effect of nitrate-rich foods was more difficult to predict; vegetables contribute the majority of nitrates in the diet, but also contain vitamin C, an inhibitor of nitrosation. High intakes of vitamins C and E were hypothesized to decrease the risk because of their action as inhibitors of nitrosation reactions.

3. BRAIN TUMORS

Brain tumors are the second most common type of cancer among children in the United States, accounting for about 20% of these cancers. The annual incidence is approx 3/million children under the age of 15 and appears to have increased substantially since the early 1970s (1). A recent analysis suggests that the observed trend does not reflect a genuine increase, but rather a change in reporting and/or improved diagnosis (26). Some brain tumors occurring in children can be cured surgically, but others require a combination of surgery, radiation, and chemotherapy. With the use of multimodality therapy, survival from childhood brain tumors has improved to almost 50% (2), but survivors are often left with neurologic, cognitive, or endocrinologic problems.

Many different histologic types of brain tumor occur in childhood. The major categories are astrocytic glioma and medulloblastoma (sometimes referred to as primitive neuroectodermal tumor), which account for approx 50% and 20% of childhood brain tumors, respectively (27). Other types of glioma, ependymoma and oligodendroglioma, comprise another 10% of the total (27). The remaining brain tumors are soft tissue sarcomas, germ cell tumors, and tumors of unspecified type (27).

The patterns of incidence with age and gender differ among the histologic types (28). For example, astrocytic glioma affects boys and girls with equal frequency, but boys have a higher risk of medulloblastoma. The incidence of astrocytic glioma peaks between 4 and 8 yr of age compared to a peak before age three for medulloblastoma. These differences in demographic pattern suggest that the two major categories of childhood brain tumors might differ etiologically. On the other hand, the fact that all tumor types arise in the brain and, thus, share that environment, might imply a common etiology. Perhaps, some etiologic factors are common among different histologic types of
brain tumors and others are specific to particular types.

In most epidemiologic investigations, childhood brain tumors have been studied together as a single entity. If risk factors differed by type of brain tumor, one would expect the studies of all types combined to mostly reflect risk factors for astrocytic glioma, the most common type. For this reason, studies of all types and astrocytic glioma are discussed together and the single study of medulloblastoma is considered separately.

### 3.1. Maternal Diet

Epidemiologists have conducted eight case-control studies of childhood brain tumors that considered a possible role of pregnancy diet (Table 1 \(3,5–12\)). Five of the studies considered all brain tumors combined \(3,8–11\), two considered astrocytic gliomas \(5,7\) and one, medulloblastoma \(6\). In one of the studies of all brain tumors combined, analyses by tumor type were performed. The extent of the dietary data collected differs among the studies. In the earlier studies, the mothers answered questions about their frequency of consumption of a small number of foods relevant to the N-nitroso hypothesis. In later studies, investigators aimed to be able to calculate total intake of food components related to the same hypothesis, which required collecting data on many more foods, usually between 50 and 60. None of the studies has investigated overall diet, which is usually assessed by data on 100 or more foods, and thus, no data are available on macronutrients such as fat and protein and most micronutrients such as B vitamins and zinc.

When one considers the seven studies that investigated maternal diet in relation to all brain tumors or astrocytic gliomas, one finds fairly consistent results for nitrite-cured meat consumption. In the four largest studies and one small study, frequent consumption of cured meats was associated with about a doubling of risk \(3,5,7,9,12\). The two studies in which an association with cured meats as a group was not seen had small numbers of cases and therefore lower statistical power \(10,11\). Although one of these studies found no effect of cured meats as a group, it did observe an association with hot dogs \(10\). The remaining study was completely negative \(11\). In all four studies that appeared to collect information on hot dogs as an individual item, odds ratios of 1.4–2.3 were observed \(3,7,10,12\). In summary, frequent maternal consumption of nitrite-cured meats, in general, and hot dogs in particular, has been fairly consistently associated with elevated risk of all brain tumors and astrocytic glioma.

Two studies report findings on the relation between maternal cured meat consumption and medulloblastoma. One of these studies did not observe an association with cured meats as a group, although the odds ratio for bacon consumption was significantly elevated \(6\). In the other study, the results for medulloblastoma are not presented, but are reported to be similar to those for all brain tumors combined, i.e., an increased risk associated with all cured meats, bacon, and two other individual cured meats. Thus, the two studies with data on medulloblastoma both show an association with bacon consumption, but conflict for other individual cured meats and for all cured meats combined.

The evidence on the effect of fruit and vegetable consumption during pregnancy is quite limited. Statistically significant effects of maternal fruit consumption were not observed in the three studies of all brain tumors or astrocytic glioma \(7,9,11\). The only suggestion of a protective effect of fruit comes from nonsignificant findings in two of the studies. In one, investigators observed decreased risk with frequent consumption of fruit \(7\), although no individual fruit, including citrus, had a significant effect. In the
other study (11), oranges and orange juice were associated with a decrease in risk, which was not statistically significant. In contrast to the findings for all brain tumors and astrocytic glioma, fruit overall, citrus fruit, and peaches were associated with lower risk of medulloblastoma (6). The limited data on fruit eaten during pregnancy suggest a stronger association with medulloblastoma than with astrocytic glioma.

The evidence linking vegetables with lower risk of brain tumors is stronger than that for fruit. McCredie et al. observed a trend of decreasing risk with increasing consumption of vegetables (9). In another study (11), frequent consumption of carrots, leeks, and green peppers was associated with decreased risk; results were not presented for all vegetables combined. The study of astrocytic glioma observed a risk that was lower, but not significantly so for vegetable consumption (7). For medulloblastoma, a strong, significant effect for vegetables was observed with individually significant effects of green salad, spinach, and sweet potatoes (6). The evidence, although limited, suggests a possible protective effect of vegetable consumption for all types of childhood brain tumors.

Vitamin supplements are also sources of vitamin C and other nitrosation inhibitors. Several studies (3,7,10,12) observed decreased risk with multivitamin use during pregnancy, although the results have been nonsignificant generally. In the study of medulloblastoma, use of multivitamins at any time during pregnancy did not affect risk, but use in the first 6 wk of pregnancy appeared to lower the risk significantly (6).

In three studies, the investigators calculated the mother’s intake of selected vitamins and other food components from the information on the foods she ate and the frequency with which she ate them (6,7,11). Intake from supplements was not included. The food components selected were generally those relevant to the N-nitroso hypothesis, as this hypothesis motivated the choice of food items. Only in the study of medulloblastoma were any of the food components studied significantly related to risk. In that study, intakes of vitamin C and nitrate were associated with lower risk (6). The association with nitrate reflects that with vegetable consumption as vegetables are the major source of nitrate. Bunin et al. (7) and Cordier et al. (11) observed nonsignificant decreases in risk with higher vitamin C intake for astrocytic glioma and all brain tumors, respectively. The N-nitroso precursors, nitrite or nitrate, and dimethylnitrosamine were not associated with increased risk (6,7).

Although the studies of maternal diet focused on the N-nitroso hypothesis, a few unrelated foods were included. Caffeinated beverages did not appear to affect risk of either astrocytic glioma or medulloblastoma (6,7). No effect of charcoal broiled foods on risk of brain tumors overall was noted (10). In the study of medulloblastoma, intake of folic acid appeared to decrease the risk (6), but the original hypotheses were unrelated to folate and, therefore, the assessment of folic acid intake was incomplete. As cited previously, risk of medulloblastoma also appeared to decrease with maternal use of multivitamins early in pregnancy. Intriguingly, the findings for folate and multivitamins are similar to those for neural tube defects and other congenital anomalies (29–34).

The studies discussed above were motivated mainly by the N-nitroso hypothesis. The N-nitroso hypothesis predicts that cured meat is associated with higher risk and fruit, especially those types rich in vitamin C, with lower risk. Vegetables, which contain nitrates and vitamin C, might either raise or lower the risk. Vitamin supplements, which usually contain vitamin C and other inhibitors of NOC formation, would also be expected to lower the risk. The finding of an association with maternal cured meat consumption in most studies of all brain tumors or astrocytic glioma supports the hypothesis. The evi-
ence linking fruit consumption to lower risk of the same groups of brain tumors is weak, as there were no statistically significant findings. The evidence for vegetables is stronger with significant associations observed for all vegetables combined or some individual vegetables in two of the three relevant studies. For vitamin supplements, no significant protective effects were observed. In summary, current evidence on maternal diet and risk of childhood brain tumors as a group or of astrocytic glioma supports the N-nitroso hypothesis to some extent, but important inconsistencies exist.

Two studies reported findings for medulloblastoma. Strong, protective associations were observed between fruit and vegetable consumption in the one study that reported on these food groups. The reports conflict regarding cured meats as a group, with one study reporting an increase and the other reporting no association. Both studies observed an elevated odds ratio for frequent bacon consumption. For medulloblastoma, the observations regarding cured meat, fruits, and vegetables can be seen as supporting the hypothesis, although the data are quite limited and inconsistencies exist.

In some instances, inconsistencies with the hypothesis can be explained by limitations of the data. For example, calculated intake of dimethylnitrosamine has not been associated with higher risk. This nitrosamine was used as marker for all N-nitroso compounds because data on the concentration of other compounds in foods are extremely limited. However, in animal studies, nitrosoureas rather than nitrosamines induce brain tumors. Another example concerns the fact that vitamin supplements are predicted to decrease risk, but have not been observed to do so in a statistically significant way. In the populations studied, however, nearly all women took supplements, making it unlikely that a real effect could be observed. Furthermore, the animal data predict that the timing of intake of supplements and foods rich in an inhibitor of nitrosation such as vitamin C may play a role. Eating such foods or taking supplements with cured meat would inhibit the formation of N-nitroso compounds and therefore lower the risk. If the cured meat was eaten at one time and the inhibiting supplement or food later, the risk would not be as low.

Although limitations of the data collected may explain the inconsistencies with the N-nitroso hypothesis, other possibilities must also be considered. For example, the association of maternal cured meat consumption with increased risk of childhood brain tumors is compatible with causal exposures other than N-nitroso compounds. Perhaps, a dietary characteristic correlated with frequent cured meat consumption is the causal exposure. For example, high fat or low β-carotene intake may explain the observed association with cured meat consumption. Inconsistencies between the data and the hypothesis might also have occurred by chance as a result of the few studies, most of which had relatively small sample sizes. Similarly, the apparent differences in dietary findings between astrocytic glioma and medulloblastoma might reflect chance variation rather than distinct etiologies. Future research should examine maternal diet during pregnancy in a comprehensive way, collecting data on macronutrients such as fat and micronutrients such as folate, as well as on food components relevant to the N-nitroso hypothesis. With these data, epidemiologists will be able to further test the hypothesis, as well as more extensively investigate the role of maternal diet in general.

### 3.2. Child's Diet

Seven studies have investigated some aspects of the child’s diet as possible risk factors (3,4,6–8,10,11). As for maternal diet, the interest in the child’s diet is motivated by
the N-nitroso hypothesis. In all studies, the information collected on the child’s diet was limited to vitamin supplements and no more than 15 food items. Researchers considered the child’s diet in the first year of life in three of the studies and the child’s usual diet before diagnosis in the other four studies.

Little evidence on infant diet supports the N-nitroso hypothesis. The N-nitroso hypothesis predicts a protective effect of vitamin C and therefore of fruit, especially citrus, and vitamin supplements. The only statistically significant finding that supports the hypothesis comes from the study of medulloblastoma (6), in which eating fruit in the first year of life was associated with decreased risk compared to eating no fruit at all. In a study that included all types of brain tumors, McCredie et al. also observed a decreased risk associated with fruit consumption, although it was not statistically significant (8). An effect of fruit was not seen in the study of astrocytic glioma (7). None of the three studies observed a decreased risk associated with orange juice or vitamin supplements, although a few odds ratios of less than 1.0 were noted for the latter. In addition, Cordier et al. noted an apparent protective effect of supplemented powdered milk (11). The studies collected data on consumption of cured meats, but few infants eat these products and no significant associations were observed. Although no strong findings resulted, more comprehensive examination of infant diet may be a fruitful area of research.

Four studies investigated the child’s usual diet before diagnosis and could better address the cured meat question (3,4,10,11). Only one of the four observed a significant association with cured meat consumption (3) and, in that study, when child and maternal consumption were analyzed simultaneously, the child’s cured meat consumption was not associated with risk. The evidence, then, does not strongly suggest a role for cured meat consumption by the child. However, Sarasua and Savitz found evidence of a possible synergistic effect between cured meats and vitamin supplements (10). They observed odds ratios of 3.2–6.8 for the joint effect of high-cured meat consumption and lack of vitamin supplement use. The possible synergistic effect is consistent with the N-nitroso hypothesis. Animals fed an N-nitroso precursor and a nitrosating agent along with vitamin C produced smaller amounts of N-nitroso compounds and developed fewer tumors than those not given vitamin C (21,25). Of the two other studies that investigated vitamin supplement use, one observed an apparent protective effect (4,11). The studies of childhood diet illustrate the need to analyze multiple aspects of diet simultaneously and to analyze the mother’s and the child’s diet simultaneously.

To our knowledge, only one study has investigated serum micronutrient levels in children in relation to cancer risk. Malvy et al. compared serum levels of antioxidant micronutrients in children with cancer to those in controls (35) and observed that children with brain tumors had lower levels of β-carotene and vitamin E compared to controls. Whether these differences reflect a protective effect of antioxidants or metabolic and nutritional disturbances of the brain tumor itself is not known.

3.3. Comparison with Adult Brain Tumor Studies

Researchers have conducted a number of studies that investigated dietary factors relevant to the N-nitroso hypothesis in relation to brain tumors in adults. The six studies of glioma are discussed below as this category of tumors includes the astrocytic gliomas, which are the most common brain tumor at all ages (36–41). Some of these researchers have also studied meningiomas, but these studies are not discussed here as these tumors are very rare in childhood. Three studies observed an increased risk asso-
cated with cured meat consumption overall and/or individual products (38,39,41) and three did not (36,37,40). Even in the studies that observed associations, the evidence is not strong. In one study, for example, men but not women appeared to incur increased risk from frequent eating of cured meats (41). The results for fruit are similarly inconsistent. Although one study observed protective effects of several types of fruit (37), other studies have generally not replicated these results. The results for citrus fruit do not suggest a protective effect. Although one study observed an association of oranges with decreased risk (36), there was no association with orange juice in the same study and no association with citrus in four other studies (36,39–41). The evidence on vitamin supplements is not more convincing. Two studies observed no effect (40,41); one observed a protective effect of any vitamin supplement (36) and one a protective effect of vitamin C and vitamin E supplements (37). In adults, the results on cured meat and supplements are conflicting and the evidence on citrus fruit suggests a lack of association. The evidence from studies of adult glioma seems less consistent with the N-nitroso hypothesis than that for childhood brain tumors.

4. LEUKEMIA

Leukemia accounts for about one-third of all cancer in children under age 15. In the United States, the annual incidence rate of leukemia in children is 4/100,000 with about 70% surviving at least 5 yr (1,2). Three-quarters of leukemias in children are classified as acute lymphocytic leukemia (ALL) and 15% as acute myeloid leukemia (AML). Other types of leukemia and leukemias not categorized as a specific type make up the remaining 10%. Similarly to the different types of brain tumors, different types of leukemia vary in patterns of incidence (42). ALL in children is more common in males than females and in whites than blacks, and peaks in incidence between ages 3 and 5. The incidence patterns for AML are quite different; male–female and white–black differences are slight and incidence is fairly constant throughout childhood. Very few data exist on any aspect of diet in relation to childhood leukemia.

A specific hypothesis regarding diet has been put forth for leukemia, particularly in infants (42). In the great majority of infant leukemias, the leukemia cells have abnormalities in band q23 of chromosome 11. Leukemias that occur after cancer treatment with epipodophyllotoxins, a class of chemotherapeutic agents, also have 11q23 abnormalities. Epipodophyllotoxins inhibit an enzyme called topoisomerase II, which is necessary for DNA replication. If epipodophyllotoxins inhibit topoisomerase II and increase the risk of leukemias with 11q23 abnormalities, perhaps other inhibitors of this enzyme also increase the risk of the same leukemias. Other inhibitors of topoisomerase II exist in nature, including certain flavonoids and medications (43,44). Flavonoids, substances found in plants, occur in the diet in fruits, vegetables, herbs, beans, wine, beer, and other plant derived foods (45). Medications that inhibit topoisomerase II include quinolones, which are used to treat urinary tract infections (46).

The specific hypothesis of Ross et al. regarding leukemia is that maternal exposure to topoisomerase II inhibitors during pregnancy increases the risk of leukemias with 11q23 abnormalities in infants (42). According to the hypothesis, children of mothers who frequently ate fruits, vegetables, beans, and other plant-derived foods would be at higher risk of leukemia. Paradoxically, these foods and flavonoids themselves have
been associated with a decreased risk of some adult cancers (47). Perhaps, as Ross et al. speculate, flavonoids affect fetuses and adults differently. Fetuses are rapidly growing and have high rates of cell division and thus high levels of topoisomerase II, while adults have much lower rates of cell division and topoisomerase II activity. Ross et al. suggest that topoisomerase II inhibition may be detrimental to a rapidly growing fetus.

Ross et al. investigated their hypothesis in a preliminary study of leukemia from birth to 12 mo of age (15). Mothers of cases and controls who had participated in previous studies of leukemia were recontacted and asked about their intake of 26 food items. Mothers of 84 cases and 97 matched controls were interviewed. A priori, the following ten foods and beverages were considered to contain topoisomerase II inhibitors: beans, fresh vegetables, canned vegetables, fruits, soy, regular coffee, wine, black tea, green tea, and cocoa (as a beverage). Frequent intake of fresh vegetables and regular coffee was associated with increased risk. When total consumption of all ten inhibitor-containing foods was investigated, no association was observed. However, when the two subgroups of infant leukemia were analyzed separately, the results were quite different. For AML, increasing intake of foods containing topoisomerase II inhibitors was significantly associated with risk, with odds ratio of 9.8 for high consumption. Among the individual food items, beans, fresh vegetables, and fruit showed significant trends for AML. For the other subgroup, ALL, neither the inhibitor-containing foods as a group or any of the composite foods were significantly associated with risk. The authors recommend extreme caution in interpreting these results. Chance could explain the findings because of the small numbers of subjects in certain categories of intake and leukemia type. Selection bias is another possible explanation since only 84 of 303 eligible cases could be reached and interviewed for this preliminary study. However, these findings demonstrate the need to investigate the hypothesis further.

Two groups of investigators have examined the effects of a small number of dietary factors related to the N-nitroso hypothesis (10,13). Although animal data have not linked N-nitroso compounds with leukemia, the potency of these carcinogens and the possibility of enhanced potency through transplacental exposure suggested investigation. Peters et al. conducted a study in California of 232 children ages 0–10 with leukemia and the same number of controls (13). Sarasua and Savitz included all types of cancer before age 15 in their study and present analyses that compare the 56 cases of ALL to the 206 controls (10). In neither study was diet the major focus and thus, the amount of information collected on diet is limited. Peters et al. collected data on 11 food items and Sarasua and Savitz on five food items and on vitamin supplements.

The study by Peters et al. observed an increased risk associated with frequent consumption of hot dogs by the mother, father, and child. The risks were high, with relative risks of about five, which were significant for the father and child. The investigators also observed elevated risks for eating of other cured meats by the child, but not by the parents. When the diet and other factors were considered simultaneously, the father’s and the child’s frequent hot dog consumption remained strongly and significantly associated with increased risk.

In contrast to the findings of Peters et al., Sarasua and Savitz did not observe increased risk associated with the mother’s or child’s eating of hot dogs. However, for the child, when the effect of frequent cured meat consumption and not using vitamin supplements was analyzed, a threefold increased risk was observed for each of the cured
meats, including hot dogs.

Peters et al. investigated another dietary item, citrus fruit, relevant to the N-nitroso hypothesis because of its concentration of vitamin C. Consumption of citrus fruit or juice by the child or the mother did not appear to influence risk.

A few foods not related to the N-nitroso hypothesis were also studied. Both studies included hamburgers among the food items in the questionnaire. In the larger study of Peters et al., no effect was observed, although Sarasua and Savitz's smaller study observed a doubling of risk. Charbroiled meats did not affect risk of leukemia in either study. The increased risk associated with the child's cola drinking (13) disappeared when adjusted for hot dog eating and other variables.

Another study with data relevant to the question of childhood leukemia and diet was conducted in Shanghai, China (14). In a case-control study with 309 cases and twice as many controls, use of cod liver oil for more than a year appeared to decrease the risk of both ALL and acute nonlymphocytic leukemia. Cod liver oil contains vitamins A and D.

The study of serum antioxidant levels cited in the brain tumor section also presented data on leukemia (35). Compared to controls, children with leukemia had lower serum levels of β-carotene, retinol, selenium, and zinc. The interpretation is difficult, as the differences may reflect the effects of the leukemia itself rather than etiologic influences.

Knowledge of adult leukemia does not add much to this discussion. A case-control study of acute leukemia in Poland included questions on frequency of consumption of about 40 food items (48) and observed frequent drinking of milk and consumption of poultry to be associated with higher risk and frequent eating of vegetables with lower risk. To our knowledge, no other case-control or cohort study has investigated the relationship between diet and leukemia in adults. In an ecological study of data from 24 countries, total and lymphocytic leukemia incidence was significantly correlated with total calorie intake (49). Countries with high-calorie intake tended to have high leukemia incidence and those with low-calorie intake had a lower incidence. Findings from ecological studies can provide only indirect evidence, but the international correlation fits well with animal data that calorie or protein restriction reduces the incidence of leukemia (50).

The data on diet and childhood leukemia, although limited, suggest that further study may be productive. Any observed dietary association with childhood leukemia is worthy of pursuit, as we still know very little about the etiology of this disease. The preliminary results on foods containing topoisomerase II inhibitors are intriguing and deserving of further study. The findings concerning hot dogs and vitamins are consistent with the N-nitroso hypothesis, although the findings on citrus fruit do not fit. The association with hot dog consumption might also be consistent with risk factors of fat intake, total calories, infrequent vegetable consumption, and weight. Clearly, comprehensive studies of diet and childhood leukemia are required.

5. OTHER CANCERS

A few studies of other childhood cancers have reported isolated findings related to diet. A small study of rhabdomyosarcoma observed an increased risk associated with the
child’s eating of organ meats (51). In a much larger study designed to follow-up on this and other findings, investigators were unable to confirm the observation (S. Grufferman, personal communication). A decreased risk of retinoblastoma was observed in relation to use of multivitamins by the mother during pregnancy (52).

6. VITAMIN K

The role of vitamin K has been studied in relation to its administration to newborns rather than as a component of diet. In many industrialized countries, newborns are routinely given vitamin K to prevent hemorrhagic disease, unexpected bleeding in previously healthy neonates. In 1990, Golding et al. reported an association between receiving vitamin K as a newborn and development of cancer before age 10 (53). The finding of an approximate doubling of risk arose unexpectedly in a nested case-control study of children born in 1970 in Great Britain. The results of a second study in Great Britain observed an increase in risk of similar magnitude for vitamin K administered intramuscularly, but not orally (54). The finding corroborated the first study as in 1970, the year of birth of the children in that study, vitamin K was almost always given intramuscularly. These two studies raised concern as newborns in many industrialized countries receive vitamin K routinely by intramuscular injection. Oral vitamin K can be given, but is less effective at preventing hemorrhagic disease. Swedish researchers studied 1.3 million infants born full term after uncomplicated delivery between 1973 and 1989 (55). By record linkage, these children were followed until 1992; approx 2350 children in the cohort developed cancer. No increase in risk of cancer overall or of leukemia was observed with exposure to intramuscular vitamin K. Similarly, a United States study did not observe cancer risk to be associated with vitamin K given to neonates (56). When the four studies are considered together, it seems unlikely that vitamin K given neonatally is a major cause of cancer during childhood.

7. CONCLUSION

Studies of the relationship between diet and risk of childhood cancer are few and most have focused on one hypothesis. Only the two most common cancers of childhood, leukemia and brain tumor, have been studied in any detail. Our knowledge of the role of diet in the etiologies of these cancers is meager. Nonetheless, the findings of the few studies suggest that diet does play a role in at least some childhood cancers. Future research will elucidate the particulars and extent of the role.

8. RECOMMENDATIONS

Maternal cured meat consumption has been fairly consistently associated with brain tumor risk in children, but whether the association is causal is unclear. Also, the frequency of cured meat consumption that was associated with higher risk varied greatly among the studies. For these reasons, a specific recommendation is not possible or appropriate. However, since cured meats are high in salt and fat, nutritional concerns other than the child’s cancer risk, such as keeping one’s fat and salt intake within recommendations, re-
quire that cured meats be eaten in no more than moderate quantities. Women eating cured meats several times a week or more might wish to reduce their intake during pregnancy.

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1. INTRODUCTION

This chapter will focus on lifestyle factors associated with cancers of the esophagus and stomach. Unlike such major cancers as prostate and breast, whose etiologies remain obscure at the present time, hindering primary prevention, cancers of the upper gastrointestinal tract offer well-defined intervention opportunities. Epidemiologic studies have clearly established the important role of alcohol, tobacco, and diet, and recent findings have documented the relation between infection with Helicobacter pylori and cancer of the stomach. These factors and their interactions will be discussed for cancers of each of these two sites, which together account for approx 35,000 new cases and 26,000 deaths annually in the United States (1).

2. CANCER OF THE ESOPHAGUS

For many years, cancer of the esophagus in the United States, and in most areas throughout the world, was virtually synonymous with squamous cell carcinoma (2). Hence, most of the established risk factors for esophageal cancer are specific to this cell type, which comprised the vast majority of cases in studies of this cancer. Recent shifts in the histopathologic cell type have given rise to a rapid increase in the incidence of adenocarcinoma of the esophagus in the United States, particularly among white males (3). Because of the increasing importance of esophageal adenocarcinoma, a separate section will consider this entity, which may differ in etiology from squamous cell carcinoma.

2.1. Squamous Cell Carcinoma

2.1.1. TOBACCO AND ALCOHOL CONSUMPTION

Both tobacco and heavy alcohol consumption are well-established risk factors for esophageal carcinoma. In the United States and other Western countries, over 90% of the risk can be attributed to the individual and joint effects of tobacco and alcohol (4).

An early study by Wynder and Bross (5) graphically examined the interaction between alcohol and tobacco and the data suggest a multiplicative effect. Tuyns et al. (6) evaluated this relation more formally in data from a case control study in Brittany. At
the highest level of consumption of both alcohol (≥ 121 g ethanol /d) and tobacco (≥ 30 g/d) the risk of esophageal cancer was 156 relative to non- or light consumers. The increased risk associated with alcohol consumption appears exponential whereas increased tobacco smoking appears to yield a more linear increase. Saracci (7) estimates that the excess risk because of the interaction of alcohol and tobacco is about 25-fold.

Data from a recent case-control study in Italy are presented in Table 1 (8). Study subjects included 271 male cases and 1754 male controls with acute illnesses unrelated to tobacco and alcohol consumption. Even with a reference category that included moderate alcohol consumption (< 35 drinks/wk) by nonsmokers, the estimated relative risk (RR) of esophageal cancer among heavy smokers (≥ 25 cigarettes/d, ≥ 40 yr) and very heavy drinkers (≥ 60 drinks/wk) is 22. This report was updated in 1994 to include women (9). Among alcohol drinkers (any vs none), similar risks were observed for women and men, 3.0 and 4.7, respectively; however, male abstainers had a twofold increased risk while female nondrinkers had a reduced risk, 0.7, compared with light to moderate drinkers. This study of esophageal cancer fails to support the hypothesis posed by Blume (10) that women may be more susceptible to the effects of alcohol, at least for this particular cancer site.

Whether the increased risk of esophageal cancer attributed to alcohol use is a function of the dose of ethanol or whether the type of alcoholic beverage and its other constituents play a role has also been examined, most recently in a Japanese study by Hanaoka et al. (11). Their findings confirm those of others that indicate the amount of alcohol consumed, rather than any particular type, is the primary determinant of risk.

2.1.2. THERMAL IRRITATION

Thermal injury as a result of drinking very hot liquids has been suggested to increase risk of esophageal cancer by increasing susceptibility to other carcinogenic exposures (12,13). This hypothesis has some support in both ecologic and analytic studies. Persons living in regions of the world with high rates of esophageal cancer, such as northern Iran and Siberia, are reported to drink excessively hot tea (14,15).

Martinez (16) found that more cases than controls reported drinking hot, rather than warm or cold, coffee in Puerto Rico. Both Segi (17) and Hirayama (18) found an increased risk of esophageal cancer in persons consuming hot tea gruel. In Latin America, several studies have examined the role of maté drinking. DeStefani et al. found a strong association between hot mate consumption and risk of esophageal cancer in Uruguay (19). An earlier case-control study in Brazil found no such association (20). In 1994, Castelletto et al. examined the role of maté in an Argentinean case-control study (21). They found alcohol, tobacco, and barbecued meat, but not hot maté, to be the primary risks factors.

A study of chronic esophagitis, a precursor lesion for esophageal cancer, in a high-risk region in China lends support to an etiologic role of thermal injury (22). A greater than fourfold excess of mild and moderate esophagitis was found in young persons 15–26 yr of age consuming burning hot beverages (odds ratio [OR] 4.39, confidence interval [CI] 95% 1.72–11.3). This study design minimizes recall/response bias because case-control status is not known at the time of interview, and suggests that this factor may be important at a relatively early stage in the development of this cancer.

2.1.3. NUTRITION

2.1.3.1. Dietary Studies. Fruits and fresh vegetables are consistently associated in studies throughout the world with decreased of esophageal cancer, even after control-
ling for tobacco and alcohol use. Deficiencies of vitamin C, one of several micronutrients contained in fruits and vegetables, have been reported in several areas of the world with exceptionally high rates of esophageal cancer. These include northern Iran (14), Linxian County, China (23), and northern and eastern Siberia (15), among others. Other dietary deficiencies are also strongly associated with esophageal cancer risk; these include iron, riboflavin, niacin, molybdenum, zinc, and other trace elements (24).

The 1961 report by Wynder and Bross noted significantly lower consumption levels of green and yellow vegetables among male cases compared to controls, and a non-significantly lower consumption level of fruit (5). Potatoes (RR = 0.4, \( p < 0.05 \)) and bananas (RR = 0.3, \( p < 0.01 \)) were determined to be protective in a case-control study in Singapore (25). Frequent consumption of 16 different fruits and vegetables was associated with decreased risk of esophageal cancer in Iran (26). Relative risks for high vs low consumption levels ranged from 0.4–0.9 and findings for 10 of the 16 foods were significantly protective.

A significant inverse trend (\( p < 0.001 \)) was reported between monthly vitamin C consumption and esophageal cancer in white males in New York state (27). A weaker but significant inverse association was observed for vitamin A intake (\( p = 0.03 \)). A five-fold reduction in risk in the highest tertile of fruit and vegetable consumption (> 81 times/mo) was also found. A more recent report from New York found no association with vitamin C derived from vegetables (28). However, in this study only 24% of the eligible cases were included and they may not be representative of the total series of cases.

Ziegler et al. (29) found significant inverse associations between relative risk of esophageal cancer and five indicators of general nutritional status, including total fruit and vegetable consumption (RR = 0.5, \( p \)-trend < 0.05). This case-control study focused on high-risk black males in Washington, DC. An index of vitamin C intake yielded an estimated relative risk of 0.55 (\( p \)-trend < 0.05) for the highest tertile of consumption. The only other micronutrient significantly inversely associated with risk was riboflavin.

Two case-control studies conducted in the high-risk region of Calvados, France, found a protective effect of vitamin C on esophageal cancer risk (30,31). Approximately threefold significant reductions in risk were observed at the highest level of intake of

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Table 1

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Alcohol (drinks per week)</th>
<th>Adjusted Odds Ratios* for Cancer of the Esophagus by Alcohol and Tobacco Consumptionb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 35</td>
<td>35–59</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>1.0c</td>
<td>2.2</td>
</tr>
<tr>
<td>Light</td>
<td>2.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>4.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Heavy</td>
<td>8.4</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* Adjusted for age, residence, education, and profession.

b Adapted from Barón et al. (8).

c Reference category.
citrus fruits and of dietary vitamin C. Similarly, DeCarli et al. (32) reported a relative risk of 0.3 (0.1–0.6) for high-level fruit consumption and nonsignificant reductions in risk for high-level vegetable intake. In India, Notani and Jayant (33) found a more modest reduction from high-level fruit intake (RR = 0.8, 0.5–1.3), but a significant risk reduction among daily consumers of vegetables (RR = 0.4, 0.2–0.7).

Two 1988 reports support the findings of others indicative of protection from high intake of dietary vitamin C and fresh fruits (34,35). Brown et al. (34) found a significant halving in risk in the highest tertile of consumption of citrus, fruit, all fruits combined, and dietary vitamin C ($p < 0.05$). A relative risk of 0.4 (0.2–0.8) for high-level consumption of raw vegetables and fresh fruit was found in the California study of Yu et al. (35). Li et al. (36) found no reduction in esophageal cancer risk associated with fruit consumption in a high-risk region of China, but a homogeneously low level of intake of fruit in this population makes it a poor one in which to evaluate the association (37). Strong protective effects ($p$-trend < 0.001) associated with consumption of citrus fruits and other fruits were reported by Cheng et al. (38) who conducted a large case-control study in Hong Kong. The proportion of esophageal cancer cases attributable to low-consumption levels of citrus fruits in this population was estimated to be 26%. A retrospective cohort study of esophageal cancer in Linxian, China, reported a significant reduction in risk associated with regular consumption of fresh vegetables, RR = 0.66 (0.44–0.99) (39).

A large Italian study of esophageal cancer in lifelong nonsmokers afforded the opportunity to evaluate other risk factors in the absence of residual confounding by tobacco use (40). Although the major risk factor was not unexpectedly alcohol, green vegetables and fresh fruit were associated with significantly reduced relative risks of 0.6 and 0.3, respectively. Similar reductions in risk were associated with β-carotene intake. The estimated relative risk for the combination of high alcohol and low β-carotene was 8.6, with an attributable risk of approx 45%.

Several dietary factors in addition to fruits and vegetables and their constituent micronutrients have been proposed as candidate protective factors, although the epidemiologic evidence to date is considerably more limited. One such factor is green tea, *Camellia sinensis*. Experimental studies have demonstrated antimutagenic and anticarcinogenic effects, especially in the esophagus (41–44). Findings in a recent population based case control study in China provide some support to this hypothesis (45). After adjustment for confounders including tobacco and alcohol, a significant halving of risk was observed in women drinking green tea (OR = 0.50, CI 95%: 0.30–0.83) and an inverse dose response was observed. The findings in men were not statistically significant; however, a significant protective effect was observed in both men and women who did not smoke or drink alcohol. Since green tea, as well as other drinks, can be consumed at hot temperatures and since excessively hot fluids have been associated with increased risk of this cancer, the relation between drinking burning-hot fluids was also evaluated. The protective effect of green tea was limited to tea taken at normal temperatures.

Ginseng, which may be taken as a tea, powder, or as a slice of the root, has also been proposed as a potential anticarcinogen. Unlike the polyphenols in green tea, no specific component or mechanism has been elaborated (46,47). Yun and Choi (48) reported a case-control study in Korea where ginseng is commonly used. The relative risk of esophageal cancer associated with ginseng intake was 0.20 (CI 95%: 0.09–0.38) after adjustment for tobacco, alcohol, and other confounders. This large reduction in risk was observed in both smokers and nonsmokers. Additional studies are obviously necessary
2.1.3.2. Biochemical Studies. A number of studies have examined biochemical nutritional indicators in blood or tissue, with particular focus on antioxidants. Chen et al. (49) collected blood specimens from a sample of the population in 65 different countries in China and correlated the concentration of over 10 different antioxidants with county-specific mortality rates for several cancers, including esophageal. A highly significant inverse relation was found between esophageal cancer rates and both plasma ascorbic acid and selenium in men and selenium in women. Another study in a high-risk region of China found low levels of zinc (50). A recent population-based case-control study conducted in Washington state (51) found no significant difference in nail zinc concentrations in esophageal cancer cases and controls, but a large and significant reduction in risk associated with dietary intake of zinc from foods and supplements: OR of 0.5 and 0.1 for the middle and upper tertile of consumption, respectively, trend $p < 0.001$. Other elements in nail tissue associated with esophageal cancer were iron (OR $= 2.9$ high vs low levels), calcium (OR $= 2.6$) and cobalt (OR $= 1.9$). Although this study suggests a number of differences in mineral levels of cases and controls reflecting differences in intake, metabolism or both, additional investigation is warranted to determine which, if any, of these findings is etiologically meaningful.

2.1.3.3. Chemoprevention Studies. Chemoprevention as defined by Sporn and Newton (52) is prevention of cancer with pharmacological agents used to inhibit or reverse the process of carcinogenesis. In this relatively new field, which has grown in acceptance in the 1980s and 1990s, esophageal cancer is one of the few cancer sites for which results from completed trials are available.

Muñoz et al. (53) reported findings from the first short-term intervention trial in 1985. A total of 610 subjects ages 35–64 in the high-risk region of Huixian, China, were randomized to receive 15 mg (50,000 IU) retinol, 200 mg riboflavin, and 50 mg zinc or placebo once per week for 13.5 mo. Five hundred sixty-seven participants completed the trial and underwent endoscopy for histological diagnosis of premalignant lesions of the esophagus (esophagitis, atrophy, dysplasia). The combined treatment had no effect on the prevalence of precancerous lesions of the esophagus. It should be noted, however, that the dose was relatively small and the intervention period short. Micronuclei in exfoliated cells of buccal and esophageal mucosa were evaluated in 170 study subjects from this same trial as an indicator of chromosomal damage (54). No reduction in micronuclei was found in subjects after treatment, but a significant reduction in the percentage of micronucleated cells was observed in treated subjects (0.19%) compared to the placebo group (0.31%), $p = 0.04$. In a third report from this same trial, Wahrendorf et al. (55) reanalyzed data by blood levels of retinol, riboflavin, and zinc at the beginning and end of the trial because improvement in blood retinol and zinc levels had been observed in the placebo group as well as the actively treated group. Individuals who had large increases in retinol, riboflavin, and zinc blood levels were more likely to have a histologically normal esophagus at the end of the trial regardless of treatment group.

Two large intervention studies conducted in the high-risk population of Linxian, China, were recently reported (56,57). A 6-yr randomized trial of daily vitamin/mineral supplementation vs placebo found no significant reductions in cancer incidence or mortality among adults with preexisting precancerous lesions of the esophagus (56). The larger trial in this same area included 29,584 subjects from the general population randomly allocated to combinations of retinol and zinc, riboflavin and niacin, vitamin C
and molybdenum, and/or β-carotene, vitamin E and selenium in doses of one to two times US Recommended Daily Allowances. Significantly reduced total mortality (RR = 0.91, 0.84–0.99) and stomach cancer mortality (RR = 0.79, 0.64–0.99) were observed in those taking β-carotene, vitamin E, and selenium. No significant effects on mortality or cancer incidence, including esophageal cancer, were observed for any of the other vitamin/mineral combinations.

Wang et al. (58) evaluated whether any of the vitamin/mineral supplement combinations affected the prevalence of clinically silent precancerous lesions and early invasive cancers of the esophagus and stomach as determined by endoscopy and biopsy in this same trial. No significant reductions in risk of dysplasia or cancer were observed for any of the supplements, although retinol and zinc were suggestively associated with a lower risk of gastric cancer, OR = 0.38, p = 0.09. Similarly, Dawsey et al. (59) evaluated the effect of the single vitamin/mineral supplement used in the trial of persons with esophageal dysplasia to see if treatment reduced the prevalence of histological dysplasia or early cancer of the esophagus or gastric cardia. Modest, nonsignificant risk reductions were observed compared to placebo, (OR = 0.86, 0.54–1.38). The authors conclude that longer interventions with larger number of subjects are required to adequately evaluate the effectiveness of micronutrient supplementation in this high-risk population. In subjects from this same trial, Rao et al. (60) evaluated whether epithelial proliferation, an early step in carcinogenesis was reduced by treatment after 30 mo of intervention. The results were similarly inconclusive.

2.2. Adenocarcinoma

2.2.1. Barrett’s Esophagus and Medications

Barrett’s esophagus is characterized by the replacement of the lower esophagus, which is normally stratified squamous epithelium, by metaplastic columnar epithelium (61). This condition, attributed to chronic esophageal reflux, is believed to be premalignant lesion for esophageal adenocarcinoma (62).

Barrett’s esophagus displays a similar age, race, and gender distribution as does esophageal adenocarcinoma: it is most common in white males over age 40 (3,63). The reported incidence of esophageal adenocarcinoma in patients with Barrett’s is from 30 to over 100 times greater than the rate observed in the general population (63–66).

There also appears to be a familial form of this disease, inherited as an autosomal dominant trait (67–69). Two recent reports of families with the inherited form of Barrett’s provide additional support for Barrett’s as a precursor lesion (67,69).

A related hypothesis has proposed that the use of medications that relax the esophageal sphincter, and thereby promote reflux, may increase risk of adenocarcinomas of the esophagus and gastric cardia (70). Histamine H2 receptor antagonists used routinely for treatment of peptic ulcer and gastroesophageal reflux disease have also been proposed as an etiologic factor (71). In a 1995 report, Chow et al. (72) examined the relation between reflux disease and its treatment to risk of adenocarcinomas of the esophagus and gastric cardia. Significant increased risks of adenocarcinoma were associated with esophageal reflux (OR = 2.1, 1.2–3.6); hiatal hernia (OR = 3.8, 1.9–7.6); and esophagitis/esophageal ulcer (5.0, 1.5–16.4). Although a fourfold increased risk was associated with four or more prescriptions for H2 antagonists, the odds ratio was reduced to 1.5 (0.4–5.4), after adjusting for predisposing conditions. The relation with use of anticholinergics adjusted for number of conditions was actually inverse: risk de-
increased with increasing number of prescriptions (p-trend = 0.08). The study findings support the elevated risk of adenocarcinoma conferred by reflux disease, but indicate that the mechanism is not strongly related to treatment of reflux. An interesting, but as yet unconfirmed, new finding indicates an increased risk of esophageal adenocarcinoma among long-term users of theophylline-containing drugs (73). The significance of this finding is linked to the rising incidence of asthma and increasing use of asthma medications in the general population and its association with reflux disease.

2.2.2. Tobacco and Alcohol

Two population-based studies of cancers of the esophagus and gastric cardia conducted in western Washington state 1983–1990 were analyzed to evaluate risk factors for adenocarcinoma compared to squamous cell (74). Use of alcohol and cigarettes were significantly associated with increased risk of both histologic types, but the odds ratios were markedly higher for squamous cell carcinoma. For current smokers of 80+ pack-yr compared to nonsmokers, the odds ratios were 16.9 (4.1–6.91) for squamous cell carcinoma and 3.4 (1.4–8.0) for adenocarcinoma. Similarly, for persons who reported drinking 21 or more drinks/wk compared to <7/wk, the respective odds ratios were 9.5 (4.1–22.3) and 1.8 (1.1–3.1). Population attributable risk estimates found that cigarette smoking and alcohol together accounted for 87% of the squamous cell carcinomas, while for adenocarcinoma the estimate for cigarettes was 34% and 10% for alcohol consumption of seven or more drinks/wk.

Estimates of esophageal adenocarcinoma risk for alcohol and tobacco use by Kabat et al. (75) were similar: current smokers, 2.3 (1.4–3.9); 4+ oz of whiskey-equivalents per week, 1.9 (1.3–4.3). Brown et al. (76) also report that tobacco and alcohol are likely etiologic factors, but conferring lower magnitude risk than that associated with squamous cell cancers. The odds ratios at the highest level of smoking (≥ 40 cigarettes/d) and drinking (≥ 29 drinks/wk) were 2.6 (p-trend < 0.01) and 2.8 (p-trend < 0.05), respectively. Their study included white men from Atlanta, Detroit, and New Jersey. Significantly increased risks were also found associated with history of ulcer, especially duodenal, and with low social class. The authors note that alcohol and tobacco use, although associated with esophageal adenocarcinoma, does not explain the rapid increase in these tumors.

In 1997, a multicenter study of esophageal and gastric cancers reported an increased risk of squamous cell carcinoma and adenocarcinoma of the esophagus and adenocarcinomas of all sites in the stomach among smokers (77). Current smokers had a two- to threefold increased risk of adenocarcinomas of the esophagus and gastric cardia compared to a fivefold increased risk of squamous cell carcinoma of the esophagus. Although risk of these squamous cell tumors declined with duration of smoking cessation, risks of esophageal and cardia adenocarcinomas remained significantly elevated for more than 30 yr after cessation. This long lag suggests that the effect of tobacco on these tumors may be on tumor initiation.

2.2.3. Obesity and Diet

Two 1995 reports have linked obesity to adenocarcinoma of the esophagus (74,76). A threefold increased risk (p < 0.01) was observed at the highest level of body mass index (> 26.6 kg/m²) compared to the lowest in white men (76). No significant associations were found for dietary fat, total calories, meals eaten per day, or consumption of coffee and tea. A protective effect of high intake of raw fruit (OR = 0.4, p < 0.05) and
vegetables (OR = 0.4, p < 0.05) was observed. Vaughan et al. (74) report divergent associations for squamous cell and adenocarcinoma with body mass index. A significantly increased risk of adenocarcinoma was found at the highest decile of body mass index (OR = 1.9, 1.1–3.2), whereas body mass was inversely associated with squamous cell carcinoma. The population-attributable risk for body mass index above the 50th percentile was 18% for adenocarcinoma. These observations are consistent with esophageal reflux associated with obesity. These reports were confirmed in a large multicenter population-based case-control study (78) in which obesity measured by body mass index was found to be a strong risk factor for esophageal adenocarcinoma and a moderate risk factor for adenocarcinoma of the gastric cardia. The authors suggest that the increasing rates of adenocarcinomas of the esophagus and cardia may be explained in part by increasing prevalence of obesity in the United States population.

3. CANCER OF THE STOMACH

A steady decline in gastric cancer has been apparent in many countries for the past several decades. The declining rates were first noted in the United States as early as 1930 (79) and have persisted throughout this century (1). Survival rates have not appreciably changed (1,80) therefore, the decline in deaths cannot be attributed to better treatment and prolonged survival, but to actual declines in incidence that are now well documented (81). This decline, believed to reflect changes in environmental factors, has been referred to as an “unplanned triumph” since the shifts did not result from active medical or public health intervention and are believed to result from large shifts in food processing and consumption (82). It should be noted that the increase in esophageal adenocarcinoma documented in the previous section does include an increase in adenocarcinomas of the gastroesophageal junction and gastric cardia.

3.1. Histologic Types

Adenocarcinomas account for more than 97% of gastric cancers, and studies of etiology are generally limited to this histologic type (83). Building on an earlier observation that gastric carcinomas were often accompanied by features found in intestinal epithelium (84), Laurén (85) proposed a classification of adenocarcinomas into two subtypes, “intestinal” and “diffuse.” Many, but not all tumors, can be thus classified because some tumors contain characteristics of both types and others neither. Diffuse carcinomas, sometimes referred to as “endemic,” tend to occur with similar frequency throughout the world, whereas the distribution of intestinal or “epidemic” type tends to parallel the distribution of overall gastric cancer rates, i.e. this type is relatively more common in areas with high rates and lower where gastric cancer are low (86).

3.2. Risk Factors
3.2.1. Helicobacter pylori

Spiral-shaped bacteria in contact with gastric mucosa were first reported about 100 yr ago by Pel (87) and ignored for the next 90 yr. In 1983, Marshall (88) and Warren (89) reported isolating these bacteria in cultures of biopsies taken from patients with gastritis and peptic ulcers undergoing endoscopy. By 1994, the International Agency for Research on Cancer, World Health Organization, had determined that infection with H. pylori is carcinogenic to humans, and declared it a Group 1 carcinogen based on the
large body of research developed during the 11-yr period (90).

*H. pylori* infection is one of the most prevalent infections worldwide, with a range of 20–40% in developed countries and as high as 70–90% in some developing countries (91,92). Prevalence increases with age and no difference in seroprevalence has been found between males and females (93). Socioeconomic status including poor housing conditions, large family size, and low education attainment, is a predictor of prevalence of infection as well as of gastric cancer.

The role of *H. pylori* in gastric carcinogenesis has been explored in correlation and case-control studies, but this approach has yielded equivocal results, largely because of difficulties in determining temporality (90). Three different cohort studies provided material for nested case-control analyses that resolved the issue of temporality. *H. pylori* infection was determined by IgG antibodies in serum collected at the time of cohort enrollment 6–14 yr earlier. Forman et al. (96) found an approximate threefold increased risk of subsequent gastric cancer in cohort of Welsh men; Parsonnet et al. (97) reported a relative risk of 3.6 (1.8–7.3) in a cohort of men and women in California; and Nomura et al. (98) a sixfold significantly increased risk in Japanese-American men living in Hawaii.

The mechanisms by which *H. pylori* infection increases gastric cancer risk are not well-established and are the focus of ongoing investigation. *H. pylori* infection, the main cause of chronic gastritis, has been demonstrated to decrease the concentration of ascorbic acid in gastric juice (99–102). *H. pylori* infection is also associated with varying degrees of inflammation (103). In inflammatory states, nitric oxide may be generated and interact with reactive oxygen species forming new cytotoxic compounds (104,105). Thus, *H. pylori* infection has the potential to increase oxidative stress and decrease antioxidant capacity.

Because *H. pylori* infection is associated with several health outcomes in addition to gastric cancer, such as dyspepsia and peptic ulcers, the role of strain virulence factors has received attention. Risks of gastric adenocarcinoma and gastric atrophy, a premalignant condition, have been associated with *cagA*/H11001 strains compared to *cagA*/H11002 ones (106–108).

### 3.2.2. Tobacco and Alcohol

Although both tobacco and alcohol use are weakly associated with increased risk of gastric cancer, the strength and magnitude of the association is much less clear than that for esophageal cancer.

Early case-control studies of gastric cancer and alcohol intake were equivocal, with some reporting positive associations (109,110) and others none (111,112). Continued study has yielded similar mixed results. Correa et al. (113) found twofold elevations in risk of gastric cancer, at the highest level of alcohol intake for both whites and blacks in Louisiana. After controlling for other risk factors, wine (OR = 2.10, 1.13–3.89) and hard liquor (OR = 1.95, 1.14–3.34) were significantly associated with risk in whites, but not in blacks. A 1990 report of stomach cancer in Los Angeles males also found an increased risk (OR = 3.0, 1.1–8.7) at the highest level of total ethanol intake and significant risks for daily consumption of beer (114). The effect of alcohol was stronger for cancer of the gastric cardia than at other sites.

A twofold increased risk of stomach cancer was found for beer consumption in a German study, but wine and hard liquor were associated with decreased risk (115). This is in contrast to a French study that reported a very large relative risk (1.9, 3.3–14.3) as-
associated with heavy use of red wine (116).

Two cohort studies, however, suggest that alcohol is not an independent risk factor for gastric cancer. Nomura et al. (117) found no increased risk of gastric cancer associated with consumption of beer, wine or hard liquor in Japanese-American men living in Hawaii. Kneller et al. (118) likewise found no association for total alcohol or for any specific type.

More consistent findings link smoking to a 1.5 to threefold increased risk of gastric cancer (113–118); however, the overall increased risk has often failed to demonstrate a dose-response (109,117,119). The cohort study by Kneller et al. did find significant increases in risk with both increasing number of cigarettes smoked per day and pack-years of smoking (118). At the highest number of pack-years, the relation of risk was 2.3 (1.23–4.33) and for current use of 30 or more cigarettes/d the relative risk was 5.8 compared to nonsmokers. Although age at death did not significantly modify risk, the association with smoking was stronger for younger cases. The authors suggest that this finding may reflect a higher proportion of adenocarcinomas of the gastric cardia at younger ages and a stronger relation between smoking and cancers of the cardia than with cancers of other sites in the stomach.

3.2.3. SALTED, PICKLED, AND SMOKED FOODS

Salt has been demonstrated in animal studies to enhance gastric carcinogenesis (120–123). It has been suggested that the action of salt as a gastric mucosal irritant facilitates the action of carcinogens and thus salt acts as a cocarcinogen (124).

Epidemiologic studies also suggest an increased risk of gastric cancer associated with high salt intake when salted and pickled foods are included in total intake. Death rates throughout regions of Japan (125) were found to be correlated with consumption of salted fish and salted vegetables. A geographic correlation has also been demonstrated in China (126). Consumption of salt-cured meats, salted fish, and other salt-preserved foods has been associated with increased risk in case-control studies throughout the world (127–130). Several studies have also reported associations with the addition of salt to foods (127,131) or a reported “heavy intake” (132,133).

Many of the strongest findings have been noted in areas of the world where there is a wide range of intake including very high levels, such as in Korea (129). A recent nested case-control analysis reported by Friedman and Parsonnet (134) failed to find evidence that routine salting led to increased risk in a California study population. “Heavy” salt intake in US populations may be quantitatively less than “heavy” intake in other areas of the world and may not be sufficient to demonstrate an increased risk. For example, salted fish and salted vegetables in Japan may contain up to 30% NaCl compared to isotonic saline which is 0.8% (124,125).

Numerous N-nitroso compounds have demonstrated carcinogenicity (135). Based on studies of premalignant lesions of the stomach, it has been hypothesized that intragastric synthesis of N-nitroso compounds is a factor in the gastric carcinogenic process (136).

Two recently reported studies evaluated factors associated with in vivo nitrosamine formation in humans using the test developed by Ohshima and Bartsch (137) that measures urinary excretion of noncarcinogenic N-nitrosoproline after ingesting a given dose of proline. Mirvish et al. (138) found that men in rural Nebraska who drank water from private wells with a high-nitrate content excreted significantly higher N-nitroso proline than men drinking water with a low-nitrate content. Their findings parallel those of a
study in Denmark (139). Sierra et al. (140) used the nitrosoproline test in children living in high- and low-risk areas for stomach cancer in Costa Rica. They found the concentration excreted by children in the high-risk area significantly greater ($p < 0.04$) compared to children from the low-risk area. They also found that excretion was markedly reduced when ascorbic acid, an inhibitor of nitrosation reactions was given with the proline.

Associations between gastric cancer and dietary intake of nitrate, nitrite and preformed nitroso compounds are suggestive (141–146), but the validity of such indexes is not well established given the multiple sources, including food, water, and endogenous formation.

### 3.2.4. Fruits and Vegetables

Table 2 presents an extensive compendium of dietary studies of gastric cancer (111,117,118,129,132,141–165). The strong, consistent inverse association between consumption of fruits and vegetables is abundantly clear. Of the 26 studies described that specifically examined foods and food groups, 24 found a decreased risk of stomach cancer associated with high intake of one or more fruits and vegetable and the vast majority were statistically significant with up to twofold reductions in risk. Only two studies reported an increased risk of gastric cancer associated with fruits (118) or vegetables (128), and their findings do little to cast doubt on the apparent protective effect of fruits and vegetables. The findings of Tajima and Tominaga (128) stand in contrast to many case-control studies in Japan and elsewhere, and the study by Kneller et al. (118) was based on a very limited dietary questionnaire that increases the likelihood of misclassification.

### 3.2.5. Micronutrients

Consumption of fruits and vegetables serves as a dietary source of a plethora of vitamins, minerals, fiber, and less well-studied trace compounds. Many of these are highly correlated with one another, particularly when exposure is based on dietary assessment; therefore, a finding attributed to one may actually reflect the effect of another constituent from the same foods. The strongest findings, therefore, are based on biochemical studies, e.g., blood levels prior to cancer onset and chemoprevention trials, which actually test the efficacy of specific micronutrients in prevention. The micronutrients believed to be most strongly associated with reduced gastric cancer risk based on studies to date are vitamin C, β-carotene, and vitamin E/selenium.

Findings from dietary estimates of intake are also included in Table 2. Relatively high consumption of vitamin C and β-carotene is consistently associated with reduced risk of gastric cancer (132,141,143–145,147,150,154,155,157,160,163). Serological assessment also supports a role. Prospective studies that have evaluated vitamin C are scant because vitamin C deteriorates quickly unless specimens are acid stabilized prior to freezing (166). A large well-conducted cohort study, the Basel study (167), did have such material available. Mean plasma vitamin C was significantly lower in persons who died of cancer than in survivors: $47.61 \pm 1.78 \mu\text{mol}/\text{L}$ vs $52.76 \pm 0.44 \mu\text{mol}/\text{L}$, respectively, $p < 0.01$. The findings were also significant ($p < 0.05$) for persons who subsequently died of stomach cancer and their blood levels were even lower, $42.86 \pm 4.88$. Low plasma levels of vitamin C were associated with a relative risk of 2.38 for gastric cancer. Low plasma levels of carotene were similarly associated with significantly increased risk of overall mortality from cancer ($p < 0.01$) and cancer of the stomach.
### Table 2
Selected Epidemiological Studies of Diet and Stomach Cancer Risk

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Population</th>
<th>Number of cases/controls or cohort size</th>
<th>Food or nutrient</th>
<th>Relative risk high vs low intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-Control</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Meinsma (147)</td>
<td>Holland</td>
<td>340/1060</td>
<td>Vitamin C</td>
<td>Inverse association</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Citrus fruit</td>
<td>$p = 0.1$ males</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.001$ females</td>
</tr>
<tr>
<td>Higginson (148)</td>
<td>United States</td>
<td>93/279</td>
<td>Dairy foods</td>
<td>Inverse association</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fresh fruits</td>
<td>Inverse association</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raw vegetables</td>
<td></td>
</tr>
<tr>
<td>Haenszel et al. (149)</td>
<td>Japanese in Hawaii</td>
<td>220/440</td>
<td>Tomatoes</td>
<td>0.4 ($p &lt; .05$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Celery</td>
<td>0.4 ($p &lt; .05$)</td>
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<tr>
<td></td>
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<td></td>
<td>Corn</td>
<td>0.5 ($p &lt; .05$)</td>
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<td></td>
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<td></td>
<td>Onion</td>
<td>0.5 ($p &lt; .05$)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Lettuce</td>
<td>0.8 (NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Western vegetables combined</td>
<td>0.4 ($p &lt; .05$)</td>
</tr>
<tr>
<td>Graham et al. (111)</td>
<td>United States</td>
<td>276/2200</td>
<td>Lettuce</td>
<td>0.64 (trend $p &lt; 0.01$)</td>
</tr>
<tr>
<td>Bjelke (150)</td>
<td>Norway and United States</td>
<td>162/1394</td>
<td>Vegetable index (Norway)</td>
<td>Inverse association (Norway &amp; United States)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Vitamin C</td>
<td>Inverse association (Norway &amp; United States)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruits &amp; vegetables (United States)</td>
<td>Inverse association (Norway &amp; United States)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruit</td>
<td>0.7 ($p &lt; 0.05$)</td>
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<tr>
<td></td>
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<td></td>
<td>Plum and pineapple</td>
<td>0.7 ($p &lt; 0.01$)</td>
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<td></td>
<td>Celery</td>
<td>0.6 ($p &lt; 0.01$)</td>
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<td></td>
<td></td>
<td></td>
<td>Lettuce</td>
<td>0.7 ($p &lt; 0.01$)</td>
</tr>
<tr>
<td>Haenszel et al. (151)</td>
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<td>783/1566</td>
<td>Vitamin C</td>
<td>0.50 (trend $p &lt; 0.05$) whites</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>0.33 (trend $p &lt; 0.001$) blacks</td>
</tr>
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<td>Vitamin C</td>
<td></td>
</tr>
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<td>Study</td>
<td>Country</td>
<td>Cases/Controls</td>
<td>Fruit index</td>
<td>Vegetable index</td>
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<tr>
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</tr>
<tr>
<td>Risch et al. (141)</td>
<td>Canada</td>
<td>246/246</td>
<td>0.47 (trend $p &lt; 0.005$) whites</td>
<td>0.33 (trend $p &lt; 0.001$) blacks</td>
</tr>
<tr>
<td>Trichopoulos et al. (142)</td>
<td>Greece</td>
<td>110/100</td>
<td>0.24 (trend $p &lt; 0.01$)</td>
<td>0.33 (trend $p &lt; 0.01$)</td>
</tr>
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<td>Tajima and Tominaja (128)</td>
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<td>93/186</td>
<td>0.24 (trend $p &lt; 0.01$)</td>
<td>0.33 (trend $p &lt; 0.01$)</td>
</tr>
<tr>
<td>Jedrychowski et al. (153)</td>
<td>Poland</td>
<td>110/110</td>
<td>0.46 (p &lt; 0.001)</td>
<td>0.53 (trend $p &lt; 0.01$)</td>
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<tr>
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<td>0.46 (p &lt; 0.001)</td>
<td>0.53 (trend $p &lt; 0.01$)</td>
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<tr>
<td>You et al. (155)</td>
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<td>1016/1159</td>
<td>0.46 (p &lt; 0.001)</td>
<td>0.53 (trend $p &lt; 0.01$)</td>
</tr>
<tr>
<td>Study (reference)</td>
<td>Population</td>
<td>Number of cases/controls or cohort size</td>
<td>Food or nutrient</td>
<td>Relative risk high vs low intake</td>
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<td>Vitamin C</td>
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<td>Carotene</td>
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<td>Sodium</td>
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<td>α-tocopherol</td>
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<td>Protein</td>
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<td>Nitrates</td>
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<td>Beef</td>
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<td>0.5 (0.3–0.6) intestinal type</td>
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<td>0.5 (0.3–0.7) diffuse type</td>
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<td>α-tocopherol</td>
<td>0.5 (0.2–0.8) diffuse type</td>
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<td>Study</td>
<td>Country</td>
<td>Participants</td>
<td>Odds Ratio</td>
<td>Trend p</td>
</tr>
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<td>0.5 (trend p = 0.02)</td>
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<tr>
<td>Hoshiyama &amp; Sasaba (162)</td>
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<td>251/483</td>
<td>0.6 (trend p = 0.02)</td>
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<td>LaVecchia et al. (163)</td>
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<td>0.38 (trend p &lt; 0.001)</td>
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<td>213/213</td>
<td>2.40 (trend p &lt; 0.001)</td>
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<tr>
<td>Hansson et al. (164)</td>
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<td>0.58 (0.37–0.89) (trend p = 0.01)</td>
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<td>Hansson et al. (145)</td>
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<td>0.49 (0.29–0.81) (trend p = 0.004)</td>
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<td>Mexico</td>
<td>220/752</td>
<td>5.49 (2.72–11.06)</td>
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</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Population</th>
<th>Number of cases/controls or cohort size</th>
<th>Food or nutrient</th>
<th>Relative risk high vs low intake</th>
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<td>Folate</td>
<td>0.50 (trend p = 0.008)</td>
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<td></td>
<td>Vitamin C</td>
<td>0.58 (trend p = 0.017)</td>
</tr>
<tr>
<td>Nomura et al. (117)</td>
<td>Japanese in Hawaii</td>
<td>150/7990</td>
<td>Fruit index</td>
<td>0.8 (0.5–1.3)</td>
</tr>
<tr>
<td>Kneller et al. (118)</td>
<td>United States</td>
<td>75/17,633</td>
<td>Fried vegetables</td>
<td>0.8 (0.4–1.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruit index</td>
<td>1.5 (trend p, NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vegetable index</td>
<td>0.9 (trend p, NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carbohydrates</td>
<td>1.6 (trend p &lt; 0.05)</td>
</tr>
</tbody>
</table>
Haenszel et al. (168) measured serum micronutrient levels in persons with various premalignant gastric lesions. Carotene levels in both men and women and vitamin E levels in men were significantly lower in subjects with gastric dysplasia than in subjects with normal mucosa or less advanced lesions.

A recent report from Japan (169) evaluated prediagnostic serum selenium and zinc levels and found no excess risk of stomach cancer in those with the lowest levels of selenium (OR = 1.0) or zinc (OR = 1.2).

The most compelling evidence to date for specific micronutrients in chemoprevention of gastric cancer comes from the previously described population trial in China (57) that found a significant reduction in stomach cancer mortality among persons taking a combination of β-carotene, vitamin E, and selenium. No reduction in risk was observed among persons taking vitamin C; however, there was no attempt in this trial to eradicate *H. pylori* that is known to decrease the concentration of ascorbic acid in gastric juice, either by increased oxidation, impaired secretion from blood into the gastric cavity, or both (99–102).

4. RECOMMENDATIONS

Primary prevention of esophageal cancer obviously begins with prevention of tobacco use by teenagers and cessation among addicted adults. Use of nicotine patches and gum in conjunction with behavioral modification may improve the success rate for smokers attempting to quit. A reduction in tobacco use by teenagers has proven a persistent challenge because education programs are offset by well-funded, effective, targeted marketing by tobacco companies. The terms of the 1998 Tobacco Settlement have the potential to reduce if not eliminate these marketing approaches. Limiting alcohol consumption to moderate levels is particularly important in smokers, and physicians should actively counsel patients accordingly. Physician prompting and participation in smoking cessation efforts have proven effective.

Intake of fresh fruits and vegetables in the United States continues to fall short of the recommended “5-A-Day” (170). Increased consumption should continue to be promoted and benefits are expected to accrue in reduced rates of both of these upper digestive tract cancers as well as other epithelial tumors. Effective population-based approaches are important, and since dietary patterns are often established in childhood, promotion of healthy choices in school-based food service programs is an opportunity that should not be missed.

The current dietary recommendations of the American Cancer Society, the American Heart Association, and the National Cancer Institute are remarkably similar in direction, but differ in specificity. They are included for reference in Table 3.

The efficacy of vitamin/mineral supplements has not yet been established in clinical trials; however, in case-control and cohort studies the individuals in the highest level of intake of specific micronutrients often combine high dietary intake with supplements. With the obvious caution to avoid excessive intake, a multivitamin/mineral supplement, or specific antioxidant supplement may complement dietary intake, particularly among persons with excessive oxidative stress, such as smokers.

In the United States, the treatment and eradication of *H. pylori* is currently recom-
<table>
<thead>
<tr>
<th>Agency</th>
<th>Obesity</th>
<th>Fat</th>
<th>Fruits and vegetables</th>
<th>Dietary fiber</th>
<th>Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Cancer Society, 1996</td>
<td>Be physical active: achieve and maintain a healthy weight</td>
<td>Limit your intake of high fat foods, particularly from animal sources:</td>
<td>Choose most of your foods from plant sources:</td>
<td>Limit consumption of alcoholic beverages, if you drink at all</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Be at least moderately active for 30 min or more on most days of the week</td>
<td>- Choose foods low in fat and saturated fat</td>
<td>- Eat five or more servings of fruits and vegetables each day</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Stay within your healthy weight range</td>
<td>- Limit consumption of meats, especially high fat meats</td>
<td>- Eat other foods from plant sources such as breads, cereals, grain products, rice, pasta, or beans several times each day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>National Cancer Institute, 1987</td>
<td>Avoid obesity</td>
<td>Reduce fat intake to 30% of calories or less</td>
<td>Include a variety of fruits and vegetables in the daily diet</td>
<td>Increase fiber to 20–30 g/d with an upper limits of 35 g</td>
<td></td>
</tr>
<tr>
<td>American Heart Association, 1996</td>
<td>Total calories should be adjusted to achieve and maintain a healthy body weight</td>
<td>Total fat intake should be no more than 30% of total calories</td>
<td>None</td>
<td>Carbohydrate intake should make up 55–60% or more of calories, with emphasis on increasing sources of complex carbohydrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Saturated and polyunsaturated fatty acid intakes should be up to 10% of total calories</td>
<td>Total dietary fiber intake should be 25–30 g a day from food, not supplements</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Monounsaturated fatty acids make up to 15% of total calories</td>
<td></td>
<td>If you drink do so in moderation (no more than two drinks/d)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cholesterol intake should be less than 3000 mg/d</td>
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</tr>
</tbody>
</table>
mended only for persons with gastric and duodenal ulcers, but not for persons with nonulcer dyspepsia (171). Although the spectrum of clinical outcomes associated with *H. pylori* infection is wide ranging, from asymptomatic to gastric cancer, treatment and eradication of infection when possible seems prudent because the cofactors that predispose an infected individual to gastric cancer have not yet been established. This approach is currently followed in Europe.

REFERENCES


87. Pel PK. Ziekten van de Maag (Diseases of the Stomach). De Erven F Bohn, Amsterdam, 1899.


94. The EuroGast Study Group. Epidemiology of, and risk factors for, Helicobacter pylori infection among 3194 asymptomatic subjects in 17 populations.


Diet and Nutrition in the Etiology and Primary Prevention of Colon Cancer

Roberd M. Bostick

1. INTRODUCTION

Cancer of the colon and rectum is the second most common cause of cancer mortality in Westernized countries (1,2). Incidence rates vary approx 20-fold around the world (1,2). The international differences, migrant data, and recent rapid changes in incidence rates in Italy, Japan, urban China, and Male Polynesians in Hawaii (1,2) show that this cancer is highly sensitive to changes in diet and other aspects of environment. Diet appears to have a particularly strong association with occurrence of this cancer (1–3) and thus offers promise for intervention. Furthermore, mortality from colon cancer in the United States has not changed substantially over the past 50 yr (4) suggesting that prevention may offer the best opportunity to control the disease.

In this chapter on diet and nutrition in the etiology and primary prevention of colon cancer, factors associated with either increased or decreased risk of this disease are reviewed. Both older, well-established (fat, meat, fiber, vegetables, and fruit) and newer, intriguing, but less well-established hypothesized associations are addressed (sucrose, calcium, vitamin D, milk products, antioxidants and antioxidant-enzyme-associated micronutrients, folate, tea). The former are covered first, but because they are reviewed extensively elsewhere (1–2,5), are summarized in less detail than the latter. That the newer hypotheses receive more space in the review should not be construed as a suggestion that they are thought more likely to be causal. Alcohol, which is reviewed extensively elsewhere (1,5), is not reviewed except in context with the folate–colon cancer association. Likewise, for a review of energy balance, including total energy intake and physical activity, the reader is referred to refs. 1 and 5.

2. FAT AND MEAT

The hypothesis that dietary meat and/or fat increases risk of colon cancer has been one of the dominant hypotheses related to colon carcinogenesis for the past generation. The observation that higher colon cancer mortality rates occurred in countries where fat and meat consumption was higher led to the hypothesis that these food items contributed to an individual’s risk of developing colon cancer and was a stimulus for the current intense interest in dietary intake in most analytic studies of the etiology of colon cancer (3,6–8).
Highly plausible explanatory hypotheses have been developed in support of a causal relationship between meat and fat intake and colon cancer (see Table 1) and the data from animal and metabolic studies in support of these hypotheses have been substantial. The oldest hypothesis asserts that fat intake increases bile acid production, ultimately increasing the exposure of the bowel mucosa to the toxic, trophic, and tumor or cancer-promoting effects of bile acids (6). High-fat diets increase excretion of bile acids in both animals (9,10) and humans (9,11). Bile acids have been shown to damage DNA (12). In animals, bile acids have toxic effects on colon epithelial cells, resulting in compensatory colonic epithelial cell proliferation (13) and promotion of tumorigenesis (14,15). Also in animals, a high intake of saturated (16,17) and unsaturated fat (17,18) has increased the incidence of chemically induced colon cancer [although not entirely consistently (19)]. In metabolic epidemiologic studies, increased fecal concentrations of bile acids have been found in populations with higher rates of colon cancer (9,20), as well as in patients with colon polyps (21) or colon cancer (22,23) [again, not entirely consistently (24)]. In animals, the tumor-enhancing effects of bile acids are increased after enzymatic modification by intestinal bacteria (25). Among humans, the capacity of colonic flora to transform bile acids into potential carcinogens has been found to be greater in populations with high rates of colon cancer and among meat-eating populations (9,26). Furthermore, this capacity is reduced when the intake of beef fat is reduced (26,27).

A more recent hypothesis is the cooked-food hypothesis (28), which proposes that the association with fat is misleading, at least in part. High-fat diets contain greater amounts of carcinogenic heterocyclic amines (from meat proteins) (29) and promoters as a consequence of cooking at high temperature (cooking in fat produces higher temperatures than cooking in water) (28). Thus, the argument goes, the meat hypothesis is really the high temperature:high carcinogens and promoters/low temperature:low carcinogens and promoters hypothesis. The two explanatory hypotheses are not incompatible and, if anything, enhance the plausibility of the meat/fat–colon cancer association.

A third and very recent hypothesis is that a high consumption of meat, particularly red meat, may increase fecal concentrations of iron, which catalyzes oxidative reactions, leading to increased lipid peroxidation and oxidative DNA damage, and, as described in more detail in Subheading 9., to increased risk for colon cancer (30). Also as described in Subheading 9., high-fat consumption has been associated with increased levels of oxidative damage.

Finally, in a recent, small metabolic study, meat intake, especially red meat intake, was found to be associated with increased production of N-nitroso compounds and precursors (31). G to A transitions in the gene K-ras occur in colorectal cancer and are characteristic of the effects of alkylating agents such as N-nitroso compounds (31).

Of at least 40 analytic epidemiologic studies investigating the meat–colon cancer association (see ref. 1 for a review of the earliest 23; for example, see refs. 32–42; more recent studies include refs. (43–59), 23 found a direct association (for example, refs. 33, 34, 37–39, and 44–47, 49–54, 59), one an inverse association (40), and 17, no definite association (for example, refs. 35, 41–43, 48, 55–58). The only study to find an inverse association was a prospective mortality study in Japan (40), a society generally at low risk for colon cancer. Other studies have found inverse associations with poultry or fish (for example, refs. 51, 52, 55, 57, 59). A second prospective mortality study, this one in Seventh Day Adventists (41), reported a null association; and a third, a small cohort in
white males (45), found a direct association. Of five prospective studies of incident colorectal cancer, one, the Nurses’ Health Study (39), reported a direct association; a second, the Iowa Women’s Health Study, using the same food frequency questionnaire as the Nurses’ study, reported a null association (42); a third, the Norwegian National Health Screening Service cohort with 143 cases found a null association for total meats, but a direct association for sausage limited to women (43); a fourth, in Seventh Day Adventists, in contradistinction to the earlier mortality study, found a direct association (44); and the fifth, a Finnish cohort study, found direct associations with smoked, salted fish, cured meat, and meats with N-nitroso compounds (54).

Of at least 26 studies investigating the fat–colon cancer association (see ref. 1 for a review of the earliest 18; for examples, see refs. 33, 34, 37–39, 42, and 60; more recent studies include refs. (43, 52, 56, 57, 61–64), 12 found a direct association (for example, refs. 33, 34, 37–39, and 56, 64), 2 an inverse association (60, 63), and 15, no definite association (for example, refs. 35, 42, and 43, 48, 52, 56, 57, 63, 64). The only studies to find an overall inverse association were a prospective study of Hawaiians of Japanese descent (60), and a case-control study of Montreal francophones that found nonsignificant odds ratios (ORs) of 0.78 and 0.71 for total and saturated fats, respectively (63). However, another study found a statistically significant inverse association (OR = 0.6) with the ratio of polyunsaturated to saturated fatty acids (52). Only four prospective studies have examined the fat–colon cancer association; one, a mortality study in Seventh Day Adventists (41), reported a null association; the second, the Nurses’ Health Study (39), reported a direct association; the third, the Iowa Women’s Health Study (42), using the same dietary assessment instrument, reported a null association; and the fourth, the Norwegian National Health Screening Service cohort study (43), found a null association for total fat and for various types of fat.

Of interest is that the Nurses’ Health Study (39), the Iowa Women’s Health Study (42), and one case-control study (38) used essentially the same dietary assessment instrument. The Nurses’ Health Study, and the Iowa Women’s Health Study, are both extant large prospective studies limited to women that used similar statistical techniques
in their reported analyses. The Nurses’ Health Study, however, was limited to women who were 30–55-yr-old registered nurses living in 11 large states in the United States, whereas the Iowa Womens’ Health Study was limited to 55–69-yr-old women living in a single state, but of any employment status or occupation. The case-control study, a 1989 population-based study in Los Angeles, CA limited to 45–69-yr-old men and women, found null associations for meat and fat (as did the Iowa Womens’ Health Study). Although these three studies differed on statistical significance, directions of associations with meat, types of meat, and fat were fairly consistent across studies. Of further note is that the Iowa Womens’ Health Study, the Nurses’ Health Study, and several (for example, refs. 34, 36, 51, 52, 55, 57, 59), but not all (for example, ref. 35) other studies that have investigated different types of meats in relation to colon cancer reported associations that involved higher fat meats (red meats, processed meats, and so on) were consistent with increased risk, whereas associations that involved fish, other seafoods, or skinless poultry were consistent with decreased risk.

Although associations between fat and meat and colon cancer have now been investigated in over 40 analytic epidemiologic studies, and although direct associations were found in approximately two-thirds of these studies, findings are too inconsistent to establish causal relationships. Furthermore, as pointed out by Willett et al. (39,65), the interpretation of many studies is hampered by the common finding of a direct association between total energy intake and colon cancer risk (for examples, see refs. 66–70), thus raising uncertainty as to whether it is the total amount of food consumed or the fat or meat components of the diet that is etiologically important. Differences in the findings of the many studies may be because of differences in study designs, populations [for a good illustration, see Whittemore et al. (71)], dietary assessment methodologies, and analysis procedures (including energy adjustment techniques and groupings of meat and fat); and different ranges of dietary intakes, cooking practices, genotypic or phenotypic susceptibility, and molecular characteristics (implying possible differences in etiologies) of the cancers across populations. Null associations in many studies may be related to dietary or cooking method homogeneity within populations, the lack of accuracy of currently available dietary assessment instruments, and mix of genetically susceptible individuals or tumors of given molecular characteristics/etiologies.

For example, some, but not all, studies that have examined the association of meat doneness or method of preparation have found a stronger risk with cooking at higher temperatures or to greater degrees of doneness, as surrogates of heterocyclic amine exposure (2). Even more recently, some studies have found even stronger associations with indicators of heterocyclic amine exposure from cooking meat with rapid activity of some enzymes that metabolize heterocyclic amines (48,50,53,72,73). In these studies N-acetyltransferase activity was indicated by either phenotyping using model compounds or genotyping for polymorphisms of NAT1 and NAT2. One study suggested that risk from beef was more associated with tumors that were p53 negative (49), but a second study found no associations with various groupings of meat regardless of the p53 status of the tumors (56).

Opposite findings within many studies for higher fat meats (increased risk) vs fish or seafoods (decreased risk) may have etiologic implications and suggest the need to investigate various meat groupings more vigorously. For example, if the mechanism of the hypothesized meat/colon cancer relationship is more a matter of low-fat meats vs
high-fat meats, then the bile acid explanatory hypothesis may be more tenable than the cooked-meat hypothesis. Alternative explanations, however, include other unidentified or accounted for healthy behaviors associated with low-fat meat consumption, potential protective effects of omega-3 fatty acids in seafoods [for example, omega-3 fatty acids have reduced colonic epithelial cell proliferation in a small clinical trial in humans (74)], and different cooking methods associated with red meats vs seafoods.

3. FIBER

The hypothesis that fiber decreases the risk of colon cancer, has, in addition to the fat hypothesis, been one of the dominant hypotheses related to colon carcinogenesis for over a quarter of a century. The idea was first proposed by Burkitt in 1969 based on his clinical observations that colon cancer appeared to be rare in Africans whose diet was high in unrefined foods (75). As reviewed elsewhere (76), mechanistic hypotheses (see Table 2) include that fiber, which comes primarily from plant foods:

1. Increases stool bulk, diluting the opportunity for fecal mutagens to contact the colon mucosa;
2. Decreases stool transit time, thus providing less time for fecal mutagens to contact the colon mucosa;
3. Binds or dilutes bile acids, thereby reducing their toxic, trophic, and promoting effects (see discussion of bile acids in Subheading 2.);
4. Ferments to volatile fatty acids that may be anticarcinogenic;
5. Ferments to volatile fatty acids that decrease pH, thereby reducing the conversion of primary to secondary bile acids, and reducing the solubility of free bile acids, thus decreasing their availability for cocarcinogenic activity;
6. Ferments, thus leading to the release of bound calcium (see implications for this in Subheading 6.);
7. Binds carcinogens; and
8. Induces different patterns of colonic bacteria, thus influencing types and degrees of metabolic reactions.

It is becoming more apparent, however, that regarding dietary fiber as a single entity may be misleading and oversimplifying the fiber–colon cancer association. Fiber classifications that may be important etiologically include nonstarch polysaccharides (cel lulose, hemicelluloses, pectin, gums, mucilages) vs nonpolysaccharides (lignin), water soluble (pectin, gums, mucilages, and some hemicelluloses) vs water insoluble (cellulose, lignin, and most hemicelluloses), fermentable vs nonfermentable, cereal vs vegetable, and so on. For example, cellulose and wheat bran have been shown to decrease fecal bile acid concentrations, whereas oat and corn bran have been shown to increase concentrations. Insoluble fiber tends to increase fecal bulk and decrease transit time, whereas soluble fiber has less effect. (Cellulose is found primarily in root and leafy green vegetables and legumes; hemicellulose primarily in cereal brans; pectin in fruit, and gums in legumes and oats.)

Furthermore, results of animal studies involving fiber feeding and colon cancer have been mixed (76–78). Part of the inconsistency may be caused by feeding different types of fiber. Wheat bran, although not in every study, has been the fiber most consistently providing an apparent protective effect. Results of studies of oat bran, corn bran, and pectin have been more mixed.
The results of observational epidemiologic studies of fiber and colon cancer have been mixed (43, 57, 63, 66, 76, 79–85), but generally supportive of the fiber–colon cancer hypothesis. Of 19 case-control studies assessing fiber intake as a specific dietary constituent, eight provided strong support for a protective effect, five provided moderate support, four no support, and two suggested an increased risk with increased fiber intake. A meta-analysis of 13 case-control studies (using original data) (86), found an approximate halving of risk for those in the highest quintile of fiber intake compared to those in the lowest quintile ($p$ for trend $= 0.0001$). The results of four prospective cohort studies in women (43, 57, 83, 87) have not provided strong support for dietary fiber, on the whole, as being protective against colon cancer. In the Nurses Health Study (83), total dietary fiber, vegetable fiber, and cereal fiber, were not associated with risk. In the Iowa Women’s Health Study (87), total dietary fiber was not associated with risk, although the relative risk for cancer of the distal colon was 0.66 (95% CI 0.34–1.29) for the highest quartile compared to the lowest, but there was no suggestion of a dose-response. In the Norwegian National Health Screening Service cohort, there was no apparent association of fiber and risk of colon cancer (43). In a mammography clinics cohort in New York and Florida (57), the relative risk was 1.5, but was not statistically significant. Two recent case-control studies had the capacity to estimate intakes of a wider variety of types of fiber. In one, a large hospital-based case-control study in Italy (79), inverse, but not statistically significant, associations were found for total fiber (nonstarch polysaccharides), soluble noncellulose polysaccharides, total insoluble fiber, cellulose, insoluble noncellulose polysaccharides, lignin, vegetable fiber, and fruit fiber, but not for cereal fiber. A large population-based case-control study in a racially diverse population in Hawaii (84), found evidence for decreased risk in association with dietary fiber, nonstarch polysaccharides, soluble fiber, insoluble fiber, cellulose, and noncellulose polysaccharides, but it appeared that these associations were all primarily caused by fiber from vegetable sources. There was also evidence to suggest that there was an association of vegetable fiber independent of other potential mechanisms of vegetables as potential reducers of risk.

On the whole, then, the idea that at least some types of dietary fiber may afford protection against colon cancer is highly plausible, and the animal experimental and human observational literature is generally supportive of the hypothesis. Much work needs to

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potential Colon Anticarcinogenic Mechanisms of Dietary Fiber</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Increases stool bulk, diluting fecal mutagens</td>
</tr>
<tr>
<td>Decreases stool transit time, decreasing fecal mutagen contact time</td>
</tr>
<tr>
<td>Binds or dilutes bile acids, reducing their mutagenic and cytotoxic effects</td>
</tr>
<tr>
<td>Binds or dilutes carcinogens</td>
</tr>
<tr>
<td>Ferments to volatile fatty acids that are potentially anticarcinogenic</td>
</tr>
<tr>
<td>to volatile fatty acids that decrease pH that reduce conversion of primary to more carcinogenic secondary bile acids reduce solubility, thus carcinogenic activity, of free bile acids leading to release of bound calcium which may bind bile acids</td>
</tr>
<tr>
<td>Induces different patterns of colonic bacteria, thus influencing type and degree of relevant metabolic reactions</td>
</tr>
</tbody>
</table>

<sup>a</sup> Potential mechanisms may not be mutually exclusive, and may be additive or synergistic.
be done to: (1) sort out which type(s) of fiber, if any, are protective in animals; (2) include valid estimates of intake of different fiber types in observational epidemiologic studies; and (3) conduct fiber feeding trials in humans.

4. VEGETABLES AND FRUIT

As reviewed more extensively elsewhere (76), vegetables and fruit contain a myriad of potentially anticarcinogenic compounds, and as a food group, have been more consistently associated with a reduced risk of colon cancer than any other dietary factor. Potential anticarcinogenic agents in plants (see Table 3) include fiber (reviewed in Subheading 3.), antioxidants and antioxidant enzyme-associated micronutrients (reviewed in Subheading 9.), and folate (reviewed in Subheading 10.). Other potential anticarcinogenic compounds for which there has of yet been little study (including no clinical trials) in humans include: dithiolthiones, glucosinolates and indoles, isothiocyanates and thiocyanates, coumarins, flavonoids, phenols, protease inhibitors, plant sterols, isoflavones, saponins, inositol hexaphosphate, allium compounds, and limonene.

Plant potential anticarcinogenic compounds, including the lesser studied ones, have both complementary and overlapping mechanisms of action, including the induction of detoxification enzymes, inhibition of nitrosamine formation, provision of substrate for formation of antineoplastic agents, dilution and binding of carcinogens in the digestive tract, alteration of hormone metabolism, antioxidant effects, and others. Dithiolthiones are present in cruciferous vegetables; when administered to animals, increase levels of glutathione and increase activities of glutathione reductase, glutathione transferase, quinone reductase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase; and are thought to protect against cancer by blocking the reaction of electrophilic carcinogens with cellular macromolecules (the mechanism probably depends on the induction of glutathione and the related conjugation enzymes) (76,88,89).

Glucosinolates and indoles are both present in cruciferous vegetables, and some of these compounds increase microsomal mixed-function oxidase activity, which can lead to either activation or detoxification of carcinogenic compounds, the aggregate effect of which appears to be anticarcinogenic (76,90). Indoles have been found to protect against a variety of tumors in animals (76). Isothiocyanates and thiocyanates are present in cruciferous vegetables; inhibit DNA methylation; induce Phase II xenobiotic-metabolizing enzymes such as glutathione S-transferase; and have been shown to be inhibitors of both early and late stages of carcinogenesis in animals (76,89). Coumarins are found in vegetables and citrus fruits; induce glutathione S-transferase activity; and have inhibited tumor formation in animals (75,88). Flavonoids (for example, quercetin and others) are found in most vegetables and fruits; have antioxidant properties; influence mixed-function oxidase activity; and have produced mixed results in animal anticarcinogenesis experiments (76). Phenols are found in a variety of vegetables and fruits; some are also classified as antioxidants, flavonoids, or coumarins, others are not; induce detoxification enzymes (Phase II conjugation reactions); some inhibit N-nitrosation reactions; and have been found to decrease tumors in animals (91). Protease inhibitors are widely distributed in plants, but are particularly abundant in seeds, legumes, potatoes, and sweet corn; competitively inhibit proteases by forming complexes that block or otherwise affect their catalytic sites; and reduce the occurrence of tumors in animals (76,89,92). Plant sterols are found in vegetables; pass through the gastrointestinal tract.
Table 3: Potentially Anticarcinogenic Constituents of Vegetables and Fruit

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Common plant sources</th>
<th>Potential anticarcinogenic mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber</td>
<td>All plants</td>
<td>See Table 2</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>All plants</td>
<td>Protect against oxidative damage</td>
</tr>
<tr>
<td>Folate</td>
<td>Leafy green vegetables</td>
<td>Protects against DNA hypomethylation</td>
</tr>
<tr>
<td>Dithiolthiones</td>
<td>Cruciferous vegetables</td>
<td>Increase glutathione</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase glutathione reductase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase glutathione transferase</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>Cruciferous vegetables</td>
<td>Increase quinone reductase</td>
</tr>
<tr>
<td>Indoles</td>
<td>Cruciferous vegetables</td>
<td>Increase 6-phosphogluconate dehydrogenase</td>
</tr>
<tr>
<td>Isothiocyanates</td>
<td>Cruciferous vegetables</td>
<td>Block reaction of electrophilic carcinogens with cellular macromolecules</td>
</tr>
<tr>
<td>Thiocyanates</td>
<td>Cruciferous vegetables</td>
<td>Induction mixed-function oxidase activity</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Vegetables, citrus fruit</td>
<td>Induce glutathione S-transferase activity</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Most vegetables, fruit</td>
<td>Antioxidant properties</td>
</tr>
<tr>
<td>Phenols</td>
<td>Variety of vegetables, fruit</td>
<td>Influence mixed-function oxidase activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Some are antioxidants, flavonoids, coumarins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induce detoxification enzymes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Some inhibit N-nitrosation reactions</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Most plants, especially in seeds, legumes, potatoes, sweet corn</td>
<td>Competitively inhibit proteases</td>
</tr>
<tr>
<td>Plant sterols</td>
<td>Most plants</td>
<td>Possible beneficial effects on cell membranes</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Variety of plants, especially soybeans</td>
<td>Weak estrogenic activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibit tyrosine kinase</td>
</tr>
<tr>
<td>Saponins</td>
<td>Variety of plants, especially soybeans</td>
<td>Inhibit certain P450 enzymes</td>
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<td></td>
<td></td>
<td>Bind bile acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce colonic epithelial cell proliferation</td>
</tr>
<tr>
<td>Inositol hexaphosphate</td>
<td>Variety of plants, especially soybeans and cereals</td>
<td>Decrease lipid peroxidation</td>
</tr>
<tr>
<td>Allium compounds</td>
<td>Allium vegetables (e.g., onions, garlic)</td>
<td>Induce glutathione S-transferase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induce microsomal monooxygenase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibit bacterial conversion of nitrate to nitrite</td>
</tr>
<tr>
<td>Limonene</td>
<td>Citrus fruit</td>
<td>Induces glutathione S-transferase</td>
</tr>
</tbody>
</table>

* Potential mechanisms may not be mutually exclusive, and may be additive or synergistic.
almost completely unabsorbed; and were found to decrease occurrence of chemical carcinogen-induced tumors in rats \((76,93)\). The possible mechanism of action is unclear, but, because their structure is similar to cholesterol, may involve affecting cellular membranes. Isoflavones are found in a variety of plants, but genistein, daidzein, and equol are particularly abundant in soybeans; have weak estrogenic activity (may bind to estrogen receptors, thus blocking more potent estrogens, but without eliciting a major estrogenic response); some inhibit tyrosine kinases and other enzymes that are associated with the transmission of signals from cellular growth factor receptors and expressed at high levels in transformed cells; may inhibit certain \(P450\) enzymes; and have been shown to reduce tumors in rodents \((94–96)\). A high intake of soy products has been associated with a reduced risk of cancer \((76)\). Saponins are found in a variety of plants, but are found in particularly high amounts in soybeans; bind bile acids and cholesterol; and have been shown to reduce colonic epithelial cell proliferation and decrease the growth and rate of DNA synthesis of various types of tumors cells \((76,94)\). Inositol hexaphosphate is found in a variety of plant foods, but is particularly high in soybeans and cereals; decreased lipid peroxidation (perhaps by binding the oxidation catalyst, iron); and decreased colon cancer in animals \((94)\). Ecologic studies have found a strong inverse association of inositol hexaphosphate with colon cancer that is stronger than the one between fiber and colon cancer \((94)\). Allium compounds are found in the allium vegetable family, such as onions and garlic; induce detoxification enzymes such as glutathione S-transferase and microsomal monooxygenase; inhibit bacterial conversion of nitrate to nitrite; and have been shown to reduce occurrence of tumors in animals \((76,97)\). Increased consumption of garlic has been found to be associated with a decreased risk of colon cancer in a prospective study of women \((87)\). Limonene is found in citrus fruits, and induces glutathione S-transferase activity in animals \((76)\).

Potter \((98)\) postulates that a diet regularly high in plant foods is the one to which humans are most adapted. This diet, then, provides regular high amounts of substances to which human metabolism is dependent for optimum health, some of which have not been identified as essential nutrients. Many of these substances can serve to keep inducible enzyme systems “tuned” to handle occasional high intakes of carcinogens; inhibit the formation of other carcinogens; reduce the capacity of transformed cells to proliferate; and act as antioxidants, and so on. Thus, abandonment of the vegetable and fruit anticarcinogen “cocktail” to which we are adapted increases the risk of colon cancer.

The analytic epidemiologic literature on the association of vegetables and fruit and colon cancer is very consistent. Of at least 39 analytic epidemiologic studies \((see reviews of the earliest 22 in refs. 5, 82, and 87; more recent studies include refs. 44–46, 49, 51, 57, 61, and 99–102)\), including 35 case-control studies and 4 cohort studies, which investigated the possible association of vegetables and fruit and incidence of colon cancer, \((32)\) were in an inverse direction \((20\) were statistically significant), and \(2\) were in the direction of increased risk. There has been more consistency for vegetable intake than for fruit intake \((5)\). A meta-analysis of six case-control studies found a combined odds ratio of 0.48 \((95\%\ Cl 0.41–0.57)\) \((81)\). The only prospective studies to report combined vegetable and fruit findings in relation to colon cancer incidence were the Iowa Women’s Health Study, which found a relative risk of 0.73 \((95\%\ Cl 0.47–1.13)\) \((87)\); a Seventh Day Adventist cohort study that found relative risks of 1.7 for men and 0.7 for women, neither of which were statistically significant \((41)\); a large American Cancer Society cohort that found statistically significant relative risks of 0.8
for men and 0.6 for women (101); and a geriatric cohort that found relative risks of 1.5 for men and 0.6 for women, the latter of which was statistically significant (102).

Among the case-control studies, analyses for specific categories of vegetables revealed inverse associations with cruciferous vegetables in six of eight studies examining this category; carrots in four of six; cabbage in two of six; and green vegetables in three of five. Intake of fruit was inversely related in four of eleven, and intake of legumes was positively associated in four of five studies. Inverse associations have been reported for garlic, lettuce, bananas, tomatoes, peppers, and others. Of the four case-control studies that found no significant or a positive association with overall vegetable and fruit consumption, all were among the first few ever conducted, and the two finding the positive associations were conducted in Japanese populations (82). There are no reported clinical trials of vegetable and fruit consumption and risk of colon cancer.

In summary, a decreased risk of colon cancer with an increased consumption of vegetables and fruit is biologically plausible and supported by the most consistent analytic observational epidemiologic literature of any diet–colon cancer association; however, more prospective data are needed. The multiplicity of potential mechanisms, rather than detracting from the plausibility of a protective effect of vegetables and fruit, make a strong case for the potential of increased vegetables and fruit in the primary prevention of colon cancer.

5. SUCROSE

It has long been known that a high intake of sucrose is a prominent distinguishing feature of the high-risk Western-style diet (103–105). However, until recently, little attention has been paid to the possibility that this historically recent prominent dietary constituent might be etiologically linked to colon carcinogenesis. It has now been shown (see Table 4) that:

1. Uncooked sucrose increases colonic epithelial cell proliferation and aberrant crypt foci formation in rodents (106);
2. Cooked sucrose contains compounds that are genotoxic under in vitro conditions (107,108);
3. Cooked sucrose increases microadenoma formation in rodents (28);
4. Cooked sucrose contains the thermolysis product, 5-hydroxymethyl-2-furaldehyde, a compound that has also increased microadenoma formation in rodents initiated with a colon carcinogen (109);
5. In humans, a high-sucrose diet increases mouth-to-anus transit time despite decreasing the mouth-to-cecum time; it also increases the fecal concentration of both total and secondary bile acids (110); and
6. Sucrose, which has a high glycemic index, can promote hypertriglyceridemia, hyperinsulinemia, and insulin resistance, in at least genetically susceptible persons, perhaps impacting various metabolic influences on risk for colon cancer.

Relatively few epidemiologic studies have investigated the sucrose–colon cancer association (32–38,42,46,58,59,111–116), only one of which has been prospective (42). To date, 17 analytic epidemiologic studies (see Table 5) have reported investigating the association of sucrose and colon neoplasia (32–38,42,46,58,59,111–116); of these, 14 (32,34–37,42,58,59,111–116) reported an association in the direction of increased risk [findings were significant in five (42,59,112,113,116); however, in one of these there was
no adjustment for total energy intake, despite a significant direct association of energy intake and colon cancer (113), one reported an odds ratio (OR) of exactly 1.0 (38), and two reported a statistically nonsignificant inverse association (33,46). Among the six studies that found a significantly increased risk with higher sucrose intakes, the prospective Iowa Women’s Health Study (42) reported a relative risk (RR) of 2.0; a Belgian case-control study (112) of incident colon cancer reported an OR of 2.31 with more evidence of a dose-response relationship; a United States multicenter case-control study (115) found an OR of 1.59; a Uruguayan case-control study (116) an OR of 2.18 (6.07 if also highest quantile of protein); and an Italian case-control study (59) an OR of 1.4. A Spanish case-control study (37) and an Italian case-control study (114) also reported risk estimates of about 2.0, but that narrowly missed statistical significance at the $p \leq 0.05$ level. Another of the studies that suggested a direct association (RR = 2.0), was the only previous prospective study to investigate the sucrose-colon cancer association (31); however, the study endpoint was colon cancer mortality, the number of cases was small ($n = 41$), and the sucrose exposure measurement was limited to consumption of cake or pie. The only studies to suggest an inverse association were a small hospital-based case-control study in Greece (33) and a hospital-based case-control study in Vaud, Switzerland (46).

The Iowa Women’s Health Study, the only prospective study to address sucrose intake and colon cancer incidence (42), found a nearly twofold increased risk of colon cancer in women associated with high intakes of sucrose and sucrose-containing foods. Among the sucrose-containing foods, no individual foods or groupings of foods appeared to contribute disproportionately to the overall association, and the association for sucrose was approximately the same as for the total sucrose-containing food grouping. No multiplicative interactions were seen between meat and sucrose-containing foods or between fat and sucrose. These observations suggest that it is increased sucrose consumption per se that is associated with increased risk of colon cancer, rather than the consumption of sucrose in combination with something else or sucrose that has been used in a certain way (e.g., cooked vs uncooked). There was a suggestion, however, that the association involving a grouping of all sucrose-containing foods was stronger after removing the calcium-rich food items (ice cream and ice milk). This suggests a hypothesis that a protective effect of calcium may have been partially negating a risk enhancing effect of sucrose.

The sucrose findings of the several studies, then, in relation to incident colon cancer are fairly consistent, and several are not only statistically significant, but are relatively strong for diet-disease associations. Taken as a whole, the findings of the analytic epi-
<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Population</th>
<th>Endpoint</th>
<th>No. of cases</th>
<th>OR/RR$^b$</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillips et al. (32)</td>
<td>Cohort</td>
<td>Seventh-Day Adventists/CA</td>
<td>Colon mortality</td>
<td>41</td>
<td>2.0</td>
<td>Cake or pie</td>
</tr>
<tr>
<td>Manousos et al. (33)</td>
<td>Case-control</td>
<td>Hospital-based/Greece</td>
<td>Incident colorectal</td>
<td>100</td>
<td>0.7</td>
<td>Sucrose-containing foods</td>
</tr>
<tr>
<td>Miller et al. (111)</td>
<td>Case-control</td>
<td>Hospital cases and neighborhood controls/Canada</td>
<td>Incident colorectal</td>
<td>348</td>
<td>1.40 (men) 1.13 (women)</td>
<td>Sugar</td>
</tr>
<tr>
<td>Pickle et al. (34)</td>
<td>Case-control</td>
<td>Hospital-based/NE</td>
<td>Incident colon</td>
<td>58</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Bristol et al. (113)</td>
<td>Case-control</td>
<td>Hospital-based/United Kingdom</td>
<td>Incident colorectal</td>
<td>50</td>
<td>3.6$^a$</td>
<td>Unadjusted, and total energy intake was directly associated</td>
</tr>
<tr>
<td>Macquart-Moulin et al. (35)</td>
<td>Case-control</td>
<td>Hospital-based/France</td>
<td>Incident colorectal</td>
<td>399</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>La Vecchia et al. (36)</td>
<td>Case-control</td>
<td>Hospital-based/Italy</td>
<td>Incident colorectal</td>
<td>575</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Tuyns et al. (112)</td>
<td>Case-control</td>
<td>Population-based/Belgium</td>
<td>Incident colon</td>
<td>453</td>
<td>2.31$^c$</td>
<td>Sugar</td>
</tr>
<tr>
<td>Benito et al. (37)</td>
<td>Case-control</td>
<td>Population-based/Majorca, Spain</td>
<td>Incident colorectal</td>
<td>286</td>
<td>1.64</td>
<td>Nearly significant at $p \leq 0.05$</td>
</tr>
<tr>
<td>Bidoli et al. (114)</td>
<td>Case-control</td>
<td>Hospital-based/Italy</td>
<td>Incident colon</td>
<td>123</td>
<td>1.6</td>
<td>Nearly significant at $p \leq 0.05$</td>
</tr>
</tbody>
</table>

$^a$ Unadjusted, and total energy intake was directly associated

$^b$ OR/RR = Odds Ratio/Relative Risk

$^c$ Sugar
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Setting</th>
<th>Type</th>
<th>Participants</th>
<th>OR (95% CI)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peters et al. (38)</td>
<td>Case-control</td>
<td>Population-based, white/Los Angeles, CA</td>
<td>Incident colorectal</td>
<td>746</td>
<td>1.0</td>
<td>Per 100 Kcal</td>
</tr>
<tr>
<td>Bostick et al. (42)</td>
<td>Cohort</td>
<td>General population, women/IA</td>
<td>Incident colon</td>
<td>212</td>
<td>2.00*</td>
<td>Sucrose-containing foods</td>
</tr>
<tr>
<td>Shannon et al. (58)</td>
<td>Case-control</td>
<td>Population-based/Seattle, WA</td>
<td>Incident colon</td>
<td>424</td>
<td>1.48 (men) 1.56 (women)</td>
<td>Sweets + table sugar</td>
</tr>
<tr>
<td>Franceschi et al. (59)</td>
<td>Case-control</td>
<td>Hospital-based/Italy</td>
<td>Incident colorectal</td>
<td>1953</td>
<td>1.4*</td>
<td>Refined sugar</td>
</tr>
<tr>
<td>Slattery et al. (115)</td>
<td>Case-control</td>
<td>Population-based/UT, MN, CA</td>
<td>Incident colon</td>
<td>1993</td>
<td>1.14</td>
<td>Cakes/desserts</td>
</tr>
<tr>
<td>De Stefani et al. (116)</td>
<td>Case-control</td>
<td>Hospital-based/Montevideo, Uruguay</td>
<td>Incident colorectal</td>
<td>289</td>
<td>2.18* 1.94* 6.07*</td>
<td>Younger men All men</td>
</tr>
<tr>
<td>Levi et al. (46)</td>
<td>Case-control</td>
<td>Hospital-based/Vaud, Switzerland</td>
<td>Incident colorectal</td>
<td>223</td>
<td>0.84</td>
<td>Cakes and desserts</td>
</tr>
</tbody>
</table>

* Exposure is sucrose as a macronutrient score unless otherwise specified in comments column of table.
* Odds ratio or relative risk, highest quantile of intake vs lowest.
* 95% CI does not include 1.0.
demographic studies are generally supportive of the possibility of a causal relationship, and as aforementioned, are biologically plausible.

6. CALCIUM

6.1. Overview of Plausibility of a Preventive Role for Calcium

The estimated average intake of calcium in modern Western diets is 740 mg daily, an amount that from the evolutionary historical perspective is low (103). The calcium intake of all mammalian species (including chimpanzees) other than modern man is equivalent to a human intake of 1500–2000 mg daily, an amount that corresponds to the estimated intake of Paleolithic man (103). Given that, on average, only about 30% of calcium consumed is absorbed from the gut, it is plausible that the mammalian gut is best adapted for high enteral levels of calcium (117). If enough calcium is consumed (estimated at 1500–2000 mg daily) for absorption for physiologic needs and to bind free phosphate in the gut, there will be sufficient free enteral calcium to bind bile acids, thereby preventing their mutagenic and cytotoxic effects (118). Furthermore, in human colonic cell culture, calcium has been shown to reduce cell proliferation and increase differentiation (117). The mechanisms by which calcium affects cell cycle are not clear; however, several lines of research indicate that calcium may exert such effects by interacting with cyclic AMP (119), calmodulin (120,121), tyrosine kinase (122), and ornithine decarboxylase (118,122). In addition, calcium may influence other mechanisms; for example, cell-adhesion mechanisms involving E-cadherin, a calcium-dependent cell adhesion molecule that interacts in complex fashion with the APC (adenomatous polyposis coli) gene product (3) (123,124). Calcium has been consistently shown to reduce colon carcinogenesis in animals (120).

6.2. Epidemiology of Calcium and Colorectal Cancer

The analytic observational epidemiologic literature on the association of calcium and colon cancer is somewhat inconsistent, but inverse associations have more frequently been found (see Table 6). Of at least 27 analytic epidemiologic studies (35,38,43,47,57,63,64,125–143) [17 case-control studies (35,38,47,63,64,69,127–131,133,137,139–142) and 9 cohort studies (43,57,125,126,130,134–136,138)] that investigated the possible association of calcium and colon cancer, 18 suggested inverse associations and five, positive associations (130–132,136,138) [no direction of association was reported in one study (68)]. None of these studies found a statistically significant increased risk associated with higher calcium intake. Statistically significant decreased risk associated with higher calcium intake was found in five of the case-control studies (38,63,64,128,142) and in three of the cohort studies (125,126,143) [limited to the sigmoid colon in one (126) and to those with no family history of colon cancer in another (143)]. The cohort studies that reported no association were the Nurses’ Health Study (RR = 0.7) (136), the Health Professionals Follow-up Study (RR = 0.75) (138), the Iowa Womens’ Health Study (RR = 0.68) (134) (all three used virtually identical food frequency questionnaires; also note the nearly identical risk estimates), and a prospective study in the Netherlands (nested case-control OR = 0.92) (135). A recent updated analysis of the Iowa Women’s Health Study data (additional accrued cases and stratified according to a history of a
first-degree relative with colon cancer) found a statistically significant relative risk of 0.5 among persons who were family history negative, but a relative risk of 1.1 for those who were family history positive (143).

Most of the studies that reported significant inverse associations (38,63,64,125,126,128,142,143) focused on delineating the association of calcium, vitamin D, and/or milk products with colon cancer. The strongest associations were reported by Garland et al. (125), Slattery et al. (128), Peters et al. (38), and De Stefani et al. (64). Data reported by Garland et al. (125) from the Western Electric Study indicated a relative risk of 0.32 (my calculations from the published quartile-specific incidence rates) for colon cancer for those in the highest quartile group of calcium intake compared to those in the lowest quartile group. This study is important because of its 19-yr prospective design and the careful dietary methodology (two 28-d dietary histories 1 yr apart). Slattery et al. (128), using an extensive food frequency questionnaire in a population-based case-control study in Utah, reported a halving of colon cancer risk (adjusted-odds ratios of 0.41 for men and 0.50 for women) for those in the highest quartile group of calcium intake compared to the lowest quartile group of intake. Peters et al. (38) in a large population-based case-control study in California using essentially the same food frequency questionnaire as in the Nurses' Health Study and the Iowa Women's Health Study, also reported a more than halving of colon cancer risk for both men and women (adjusted OR = 0.42 for men and women combined). De Stefani et al. (64), in a hospital-based case-control study in Montevideo, Uruguay, found a risk estimate (OR = 0.41) nearly identical to that in the California study. Of the five studies suggesting positive associations of calcium intake with risk of colon cancer (131–133,137,139), one reported only univariate results even though total energy intake was positively associated with colon cancer (131); a second reported odds ratio was 1.1, a figure not meaningfully different from 1.0 (133); a third reported an OR of 1.2 for dietary calcium (supplemental calcium intake was not ascertained) (139); and the fourth, a 1987 Belgian case-control study, reported an OR of 1.34 (132); and the fifth, a French case-control study, reported an OR of 1.7 (137). Otherwise, when these analytic epidemiologic studies were compared across study type, location, endpoint (colon cancer vs colorectal cancer), size, number of cases, population age and sex, other population characteristics, and dietary methodology, no pattern distinguishing studies reporting inverse associations vs positive associations was found.

Several factors make it difficult to draw strong conclusions from these reported analytic observational epidemiologic studies investigating the association of calcium with colon cancer. In general, observational studies are hampered by the relative homogeneity of diets within populations, the multitude of dietary variables, the problem of unmeasured nutrient–nutrient interactions, and by the lack of precision in current dietary measures used in large population studies. Although the majority of the studies reviewed here reported associations suggesting decreased risk of colon cancer with relatively high intakes of calcium, reporting and publication biases cannot be ruled out. Residual confounding in the reviewed studies also cannot be ruled out. Although the epidemiologic appearance of colon cancer is different from that of rectal cancer, many of these studies combined the two for reported analyses, possibly attenuating observed associations. Furthermore, few of the studies reported taking into account supplement
## Table 6
Comparisons of Selected Characteristics of Analytic Epidemiologic Studies
Investigating Dietary Calcium Intake in Relation to Colorectal Cancer Incidence

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Population</th>
<th>Endpoint</th>
<th>No. of cases</th>
<th>OR/RR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garland et al.</td>
<td>Cohort</td>
<td>Western Electric Study/Chicago, IL</td>
<td>Colorectal</td>
<td>49</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Macquart-Moulin</td>
<td>Case-control</td>
<td>Hospital-based/France</td>
<td>Colorectal</td>
<td>339</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Wu et al. (130)</td>
<td>Cohort</td>
<td>Retirement community/Los Angeles, CA</td>
<td>Colorectal</td>
<td>126</td>
<td>0.86 (men) 0.89 (women)</td>
<td>Dairy sources</td>
</tr>
<tr>
<td>Kune et al. (127)</td>
<td>Case-control</td>
<td>Community-based/Australia</td>
<td>Colorectal</td>
<td>715</td>
<td>0.56 (women)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Tuyens et al. (132)</td>
<td>Case-control</td>
<td>Population-based/Belgium</td>
<td>Colon</td>
<td>453</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Slattery et al. (128)</td>
<td>Case-control</td>
<td>Population-based/Utah</td>
<td>Colon</td>
<td>231</td>
<td>0.41 (men)&lt;sup&gt;b&lt;/sup&gt; 0.50 (women)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Graham et al. (69)</td>
<td>Case-control</td>
<td>Hospital &amp; neighborhood-based/Western New York Hospital-based/Chinese in Singapore</td>
<td>Colon</td>
<td>428</td>
<td>NS/not given</td>
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<tr>
<td>Lee et al. (129)</td>
<td>Case-control</td>
<td>Japanese descent Hawaiians</td>
<td>Colon</td>
<td>132</td>
<td>0.88</td>
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<tr>
<td>Stemmerman et al. (126)</td>
<td>Cohort</td>
<td>Japanese descent Hawaiians</td>
<td>Colon</td>
<td>189</td>
<td>0.77 (total) 0.59 (sigmoid)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Negri et al. (133)</td>
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<td>Hospital-based/Italy</td>
<td>Colon</td>
<td>558</td>
<td>1.1</td>
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<tr>
<td>Benito et al. (131)</td>
<td>Case-control</td>
<td>Population &amp; hospital-based/Majorca, Spain</td>
<td>Colon</td>
<td>286</td>
<td>1.48</td>
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<td>Peters et al. (38)</td>
<td>Case-control</td>
<td>Population-based, white/Los Angeles, CA</td>
<td>Colon</td>
<td>746</td>
<td>0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Univariate estimate</td>
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<td>Bostick et al. (134)</td>
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<td>General population, women/IA</td>
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<td>212</td>
<td>0.68</td>
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<tr>
<td>Kampman et al. (135)</td>
<td>Cohort</td>
<td>General population/ Netherland</td>
<td>Colorectal</td>
<td>326</td>
<td>0.92</td>
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</tr>
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<td>Study</td>
<td>Design</td>
<td>Type</td>
<td>Population</td>
<td>Disease Type</td>
<td>Cases</td>
<td>Controls</td>
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<tr>
<td>Martinez et al. (136)</td>
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<td>US female nurses</td>
<td>Colorectal</td>
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<td>501</td>
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<tr>
<td>Boutron et al. (137)</td>
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<td>Population-based/</td>
<td>Colorectal</td>
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<td>Burgundy/France</td>
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<td>Gaard et al. (43)</td>
<td>Cohort</td>
<td>Norwegian National Health</td>
<td>Colorectal</td>
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<tr>
<td>Kearney et al. (138)</td>
<td>Cohort</td>
<td>US male health professionals</td>
<td>Colorectal</td>
<td></td>
<td>203</td>
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</table>

a Odds ratio or relative risk, highest quantile of intake vs lowest.
b 95% CI does not include 1.0.
c Not statistically significant, i.e., 95% CI includes 1.0.
d Family history negative for colon cancer in first degree relatives.
e Family history positive for colon cancer in first degree relatives.
intake—a factor that is especially important given the homogeneity of diets within most populations. Many of the studies were reported before the emergence of strong theoretical and experimental support for calcium as a potential protective factor against colon cancer and did not focus specifically on calcium intake; consequently, they did not fully address all confounding and interaction issues related to current hypotheses regarding this dietary constituent. In only a few studies were associations with colon subsite investigated. Overall, however, despite these limitations, the observational epidemiologic literature can be considered weakly supportive of the hypothesis that a relatively high intake of calcium may provide modest protection against colon cancer.

Perhaps the strongest evidence that a higher consumption of calcium may reduce the risk of colon cancer comes from one of the few clinical trials to test the efficacy of any intervention for the primary prevention of any neoplasms. A United States multicenter, randomized, double-blind, placebo-controlled clinical trial (144) of calcium supplementation and adenoma recurrence was recently completed. A total of 913 persons with at least one histologically confirmed adenoma at a baseline colonoscopy were randomized to either placebo or 1200 mg of elemental calcium (as 3.0 g of calcium carbonate) daily. Colonoscopies were performed 1 and 4 yr later (and between, if clinically indicated). Any polyps found on the 1-yr follow-up colonoscopy were removed, but not considered recurrent. Adenomas detected after the 1-yr follow-up colonoscopy and up to and including a 4-yr follow-up colonoscopy were considered recurrent. The relative risk for any recurrence of adenoma was 0.85 (95% CI 0.74–0.98), and for the average number of adenomas, 0.76 (95% CI 0.60–0.96). These risk-reduction figures of approx 20% are remarkably similar to those for higher calcium intake and incidence of colon cancer from the United States cohort studies.

Other supportive, though less direct and less convincing, evidence for a protective role for calcium against colorectal cancer comes from clinical trials of calcium and a putative biomarker of risk, colorectal epithelial cell proliferation patterns (117). This line of evidence is considered further below, in part because of its supportive role, but largely because of its potential to elucidate possible protective mechanisms of action of calcium and because the use of biomarkers represents a growing direction in assessing the potential efficacy and mechanisms of action of various dietary and other interventions for reducing the risk of colorectal neoplasms.

6.3. Calcium and Colorectal Epithelial Cell Proliferation

6.3.1. Colorectal Epithelial Cell Proliferation as an Intermediate Endpoint for the Study of Colon Cancer Prevention

It has been hypothesized that calcium may reduce the risk of colon cancer by normalizing colonic crypt cell proliferation kinetics (118). Several studies (145–159) have reported that, compared to patients at low risk for colon cancer, patients with colon cancer (145–154) and patients in every category known to be at higher risk for colon cancer [those with a history of sporadic adenoma (145,148,150–154), familial polyposis (149,155), ulcerative colitis (245,156,157), or a family history of colon cancer (149,150,158), and the elderly (146,159), on average, exhibit in their normal-appearing mucosa both an increased colonic epithelial cell proliferation rate and an extension of the colon crypt proliferative zone from the lower (basal) 60% of the crypt to include the upper (luminal) 40% of the crypt. In patients with previous colon cancer or sporadic adenomas, these changes also predict adenoma recurrence (160,161). In large bowel tu-
mors in humans, an upward shift in the proliferative zone is found in colon cancers and adenomas, but not in hyperplastic polyps (162). As reviewed elsewhere (120,163,164), proliferative changes in normal-appearing mucosa have been shown to be a consequence of both cancer-initiating and cancer-promoting agents: proliferative changes both precede and accompany colonic neoplasms in rodents given chemical carcinogens, and a high-fat diet produces proliferative changes in both rodents and humans. Animal experimental evidence and preliminary evidence in humans suggest that these two proliferation abnormalities (hyperproliferation and upward shift of the proliferation zone) are reversible biomarkers or precursors for colon neoplasia (120,163–165). In humans, the two proliferation abnormalities appear to be independent variables (152,166), and rectal biopsy findings on both measures reflect those throughout the colon (154,167).

6.3.2. Calcium and Colorectal Epithelial Cell Proliferation Interventions in Animals and Humans

Calcium administration has ameliorated the proliferative changes in rodents (167–170), and the findings in several (171–175), but not all (164,176), small preliminary clinical trials, and a recently completed full-scale clinical trial (177) suggest similar effects in humans. Some preliminary clinical trial evidence suggested, and the recently completed full-scale trial confirmed, that calcium normalizes the distribution of proliferating cells within the colon crypts without affecting the proliferation rate (174,177). Hypothesized mechanisms have included the binding of calcium with bile acids (thought to be promoters) to form inert soaps (118), and the direct induction by calcium of terminal differentiation of the colonic epithelial cells (120,163–165).

The only full-scale randomized clinical trial dedicated to assess the efficacy of higher calcium consumption in normalizing cell proliferation kinetics in humans (177) was a randomized, double-blind, placebo-controlled, three-armed (two doses of calcium: 1.0 g and 2.0 g), parallel group clinical trial (n = 193) to determine whether calcium supplementation can reduce the colorectal epithelial cell proliferation rate and normalize the distribution of proliferating cells within colorectal crypts (i.e., shift the zone of proliferation from one that includes the entire crypt to one that is confined to the lower 60%, or normal proliferative zone, of the crypt). Data from this trial provided evidence for a relative downward shift of the proliferative zone in colorectal crypts of sporadic adenoma patients in response to calcium supplementation, and thus were consistent with the hypothesis that a higher consumption of calcium may reduce the risk of colorectal cancer. The data provided no evidence that the overall colorectal epithelial cell proliferation rate can be reduced by calcium supplementation.

As a result of these data, the mechanistic hypotheses for how calcium affects cell proliferation in vivo and may reduce colon cancer need to be re-examined. The first and most cited hypothesis for how calcium might reduce colorectal cancer risk has been that calcium binds intraluminal bile acids, thus preventing their toxic effects with their resultant promotion of compensatory hyperproliferation (118). However, the data from this trial provide little evidence for this explanatory hypothesis. First, there was no evidence for an effect on the overall cell proliferation rate. Second, the hypothesis would predict that for a person consuming a level of fat and calcium in the Western-style diet range, that the 1.0 g calcium dose (resulting in a total calcium intake of 1.5–2.0 g daily) would have provided as great as an effect as the 2.0 g dose. The data, however, were consistent with a greater effect provided by the higher dose. Further, recent reports of
studies that have examined the effects of calcium supplementation on stool bile acids in humans are inconsistent with one another (178–181). These lines of evidence do not rule out a beneficial effect of calcium via bile acid-binding, but do suggest that, if this is involved, that the mechanism is more complex than previously thought.

Based on in vitro data showing that calcium directly affects the cell cycle, modulating cell proliferation, and inducing terminal differentiation, it has also been hypothesized that calcium may exert a similar influence in vivo in the cells of the colon mucosa (163,164,182). The data from the full-scale calcium and colorectal epithelial cell-proliferation trial (177) are consistent with this hypothesis. Even when proliferation rates are fast, if differentiation occurs rapidly as cells migrate up the crypt, they are more likely to have completed proliferation lower in the crypt. A consequence of this may be that because the DNA of a cell undergoing replication is more vulnerable to damage by various agents, and because cells proliferating lower in the crypt may be less likely to be exposed to injurious intraluminal agents, such cells may be less likely to be involved in colorectal carcinogenesis.

All calcium and colorectal epithelial cell proliferation trials, except the recent full-scale trial, were small and most were uncontrolled (164,171–176). Most examined only the overall cell-proliferation rate. The uncontrolled trials each suggested that calcium would reduce the overall cell-proliferation rate (171–174), however, two (164,185) of the three (164,174,176) preliminary controlled trials did not. The data of the full-scale trial indicate that the findings of uncontrolled trials of calcium and the overall cell-proliferation rate (termed the labeling index, or LI) were likely the result of regression to the mean and/or the Hawthorne effect (i.e., participants under the intense scrutiny characteristic of the clinical trial setting unconsciously change their behavior). Alternatively, based on the results of one uncontrolled trial, the authors speculated that patients with relatively high-proliferation rates may be calcium responsive, and that those with relatively low rates may be unresponsive (173). However, in the full-scale trial, relative changes (calcium vs placebo) in the LI were similar in both those with high and low proliferation at baseline, thus indicating that the findings of the uncontrolled trial were likely the result of regression to the mean. Previous studies (152,166) have shown that the LI and $\phi_h$ (an indicator of distribution of proliferating cells in the crypt; defined as the proportion of labeled cells in the entire crypt that fall in the upper 40% of the crypt) are statistically independent variables, and other preliminary controlled trials testing other agents hypothesized to normalize colorectal proliferative kinetics and to reduce risk of colon cancer have found reductions in the $\phi_h$ without reductions in the LI (74,183).

Although studies in humans have found that cell-proliferation kinetics found on rectal biopsies reflect those found throughout the colon (154,166), and studies in rodents have found that calcium affects cell-proliferation kinetics throughout the colon (168–170), there is no direct evidence from this or any other study that calcium affects cell proliferation throughout the colon in humans. This remains an important question, especially because the epidemiology of rectal cancer appears different in several respects from that of colon cancer (1). In addition, colorectal epithelial cell-proliferation kinetics remain unproven, but logical and well-supported intermediate end points for colon cancer. The existing cell-proliferation studies in humans therefore cannot prove that, because calcium normalizes one of the cell-proliferation endpoints in the rectum, that calcium can reduce the risk of colon cancer; they do, however, provide justification
for further study of the calcium–colon cancer association.

6.4. Summary

In summary, biologically plausible mechanisms of action for protective effects of calcium against colon cancer exist. Currently, animal experimental data are strongly supportive. Observational epidemiologic data are inconsistent, but overall, are weakly supportive as well. A major clinical trial found that calcium supplementation reduced adenoma recurrence by approx 20% \((144)\), a figure in line with three large United States cohort studies of calcium and incidence of colon cancer \((134,136,138)\). A full-scale intermediate endpoint chemoprevention trial found that calcium supplementation, without affecting the proliferation rate, normalized the distribution of proliferating cells in the rectal mucosa of sporadic adenoma patients \((177)\). The results of this trial support the hypothesis that higher calcium consumption may reduce the risk of colon cancer. They also support the hypothesis that the possible chemoprotective action of calcium may not be by simply binding bile acids and thereby reducing compensatory hyperproliferation, but they are consistent with the hypothesis that calcium exerts its possible chemoprotective effect by directly affecting cell cycle and increasing rates of cell differentiation. The mechanism(s) by which calcium affects colorectal epithelial cell proliferation in humans remain(s) to be resolved, and a causal relationship between calcium intake and colorectal cancer incidence, though increasingly supported, cannot be considered firmly established.

7. VITAMIN D

Compared to calcium, similar, but less-extensive evidence exists to support a role for vitamin D in lowering colorectal cancer risk. Vitamin D is intimately related to calcium metabolism \((184)\), has reduced cell proliferation in human colon cell lines in vitro \((185)\), has reduced colonic epithelial cell proliferation in rodents \((186)\), was a necessary cofactor in reducing k-ras G to A mutations in colorectal neoplasms in rats \((187)\), and has reduced tumorigenesis in rats \((188,189)\).

Of 13 analytic epidemiologic studies \((38,125,131,134,136–140,142,143,190,191)\) [six cohort studies \((125,134,136,138,190,191)\) and six case-control studies \((38,131,137,139,140,142)\)] that investigated the possible association of vitamin D and colon cancer (see Table 7) 11 suggested an inverse association \((125,131,134,136–140,142,190,191)\), one reported a null association without providing a specific risk estimate \((38)\), and none reported a direct association. All six of the cohort studies found inverse associations ranging from RRs of 0.3–0.73; of these, four \((125,138,143,190)\) were statistically significant. Five of the case-control studies that reported risk estimates found inverse associations ranging from ORs of 0.4–0.77; of these, three \((139,140,142)\) were statistically significant. Among the cohort studies, in one, the 19-yr prospective Western Electric Study, the data indicated a relative risk of 0.55 (my calculations from the published quartile-specific incidence rates) for colon cancer for those in the highest quartile group of intake as opposed to those in the lowest quartile group of intake of vitamin D \((125)\). In the second, the Washington County, MD, prospective cohort study, a nested case-control analysis showed an odds ratio of 0.3 for colon cancer for those with a 25-hydroxyvitamin D serum level of \(\geq 20\) ng/mL compared to those with a serum level \(< 20\) ng/mL \((190)\). In the third, the Iowa Women’s Health Study, the data indi-
Table 7
Comparisons of Selected Characteristics of Analytic Epidemiologic Studies
Investigating Vitamin D in Relation to Colorectal Cancer Incidence

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Population</th>
<th>Endpoint</th>
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<th>OR/RR&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Western Electric Study/Chicago, IL</td>
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<td>0.55&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Colon</td>
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<td>0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Population &amp; hospital-based/Majorca, Spain</td>
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<td>NS&lt;sup&gt;c&lt;/sup&gt;/not given</td>
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<td>RR</td>
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<td>0.66</td>
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<td>IA women</td>
<td>Colon</td>
<td>241</td>
<td>0.6b</td>
<td>−FH^{e}</td>
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*a Odds ratio or relative risk, highest quantile of intake vs lowest.

*b 95% CI does not include 1.0.

*c Not statistically significant, i.e., 95% CI includes 1.0.

*d Family history negative for colon cancer in first degree relatives.

*e Family history positive for colon cancer in first degree relatives.
cated a relative risk of 0.73 (134); in an updated analysis with a larger number of cases and stratified by a family history of colon cancer, a statistically significant relative risk of 0.6 was found among those who were family-history negative, but a not statistically significant relative risk of 0.8 was found among those who were family-history positive (143). In the fourth, the United States Male Health Professionals Follow-up Study, a relative risk of 0.66 (p for trend = 0.0006) was found (138). In the fifth, the US Nurses' Health Study (136), a relative risk of 0.88 was found. Finally, in the sixth, a cohort study of older Finnish male smokers participating in an antioxidant chemoprevention clinical trial (191), odds ratios for serum 25-hydroxyvitamin D and 1,2-dihydrovitamin D were 0.6 and 0.9, respectively.

8. MILK PRODUCTS

Although milk products have received little attention in laboratory experiments related to colon cancer, they are major sources of calcium and vitamin D in the American diet.

Of at least 23 analytic epidemiologic studies (32–35,37,38,43,44,46,57,58,111,127–129,133–135,137,192,193,194) [15 case-control studies (33–35,37,38,46,58,111,127–129,133,137,192,194) and eight cohort studies (32,43,44,57,134,135,138,193)] that investigated the possible association of milk and colon cancer (see Table 8), 15 suggested an inverse association (35,38,43,44,46,57,58,127–129,134,135,192,193,194); six, a positive association (33,34,37,111,137,138); and one, no association (OR = 1.0) (133). None of these studies found a statistically significant positive association. The inverse associations were statistically significant in three case-control studies (35,127,128). One of the eight cohort studies reported a positive association (RR = 1.09) (138) and most other studies reporting positive associations were hospital-based case-control studies. The only (two) studies that reported inverse associations that were strong were the population-based Utah (128) and Seattle, WA (58) case-control studies.

9. ANTIOXIDANTS AND ANTIOXIDANT ENZYME-ASSOCIATED MICRONUTRIENTS

9.1. Biological Plausibility of Antioxidants Protecting Against Colon Cancer

9.1.1. OXIDATIVE DAMAGE AND CANCER

The plausibility of a role in carcinogenesis for reactive oxygen molecules and oxygen-derived free radicals (see Fig. 1) is backed by an extensive basic science literature, the subject of several reviews (195–206). Briefly, these compounds apparently act as both initiators and promoters of carcinogenesis: they are known to:

1. Alter nucleic acids, leading to mutations, sister chromatid exchanges, and chromosome aberrations, which can lead to propagated initiated cells or to cell death (the latter leading to the promoting effects of compensatory cell hyperproliferation);

2. Damage cells by reacting with unsaturated bonds in membrane lipids and by denaturing proteins, which can lead to cell death, thereby increasing compensatory cell proliferation (also, both the breakdown of cell membrane barriers and increased cell division may in turn expose DNA to easier damage, and thus to initiation or to further promotion from cell death-induced compensatory cell proliferation. Furthermore, some of the cell-membrane breakdown products are themselves mutagenic); and
3. Modulate gene expression of initiated cells by affecting genes that regulate cell differentiation and growth.

Sources of reactive oxygen molecules and oxygen-derived free radicals include endogenous production from normal metabolic reactions, as well as exogenous sources. These compounds may also be important in aging and in the pathogenesis of coronary heart disease and other chronic diseases.

Oxidative damage may be of particular relevance to colon cancer (see Fig 2). It has been recently discovered that feces contain large quantities of oxygen-derived free radicals and that the rate of formation corresponds to that which would be produced by over 10,000 rads of gamma radiation per day (30). As reviewed elsewhere (30), the respiratory activity of fecal bacteria is an abundant source of oxygen-derived free radicals. Another source in the colon is the lipo-oxygenase activity of normal or sloughed colon epithelial cells. Iron, which is present in relatively high concentrations in feces, facilitates the production of oxygen-derived free radicals (the Fenton reaction); furthermore, bile pigments, also present in feces, keep iron soluble thereby enhancing iron’s availability for the Fenton reaction. Radicals formed in the colon can cause oxidative DNA damage; can initiate lipid peroxidation leading to formation of substances shown to stimulate DNA synthesis and cell proliferation in colonic epithelium; can be involved in free-radical oxidations that mimic those produced by the cytochrome P450 system of the liver (which is known to convert procarcinogens to active carcinogens); and can participate in aromatic hydroxylation reactions (involving ingested organic compounds) to form carcinogenic products. These observations are consistent with the high incidence of cancer of the colon and rectum compared to other regions of the gastrointestinal tract. They are also consistent with the association of a higher incidence of colon cancer with a high intake of red meat (which increases stool iron), and with a high intake of fat [which has been associated with increased levels of oxidative damage in humans (207), and which may increase the fecal content of bile pigments and fecal procarcinogens (30)]. More recently, it has been found that oxygen-derived free radicals increase expression of cyclo-oxygenase-2 (COX-2) in colon cell culture (208). Inhibition of COX-2 (which can modulate the colon carcinogenesis gateway gene, APC) by non-steroidal antiinflammatory drugs has been shown to reduce colon tumorigenesis in animals and to cause regression of adenomas in patients with familial adenomatous polyposis (FAP) (2).

9.1.2. ANTIOXIDANTS AND CANCER

As reviewed elsewhere (204,209), vitamin E, vitamin C, and the carotenoids are micronutrients that act as antioxidants, trapping reactive oxygen molecules and oxygen-derived free radicals, and as such are a prominent part of the body’s primary defenses against these damaging agents. The micronutrients selenium, riboflavin, niacin, zinc, and manganese, are essential components of various important antioxidant enzymes. Vitamin E is the major lipid-soluble antioxidant found in all cellular membranes, where it protects against lipid peroxidation (i.e., oxidative cell-membrane damage). Vitamin E acts directly with oxygen-derived free radicals, as well as with reactive oxygen molecules. β-carotene, the major carotenoid precursor of vitamin A, is also fat-soluble and present in cell membranes, and is one of the most effective quenchers of reactive oxygen molecules known in nature. (Vitamin A has minimal antioxidant properties.) Vitamin C is water soluble and can quench both reactive oxygen molecules and oxygen-
Table 8
Comparisons of Selected Characteristics of Analytic Epidemiologic Studies Investigating Milk-Product Intake in Relation to Colorectal Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
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<th>No. of cases</th>
<th>OR/RR&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Hospital &amp; neighborhood-based/Canada</td>
<td>Incident colon</td>
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<td>1.2 (men)</td>
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<td></td>
<td></td>
<td></td>
<td>1.3 (women)</td>
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<td>Manousos et al. (33)</td>
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<td>Hospital-based/Greece</td>
<td>Incident colorectal</td>
<td>100</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Pickle et al. (34)</td>
<td>Case-control</td>
<td>Hospital-based/NE</td>
<td>Incident colon</td>
<td>58</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>Phillips et al. (32)</td>
<td>Cohort</td>
<td>Seventh-Day Adventists/CA</td>
<td>Colon mortality</td>
<td>147</td>
<td>0.5 (men)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.1 (women)</td>
<td></td>
</tr>
<tr>
<td>Tajima and Tominga</td>
<td>Case-control</td>
<td>Hospital-based/Japan</td>
<td>Incident colon</td>
<td>50</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>(192)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macquart-Moulin et al. (35)</td>
<td>Case-control</td>
<td>Hospital-based/France</td>
<td>Incident colorectal</td>
<td>399</td>
<td>0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Kune et al. (127)</td>
<td>Case-control</td>
<td>Community-based/ Australia</td>
<td>Incident colorectal</td>
<td>715</td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;  (women)</td>
<td>0.89 (sexes combined)</td>
</tr>
<tr>
<td>Slattery et al. (128)</td>
<td>Case-control</td>
<td>Population-based/UT</td>
<td>Incident colon</td>
<td>231</td>
<td>0.44&lt;sup&gt;c&lt;/sup&gt; (men)</td>
<td>0.55&lt;sup&gt;c&lt;/sup&gt; (women)</td>
</tr>
<tr>
<td>Lee et al. (129)</td>
<td>Case-control</td>
<td>Hospital-based/Chinese in Singapore</td>
<td>Incident colon</td>
<td>132</td>
<td>0.81</td>
<td></td>
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<tr>
<td>Ursin et al. (193)</td>
<td>Cohort</td>
<td>Norway</td>
<td>Incident colon</td>
<td>92</td>
<td>0.85</td>
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<tr>
<td>Benito et al. (37)</td>
<td>Case-control</td>
<td>Population-based/ Majorca, Spain</td>
<td>Incident colon</td>
<td>148</td>
<td>1.07</td>
<td></td>
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<tr>
<td>Negri et al. (133)</td>
<td>Case-control</td>
<td>Hospital-based/Italy</td>
<td>Incident colon</td>
<td>558</td>
<td>1.0</td>
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</tr>
<tr>
<td>Peters et al. (38)</td>
<td>Case-control</td>
<td>Population-based/UT</td>
<td>Incident colon</td>
<td>746</td>
<td>0.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Bostick et al. (134)</td>
<td>Cohort</td>
<td>General population, women/Iowa</td>
<td>Incident colon</td>
<td>212</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Population/ Location</td>
<td>Code</td>
<td>Cases</td>
<td>Odds Ratio or Relative Risk</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Kampman et al. (135)</td>
<td>Cohort</td>
<td>General population/ Netherlands</td>
<td>Incident colorectal</td>
<td>326</td>
<td>0.86 Unfermented milk</td>
<td></td>
</tr>
<tr>
<td>Boutron et al. (137)</td>
<td>Case-control</td>
<td>Population-based/ Burgundy, France</td>
<td>Colorectal</td>
<td>Sm. adenoma</td>
<td>154</td>
<td>0.89 Fermented milk</td>
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<tr>
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<td>Lg. adenoma</td>
<td>208</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cancer</td>
<td>171</td>
<td>0.88</td>
</tr>
<tr>
<td>Gaard et al. (43)</td>
<td>Cohort</td>
<td>Norwegian National Health Scoring Service</td>
<td>Incident colon</td>
<td>1953</td>
<td>0.83</td>
<td>Hard cheese</td>
</tr>
<tr>
<td>Kato et al. (57)</td>
<td>Cohort</td>
<td>Mammography clinics/ NY, FL</td>
<td>Incident colorectal</td>
<td>100</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Kearney et al. (138)</td>
<td>Cohort</td>
<td>US male health professionals</td>
<td>Incident colon</td>
<td>203</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Shannon et al. (58)</td>
<td>Case-control</td>
<td>Population-based/ Seattle, WA Hospital-based/Italy</td>
<td>Incident colon</td>
<td>424</td>
<td>0.40&lt;sup&gt;a&lt;/sup&gt; (women) 0.92&lt;sup&gt;b&lt;/sup&gt; (men)</td>
<td>1.09</td>
</tr>
<tr>
<td>Franceschi et al. (194)</td>
<td>Case-control</td>
<td>Incident colon</td>
<td>1953</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh &amp; Fraser (44)</td>
<td>Cohort</td>
<td>Seventh Day Adventists/ CA</td>
<td>Incident colon</td>
<td>166</td>
<td>0.78 Skim milk 0.97 Lowfat milk 1.06 Whole milk 1.31 Cheese 0.74 Cottage cheese 1.66&lt;sup&gt;c&lt;/sup&gt; Cheese</td>
<td>0.72 Milk</td>
</tr>
<tr>
<td>Levi et al. (46)</td>
<td>Case-control</td>
<td>Hospital-based/ Vaud, Switzerland</td>
<td>Incident colorectal</td>
<td>223</td>
<td>0.72 Milk 1.66&lt;sup&gt;c&lt;/sup&gt; Cheese</td>
<td>0.74 Cottage cheese 1.66&lt;sup&gt;c&lt;/sup&gt; Cheese</td>
</tr>
</tbody>
</table>

<sup>a</sup> Odds ratio or relative risk, highest quantile of intake vs lowest.
<sup>b</sup> 95% CI does not include 1.0.
<sup>c</sup> Per 10 servings/mo of yogurt. For milk was 0.97 without calcium in model, null with calcium in model.
derived free radicals. Selenium is an essential component of the enzyme, glutathione peroxidase, which reduces oxygen-derived free radicals and thus prevents damage to intracellular membranes. Riboflavin, niacin, zinc, and manganese are also essential constituents of various intracellular antioxidant enzyme systems (as are iron, copper, and molybdenum, which, however, also have prooxidant properties). Of particular importance is that various antioxidant micronutrients have been shown to enhance or even be essential to the antioxidant effects of one another (204,210–221). Vitamin E, the carotenoids, vitamin C, and selenium can also stimulate the immune system and may protect against the development of cancer by enhancing immune surveillance (209,222,223). Vitamins E and C can also reduce nitrite, inhibiting the production of nitrosamines and nitrosamides (223), compounds that induce tumors in experimental animals and possibly in humans. β-carotene (224) and selenium (225,226) may also inhibit cell proliferation by effects independent of their antioxidant activities. More recently, it has been found that antioxidants can block the increased expression of COX-2 provoked by oxidizing agents in colon cell culture (208).

Specific antioxidant micronutrients, including vitamin E, carotenoids, vitamin C, and selenium, have been shown to protect against colon cancer in animals (209,227,228). Furthermore, in several studies, antioxidant micronutrients enhanced the effects of one another (212,213,215,220,221), emphasizing their interdependence.

9.2. Epidemiology of Antioxidants and Colon Cancer

The analytic observational epidemiologic evidence for an association of vitamin E and risk for colorectal cancer is mixed, but the evidence from prospective studies is consistent with an inverse association. In the prospective Iowa Women’s Health Study (n = 35,215) (229), an adjusted RR of 0.32 (95% CI 0.19–0.54) was found for those in the
highest quintile of intake of total vitamin E compared to those in the lowest quintile of intake. The association was even more striking in the youngest age group (55–59 yr old) for which the RR was 0.16 (95% CI 0.04–0.70). Several years later, the association was again investigated, this time according to a family history of colon cancer (143). An inverse association was found among persons without a history of a first degree relative who had colon cancer, but not among persons with a positive family history. Findings in five other prospective studies suggested that the prediagnostic serum level of α-tocopherol was lower in subjects who subsequently developed colorectal cancer than in noncases (230). Differences were not statistically significant in any one of the five studies, but when the original data from the five studies were pooled and analyzed (230), the OR for the highest quartile of serum α-tocopherol concentration compared to the lowest was 0.6 (95% CI 0.4–1.0) with, and 0.7 (95% CI 0.4–1.1) without, adjustment for serum cholesterol. Three recent case-control studies all suggested decreased risk with higher intakes of vitamin E (63,140,141).

Assessment of dietary intake of carotenoids until recently has been limited to estimates of β-carotene and vitamin A. Furthermore, human study of carotenoids as potential anticarcinogenic agents has been somewhat inhibited by findings of increased risk of lung cancer in smokers in two clinical trials of β-carotene (231,232). Findings in older analytic observational epidemiologic studies are mixed (1,5). Findings in two prospective studies were null (229). In more recent studies, all case-control studies, five (51,63,84,140,141) of six found inverse associations and one (85), no association. One of the studies was able to examine various types of carotenoids (63); inverse associations were found for multiple carotenoids, but not for β-carotene.

The analytic observational epidemiologic evidence for vitamin C has also been mixed, with most studies finding either weak inverse associations or no association (1,5,51,85,140,141).

Selenium has also been found to be inversely associated with colon cancer. Because the selenium content of food varies with soil and growing conditions, dietary intake of selenium cannot be measured accurately in larger, analytic epidemiologic studies. In ecologic studies (233,234), internationally and within the United States, dietary selenium, local plant selenium levels, and blood selenium concentrations were significantly inversely correlated with age-adjusted mortality from cancer of the colon-rectum. In three cohort studies that measured serum selenium levels (209), a marginal association was observed in one; lower mean levels of selenium were found in individuals who de-
veloped colon cancer in a second; and a null association was found in the third. In another prospective cohort study, toenail selenium levels were marginally, but not statistically, associated with risk of colon cancer (235). In the prospective Iowa Women’s Health Study, the adjusted relative risk of colon cancer for those taking selenium supplements compared to those who were not was 0.6 (95% CI 0.27–1.32) [3] (229).

Despite the strong plausibility for a protective effect against cancer, riboflavin, niacin, zinc, and manganese have received little attention in animal or human studies; however, the limited data are generally (236,237), but not entirely (238), supportive for a protective effect for them too.

There are few clinical trial data pertaining to the efficacy of antioxidants in reducing incidence or mortality of cancer, and the colon cancer data are even more limited. There have been two reported clinical trials testing the efficacy of antioxidants in reducing cancer incidence or mortality in which colon cancer incidence was monitored. One study, a randomized, double-blind, placebo-controlled trial in 29,133 50–69-yr-old Finnish male smokers, tested α-tocopherol 50 mg daily and β-carotene 20 mg daily, each alone and in combination, vs placebo over 5–8 yr (231). The primary endpoint of the trial was lung cancer incidence, but colon cancer incidence was monitored. Although there was an 18% increase in the incidence of lung cancer in men on β-carotene alone (but no increase in those on α-tocopherol alone or in combination with β-carotene, perhaps again emphasizing their interdependence), there was a nonstatistically significant decrease in the incidence of colon cancer in those on α-tocopherol, but no apparent effect from β-carotene alone. Data on α-tocopherol and β-carotene in combination in relation to colon cancer were not reported. In the second trial (239), a randomized, double-blind, placebo-controlled clinical trial (n = 1312) of selenium supplementation (200 mcg daily) and skin cancer in which colon cancer incidence was also monitored, there was a statistically significant decrease in the risk (RR = 0.42; 95% CI 0.18–0.95) of colorectal cancer with selenium supplementation relative to placebo.

Although five preliminary clinical trials of antioxidant micronutrient supplements and adenoma recurrence all suggested beneficial effects, a large-scale well-conducted randomized, controlled trial (240) found no efficacy of administering a combination of vitamin E 400 mg, β-carotene 25 mg, and vitamin C 1000 mg in reducing adenoma recurrence over a 4-yr period. In the most striking small trial, a randomized, placebo-controlled clinical trial of polyp recurrence, sporadic adenoma patients (n = 209) treated with vitamin E 70 mg plus vitamin C 1.0 g plus vitamin A 30,000 IU daily over 18 mo had a polyp recurrence rate of 5.7% vs 35.9% for those on placebo (p < 0.001) (241). In a pilot-randomized placebo-controlled trial, sporadic adenoma patients (n = 129) treated with vitamin E 400 mg plus ascorbic acid 400 mg daily over 2 yr had a polyp recurrence rate of 41.4% vs 50.7% for those on placebo (not statistically significant) (242). In three small trials of polyp recurrence in familial polyposis patients, two testing ascorbic acid 3.0 g daily (243,244) and the other testing ascorbic acid 4.0 g plus α-tocopherol 400 mg daily (245), small reductions in polyp formation were suggested. On the other hand, in an analysis of adenoma incidence (n = 146 cases) in the Finnish ATBC trial (231) in older male smokers, there was an OR of 0.98 for 20 mg of supplemental β-carotene daily, and a statistically significant OR of 1.66 for 50 mg of α-tocopherol supplements (246). However, as pointed out by the authors, this may have been the result of a detection bias, especially because the adenoma results are contrary
to the colon cancer results.

One reason that the large-scale Greenberg et al. trial (240) may not have yielded hypothesized results is that, when originally designed, it did not take into account the not-yet-published findings of Vogelstein et al. and others (247) that multiple slowly accumulated genetic alterations are usually required to produce a colonic neoplasm in the non-FAP patient. It would be unrealistic to think that every potential protective factor can protect against cancer by inhibiting every step in this “pathway,” and antioxidants are no exception. If a patient already has several cell lines with the requisite accumulated genetic changes to commit the cell lines to adenoma formation, there may be no mechanism whereby antioxidants could block the growth of the committed lines. Patients with incident adenoma are likely to form recurrent adenoma; thus, it is likely that by the time a person has an incident adenoma, other cell lines are already genetically altered/committed to form adenomas also. We also now know that the natural history of adenoma growth is rather slower in general than originally thought, so slow in fact that the recommended interval for colonoscopic follow-up surveillance for polyp recurrence has now been extended to 5 yr. What this all means, then, is that if a protective agent exerts its action prior to full genetic commitment of a cell-to-adenoma formation, a reduction in polyp recurrence may not be seen until a substantial proportion of the already committed cell lines have “played out,” and this may take longer than the 4 yr of follow-up employed in the Greenberg et al. study.

Epidemiologic data are available to support this line of reasoning. In a Finnish male cohort study, prediagnostic serum vitamin E levels were associated with a decreased risk of all cancers combined, but the inverse association was limited to those younger than 70 yr of age (248). In an American female cohort study, vitamin E intake was associated with a decreased risk of colon cancer, and the inverse association was limited to those younger than 65 yr of age in a monotonic fashion (229). Most of the association was attributable to supplemental vitamin E intake, a relatively recent population phenomenon. Older members of the cohort were more likely to have accumulated the requisite number of genetic changes to form polyps, and thus cancer, than the younger members. Beginning vitamin E supplements at a young age may maximize the potential for protecting against colon cancer because, at that point, the progression of genetic changes is at a point at which vitamin E is effective. Conversely, beginning vitamin E at an older age, for some persons, may simply be too late for it to do much good. The fact that Greenberg et al. did not find treatment differences by age does not negate this argument because all patients in the trial were already adenoma formers, regardless of age.

Despite these comments, it should be pointed out that adenoma patients are still very appropriate subjects for studying the efficacy of various interventions on endpoints earlier in the carcinogenic process than adenomas. Because a sporadic adenoma patient has had a polyp does not mean that all cell lines have been committed to polyp formation. Earlier endpoints, such as colonic epithelial cell-proliferative abnormalities, still exist and can be normalized. Normalization of such early endpoints can still be used as evidence that the tested intervention may be effective for cancer prevention in patients who have not yet formed polyps, as well as in polyp patients once they have been treated long enough and all the cell lines already committed to polyp formation have played out.

Finally, it should be remembered that another, albeit smaller, trial similar to the Greenberg et al. trial found a statistically significant reduction in polyp recurrence (241). Chance always remains a possible explanation for the results of any study in
which inference is required.

9.3. Colon Cancer Intermediate Endpoint Trials

As for calcium, there have been several clinical trials of antioxidants and biomarkers of risk, including colorectal epithelial cell proliferation. In humans given supplemental vitamin E 400 mg plus ascorbic acid 400 mg daily, fecal mutagenicity was reduced by 26% ($p < 0.01$) (249). A higher fat diet increased lipid peroxidation (250,251), but this increase was blocked by higher vitamin E intake (250,251). In small trials, levels of lipid peroxidation products in both exhaled air and in serum were reduced in a dose-response manner by β-carotene in a controlled trial in young healthy adults (252,253); were reduced in serum by an antioxidant micronutrient combination in a randomized placebo-controlled trial in geriatric patients (254); and by selenium in a controlled trial in top athletes (255).

Four small clinical trials in humans suggest that antioxidants can reduce colorectal epithelial cell proliferation. In a small randomized, placebo-controlled clinical trial in adenoma patients ($n = 41$), those given α-tocopherol 70 mg plus ascorbic acid 1000 mg plus vitamin A 30,000 IU had a 45% greater reduction than those given placebo in the LI of the upper 40% of the colonic crypts (183). In an uncontrolled trial ($n = 10$), the LI dropped 33% in sporadic adenoma patients given 200 mcg of selenium for 1 mo (256). In a 1-mo randomized placebo-controlled four-armed parallel group trial in sporadic adenoma patients ($n = 48$), those given vitamin C 750 mg had a 54% drop in LI, those given β-carotene 9 mg had a 41% drop, and those given α-tocopherol 160 mg or placebo had no change (257). Finally, in a trial in familial polyposis patients ($n = 17$), those given ascorbic acid 3.0 g daily had a 19% greater reduction in the labeling index than those on placebo (243).

9.4. Summary

In summary, the rationale for a causal role of oxidative damage and a protective role of antioxidant micronutrients in colon carcinogenesis is quite strong. The existing data from experimental animal studies are strong but are of uncertain relevance to humans. Although a few consistent patterns appear to be emerging from the observational epidemiologic studies, by and large, the results of these studies have been unclear. These studies are hampered by the homogeneity of dietary constituents within populations, the multitude of dietary factors and their interactions, and the limited accuracy of current dietary measures. It would, therefore, appear that carefully designed, sufficiently long clinical trials will be the best way to evaluate this group of potential protective factors. Two recent clinical trials directed at other endpoints found reduced risk for colon cancer with antioxidant micronutrient supplementation [the results of one, a trial of vitamin E, β-carotene, and vitamin C were not statistically significant (231), but the results of the second, a trial of selenium supplementation were (239)]. The conservative interpretation of the results of adenoma recurrence trials is that antioxidants do not reduce recurrence in the relative short term ($< 5$ yr). The clinical trials of antioxidants and biomarkers have largely been small and of a preliminary nature. Future clinical trials would address the following gaps of knowledge, whether antioxidants can:

1. Effectively modulate endpoints early in the carcinogenic process (e.g., colorectal epithelial cell proliferation, COX-2 expression, and so on;
2. Reduce colon polyp recurrence after 5 yr of treatment;
3. Reduce incidence of adenomatous polyps after 5 yr of treatment;
4. Reduce incidence of, or mortality from, colon cancer after 5 or more years of treatment.

Clinical trials to address gaps 2–4, the adenoma or carcinoma endpoints, would require extremely large sample sizes, prolonged follow-up, or both; consequently, they would be extremely expensive and may not be justified by the current level of evidence. However, full-scale clinical trials using biomarkers or precursors for colon cancer, such as colorectal epithelial cell proliferation, that can be measured easily and allow the use of small sample sizes and short interventions are indicated to determine whether adenoma or colon cancer trials of sufficient duration and sample size/statistical power should be undertaken.

10. FOLATE, METHIONINE, VITAMIN B₆, AND VITAMIN B₁₂

Folate intake as a potential protective factor against colon carcinogenesis has been of recent interest. As reviewed elsewhere (258), dietary folate, methionine, vitamin B₆, vitamin B₁₂, and alcohol have been associated with colon cancer in some, but not all, epidemiologic studies. It has been hypothesized that these dietary factors work together through their involvement in DNA methylation processes. Methylation plays an important role in gene regulation. In colonic neoplasms, generalized hypomethylation, as well as hypermethylation of cytosine-rich areas can be frequently found. This imbalance in methylation of DNA is thought to result in abnormal expression of oncogenes and tumor suppressor genes. The methylation process involves several steps and several dietary factors such as folate, methionine, vitamin B₆, and vitamin B₁₂ are potentially involved in these processes. The folate pathway is also important in determining the availability of nucleotides for DNA synthesis. 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrolate to 5-methyltetrahydrofolate, the major circulatory form of folate and carbon donor for remethylating homocysteine to methionine. Folate in the form of methyltetrahydrofolate and B₁₂, transmethylase, is involved in these pathways, as is vitamin B₆, the co-factor for serinehydroxymethyltransferase, which could have an impact on the availability of 5,10-methylenetetrahydrofolate (MTHFR). Methionine is a precursor for S-adenosylmethionine, the methyl donor for most biological transmethylation reactions in the body, including that of DNA. Alterations in plasma homocysteine can result from genetic or nutrient-related disturbances in the transsulfuration or remethylation pathways of homocysteine metabolism. Thus, MTHFR is involved in regulating plasma homocysteine concentrations and maintaining an adequate methionine pool. A variant of the MTHFR gene has been described: the variant form results in elevated plasma homocysteine concentrations and maintaining an adequate methionine pool. Persons homozygous for the variant have 30% of normal enzyme activity, and heterozygotes have 65% of normal activity.

Global DNA hypomethylation is consistently found in colon neoplasms (259–261). It also appears to be an early event in the multistep process of colon carcinogenesis, occurring in nonneoplastic tissue prior to the development of the neoplasm. Diets deficient in folate and methionine (and possibly vitamins B₆ and B₁₂) may cause DNA hypomethylation (262). In addition, alcohol, a methyl group antagonist, may cause DNA hypomethylation (262). Thus, a high alcohol intake, in combination with a diet low in folate and methionine, may cause even greater DNA hypomethylation. A major
source of dietary folate and vitamin B₆ is plant foods, and that of methionine and B₁₂, animal products, including red meat as well as poultry, fish, seafood, and dairy products. Finally, the impacts of these dietary factors may be modified by a person’s MTHFR genotype. In support of these biochemical findings providing biological plausibility for the hypothesis that folate and methionine may protect against colon cancer, is that methyl-deficient diets have been shown to cause various cancers in animals (263–267).

Among 11 recent human analytic observational epidemiologic studies (131,140,141,143,258,268–273), an inverse association between folate and risk of colon cancer was reported in five case-control studies (131,140,141,258,268,273) and five cohort studies (143,269–272). The authors of one cohort study also found, using the same food frequency questionnaire, an inverse association with colon adenoma in two cohorts (274). In the cohort studies, the association was strongest in those with high alcohol consumption. In the Nurses’ Health Study (270), a statistically significant overall relative risk of 0.69 was found; for persons taking folate-containing vitamin supplements, substantial reductions in risk were not seen until after 14 yr of use (RR = 0.25, 95% CI 0.13–0.51). In the Male Health Professionals Follow-up Study (272), a relative risk of 0.56 was found with high-folate intake; among persons who were homozygous mutant MTHFR, the relative risk was a statistically significant 0.32, whereas there was no evidence of an association among persons with homozygous normal MTHFR. In the Iowa Womens’ Health Study (143), the relative risk was a statistically significant 0.7 among persons without a history of a first degree relative with colon cancer, but 0.9 among persons without such a history. In a clinical trial cohort of older Finnish male smokers (271), a relative risk of 0.51 was found with folate; the relative risk was a statistically significant 4.79 among those with a diet low in folate and high in protein and alcohol. A population-based case-control study in Seattle, WA found a halving of risk with higher folate intake (141), and a hospital-based case-control study in Italy found an odds ratio of 0.83 (not significant) (140). A population-based case-control study in areas of Utah, Minnesota, and California (258) found no independent associations of folate, methionine, vitamin B₆, vitamin B₁₂, and alcohol and risk for colon cancer. However, high levels of folate, vitamin B₆, and vitamin B₁₂ were associated with a 30–40% reduction in risk among those who were homozygous for mutant MTHFR vs those who were homozygous for normal MTHFR. Lowest risk was seen among persons homozygous for mutant MTHFR and who consumed no alcohol and the highest levels of folate and methionine (OR = 0.4, 95% CI 0.1–0.9).

11. TEA

Although associations of tea with risk for colorectal cancer have been investigated previously, only recently have strong, biologically plausible mechanisms been articulated (275). Polyphenols, such as epigallocatechin gallate, have been proposed as potential protective constituents in tea. Polyphenols have antioxidant activity. It has also been proposed that tea polyphenols selectively inhibit growth of intestinal Clostridia and reduce the biotransformation of procarcinogens to carcinogens. Others have proposed that tea inhibits protein kinase C activation. A few studies in animals have found reductions of colon tumorigenesis in animals with green tea polyphenol fractions and green tea extracts.

The findings from the observational epidemiologic literature are quite mixed. Of
at least 15 analytic observational epidemiologic studies \((46,58,59,112,192,276–285)\) [11 case-control \((47,58,59,112,192,276,278,280,283,284,285)\) and four cohort \((277,279,281,282)\)] that investigated tea, nine, \((46,59,112,192,276,279,280,282,285)\) including two cohort studies \((279,282)\), found inverse associations of which only three \((59,112,276)\), all case-control studies, were statistically significant. Six studies \((58,277,278,281,283,284)\), including two cohort studies \((277,281)\), found direct associations of which four [two cohort \((277,281)\) and two case-control \((283,284)\)] were statistically significant. The studies were based in a wide variety of countries including the US (Washington and Hawaii), China, Japan, Italy, Switzerland, Sweden, the Netherlands, Belgium, Finland, Argentina, and the United Kingdom. The level of exposure in the different studies varied considerably. The exposure in most studies was black tea. Two studies, both case-control, had good measures of green tea exposure \((192,276)\) and both found inverse associations [statistically significant in the one from Shanghai, China \((276)\)]. In the study finding the not statistically significant inverse association, a not statistically significant direct association was found for black tea \((192)\).

There is no clear pattern of differences according to colon sites. The Shanghai case-control study of green tea found stronger statistically significant associations for rectal cancer and among women \((276)\); whereas the Finnish clinical trial cohort study found a statistically significant direct association for colon cancer and a not statistically significant inverse association for rectal cancer \((277)\); and the Hawaiian cohort study found a statistically significant direct association for rectal cancer, but no association for colon cancer \((281)\).

At this point in time, the limited observational epidemiologic literature does not support a role for tea in the etiology or prevention of colon cancer.

12. OTHER ASPECTS

Other aspects of diet have been examined in basic, animal, and a few human studies. Investigated factors have included dietary diversity \((286,287)\); dietary patterns \((288)\); other specific foods, coffee, nutrients, micronutrients, and nonnutrients; cooking vs raw foods; \(N\)-nitroso compounds \((31,54)\); and others. There is as yet not enough human evidence to discern a pattern or lack thereof for many of these aspects of diet.

13. A MODEL OF DIET AND NUTRITION IN THE ETIOLOGY AND PRIMARY PREVENTION OF COLON CANCER

Colon carcinogenesis can be thought of as a long-term, multistep process, in which multiple somatic genetic defects are accumulated (see Fig. 3) \((2,247)\). The possibility exists that various factors can influence the occurrence or even the reversal of each of the steps (see Fig. 4; then refer back to Fig. 3 for specific points in the molecular carcinogenesis of colon cancer that diet could impact that could be currently studied). By inference, the possibility exists that, even after several steps have occurred, the entire process can be reversed. It would seem that the likelihood of reversing some steps would be greater than that of others, and that the likelihood of reversing the entire process would be greater when few rather than many steps have occurred. Naturally occurring foods, food processing, and food preparation methods introduce into the diet multiple factors hypothesized to either increase or decrease the risk of colon cancer. Considering the multistep process of colon carcinogenesis, and the multiple biologi-
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... cally plausible and well-supported dietary factors (although none have acquired consensus status for being established as causal), it would seem likely that risk is a balance of genetic susceptibility, levels of several dietary and other risk factors that increase risk, and levels of several dietary and other risk factors that decrease risk. Some dietary components may influence risk at different steps than do other dietary components; some may influence risk at more than one step; some, because of genetic reasons, may influence risk in some individuals and not in other individuals; some may influence risk only in association with other dietary components; and some may interact with other dietary components in other ways.

Thus, a magic bullet approach to prevention, particularly from the public health standpoint, would seem naive and inadequate. A multifactorial approach to prevention would seem most prudent at this time. The diet that would appear ideal, based on current knowledge, for preventing colon cancer would be most like the Paleolithic diet (described in ref. 103), the diet of the earliest evolving/evolved Homo sapiens. It is mostly current deviations from this diet that are associated with increased risk for colon cancer: diets high in fat, sucrose, and alcohol, and low in vegetables and fruit and all their associated constituents, including fiber, antioxidants, folate, calcium (wild plant foods were a high source of calcium, thus providing 1500–2000 mg daily compared to the current American average intake of 740 mg), and other nutrients and non-nutrients. The role of meat, apart from fat, is unclear. Meat was clearly a component of the Paleolithic diet, and

Fig. 3. A model of the molecular biology of colorectal cancer showing potential points of action and study of the effects of dietary agents on colorectal carcinogenesis (abbreviations: ACF, aberrant crypt foci; β-Cat., β-catenin; LOH, loss of heterozygosity).
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is a source of methionine and vitamin B12. Wild meat, even red wild meat, is low in fat. The observational epidemiologic literature would suggest increased risk with modern red meat, which is largely high in fat, but no or decreased risk with seafood and skinless white poultry, probably the closest approximations to the wild meat of the Paleolithic period. Contributions of current cooking methods remain to be determined.

The diet that protects, then, is likely to be low in fat (and thus in domesticated high-fat red meat), sucrose, and alcohol, and dense in micronutrients (calcium, antioxidants, folate, and others), fiber, and various plant-derived non-nutrient compounds. Because of modern agri-business practices designed to produce vegetables and fruit for transportability and shelf-life, for more succulence and less fiber, for more sweetness and less tartness, and so on many are concerned that even with a vegetarian diet, that the modern American eating these commercially grown foods cannot achieve the nutrient and fiber density of the Paleolithic diet. The optimum solution would be to, as is already occurring in the red meat industry and others, to move to foods that more closely approximate wild foods in being low in fat and dense in micronutrients. An obvious, but unanswered, question is whether nutritional supplements, such as vitamins and minerals, can overcome some of the nutritional deficiencies (relative to the Paleolithic diet) in diets based on modern commercial vegetables and fruit, and thus provide some protection against colon cancer, especially in high-risk persons who fail to increase their intake of vegetables and fruit or change other high-risk characteristics of their diets.

14. NEEDED RESEARCH
Some areas of needed research have been alluded to throughout this chapter and are summarized in Table 9. Increased understanding of the molecular basis of colon carcinogenesis is opening up opportunities for increasing the understanding of the environmental determinants of colorectal cancer. Fig. 3 shows a schema summarizing current knowledge of molecular events involved or associated with colon carcinogenesis (2), where diet may have an impact, and what genes/proteins and gene expressions could be assessed for impact.

15. SUMMARY

Diet and nutrition clearly play a role in the etiology and primary prevention of colon cancer. The most consistent dietary factor associated with colon cancer is vegetable and fruit intake, with a high intake being associated with a decreased risk. Many of the dietary constituents that have been less well studied, but are emerging as at least fairly consistently associated with a decreased risk of colon cancer are nutritive and nonnutritive substances that are most abundant (or could be most abundant; e.g., calcium) in vegetables and fruit, including fiber, calcium, antioxidants, and folate. In addition to many of these relatively well-studied constituents of vegetables and fruit, there is a myriad of other compounds in vegetables and fruit that plausibly may reduce the risk of colon cancer that have been scarcely studied. Many of the dietary constituents of the modern American diet that are most consistently associated with an increased risk of colon cancer are found in abundance in diets low in vegetables and fruit, including fat, sucrose, and high-fat red meat cooked at high temperatures. An explosion in understanding of the molecular basis of colon carcinogenesis is beginning to fuel a leap forward in understanding the contributions of diet to modulation of risk. Individualization of prescriptions for risk reduction, as well as substantial reductions in population risk, are within sight.

16. RECOMMENDATIONS
Based on current knowledge, perhaps the most prudent diet would be one modeled after the Paleolithic diet: a diet high in plant foods (vegetables, fruit, nuts, seeds, whole grains) and low in fatty meats and other sources of fat, alcohol, and sucrose. Evidence from studies of several dietary constituents that are found in most abundance in vegetables and fruit would suggest that changes in agricultural practices to return to more micronutrient rich vegetable and fruit varieties may be helpful. Until that time, dietary supplementation with micronutrients such as calcium, antioxidants, and folate should receive further study as measures for high-risk individuals unable or unwilling to achieve the ideal diet, realizing that this would not be optimum because current supplements would not be able to address all of the deficiencies of a low vegetable and fruit diet. The current literature provides optimism that colon cancer can be prevented and that diet and nutrition will be key.

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1. INTRODUCTION

In searching for the causes of any cancer, it is natural to think first of substances that come into direct contact with the organs and cells that are involved. For lung cancer, the early suspects were smokes, dusts, and gases, particularly smoke from burning tobacco; dusts containing carcinogens such as arsenic or asbestos; and radon gas. At present, these and other airborne substances have become accepted carcinogens (1). Tobacco smoke is by far the most important because of the intensity, prevalence, and ubiquity of exposures to this complex mixture of known and suspected toxins and carcinogens. Not everyone exposed to even high concentrations of these inhalants develops lung cancer, however, and lung cancer occurs among some persons with little or no known exposures. These observations suggest that there are other causes of lung cancer and perhaps substances that prevent or inhibit carcinogenesis. Neither carcinogens nor protective substances have to arrive at the lungs by way of the airways. Anything that is absorbed into the bloodstream has the potential to reach every cell in the body. In this way, dietary components can also affect the tracheo-bronchial tree and the pulmonary parenchyma.

2. CELL TYPES

A potential problem with much of the literature on lung cancer is that this disease is treated as a single entity. Reports usually ignore the possibility that different histologic cell types might have different causes. One reason for lumping cell types together is the heterogeneity of histologic features even within cell types and the consequent unreliability of cell-type classification (2). Fortunately, the associations of each reported cell type with sex, smoking, most occupational exposures, and nutritional factors appear to differ mainly in degree and not in direction. In any case, very few reports dealing with nutrition specify cell types; hence, cell type will have to be ignored in this chapter.

3. STUDY DESIGNS

3.1. Types

Nutritional studies that relate to the prevention of lung cancer involve many study designs. Those that produce the clearest evidence are experiments on animals in which virtually all known variables can be controlled. Such studies have led to numerous advances in human nutrition. But appealing as they may be, they can be considered only
rough guides to human studies of cancer. Animals often metabolize carcinogens and nutrients differently than humans; it is difficult to mimic most human environments and no suitable laboratory animal survives long enough to show the effects of long-term exposures.

Similar experimental studies on humans have to be conducted on metabolic wards, are expensive, and rarely involve more than a few subjects. Most important, carcinogens cannot be administered and long-term observations are not feasible. Experimentally, about the most that can be done with adequate numbers of people is to assess the effects of various dietary components on the endogenous production of various harmful oxidation products or their markers, such as breath ethane. The most viable alternative is to conduct observational studies among free-living general populations to see what outcomes are associated with what people eat.

The simplest observational studies among humans are ecologic in nature. In these studies, population characteristics of various sized geographic units are correlated with the incidence or mortality of lung cancer. Their major advantage lies in the fact that nutritional exposures may differ much more between populations than within them, thereby producing sharper contrasts. On the other hand, data on production, sales, or consumption in geographic units are available for only a few nutrients, and none of these indexes can accurately reflect what is actually eaten by individuals in the populations.

Cross-sectional surveys and case-control studies come closer to being able to evaluate differences in dietary intakes of persons with lung cancer and similar persons who have not developed lung cancer. Cross-sectional surveys suffer from the fact that, at the time of study, the case group is heavily weighted with survivors. The experience of those who survive may or may not be typical of those who have already died. Cross-sectional studies, in which information about diet and cancer are obtained for the same time period, also suffer from the fact that dietary histories tend to be influenced by current diet, which may be altered considerably by symptoms of the disease or the effects of treatment. Case-control studies of newly diagnosed cases not only can have the same problems, but are beset by the difficulties of obtaining accurate and unbiased histories of dietary habits that existed a long time prior to diagnosis.

Prospective studies, such as cohort or nested case-control studies, are more suitable for investigating the associations between nutrients and lung cancer than cross-sectional or case-control studies. Because dietary histories in these types of studies are obtained before cancer has been diagnosed, they cannot be influenced by the presence of disease.

Such considerations are even more pertinent for investigating associations of serum components with cancers that cause symptoms or systemic effects that could affect concentrations of nutrients in the serum. In both cross-sectional and case-control studies, serum is drawn after lung cancer is diagnosed, and it is impossible to tell how much of the difference in serum concentrations between cases and noncases is because of differences that existed long before the cancer was recognized and how much is due to the effect of a manifest cancer and consequent illness on serum concentrations. As a result, cross-sectional and case-control studies of associations of serum components with lung cancer will not be considered further.

*“Serum” will be used to denote either serum or plasma.*
In all of these observational studies, it is possible to conclude only that dietary intake or serum concentrations are associated with lung cancer prevalence, incidence, or mortality. A statistically significant association merely indicates that chance was unlikely to have produced the observation. It tells nothing about the likelihood that some unsuspected flaw in design or some unknown confounder might have been responsible. Only when the result is replicated in different populations and by different study designs is one entitled to believe that the association is true and general. Even so, there is always the possibility that the association with nutrient X came about because it was a “fellow-traveler” with the true, but unsuspected causal agent.

Controlled trials produce the most convincing evidence of causation. But even with this study design, replication in a different population is highly desirable. Rare events, such as statistically significant but false outcomes, can occur. Replication in other populations can reduce such a probability almost to the vanishing point and also reduce the possibility that the initial finding resulted from some unsuspected interaction of the treatment with a peculiarity of a particular study population. But there are disadvantages to controlled trials. For relatively uncommon outcomes, the study population must be very large. Dosages and methods of administration have to be fixed and only a few preventive agents or regimens can be tested. Most important, if the event that initiates carcinogenesis occurs long before cancer becomes manifest, or if the initiating event occurs prior to adult life, a controlled preventive trial must continue over a very long period.

In summary, controlled trials are the only study design capable of proving causation. They are limited to investigating only a few preventive agents and regimens, and may require very long periods to study effects on initiation of carcinogenesis. Prospective studies also require long periods of observation and can only determine associations. Case-control and cross-sectional studies are not suitable for most major cancers; cross-sectional studies cannot clearly differentiate whether the tumor affected the diet or vice versa. Ecologic studies are quick and inexpensive, but do not lend themselves to studies of minor nutrients and are rarely more than suggestive. The most reliable information comes from careful consideration of all available evidence.

For all observational studies, consistent failure of a nutrient to be associated with prevention is probably good evidence that it is not a protective agent. In contrast, demonstration that a nutrient is associated with a decrease in cancer, even if consistently observed in a variety of circumstances, does not mean that the observed protection is owing to that particular nutrient. It may only be associated with the true protective factor. It behooves us always to think about what might accompany the substances we are studying.

### 3.2. Smoking and Diet

Because smoking is so strongly implicated in the pathogenesis of lung cancer, many authors have felt compelled to remove the effects of smoking by matching or statistical adjustment. The wisdom of this approach is debatable. Numerous studies have shown that the diets of smokers and nonsmokers differ. The diets of nonsmokers married to spouses that smoke are also likely to be different from nonsmokers married to non-smokers. Both active and passive smokers (exposed nonsmokers) are usually reported to eat fewer fruits and vegetables than nonsmokers (3–11). Dietary intake is an important determinant of the level of nutrients that reach the cells. If smoking is the cause of
a lower intake of fruits and vegetables, then the dietary deficit associated with smoking is a link in the chain of causation with respect to the protective effect of fruits and vegetables (or any specific nutrients they contain) against lung cancer. Under such circumstances, adjustment or matching is usually not appropriate. The adjusted values merely show the effect of a nutrient after the effect of the reduced value because smoking, and its associated dietary changes, has been removed. If, on the other hand, one considers smoking to be independent of dietary change, then smoking is a confounder and adjustment is entirely appropriate. In any case, the most informative way to report results with respect to smoking is to show the findings both unadjusted and adjusted for smoking, or much better, separately for smokers and nonsmokers. Unfortunately, neither approach is common.

4. NUTRIENTS

4.1. Cholesterol and Fat

Interest in the possible effects of diet on the development of lung cancer appears to have arisen from several sources: trials of cholesterol-lowering diets (12); a general belief in the health promoting effects of fruits and vegetables (13–14); and possibly an early animal study of vitamin A deficiency (15). One of the first trials of the effects of substituting polyunsaturated for saturated fats in the diet showed an encouraging reduction in mortality from causes related to atherosclerosis, although this finding was not confirmed in similar trials (12). An alarming finding was that an excess of cancer developed during the course of the study among the group fed polyunsaturated fats (16). Case-control studies designed to confirm and elucidate that finding have tended to show, contrary to the finding of the early trial, that persons with the highest intake of cholesterol had an increased risk of lung cancer, even after adjustment for smoking (17–20). In two of the studies, the association was strongest among men and for squamous and small cell cancers (17,18). Cohort studies have yielded divergent results: one result in Illinois found a significant association of lung cancer risk with increased cholesterol intake (21) and studies in Hawaii and Finland failed to demonstrate this association (22,23).

The frequency of lung cancer has been reported among participants in two large cohort studies and in five controlled trials of regimens designed to reduce serum cholesterol. Among 160,135 participants in a screening program, 528 cases of lung cancer developed among men and 315 among women two or more years after serum cholesterol was determined (24). Among men, those in the lowest fifth of the cholesterol distribution had a risk of developing lung cancer 1.92 times that of men in the highest fifth; for women, the relative risk was 2.28.

Among 151 men and 59 women with lung cancer reported in the NHANES I follow-up study, the odds ratio for developing this disease was 1.66 times greater among men in the lowest fourth of the cholesterol distribution than among men with cholesterol levels of 190–216 mg/dL. Among women, the odds ratio was 2.58 compared to those with cholesterol levels of 186–216 mg/dL (25). However, in neither of the two studies was the dose-response-trend monotonically linear. In three controlled trials, lung cancer death rates were higher in the group under treatment designed to lower cholesterol (26–28), in another there was no difference between the two groups (29), and in two, lung cancer deaths were more frequent among the controls (30).
Closely related to the cholesterol studies are those that dealt with dietary fat intake. Ecologic studies have shown strong correlations between per capita fat consumption and lung cancer mortality, more marked among men than among women (31,32). Some case-control studies have also found that high fat intake was associated with lung cancer (17,33–35). The degree of saturation of the fat was rarely mentioned. One case-control study among nonsmoking women found a much stronger association of saturated fat in the diet with subsequent lung cancer than total fat, cholesterol, oleic acid (monounsaturated) or linoleic acid (polyunsaturated) (35). Confirmation came from a 20-yr follow-up study among Finnish men (36). High intakes of milk, butter, and meat were associated with higher rates of subsequent lung cancer. A protective association with margarine was based on a small number of users and was attributed to a lifestyle consistent with a high degree of health consciousness. Another case-control study among older women in Iowa came to the opposite conclusion (37). Women with the highest intakes of fat had lower lung cancer risks. The results achieved statistical significance only for plant fats.

Two reviews have concluded that there was an association of high dietary intakes of cholesterol and fat with lung cancer (14,38). Another commentary listed a number of potential biologic mechanisms by which fat might contribute to cancer risk (39). Equally appealing as the hypothesis that cholesterol and fat in diets might contribute in some way to carcinogenesis is the possibility that such diets are low in nutrients that might be protective (3,5–10). Methodologic problems also affect estimates of risk associated with fat intake. In addition to the difficulties in separating the effects of fat from those of total energy intake, Swanson et al. has pointed out that the methods used for adjustment also affect the estimate (40). The risk of high vs low-fat intake in the Missouri study (35) was 6.3 using the standard multivariate regression model, but only 1.8 using the method of nutrient residuals.

Serum cholesterol is closely related to fat and cholesterol in the diet. Summaries of prospective studies of serum cholesterol and lung cancer, representing 10 different populations, are shown in Table 1. Baseline levels of serum cholesterol tend to be slightly, but not significantly, lower among persons who subsequently developed lung cancer. The study in Hawaii, which showed the greatest difference, was based on lung cancer deaths and probably included a few prevalent cases at the time serum cholesterol was determined (43). If there is an association of serum cholesterol with subsequent lung cancer, it does not appear to be of much importance.

4.2 Alcohol

Relatively little attention has been paid to alcohol intake as a risk factor for lung cancer. Studies in Europe, Asia, and North America have agreed that there is an excess risk of lung cancer among moderate to heavy users of alcohol (34,48–54). In most of them, the findings were adjusted for the effects of smoking and no mention was made of any interactions of smoking and alcohol intake. In the study involving women in Hong Kong who had never smoked, the risk associated with moderate use was close to twice that for nonusers (49).

4.3 Fruits and Vegetables

Numerous investigators have examined the possibility that fruits and vegetables might not only be “good for you,” but that they might in some way protect against cancer. A thorough review of this topic was published in 1991 (13). It concluded that “con-
Consumption of higher levels of vegetables and fruit is associated consistently, although not universally, with a reduced risk of cancer at most sites. Included in this review were five cohort and eight case-control studies of lung cancer. A weak to moderate association was found between a low intake of vegetables and/or fruit and an increased risk of lung cancer in most of these studies.

In the eight years since that review was published, the numbers of papers on this topic has more than doubled. Included in this number is a recent comprehensive review of nutritional factors related to lung cancer (14). A small ecologic study of lung cancer in northern and southern Italy showed that death rates were lower in southern Italy where the diet was low in saturated and polyunsaturated fats and high in vegetables, even though smoking habits were similar in the two areas (32).

Among the recent studies, there is a nested case-control study from Finland (36), three cohort studies (52,55,56), and updated reports of two previously published studies (53,57). These results are summarized in Table 2. Evidence from the cohort studies favors a decreased risk of lung cancer associated with an increased intake of fruits. For vegetables, the evidence points in the same direction, but less strongly and consistently. In the Zutphen study, the associations with intakes of fruits and vegetables were strongest among the men who were in the same third of dietary intake on each of three occasions, 5 yr apart.

In addition to the eight case-control studies summarized by Steinmetz and Potter (13), findings from 13 later reports are shown in Table 2. In these studies, evidence regarding the intake of fruits is split between positive and negative associations with lung cancer. However, those favoring a negative (protective) association are both more numerous and the associations are stronger. Nearly all the case-control studies agree in finding that increased intakes of vegetables are associated with a decreased lung cancer risk.

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* Includes some prevalent cases.
* Cancer deaths in first 5 yr of follow-up excluded.
* Cases in first year of follow-up excluded.
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Table 2 (continued)

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</tr>
<tr>
<td></td>
<td>Green vegetables</td>
<td>M All</td>
<td>Negative</td>
</tr>
<tr>
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<td>Fruits</td>
<td>WM All</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WF All</td>
<td>Negative</td>
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<td>BM All</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BF All</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>WM All</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
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<td>WF All</td>
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</tr>
<tr>
<td></td>
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<td>BM All</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>BF All</td>
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</tr>
<tr>
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<td>Non</td>
<td>——</td>
</tr>
<tr>
<td></td>
<td>All</td>
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<td>Negative</td>
</tr>
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<td></td>
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<td>Negative</td>
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<tr>
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<td>Fruits</td>
<td>F Non</td>
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</tr>
<tr>
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<td>Vegetables</td>
<td>F Non</td>
<td>Negative</td>
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<tr>
<td>----------</td>
<td>----------------------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Gao (65)</td>
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<tr>
<td></td>
<td>Fruits</td>
<td>M</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>Ex</td>
</tr>
<tr>
<td></td>
<td>Green vegetables</td>
<td>M</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Ex</td>
</tr>
<tr>
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<td>M</td>
<td>Current</td>
</tr>
<tr>
<td></td>
<td>Mayne (66)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Fruit/fruit juices</td>
<td>M</td>
<td>Non</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>M</td>
<td>Non</td>
</tr>
<tr>
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<td>Fruits</td>
<td>F</td>
<td>Non</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>F</td>
<td>Non</td>
</tr>
<tr>
<td></td>
<td>Raw fruits and</td>
<td>M&amp;F</td>
<td>Ex</td>
</tr>
<tr>
<td></td>
<td>vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sankaranarayanan</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>M</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nyberg (68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citrus fruits</td>
<td>M&amp;F</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>Other fruits</td>
<td>M&amp;F</td>
<td>Never</td>
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<tr>
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<td>Carrots</td>
<td>M&amp;F</td>
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<tr>
<td></td>
<td>Other vegetables</td>
<td>M&amp;F</td>
<td>Never</td>
</tr>
</tbody>
</table>

NC: Not calculable.

* All = Nonsmokers and smokers; Never = Never smoked; Non = Never and former smokers; Ex = Former smokers; Current = Smoking at time of study.

+ Positive = Level higher in cases; Negative = Level higher in noncases.

<table>
<thead>
<tr>
<th>Strength</th>
<th>Relative risks or odds ratios</th>
<th>% difference in means</th>
</tr>
</thead>
<tbody>
<tr>
<td>±</td>
<td>(0.91–1.09) or (1.10–1.25)</td>
<td>± 0–4.9</td>
</tr>
<tr>
<td>+</td>
<td>(0.60–0.79) or (1.26–1.67)</td>
<td>± 10–19.9</td>
</tr>
<tr>
<td>++</td>
<td>(0.40–0.59) or (1.68–2.50)</td>
<td>± 20–29.9</td>
</tr>
<tr>
<td>+++</td>
<td>(&lt;0.40) or (&gt;2.50)</td>
<td>± 30+</td>
</tr>
</tbody>
</table>

* Adjusted for smoking.

* Never and former light smokers.

* Former heavy smokers.

* Stable intake, 3 periods.
Few of these studies have looked at interactions between dietary intakes and sex or smoking. When both sexes and all smoking groups have been included in the same study, the cases among women and nonsmokers have usually been too few for meaningful comparisons. When studies are limited to women and nonsmokers, comparisons with men and smokers within the same setting are impossible. As a result, meaningful conclusions cannot be drawn about interactions of diet with sex or smoking at the present time.

Although carrots and cruciferous vegetables are often among the individual food items that are mentioned as being associated with decreased risk, in most instances the broader, more inclusive categories such as “vegetables” or “green and yellow vegetables” tend to be more strongly associated with decreased risk than individual vegetables.

The findings summarized here and earlier (13,14) show that a diet that includes a high proportion of fruits and vegetables is likely to be associated with protection against lung cancer. On balance, the results are more than sufficient to support public health action, such as the recommendations for increasing intake of fruits and vegetables in the dietary guidelines issued by governmental and private health agencies (69–70).

4.4. Retinol

In 1925, it was reported that rats deprived of “fat-soluble A vitamin” showed “replacement of various epithelia by stratified squamous keratinizing epithelium” (15). These changes, generally considered to be precancerous, were noted throughout the respiratory tract, including the bronchi. When butter, a source of retinol, was added to the diet, these changes were prevented. It was 50 yr later before a similar study was done among humans. It found that lung cancer was associated with diets deficient in “vitamin A” as indicated by the consumption of carrots (a source of β-carotene) and milk and eggs (sources of retinol) (71).

Many of the earlier dietary reports referred to “vitamin A” without specifying whether this was retinol, some of the carotenoids (mainly β-carotene), or both. Such studies have been omitted from further consideration. Fourteen that specifically dealt with dietary retinol are summarized in Table 3. Only two showed a strong negative association indicating possible protection (72,74). Most of the others showed weak positive associations.

As a serum biomarker, retinol has a number of advantages for cancer studies. It is relatively stable when stored in serum at –20°C (76). Serum levels tend to be constant for individuals, falling appreciably only when liver stores are badly depleted and rising only temporarily after ingestion of large doses. These characteristics make it unsuitable as an index of adequacy of individual body stores. But because most individuals’ serum retinol concentrations are likely to be constant over long periods of time, the serum level to which cells are exposed at the time of blood drawing is likely to represent a long-term exposure. How retinol might act in protecting against cancer is uncertain, but it seems more likely to be involved in repair of oxidative damage than in its prevention.

Ten studies that compared serum retinol concentrations at baseline among persons who subsequently developed lung cancer and persons who did not are summarized in Table 4. Negative associations are more common than positive associations, suggesting that higher serum concentrations are associated with decreased risk. However, few are strong and only one is statistically significant. Only in the Finnish study were findings
Table 3

Association of Dietary Retinol with Lung Cancer in Selected Observational Studies, by Type of Study, Characteristics of Study Participants, and Nature of Association

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Type of study</th>
<th>Characteristics of participants</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knekt (36)</td>
<td>Cohort</td>
<td>Number 2121, Finland M Non</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2417, Finland M Current</td>
<td>Positive</td>
</tr>
<tr>
<td>Chow (52)</td>
<td>Cohort</td>
<td>Number 17,633, United States M All</td>
<td>Negative</td>
</tr>
<tr>
<td>Bandera (34)</td>
<td>Cohort</td>
<td>Number 27,544, New York M All</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20,456, New York F All</td>
<td>Positive</td>
</tr>
<tr>
<td>Yong (56)</td>
<td>Cohort</td>
<td>Number 10,068, United States M&amp;F All</td>
<td>Positive</td>
</tr>
<tr>
<td>Pastorino (72)</td>
<td>Case-control</td>
<td>Number 206, Italy F All</td>
<td>Negative</td>
</tr>
<tr>
<td>LeMarchand (73)</td>
<td>Case-control</td>
<td>Number 827, Hawaii M All</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>370, Hawaii F All</td>
<td>Positive</td>
</tr>
<tr>
<td>Dartigues (74)d</td>
<td>Case-control</td>
<td>Number 318, France M&amp;F All</td>
<td>Negative</td>
</tr>
<tr>
<td>Kallandidi (58)</td>
<td>Case-control</td>
<td>Number 211, Greece F Non</td>
<td>Positive</td>
</tr>
<tr>
<td>Harris (59)</td>
<td>Case-control</td>
<td>Number 193, England M All</td>
<td>Positive</td>
</tr>
<tr>
<td>Candelora (61)</td>
<td>Case-control</td>
<td>Number 387, Florida F Never</td>
<td>Positive</td>
</tr>
<tr>
<td>Alavanja (64)</td>
<td>Case-control</td>
<td>Number 1450, Missouri F Non</td>
<td>Positive</td>
</tr>
<tr>
<td>Mayne (66)</td>
<td>Case-control</td>
<td>Number 424, New York M Non</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>402, New York F Non</td>
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<td></td>
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<td>364, New York M&amp;F Never</td>
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<tr>
<td></td>
<td></td>
<td>462, New York M&amp;F Ex</td>
<td>Positive</td>
</tr>
<tr>
<td>Ziegler (75)</td>
<td>Case-control</td>
<td>Number 1327, New Jersey M All</td>
<td>Positive</td>
</tr>
<tr>
<td>Nyberg (68)</td>
<td>Case-control</td>
<td>Number 359, Sweden M&amp;F Never</td>
<td>Positive</td>
</tr>
</tbody>
</table>

a All = Nonsmokers and smokers; Never = Never smoked; Non = Never and former smokers; Ex = Former smokers; Current = Smoking at time of study.
b Positive = Level higher in cases; Negative = Level higher in noncases.
c Strength = Relative risks or odds ratios.
d Adjusted for smoking.

0 (0.91–1.09) ±0–4.9
± (0.80–0.90) or (1.10–1.25) ±5–9.9
+ (0.60–0.79) or (1.26–1.67) ±10–19.9
+++ (0.40–0.59) or (1.68–2.50) ±20–29.9
++++ (<0.40) or (>2.50) ±30+
### Table 4
Serum Retinol and Subsequent Lung Cancer: Differences Between Cases and Comparison Groups and/or Risk Ratios of Highest to Lowest Category of Serum Retinol Concentrations

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Area</th>
<th>No. of cases</th>
<th>Sex and smoking</th>
<th>Difference (case mean-comparison mean)</th>
<th>Risk ratio</th>
<th>Group</th>
<th>Ratio</th>
<th>p-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Willett (77)</td>
<td>United States</td>
<td>17</td>
<td>M&amp;F</td>
<td>-1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fifths</td>
<td>1.1†</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Nomura (78)</td>
<td>Hawaii</td>
<td>74</td>
<td>M</td>
<td>+7.0</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Wald (79)</td>
<td>England</td>
<td>656</td>
<td>M</td>
<td>-0.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Friedman (80)</td>
<td>California</td>
<td>151</td>
<td>M&amp;F</td>
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<td></td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Fifths</td>
<td>0.8†</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Menkes (45)</td>
<td>Maryland</td>
<td>99</td>
<td>M&amp;F</td>
<td>-1.1</td>
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<td></td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>Kok (46)</td>
<td>Netherlands</td>
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<td>M&amp;F</td>
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</tr>
<tr>
<td>Connett (81)</td>
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<td>M</td>
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<td>0.25</td>
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<td>Fifths</td>
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<td>0.02</td>
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<tr>
<td>Stähelin (47)</td>
<td>Switzerland</td>
<td>68</td>
<td>M</td>
<td>+3.4</td>
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<td>121</td>
<td>M, Current</td>
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<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* If smoking is not specified, data were not stratified by smoking status.

† A risk ratio below 1.0 signifies that the group (third, fifth, and so on) with highest concentration of serum retinol had less lung cancer develop after baseline than the group with the lowest concentration, i.e., this is a protective association.

* Adjusted for smoking.


Current = Smokers at baseline.
among smokers and nonsmokers reported. The evidence for a protective association was stronger among nonsmokers than smokers (83). More decisive, though limited to smokers, former smokers, and persons exposed to asbestos, is the β-carotene and retinol efficacy trial (CARET) (84). Approximately 9000 persons were assigned to a daily regimen of 25,000 IU of retinol and 30 mg of β-carotene; a similar number was assigned to placebo. After 4 yr of follow-up, the incidence of lung cancer was 28% higher among the treated group.

It seems unlikely that retinol protects against lung cancer in humans, although such a possibility exists for nonsmokers. If there is a protective effect of retinol against lung cancer in humans, it is not yet demonstrable, and seems to be no more than weak, if indeed there is any protective effect at all.

4.5. Carotenoids

For many years, β-carotene was considered to be important in human nutrition only because it is a precursor of retinol. That it might play an important role in protection against cancer was brought to wide attention by Peto and Doll (85,86). Study of its potential role was delayed because earlier food composition tables did not distinguish between retinol and β-carotene and because the carotenoids are not stable when stored at temperatures appreciably warmer than −70°C (76).

Hundreds of carotenoids have been identified but only six have been found to exist in appreciable concentrations in human serum: α-carotene, β-carotene, cryptoxanthin, lutein, lycopene, and zeaxanthin (87). Table 5 summarizes the associations of lung cancer with dietary carotenoids. Almost all are negative, signifying that higher levels are associated with decreased risk. These negative associations tend to be strongest with α- and β-carotene intake. Only the Finnish study (88) examined the association among current smokers, finding essentially none; the protective association was moderately strong among nonsmokers.

Two of the earlier studies of serum carotenoids, those done among participants in the Hypertension Detection and Follow-up Program (HDFP) and the Multiple Risk Factor Intervention Trial (MRFIT), measured total serum carotenoids (77,81). The results were discrepant (Table 6). In the former, the association was positive but nonsignificant; in the latter, the association was negative, moderately strong, and significant. The two populations differed in several ways. The HDFP enrolled both women and men, and a considerable proportion of blacks; the MRFIT was limited to men and was heavily weighted with smokers. Unfortunately, neither group stratified their findings on these characteristics, a failing common to many studies of nutritional relationships with lung cancer.

Serum β-carotene has been more extensively studied than total carotenoids (Table 6). Most found strong and significant negative associations. Current smokers, in an analysis limited to men, also showed a nonsignificant positive association, whereas a strong negative (protective) association was found among nonsmokers (83).

More definitive answers regarding the ability of β-carotene supplementation to prevent lung cancer are the results of three controlled trials. The first to be reported was the α-tocopherol β-carotene Study, a controlled trial involving more than 29,000 male smokers in Finland (95). After 5–8 yr of follow-up, the group receiving 20 mg β-carotene per day had a cancer rate 18% higher than those given placebo. As previously noted, the CARET study found that persons receiving 30 mg of β-carotene and
### Table 5
Association of Dietary Carotenoids with Lung Cancer in Selected Observational Studies, by Type of Study, Characteristics of Study Participants, and Nature of Association

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Type of study</th>
<th>Characteristics of participants</th>
<th>Association</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Residence</td>
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<tr>
<td><strong>Total carotenoids</strong></td>
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<td></td>
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<td>Finland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2417</td>
<td>Finland</td>
</tr>
<tr>
<td>Chow (52) Cohort</td>
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<td>17,633</td>
<td>United States</td>
</tr>
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</tr>
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<td>Bandera (34) Cohort</td>
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<td>27,544</td>
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<td>20,456</td>
<td>New York</td>
</tr>
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<td>Yong (56) Cohort</td>
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<td>10,068</td>
<td>United States</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Candelora (61) Case-control</td>
<td></td>
<td>387</td>
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</tr>
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<td>Alavanja (64) Case-control</td>
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<td>Missouri</td>
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<td>New Jersey</td>
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<td>New Jersey</td>
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<td>New Jersey</td>
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<td>Nyberg (68) Case-control</td>
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<td>Sweden</td>
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<td>Florida</td>
</tr>
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<td>LeMarchand (89) Case-control</td>
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<td>827</td>
<td>Hawaii</td>
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</tr>
<tr>
<td>Ziegler (75) Case-control</td>
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<td>New Jersey</td>
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### β-Carotene

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Country</th>
<th>Gender</th>
<th>Status</th>
<th>Outcome</th>
<th>RR</th>
</tr>
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<tbody>
<tr>
<td>Kromhout (90)</td>
<td>Cohort</td>
<td>Netherlands</td>
<td>M</td>
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<td>+</td>
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</tr>
<tr>
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<td>Finland</td>
<td>M</td>
<td>All</td>
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<td>+</td>
</tr>
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<td>M&amp;F</td>
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<td>++</td>
</tr>
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<td>All</td>
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<td>+</td>
</tr>
<tr>
<td>LeMarchand (89)</td>
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<td>Hawaii</td>
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<td>+</td>
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<td>+</td>
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<tr>
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<td>Sex</td>
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<td>Direction&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Positive</td>
</tr>
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<td>Negative</td>
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<td>Study</td>
<td>Design</td>
<td>Location</td>
<td>Gender</td>
<td>Status</td>
<td>Lymphangioma Status</td>
<td>Odds Ratio</td>
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<td>Never</td>
<td>Negative</td>
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<td>New Jersey</td>
<td>M</td>
<td>All</td>
<td>Negative</td>
<td>6</td>
</tr>
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</table>

- **All** = Nonsmokers and smokers; Never = Never smoked; Non = Never and former smokers; Ex = Former smokers; Current = Smoking at time of study.
- **Positive** = Level higher in cases; Negative = Level higher in noncases.
- **Strength** = Relative risks or odds ratios; % difference in means.
- **0** = (0.91–1.09) or (1.00–1.09) when comparing never smokers to all.
- **±** = (0.80–0.90) or (1.10–1.25) when comparing current smokers to all.
- **+** = (0.60–0.79) or (1.26–1.67) when comparing former smokers to all.
- **++** = (0.40–0.59) or (1.68–2.50) when comparing ever smokers to all.
- **+++** = (<0.40) or (>2.50) when comparing ever smokers to noncases.

- **Adjusted for smoking.**
- **p value for difference in means.**
- **Stable intake, three periods.**
Table 6
Serum Carotenoids and Subsequent Lung Cancer:
Differences Between Cases and Comparison Groups and Risk Ratio of Highest to Lowest Category of Serum Carotenoid Concentrations

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Area</th>
<th>No. of cases</th>
<th>Sex and smoking$^a$</th>
<th>Difference (case mean-comparison mean)</th>
<th>Risk ratio</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>p</td>
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<tr>
<td><strong>Total carotenoids</strong></td>
<td></td>
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<tr>
<td>Willett (77)</td>
<td>United States (HDFP)</td>
<td>17</td>
<td>M&amp;F</td>
<td>±$^a$</td>
<td>0.59</td>
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<tr>
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<td>United States (MRFIT)</td>
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<td>0.03</td>
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<td></td>
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<tr>
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<td>Maryland</td>
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<td>M</td>
<td>−11.9</td>
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<tr>
<td></td>
<td></td>
<td>97</td>
<td>F</td>
<td>−14.5</td>
<td>0.15</td>
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<td></td>
</tr>
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<td>Nomura (78)</td>
<td>Hawaii</td>
<td>74</td>
<td>M</td>
<td>−31.0</td>
<td>&lt;0.01</td>
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<tr>
<td>Menkes (45)</td>
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<td>99</td>
<td>M&amp;F</td>
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<td>0.04</td>
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<td>Wald (93)</td>
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<td>50</td>
<td>M</td>
<td>−22.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Connnett (81)</td>
<td>United States (MRFIT)</td>
<td>66</td>
<td>M</td>
<td>−22.4</td>
<td>0.07</td>
</tr>
<tr>
<td>Pastorino (72)</td>
<td>Italy</td>
<td>47</td>
<td>F</td>
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<td></td>
</tr>
<tr>
<td>Name</td>
<td>Location</td>
<td>Sex</td>
<td>Age</td>
<td>Retention</td>
<td>Unit</td>
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<td>F</td>
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<td>-39.5</td>
<td>0.14</td>
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<td>Stähelin (47)</td>
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<td>M</td>
<td>68</td>
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<td></td>
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<td>M, Current</td>
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<td>0.01</td>
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<tr>
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<td>M</td>
<td>157</td>
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<td>F</td>
<td>101</td>
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<tr>
<td><strong>β-Cryptoxanthin</strong></td>
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<td>M</td>
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<td>-8.2</td>
<td>0.07</td>
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<td></td>
<td>F</td>
<td>101</td>
<td>-12.6</td>
<td>0.01</td>
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<td>Lutein/zeaxanthin</td>
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<td>M</td>
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<td>-1.2</td>
<td>0.83</td>
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<td></td>
<td>F</td>
<td>101</td>
<td>-1.4</td>
<td>0.83</td>
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</table>

* Adjusted for smoking.
25,000 IU of retinol daily had a lung cancer increase rate 28% higher than the placebo group (84). The third trial, the Physicians' Health Study, found essentially no differences between the 11,000 men receiving 50 mg of β-carotene every other day and those receiving placebo (96). There were slight but far from significant reductions in lung cancer among smokers and nonsmokers.

A potential problem with these three trials has been pointed out (97). If the effect of β-carotene (or other antioxidants) is to protect against the initiation of carcinogenesis, all three trials were much too short in duration. More cogent is the possibility that β-carotene in the observational studies has merely been a “fellow-traveler” with some other substance or substances that are the real causes of the observed protective associations so that the trial did not test the proper dietary component. None of these explanations accounts for the increased risk of lung cancer among persons who took β-carotene supplements. There is a possibility that a large intake of β-carotene might inhibit the absorption of other protective substances. The evidence on this possibility is still conflicting. Two studies have shown that supplementary β-carotene was associated with a decrease in serum levels of lycopene (98) and lutein (99). In contrast, another found that the increase in serum β-carotene following supplementation was accompanied by a rise in the serum concentration of α-carotene and lycopene, and essentially no change in the concentrations of cryptoxanthin or lutein/zeaxanthin (100).

Some have speculated that the presence of smoke in the lungs may enhance tumor promotion, either by favoring the prooxidant properties of β-carotene, or by interfering with retinoid signaling (101–102). In any case, the present evidence strongly suggests that heavy smokers should not take large doses of β-carotene.

4.6. Vitamin E

Four tocopherols and four tocotrienols have vitamin E activity, but α-tocopherol is the major form found in human tissues. Vitamin E (almost always assayed as α-tocopherol) is present in human serum at much greater concentrations than retinol or the carotenoids, being reported in milligrams per deciliter rather than micrograms per liter. Six studies of the association of vitamin E intake with lung cancer are summarized in Table 7. Of considerable interest are the strong associations reported with γ- and δ-tocopherol from the study in Finland (36), raising questions of whether or not the usual emphasis on α-tocopherol is justified. Serum concentrations of vitamin E have been studied more extensively than dietary intakes (Table 8). In this instance, the results are almost evenly split between negative and positive associations, with only a few being statistically significant. It is once more noteworthy that current smokers in Finland showed no evidence of a protective association either with dietary intake or serum levels of vitamin E (36,83).

Again, the strongest evidence is provided by the α-Tocopherol β-Carotene Trial among smokers (95). In this instance, lung cancer rates were essentially the same among persons taking vitamin E supplements and those taking placebo. Essentially, the same criticisms can be raised with respect to the α-tocopherol arm as were mentioned for the β-carotene arm of the trial. In addition, some critics feel that the dose of α-tocopherol was too low to be effective (97).

Based on currently available evidence, it does not appear that α-tocopherol is likely to have a protective effect against lung cancer. Further studies with the γ and δ forms
## Table 7

Association of Dietary Vitamin E and Specific Tocopherols with Lung Cancer in Selected Studies, by Type of Study, Observations of Participants, and Nature of Association

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
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<th>Characteristics of participants</th>
<th>Association</th>
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<td>Sex</td>
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<td>2417</td>
<td>Finland</td>
<td>M</td>
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<td></td>
<td>4538</td>
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</tr>
<tr>
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<td>4538</td>
<td>Finland</td>
<td>M</td>
</tr>
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<td>Bandera (34) Cohort (E)</td>
<td>27,544</td>
<td>New York</td>
<td>M</td>
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<tr>
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<td>20,456</td>
<td>New York</td>
<td>F</td>
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<td>10,068</td>
<td>United States</td>
<td>M&amp;F</td>
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<td>2011</td>
<td>United States</td>
<td>M&amp;F</td>
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<td>M</td>
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<td>F</td>
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<tr>
<td>Alavanja (64) Case- (E)</td>
<td>1450</td>
<td>Missouri</td>
<td>F</td>
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</tbody>
</table>

<sup>a</sup> All = Nonsmokers and smokers; Never = Never smoked; Non = Never and former smokers; Ex = Former smokers; Current = Smoking at time of study.

<sup>b</sup> Positive = Level higher in cases; Negative = Level higher in noncases.

<sup>c</sup> Strength = Relative risks or odds ratios

<sup>d</sup> Adjusted for smoking.

<sup>e</sup> Total vitamin E; α, β, γ; Specific tocopherols.

<sup>f</sup> p value for difference in means.

<sup>g</sup> Stable intake, three periods.
<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Residence</th>
<th>No. of cases</th>
<th>Sex and smoking</th>
<th>Difference (case mean-comparison mean)</th>
<th>Risk ratio</th>
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<td>Willett (77)</td>
<td>United States (HDFP)</td>
<td>17</td>
<td>M&amp;F</td>
<td>-7.9</td>
<td>0.23</td>
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<td>Nomura (78)</td>
<td>Hawaii</td>
<td>74</td>
<td>M</td>
<td>+7.0</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Menkes (45)</td>
<td>Maryland</td>
<td>99</td>
<td>M&amp;F</td>
<td>-11.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kok (46)</td>
<td>Netherlands</td>
<td>18</td>
<td>M&amp;F</td>
<td>-9.4</td>
<td>0.33</td>
</tr>
<tr>
<td>Knekt (103)</td>
<td>Finland</td>
<td>144</td>
<td>M</td>
<td>-3.6</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>+2.2</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connett (81)</td>
<td>United States (MRFIT)</td>
<td>66</td>
<td>M</td>
<td>-4.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Stähelin (47)</td>
<td>Switzerland</td>
<td>68</td>
<td>M</td>
<td>+1.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Orentreich (94)</td>
<td>California</td>
<td>123</td>
<td>M&amp;F</td>
<td>+4.3</td>
<td></td>
</tr>
<tr>
<td>Knekt (83)</td>
<td>Finland</td>
<td>22</td>
<td>M, Non</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>121</td>
<td>M, Current</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comstock (92)</td>
<td>Maryland</td>
<td>157</td>
<td>M</td>
<td>-6.9</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101</td>
<td>F</td>
<td>-1.9</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Adjusted for smoking.
need to be done.

4.7. Vitamin C

There has been considerable public interest in the possible benefits of taking large doses of vitamin C, largely owing to the influence of the Nobel Laureate, Linus Pauling (104). Seven prospective and eight retrospective (case-control) dietary studies are summarized in Table 9. Although most showed a negative, protective association, only five were statistically significant, one being the study among Finnish nonsmokers. Again, the finding among Finnish smokers was discrepant.

Only two studies have reported on the association of serum concentrations of vitamin C with the subsequent development of lung cancer. In the Prospective Basel Study (Switzerland), there was virtually no difference in mean serum concentrations at baseline between the 69 men who subsequently died from lung cancer and the 2421 survivors after 12–14 yr of follow-up (47). In the nested case-control study in Washington County, MD, plasma collected in 1989 was treated to preserve vitamin C on storage. In the subsequent 8 yr, 26 men and 18 women developed lung cancer. Mean concentrations were 15% lower among cases in both groups than among matched controls, with the most marked differences occurring among persons who had never smoked (92).

The available evidence suggests that vitamin C is associated with only a modest decrease in lung cancer risk, and that this association is most marked among persons who have never smoked.

4.8. B Vitamins

Serum levels of vitamins B1, B2, and B6 were assayed in the cohort study in Switzerland (48). There were essentially no differences in the concentrations of these vitamins between cases and noncases in a nested case-control study. On the other hand, there is an indication that a daily regimen of 10 mg of folate plus 500 μg of vitamin B12 may reduce the severity of atypical bronchial squamous metaplasia among smokers and thereby reduce the risk of subsequent lung cancer (108). Among 36 smokers with metaplasia on this treatment regimen, 39% showed improvement compared to only 16% among 37 smokers on placebo. If this protective effect of folate is confirmed by observational studies, a more definitive trial would be indicated.

4.9. Selenium

The trace metal selenium was discovered in 1818 by Berzelius who named it for the moon because of its companionship with tellurium, named for the earth (109). Its importance with relation to cancer comes from the fact that it is a component of the antioxidant enzyme, glutathione peroxidase. Selenium is a convenient marker for this enzyme because it is much easier to assay serum for selenium than for the entire enzyme. The concentration of selenium in soils and crops varies considerably from place to place, making dietary assays difficult when foods are imported from a variety of regions. Only in Finland has such a study been attempted, probably because when the study started, the selenium content of available foods was low, a situation that has changed during the course of the study. In this instance, there was only a slight, nonsignificant negative association among smokers, and essentially no association among nonsmokers (36).

In contrast to the dietary assay situation, 14 prospective studies of selenium as a bio-
## Table 9

Association of Dietary Vitamin C with Lung Cancer in Selected Studies, by Type of Study, Characteristics of Participants, and Nature of Association

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Type of study</th>
<th>Characteristics of participants</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Residence</td>
<td>Sex</td>
<td>Smoking&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kvale (105)</td>
<td>Cohort</td>
<td>10,602</td>
<td>Norway</td>
</tr>
<tr>
<td>Kromhout (90)</td>
<td>Cohort</td>
<td>878</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Knekt (36)</td>
<td>Cohort</td>
<td>2121</td>
<td>Finland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2417</td>
<td>Finland</td>
</tr>
<tr>
<td>Enstrom (106)</td>
<td>Cohort</td>
<td>4479</td>
<td>United States</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6869</td>
<td>United States</td>
</tr>
<tr>
<td>Shibata (55)</td>
<td>Cohort</td>
<td>24,218</td>
<td>California</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45,941</td>
<td>California</td>
</tr>
<tr>
<td>Chow (52)</td>
<td>Cohort</td>
<td>17,633</td>
<td>United States</td>
</tr>
<tr>
<td>Bandera (34)</td>
<td>Cohort</td>
<td>27,544</td>
<td>New York</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20,456</td>
<td>New York</td>
</tr>
<tr>
<td>Ocké (57)</td>
<td>Cohort</td>
<td>561</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Yong (56)</td>
<td>Cohort</td>
<td>10,068</td>
<td>United States</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2982</td>
<td>Iowa</td>
</tr>
<tr>
<td>Steinmetz (53)</td>
<td>Nested</td>
<td>705</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Hinds (107)</td>
<td>Case-control</td>
<td>286</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Study</td>
<td>Study Type</td>
<td>N</td>
<td>Location</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>------</td>
<td>----------</td>
</tr>
<tr>
<td>Byers (33)</td>
<td>Case-control</td>
<td>991</td>
<td>New York</td>
</tr>
<tr>
<td>Byers (17)</td>
<td>Case-control</td>
<td>883</td>
<td>New York</td>
</tr>
<tr>
<td>Koo (49)</td>
<td>Case-control</td>
<td>469</td>
<td>New York</td>
</tr>
<tr>
<td>LeMarchand (70)</td>
<td>Case-control</td>
<td>137</td>
<td>Hong Kong</td>
</tr>
<tr>
<td>Kallandidi (58)</td>
<td>Case-control</td>
<td>827</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Candelora (61)</td>
<td>Case-control</td>
<td>370</td>
<td>Hawaii</td>
</tr>
<tr>
<td>Alavanja (64)</td>
<td>Case-control</td>
<td>211</td>
<td>Greece</td>
</tr>
<tr>
<td>Nyberg (68)</td>
<td>Case-control</td>
<td>1450</td>
<td>Missouri</td>
</tr>
<tr>
<td></td>
<td></td>
<td>359</td>
<td>Sweden</td>
</tr>
</tbody>
</table>

a All = Nonsmokers and smokers; Never = Never smoked; Non = Never and former smokers; Ex = Former smokers; Current = Smoking at time of study.
b Positive = Level higher in cases; Negative = Level higher in noncases.
c Strength |
| Relative risks or odds ratios | % difference in means |
| 0        | (0.91–1.09) | ±0–4.9  |
| ±        | (0.80–0.90) or (1.10–1.25) | ±5–9.9 |
| +        | (0.60–0.79) or (1.26–1.67) | ±10–19.9 |
| ++       | (0.40–0.59) or (1.68–2.50) | ±20–29.9 |
| +++      | (<0.40) or (>2.50) | ±30+    |
d Adjusted for smoking.
e p value for difference in means.
f Stable intake, three periods.
Table 10
Serum or Toenail Selenium and Lung Cancer: Differences Between Cases and Comparison Groups and/or Risk Ratios of Highest to Lowest Category of Serum Selenium Concentrations

<table>
<thead>
<tr>
<th>Author and ref. no.</th>
<th>Area</th>
<th>No. of cases</th>
<th>Sex and smoking</th>
<th>Difference (case mean-comparison mean)</th>
<th>Risk ratio</th>
<th>Group</th>
<th>Ratio</th>
<th>p-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Willett (110)</td>
<td>United States (HDFP)</td>
<td>18</td>
<td>M&amp;F</td>
<td>−6.9</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salonen (111)</td>
<td>Finland</td>
<td>23</td>
<td>M&amp;F</td>
<td>−6.5</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menkes (45)</td>
<td>Maryland</td>
<td>99</td>
<td>M&amp;F</td>
<td>+2.7</td>
<td>0.16</td>
<td>Fifths</td>
<td>1.5a</td>
<td>0.07</td>
</tr>
<tr>
<td>Nomura (112)</td>
<td>Hawaii</td>
<td>71</td>
<td>M</td>
<td>+0.4</td>
<td>&gt;0.50</td>
<td>Fifths</td>
<td>0.9a</td>
<td>0.46</td>
</tr>
<tr>
<td>Kok (46)</td>
<td>Netherlands</td>
<td>18</td>
<td>M&amp;F</td>
<td>−4.0</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coates (113)</td>
<td>Washington</td>
<td>11</td>
<td>M&amp;F</td>
<td>−8.2</td>
<td>0.25</td>
<td>Thirds</td>
<td>0.8</td>
<td>0.73</td>
</tr>
<tr>
<td>Ringstad (114)</td>
<td>Norway</td>
<td>7</td>
<td>M&amp;F</td>
<td>+0.7</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criqui (115)</td>
<td>California</td>
<td>27</td>
<td>M&amp;F</td>
<td>−3.3</td>
<td>0.13</td>
<td>Fifths</td>
<td>0.5a</td>
<td>0.01</td>
</tr>
<tr>
<td>Van den Brandt (116)b</td>
<td>Netherlands</td>
<td>285</td>
<td>M</td>
<td>−6.6</td>
<td>&lt;0.01</td>
<td>Fifths</td>
<td>0.4a</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>F</td>
<td>−10.1</td>
<td>0.25</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>Never</td>
<td>−2.6</td>
<td>0.16</td>
<td>Fifths</td>
<td>0.5</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>122</td>
<td>Ex</td>
<td>−1.7</td>
<td>0.58</td>
<td>Fifths</td>
<td>0.6</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>189</td>
<td>Current</td>
<td>−4.4</td>
<td>0.16</td>
<td>Fourths</td>
<td>0.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Kabuto (117)</td>
<td>Japan</td>
<td>77</td>
<td>M&amp;F</td>
<td>−9.6</td>
<td>0.03</td>
<td>Thirds</td>
<td>0.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Garland (118)b</td>
<td>United States</td>
<td>47</td>
<td>F</td>
<td>−1.8</td>
<td>0.23</td>
<td>Fifths</td>
<td>0.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Comstock (92)</td>
<td>Maryland</td>
<td>157</td>
<td>M</td>
<td>−1.8</td>
<td>0.24</td>
<td>Fifths</td>
<td>0.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Knekt (119)</td>
<td>Finland</td>
<td>91</td>
<td>M&amp;F</td>
<td>−8.0</td>
<td>0.14</td>
<td>Thirds</td>
<td>0.4a</td>
<td>0.04</td>
</tr>
</tbody>
</table>

a Adjusted for smoking.
b Toenail selenium was exposure biomarker.
marker for lung cancer risk have been done and are shown in Table 10. These include two in which selenium was measured in toenail clippings rather than in serum (116,118). Selenium is concentrated in hair and nails. Hair is unsuitable because of selenium-containing shampoos, so that clippings from the nail of the big toe are used. Depending on their length, these reflect the circulating selenium levels over a period of days or even weeks. As can be seen in Table 10, in almost all instances the associations are negative, consistent with a protective association with higher levels of selenium. With respect to smoking, the findings in the Netherlands and Finland were contradictory (116,119).

The most persuasive bit of evidence comes from a controlled trial of selenium supplementation for the prevention of recurrences of skin cancer (120). Although selenium did not prevent such recurrences, the selenium group experienced a 46% reduction in lung cancer \( p = 0.04 \). Because there were numerous post-hoc analyses and the reduction in lung cancer was at the borderline of statistical significance, this encouraging result needs to be treated with caution. Large doses of selenium are to be avoided because selenium in excess can be toxic (109). This is one instance where the overused phrase, “more research is needed,” is appropriate.

4.10. Zinc

Zinc is reported to be one of the factors associated with mobilization of retinol from the liver. In a single cohort study from Japan, a slight and nonsignificant negative association was reported (117).

4.11. Flavonoids

Because of the persistent reports of decreased cancer risks among persons whose diet includes a high proportion of fruits and vegetables, decreases that tend to be more consistent and larger than those found with the nutrients discussed thus far, investigators have turned to other antioxidants in foods. Flavonoids, in particular, have attracted recent attention. Three recent studies have dealt specifically with lung cancer. In the Zutphen cohort, tea intake was used as a surrogate for flavonoid consumption (121). With adjustments only for age and sex, there was a strong and significant association of tea intake with decreased lung cancer risk. This association disappeared with adjustment of several additional factors, most of which seem likely to be associated with both tea drinking and lung cancer, and hence, dubiously appropriate for use in adjustment. In a Finnish cohort, there was also a strong and significant inverse association of flavonoid intake with subsequent lung cancer, but this was confined to nonsmokers (122). In contrast, among Swedish persons who had never smoked, frequent tea drinking was associated with an increased risk of lung cancer (68). It should be noted that the sources of flavonoids, and hence, the individual substances, vary considerably among populations. It will require studies specifically oriented at individual flavonoids or flavonoid-containing foods to determine whether or not any of these substances might be protective.

5. CONCLUSIONS/RECOMMENDATIONS

The available evidence is strong that certain factors in fruits and vegetables inhibit the initiation or promotion of lung cancer. If inhibition depends largely on a single substance, it is likely to be something associated with the carotenoids or vitamin C rather than
with vitamin E. The strong protective associations of γ- and δ-tocopherol in a single study suggest they need further investigation, as does selenium. Of great potential importance, both for indicating mode of action and for public health application, is the possible interaction of smoking and potentially protective substances. Future studies should stratify their results by smoking status, and possibly also by sex.

The Zutphen study showed that associations of various micronutrients with lung cancer were much stronger when the analyses were limited to persons who were repeatedly in the same third of dietary intake (57). This finding underscores the probability that considerable misclassification occurs with baseline characteristics that are recorded only once. Similar repetitions of dietary questionnaires and serum assays in cohort studies could be very informative and should be encouraged.

Collaboration with cellular biologists is highly desirable. Knowledge of oxidative reactions within cells that could damage DNA might point to the class of antioxidants most likely to yield protection. How such antioxidants penetrate into cells will also indicate which ones are most likely to affect intracellular reactions.

With respect to dietary recommendations, the findings at present confirm general advice to decrease the ingestion of fat to less than 30% of caloric intake and to increase the amount of fruits and vegetables in the diet. Specific dietary supplements do not seem indicated at this time. Finally, the available evidence, admittedly scanty, suggest that dietary changes are unlikely to appreciably reduce the risk of lung cancer among persons who continue to smoke.

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111. van den Brandt PA, Goldbohm RA, van’t Veer P, Bode P, Dorant E, Hermus RJJ, Sturmans F. A
1. INTRODUCTION

Cancer, once thought to be an inevitable consequence of aging, is now considered to be primarily determined by the interaction of environmental factors, including dietary habits, with genes involved in the cancer process (1–3). Because dominantly inherited or familial cancers probably contribute only a small percent of total cases, it is of paramount importance to identify those environmental modulators influencing nonfamilial risks (1,2). Dietary habits are possibly a variable that markedly influences nonfamilial cancer risk. Some have estimated that dietary habits are instrumental in about 60% of cancers in women and about 40% in men (4). Although these are significant contributions, the true effect depends on the individual’s genetic profile, the particular neoplasms, and the composition of the entire diet.

Although variability exists, fruit and vegetable consumption is frequently inversely linked with cancer risks (5–8). The reason for variability remains obscure but may relate to oxidative balance (9,10). Variations in pro- and antioxidant conditions are recognized to influence several essential cellular functions, including gene expression (11,12). This homeostasis is unquestionably complex as evident by the sensitivity of several kinases and transcription factors.

Almost 5 yr ago, Serdula et al. (13) reported men and women living in various regions of the United States consumed an average of 3.3 and 3.7 servings of fruit and vegetable per day, respectively. More recently, mean daily servings of fruits and vegetables in rural African Americans were found to be 3.7, based on a telephone survey (14). Last year, Thompson et al. (15) found daily consumption for 15,060 adults averaged 3.6 servings based on a 7-item food frequency questionnaire. Collectively, although there is a fair amount of agreement about average intakes, the actual number of servings depends on several societal factors including cost and availability, as well as several personal determinants such as age, education, and race.

Unquestionably, variation in fruit and vegetable consumption is recognized to influence an individual’s redox status (10,16). Unfortunately, few Americans (10–20%) consume the recommended five or more daily servings of fruits and vegetables (13). Thompson et al. (10) suggests even these individuals may not be able to meet all oxidative challenges. Their studies revealed that women whose daily vegetables and fruit...
consumption increased from 5.8 to 12.0 servings exhibited marked reduction in markers of oxidative cellular damage, i.e., 8-hydroxydeoxyguanosine in DNA isolate from peripheral lymphocytes. Overall, the linkages existing between fruit and vegetable consumption and reduced cancer risk serves as ample justification for the continued examination of individual foods or dietary components as modulators of the initiation, promotion, or progression stages of carcinogenesis.

A large number of agents with antioxidant properties are found in fruits and vegetables, including carotenoids, dithiolthiones, flavonoids, glucosinolates, indoles, isothiocyanates, monoterpenes, phenols, selenium, sterols, sulfhydryls, and vitamins C and E. These dietary components likely have both complementary and overlapping mechanisms of action, including the induction of detoxification enzymes, blockage of carcinogen formation (such as nitrosamines), shifts in hormone homeostasis, slowing of cell division, induction of apoptosis, and possibly others.

Whereas several macronutrients are likely involved in the cancer process, they do not appear to totally explain the worldwide variance in cancer risk. Furthermore, it is likely that their impact is markedly influenced by several physiologically important dietary constituents. A host of nonessential constituents continue to emerge as key modulators of cancer risk. Collectively, these findings have highlighted a new and intriguing term, “functional foods;” based on the ability of selected foods to have health benefits above and beyond the basis nutrure provided. These so-called functional foods continue to captivate the interest of scientists, legislators, and most importantly, the consumer (17).

Although the term functional foods has no legal meaning, it nevertheless signifies a proactive movement that deals with the health benefits of foods. Certainly, this is a refreshing change to the largely negative campaigns that have been the norm for providing diet and health information to the general public during the past few decades.

Clearly, variability in detecting an association between dietary practices and cancer risk is logical because such a relationship must depend on a host of environmental and genetic factors. The complexity of this issue becomes evident when one considers the multitude of interactions that can occur among the many food components. Likewise, because all metabolic and phenotypic characteristics are linked by heredity, it is almost inconceivable that a simple solution will alter susceptibility equally in all individuals. Obviously, a greater understanding of the interrelationships among diet, environment, and genetics is needed to determine who might benefit most from a change in one’s eating behavior. Acquiring this information will be key in permitting tailored dietary recommendations that can assist in minimizing cancer risk in target populations.

To date, more than 500 compounds have been identified as potential modifiers of cancer. Some of the major antioxidant constituents of fruit, vegetables, and beverages are derived from phenolic phytochemicals synthesized through the shikimate pathway from tyrosine and phenylalanine (18,19). Many of these exist as O-glycosides and O-methyl conjugates (see Table 1).

Cinnamic acid found widely in fruits and vegetables is a transformation product of phenylalanine produced by the action of phenylalanine-ammonia lyase. Isoflavonoids, flavonoids, and lignans are additional plant constituents that make up the three principal classes of phytoestrogens consumed by humans. A major source of the isoflavonoids, daidzein, and genistein, is soy, a staple for many Asians. Flavonoids are widely present in fruits. Quercetin and kempferol are two commonly found flavonoids, although they are particularly rich in apples, onions, and tea leaves. Plant lignans are
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present in many cereal grains, fruits, and vegetables, and give rise to the mammalian lignans, enterodiol and enterolactone. The richest source of lignans is linseed (flaxseed) and other oil seeds. Allium foods, including garlic, onions, and leeks, provide a host of organosulfur compounds that may influence health.

Terpenes are a group of hydrocarbons made up of building blocks of isoprene \((\text{C}_5\text{H}_8)\) units that are widespread in nature. Most occur in plants as constituents of essential oils. Monoterpene are made up of two units such as limonene, citral, and camphor, whereas sesquiterpene are made up of three units and include such compounds as humulene, which is a Hops aromatic. Vitamin A1 is an example of a 4-isoprene unit or dieterpene, whereas carotene is an 8-isoprene or tetraterpene unit.

Fruit and vegetable consumption is not the only dietary factor that can influence cancer risk. Ingestion of green and black tea, herbs, and spices has been reported to be inversely associated with cancer risk \((16,20–22)\). Some of these food items and their associated nonnutritive components are also addressed in subsequent parts of this chapter.

This chapter is limited to a few nonessential dietary components where ample documentation exists about an effect on the cancer process and for those where a plausible mechanism of action can be postulated. It must be noted that the response to individual components is assumed to be consistent with that occurring in a complex food matrix. Whether this is true or not remains to be adequately verified!

2. CINNAMIC ACID

Cinnamic acid, chemically related to benzoic acid, is ubiquitous in plants and fruits, providing a natural protection against infections by pathogenic microorganisms. Products containing cinnamon oil are particularly rich sources of cinnamic acid. Its relatively low toxicity, coupled with its flavoring characteristics, has fostered its commercial use \((23)\).
Cinnamic acid and associated derivatives posses a broad spectrum of antifungal and antibacterial activities (24,25). Cinnamic acid decreases revertants in eukaryotic murine FM3A cells induced by ethyl methanesulfonate (an alkylating agent), hydrogen peroxide (an oxidizing agent), and quinacrine (a frameshift mutagen) (26). Additionally, cinnamic acid has antitumor activity against a wide range of neoplasms (27,28). Cinnamic acid may exert its antiproliferative effects by inhibition of protein isoprenylation, which in turn inhibits mitogenic signal transduction (27,29). Likewise, cinnamyl analogs are recognized to act as specific protein tyrosine kinase inhibitors, also possibly accounting for their ability to suppress tumor cell growth (30). It must be emphasized that the concentrations of cinnamic acid necessary to bring about antitumorigenic properties are rather massive (2–8 mM). Thus, it remains to be determined their true physiological important in modifying cancer risk. Additional studies are needed to determine what effects, if any, that cinnamic acid and associated compounds have on other models for cancer, especially those where dietary manipulation is possible.

3. FLAVONOIDS

The flavonoids are a group of organic molecules ubiquitously distributed in vascular plants. Approximately 4000 individual members of flavonoids are known to exist. Typical dietary intakes of flavonoids are not known with any certainty, but are estimated to be several hundred milligrams per day (31). As modified phenolic compounds, these compounds can act as potent antioxidants and metal chelators. The cytochrome P450 (CYP) 1A isoyme appears to be predominately involved in flavonoid hydroxylation, whereas other cytochrome CYP isozymes are involved in their demethylation (32). Overall, several of these flavonoids appear to be effective antipromoters of the cancer process. In addition, some appear to be effective inhibitors of the bioactivation of selected carcinogens. However, depending on the flavonoid, as well as the specific foreign compound, the influence can sometimes be stimulatory, whereas in other cases, it is inhibitory (33).

Flavonoids are generally classified on the basis of substitutions occurring on one or more of the rings. These classes include flavones, flavonols, flavanones, and isoflavones. Table 2 lists some compounds within each class that have been examined for their anticarcinogenic properties.

3.1. Flavones

Tangeretin and nobiletin are two polymethoxylated flavonoids in citrus foods that have been examined for their anticarcinogenic properties. These unhydroxylated compounds have been shown to modify several CYP isoymes. Canivenc-Lavier et al. (34) reported tangeretin increased rat liver CYP 1A1, 1A2, and 2B1, 2. The ability of tangeretin to inhibit unscheduled DNA synthesis induced in cultured human liver slices by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-acetylaminofluorene (2-AAF) may be attributable to the these CYP changes (35). Nevertheless, it remains to be determined how important these changes are in vivo in determining tumor incidence.

Kandaswami et al. (36) found that both Nobiletin and tangeretin are effective in inhibiting the growth of a variety of neoplastic cells in culture (36,37). Both markedly inhibited the proliferation of a squamous cell carcinoma (HTB 43) and a gliosarcoma (9L) cell line when added to the medium at relatively high concentrations 2–8 μg/mL (36). Some cells such as the human lung fibroblast-like cell line (CCL 135) were relatively
resistant to these flavonoids. Kawaii et al. (37) provided evidence that the ortho-
catechol moiety in ring B and a C2-C3 double bond was important for the antiprolifer-
ative activity. Most recently, Bracke et al. (38) found that although Tamoxifen and the
tangeretin exhibit similar inhibitory effects on the growth and invasive properties of
human mammary cancer cells in vitro, only Tamoxifen was effective in a xenograph-
transplant model, suggesting that absorption may be different between the two com-
pounds. Of greater concern, however, was that when the two were combined, an
interference with the effectiveness of tamoxifen emerged. Clearly, such evidence re-
veals that it is unwise, and possibly counterproductive, to use flavonoids as adjuvant
therapy until their true impact on classical therapies are better understood.

3.2. Flavonols

3.2.1. QUERCETIN AND KEMPFEROL

Although two of the most common flavonols, quercetin (3,5,7,3',4'-pentahydroxy-
flavone), and kempferol (3,5,7,4'-tetrahydroxyflavone), do exhibit some mutagenicity
in the Ames assay (39), they also appear to be effective modifiers of cancer risk. Conjugates
including quercitrin (quercetin-3-L-rhamnoside), quercetin-3-glucoside,
and rutin (quercetin-3-beta-D-rutinoside are known to occur in nature and to, at time,
have biological activity. Although flavonols can be absorbed into the bloodstream prin-
cipally as glucosides, minor structural differences can markedly influence both the level
of accumulation and the extent to which the conjugates are excreted (40).

Quercetin is recognized as a potent modifier of CYP1A1 reactions (41). In studies by
Verma et al. (42), up to 5% quercetin was fed without apparent ill consequences to the rat.
However, this quantity of quercetin significantly reduced the incidence of both DMBA and
MNU-induced mammary tumors (42). Such data suggest that bioactivation is not essential
for the protection provided. Evidence exists that quercetin can inhibit the promotion phase
of DMBA and MNU-induced skin tumors (43). Likewise, considerable evidence points to
the ability of quercetin and related compounds to modify the proliferation of existing neo-
plasms. These growth-inhibitory effects frequently coincide with a block in the G0/G1

Table 2

<table>
<thead>
<tr>
<th>Class</th>
<th>Food Sources</th>
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<tbody>
<tr>
<td>Flavones</td>
<td></td>
</tr>
<tr>
<td>Tangeretin</td>
<td>Citrus</td>
</tr>
<tr>
<td>Nobiletin</td>
<td>Citrus</td>
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<tr>
<td>Flavonols</td>
<td></td>
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<tr>
<td>Quercetin</td>
<td>Fruit, vegetables, cereal</td>
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<tr>
<td>Grains</td>
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<tr>
<td>Kaempferol</td>
<td>Fruit, vegetables</td>
</tr>
<tr>
<td>Catechins</td>
<td>Tea</td>
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<tr>
<td>Flavanones</td>
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<tr>
<td>Naringenin</td>
<td>Grapefruit</td>
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<td>Isoflavones</td>
<td></td>
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<tr>
<td>Genistein</td>
<td>Soybeans</td>
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</tbody>
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phase of the cell cycle. Ranelletti et al. (44) found that the quercetin (10 nM and 10 μM) addition to cultures of colon cancer cell lines produced a dose-dependent, reversible growth inhibition. More recently, Ranelletti et al. (45) found that 10 μM quercetin reduced p21-ras proteins by about 50% in both cultured colon cancer cell lines and primary colorectal tumors. Likewise, it inhibited the expression of K-, H-, and N-ras proteins.

Estrogenic potency of some flavonoids can be significant, especially for estrogen-receptor-β. Thus, hormonal changes may trigger some of the biological responses that are evoked by some flavonoids (46). Quercetin can also induce apoptosis in tumor cells (47). In human promyelocytic leukemia HL-60 cells, apoptosis induced by adding quercetin was correlated with a loss of mitochondrial transmembrane potential, cytochrome c release, and caspase-9 and caspase-3 activation (48). Nevertheless, it must be emphasized that not all tumors are equally sensitive to flavonols and those that are sensitive vary in their sensitive (36).

3.2.2. GREEN AND BLACK TEA FLAVONOLS

Tea, grown in about 30 countries, is consumed with considerable variability as a beverage among individuals (49). Although tea continues to be the most widely consumed beverage other than water, consumption is only about 0.12 L/yr. Tea is manufactured in three basic forms: unoxidized or green tea, oxidized or black tea, and partially oxidized or oolong tea. Only about 20% of the tea produced is green, whereas less than 2% is oolong. Green tea is consumed primarily in China, Japan, and a few countries in North Africa and the Middle East, although it is gaining in popularity in other parts of the world.

Fresh tea leaves are unusually rich in polyphenols. These catechins may constitute up to 30% of the dry-leaf weight. Other polyphenols include flavonols and their glycosides, and depsides such as chlorogenic acid, coumarylquinic acid, and one unique to tea, theogallin (3-galloylquinic acid). Various quinones are produced by oxidation and condense to form a series of compounds, including bisflavanols, theaflavins, epitheaflavic acids, and thearubigens, which give rise to the characteristic taste and color properties of black tea (49). Green tea composition is very similar to that of the fresh leaf, except for a few enzymatically catalyzed changes that occur following harvesting. Thearubigens constitute the largest mass of the extractable matter in black tea. Oolong tea is intermediate in composition between green and black teas.

Although inconsistencies exist, green tea consumption has frequently been associated with a reduction in cancer (50,51). However, exaggerated intakes may also pose some problems at least in some individuals. Recently, Lu et al. (52) suggested tea consumption is associated with an increased risk of bladder cancer. Obviously, additional attention is need to determine if, and under what circumstances, that tea consumption may modify one or more phase of the cancer process.

Several investigations with a variety of animal model bioassays have found that treatment with the polyphenolic fraction isolated from green tea leaves protects against chemically induced cancers (20). Chemically induced tumors in the large intestine, forestomach, liver, lung, and mammary tissue have all been reported to be suppressed by exaggerated exposure to tea or associated polyphenolics (53–57).

Several tea preparations can inhibit nitrosamine formation (58). Because the vast majority of nitrosamines are known carcinogens, at least in animal models, these studies may have particular significance (59). Wu et al. (60) demonstrated that the amounts of N-nitrosomorpholine, a hepatocarcinogen, formed in vitro depended on the molecular
structure of tea catechin derivatives and their molar ratios to nitrite. The depression in nitrosamine formation thus relates to a sequestering of nitrite making it unavailable for the formation of the carcinogen. Not only can various tea preparations decrease the formation of nitrosamines, they can also depress their bioactivation. Various teas have been reported to block nitrosamine-induced tumorigenesis in skin (61). However, recent studies by Rogers et al. (62) suggest that black tea may not be as effective in mammary tissue.

The cancer chemopreventive effects of green tea may be attributed to several polyphenolic compounds, specifically the catechins epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin-3-gallate (ECG), which account for 30–40% of the extractable solids of tea leaves. It is likely that several mechanisms of action may contribute to the observed anticancer effects of tea including serving as a free-radical scavenger (63), or induction of detoxification systems through altered phase I and phase II enzymes (20,53,64). Shi et al. (65) found (-)-epigallocatechin-3-gallate inhibited the catalytic activities of several CYP isozymes and was more potent against CYP 1A and 2B1, than it was against 2E1. Several tea preparations have been reported to induce phase II enzymes such as glutathione-S-transferase (66). Thus, a reduction in carcinogen exposure may partially account for some of the protection provided by supplemental tea and its polyphenols under experimental conditions.

In addition to an effect on chemically induced tumors, researchers have found that chronic feeding of green tea polyphenols or water extract of green tea protection against ultraviolet B radiation-induced skin tumorigenicity (61). This protection may be mediated by several physiological changes including a protection against UVB-induced immunosuppression by blocking UVB-induced infiltration of CD11b+ cells into the skin; reduction in IL-10 production in skin; and a markedly increasing IL-12 production in associated lymph nodes (67). Black tea is also effective in retarding UVB-induced skin carcinogenesis. The reduced ability of decaffeinated black tea to suppress UVB skin carcinogenesis suggests that caffeine may be involved with the observed protection (68).

Green tea extract appears to have additional benefits in retarding selected cancers. Komori et al. (69) found EGCG and green tea extracts blocked the growth of lung and mammary cancer cell lines with similar potencies. Part of the antiproliferative effects of green tea may relate to its ability to modify estrogen binding to membrane receptors. EGCG has been reported to suppressed autophosphorylation of epidermal growth factor (EGF). Recently, black tea polyphenols have been reported to lead to a similar autophosphorylation (70). The inhibition of six-transfected NIH cells caused by EGCG in mouse epidermal JB6 cell line transfected with a mutant H-ras gene was found to correlate with an inhibition of AP-1 activity and the mitogen-activated protein kinase pathway (71). The activity of receptor tyrosine kinases, such as the PDGF β-receptor and EGF receptor, is implicated in the development of malignant proliferative diseases. Recently, EGCG has been found to serve as a selective inhibitor of the tyrosine phosphorylation of PDGF-Rβ and its downstream signaling pathway in vascular smooth muscle cells from rat aorta (72). Okabe et al. (73) proposed that the interaction of tea polyphenols with various transcription factors, in addition to AP-1 and NF-κB might account for the observed inhibition of TNF-α gene expression and TNF-α release.

Collectively, considerable information supports a preventative role of green and black tea against each stage of carcinogenesis. The mechanism by which these tea afford these diversifies effects appear to be multifold, with an alteration in both phase I and II enzymes and alterations in cell signaling.
3.3. Flavanones in Grapefruit

Naringin is the most abundant flavonoid in grapefruit. It has been shown to inhibit the activation of aflatoxin B1 (74), yet was relatively ineffective in blocking unscheduled DNA synthesis in liver slices exposed to PhIP (35). Part of the modest protection provided by naringin against cancer models may relate to its ability to induce phase II enzymes. These changes can have unexpected consequences as evident by the ability of grapefruit juice to markedly augment oral drug bioavailability. The ability of naringin to posttranslationally downregulate CYP 3A4 expression in the intestinal wall serves to illustrate important drug nutrient interactions that can occur. It will become increasingly important as consumers consume more fruits and vegetables to understand their impact on a host of drugs that may be used for a variety of health conditions.

3.4. Isoflavones in Soybeans

Considerable evidence points to the ability of isoflavonoids in soybeans to alter the cancer process (75,76). Soybeans, compared to several other foods, supply relative large amounts of four different types of compounds that may have anticarcinogenic properties: glycosides, phytosterols, protease inhibitors, and phytic acid. Soybeans are known to contain about 2% glycosides, which are composed of soya saponins and isoflavonoids. Plant isoflavonoid glycosides are generally converted by intestinal bacteria to hormone-like compounds with weak estrogenic and antioxidative activity. Much of the attention of soybean isoflavonoids has been directed at daidzein, equol, and genistein. Although soybean isoflavones are weak estrogens, they can function both as estrogen agonists and antagonists depending on the hormonal milieu and the target tissue and species being examined. Evidence now points to the ability of these compounds to not only influence sex hormone metabolism and associated biological activity, but also influence intracellular enzymes, protein synthesis, growth factors, malignant cell proliferation, differentiation, and angiogenesis (77,78).

Considerable evidence points to the ability of isoflavonoids to inhibit chemically induced carcinogenesis. Part of this response may relate to selected changes in selected CYP enzymes. Recently, the isoflavones genistin and daidzin, and their respective aglucone forms daidzein and genistein, were found to block 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; dioxin)-induced CYP1A1 enzyme activity. The response was independent of aromatic hydrocarbon receptor, but appeared to reveal a noncompetitive inhibition with the CYP1A1 substrate (79).

Genistein has also attracted considerable attention because of its ability to inhibit several enzymes, including protein tyrosine kinases and others involved in signal transduction (80). Genistein can attenuate both growth factor- and cytokine-stimulated proliferation of both normal and neoplastic cells. Although genistein is a potent inhibitor of tyrosine autophosphorylation of the EGF receptor this response does not coincide with a suppression in cell growth suggesting the involvement of other signal-transduction pathways as possible sites of action. The alternative sites include a retarding of DNA topoisomerase II activity, a variance in cell cycle checkpoints, and/or antiangiogenic activity (80,81). Genistein may also inhibit cell growth by modulating transforming growth factor (TGF-Î²)-1-signaling pathways (80).

Lamartiniere et al. (82) have provided convincing evidence that neonatal and prepubertal exposure to genistein has a long-lasting effect of the ability to withstand carcinogen exposure. Part of this protection may relate to a reduction in the number of terminal
end buds and increase the number of lobular structures within mammary tissue. However, soy and genistein may also increase the risk of some cancers, especially those that are estrogen sensitive (83). Whereas experimental data are rather compelling that genistein can induce the growth of human mammary tumors transplanted into nude mice (84) it remains to determine if this coincides with a more aggressive tumor that is more invasive to surrounding tissue or one that metastasizes to a greater degree. It may be that the other metabolic changes induced by soy or genistein outweigh this change in estrogen sensitive tumors. However, it would be unwise and irresponsible to dismiss this as experimental phenomena with no physiological significance. It may be that some will be placed at risk by exaggerated intakes of soy and its components.

Vasculature has an important role in several steps of the cancer metastatic process including: (1) the site of metastasis because vessels capture cancerous cells and provide the entry route into secondary organs; and (2) through angiogenesis, because vascular endothelial cells supply nutrients for tumor growth. The linings of all blood vessels are covered with endothelial cells, which can have an active role in both processes. Several studies provide evidence that the consumption of plant foods can prevent or retard angiogenesis or neovascularization. Soy products and genistein appear to be potent inhibitors of endothelial cell proliferation and in vitro angiogenesis (85). Degradation of the extracellular matrix is one of the essential steps in angiogenesis. Studies by Fajardo et al. (86) demonstrated that genistein can induce a shift toward antiproteolysis in both matrix metalloproteinase/tissue inhibitor of metalloproteinase and urokinase/plasminogen activator inhibitor proteolytic balance. Overall, the anticancer effects of soy may relate to a direct effect on tumor cells and indirect effect on tumor neovasculature.

4. INDOLES

Several indole metabolites arise during the hydrolysis of indolyl-methyl glucosinolate that occur in cabbage, broccoli, brussels sprouts, and other members of the genus Brassica (87). Dietary indole-3-acetonitrile (3-ICN), indole-3-carbinol (I3C), and 3,3'-diindolylmethane can be natural inducers of phase I enzymes, including aryl hydrocarbon hydroxylase (88). Whereas induction of CYP-dependent enzyme activities occur following the consumption of I3C, a similar response was not observed when liver slices were treated with I3C or 3-ICN, suggesting additional changes were occurring during the absorption process (89). Diindolylmethane (DIM) has emerged as a major acid-catalyzed metabolite of I3C that is formed within the gastrointestinal tract. Treatment of rat liver slices with this I3C dimer markedly influences the activities of the CYP1A, CYP2B, and CYP3A subfamilies (89). Induction of CYP1A1 gene expression probably arises from the binding of DIM to the aryl hydrocarbon receptor (AhR) (90). Induction of CYP1A1 increases 2-hydroxylation of estrogens, leading to the generation of protective 2-OHE1. DIM also decreases CYP1B1, depresses 4-hydroxylation of estradiol, and decreases carcinogenic 4-OHE1 (91). In addition to these changes in CYP activities, consumption of I3C alters several phase II enzymes including glutathione S-transferase, quinone reductase, and glutathione reductase (92,93).

Although many experimental cancer studies have utilized rather large quantities of I3C (0.5–3%), providing as little as 56 mg/kg is sufficient to alter enzymes involved in carcinogen bioactivation (93). Thus, conceivably, changes in cancer risk may occur
with very modest intakes. The protection provided likely depends on the species and tissue examined, although humans do appear to be sensitive to I3C intakes (94,95). The ability of oral I3C to enhance estradiol C-2 hydroxylation while depressing C-16 is readily apparent in females from several species (94,96).

Tissues influenced by estrogen may be particularly sensitive to I3C (97–99). However, the response may also arise because of changes in both phase I and II enzymes. I3C has been shown to reduce DNA adduct formation and reduce γ-glutamyl transpeptidase activity in mouse skin induced by DMBA and benzo(a)pyrene (100,101). Likewise, 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]quinoline (IQ) to mammary tissue (102). This response is particularly intriguing because both are recognized carcinogenic heterocyclic amines formed in proteinaceous foods during cooking and are known to be initially activated enzymatically by CYP1A1 and CYP1A2. The depression in target and nontarget organs of female rats at intakes of I3C that should have increased CYP activities suggest protection may involve other metabolic changes than the first stage of carcinogen bioactivation.

Evidence exists that I3C can influence both the initiation and promotion phases of some chemically induced cancers. In addition to indirect effects resulting from altered estrogen metabolism, I3C may directly effect cell proliferation by blocking the cell cycle and by inducing apoptosis. Whereas the response to I3C in estrogen-responsive human mammary cell line MCF-7 greater than estrogen-nonresponsive MDA-MB-231 cell, a response is still observed. A depression in the cell cycle may be attributable to a loss of cyclin-dependent kinase-6 (103). A depression in proliferation may also arise from the induction of apoptosis as has been noted in MDA-MB-231 (104). I3C induction of apoptosis in noncancerous human mammary epithelial 184-B5 cells was accompanied by enhanced p53 immunoreactivity (105). Indole-3-carbinol was found to enhance C-2 hydroxylation of estrogen and induce cytochrome P-4501A1 in MCF-7, but not in MDA-MB-231 cells. Thus, some of the antiproliferative effects of I3C probably involve selective induction of estradiol metabolism and/or the related cytochrome P-450 system that limited estrogen sensitivity.

The ability of I3C to inhibit virally induced tumors comes from studies showing that it inhibits the spontaneous occurrence of endometrial adenocarcinoma in female Donryu rats and, more recently, several studies with virally induced cervical cancer (97,106). Estrogen is known to promote the development of endometrial and cervical cancers. Jin et al. (106) provided evidence that I3C may be particularly useful in the prevention of cervical-vaginal cancer and, possibly, other cancers with a papillomavirus component. Recent studies by Yuan et al. (98) with cervical CaSki cancer cells provided evidence that I3C can abrogate estrogen-increased expression of in cells infected with human papillomaviruses oncogenes, probably by enhancing the formation of 2-hydroxyestrone (2-OHE) by the mechanism indicated above.

Consumption of indoles does not always result in a positive effect on tumorigenesis. Increased tumors have been reported (107,108). Although limited in number, these studies suggest the response to I3C is highly dependent on the timing of administration. It remains to be determined if this response relates to changes in estradiol homeostasis or not.

Collectively, considerable evidence points to the health benefits associated with enhance I3C consumption. Because some cases of enhanced carcinogenesis are evident, it is prudent that we understand who might be placed at risk before widespread recom-
mendations for large increases in I3C can be encouraged.

5. ISOTHIOCYANATES

Glucosinolates are naturally occurring constituents of cruciferous vegetables. The term actually refers to more than 100 sulfur-containing glycosides that yield thiocyanate, nitrile, and isothiocyanate derivatives upon hydrolysis. Damage to the plant cell by mastication releases the enzyme myrosinase, which transforms glucosinolates to isothiocyanates. Cruciferous vegetables are particularly rich sources of isothiocyanates. Several of these vegetables, including cabbage, brussels sprouts, and broccoli, found in the genus *Brassica*, are viewed as effective antagonists to the cancer process, at least under experimental conditions (109). Some of the naturally occurring isothiocyanates include phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), and sulforaphane. PEITC is particularly plentiful in cabbage, brussels sprouts, cauliflower, kale, and turnips.

Organic isothiocyanates have been reported to block the production of tumors in rodents induced by such diverse carcinogens as polycyclic aromatic hydrocarbons, azo dyes, ethionine, N-2-fluorenylacetamide, and nitrosamines. One of the most thoroughly examined rodent models is the block in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis by selected isothiocyanates (110,111). Nevertheless, isothiocyanates are not always found to provide protection. In some cases, they may actually accentuate tumor development. For example, Hirose et al. (112) found that phenylethyl isothiocyanate and benzyl isothiocyanate promoted the post-initiation phase of diethylnitrosamine induced bladder carcinogenesis. Likewise, Stoner et al. (113) found that dietary 6-phenylhexylisothiocyanate (PHITC) promoted N-nitrosomethylbenzylamine (NMBA)-induced esophageal carcinogenesis.

Several thiocyanates or isothiocyanates appear to be effective inhibitors of carcinogen metabolism as evident by a reduction in carcinogen binding to DNA in target tissue (114,115). Nevertheless, the overall response and its magnitude likely depend on the structure of the thiocyanate and the carcinogen examined. Overall, α-naphthyl-, β-naphthyl-, phenyl-, benzyl-, phenethyl-, and other arylalkyl isothiocyanates have been reported to inhibit tumor development in a variety of tissues including liver, lung, mammary gland, forestomach, and esophagus (114–116). Thus, widely diverse isothiocyanates may have importance in the human diet in inhibiting tumors occurring at a variety of sites.

The anticarcinogenic effects of isothiocyanates appear to be mediated through a suppression in cytochrome *P-450*-mediated carcinogen bioactivation and by an induction of phase II enzymes including glutathione transferases and NAD(P)H:quinone reductase (114,117). *CYPIA2* is known to catalyze the bioactivation of the lung carcinogen, NNK. PEITC is recognized to suppress the activity of this cytochrome. Additionally, the inactivation of cytochrome *P450 2E1* has been demonstrated with some isothiocyanates. The depression in *CYP 2E1* may relate to a modification of the apoprotein (118). Other *P450* enzymes may be enhanced by providing isothiocyanates. The *P450 2A* family of enzymes may be involved with NMBA metabolism (119). It is unclear what impact, if any, that selected isothiocyanates have on its activity.

Isothiocyanates may also modify the proliferation of some neoplasms. Early studies
revealed isothiocyanic esters inhibited the in vivo growth of transplanted Ehrlich ascites carcinoma cells (120). Part of the depression in growth is likely attributable to induction of apoptosis induction. PEITC has been found to induce p53 protein expression and p53-dependent transactivation. The significance of these finding is also evident by the ability of PEITC to induce apoptosis in p53 +/+ cells but not in p53 −/− cells (121). Recently, Sasaki et al. (122) has shown that oral administration of 5-methylthiopentyl isothiocyanate markedly reduced the pulmonary colonization of B16-F10 murine melanoma cells in syngeneic mice. This protection was not without some complications because it also resulted in thymus atrophy and a selective loss of CD4+CD8+ cells in thymocytes. Interestingly, neither the metastaticity or thymus responses were observed in mice treated with 3-methylthiopropyl isothiocyanate.

The protective effective of isothiocyanates may be highly dependent on its clearance within an individual or possible within a specific tissue. Glutathione transferase enzymes are known to conjugate isothiocyanates, leading to their excretion, which largely occurs as dithiocarbamates (123). Recent evidence suggests that individuals with a glutathione transferase M1 (GSTM1) null genotype may have a more exaggerated response to broccoli and associated isothiocyanates (124).

6. ORGANOSULFUR COMPOUNDS

Garlic, along with onions, leeks, and chives, represents the major Allium foods consumed by human beings. Considerable evidence points to their consumption, particularly garlic, as possible modifiers of cancer risk (125–127). Unlike many other foods, about 0.35% of garlic’s fresh weight or 1% of its dry weight is contributed by sulfur (128,129). A complex array of sulfur compounds including thiosulfonates, dithiins, ajoenes, and so on can occur in garlic preparations (130–132).

Although major limitations exist in defining the precise role that garlic has in the cancer process, the likelihood of its significance is underscored by both epidemiological and laboratory investigations. Laboratory-based studies with model cancers provide some of the most compelling evidence that garlic and its related sulfur components can suppress cancer risk and alter the biological behavior of tumors.

Experimentally, garlic, and its associated components, suppresses the incidence of breast, colon, skin, uterine, esophagus, and lung cancers (127). This protection may arise from several mechanisms including: blockage of nitrosamine formation, suppressed bioactivation of several carcinogens, enhanced DNA repair, decreased cell proliferation and/or the induction of apoptosis. It is possible, and quite probable, that several of these cellular events are modified simultaneously.

As aforementioned, nitrosamines are potent experimental carcinogens. Mounting evidence demonstrates the ability of several foods, including garlic, to suppress their formation both in vitro and in vivo (133–135). The reduction in nitrosamines may arise secondarily to an increase in the formation of nitrosothiols. Studies by Dion et al. (134) revealed that not all allyl sulfur compounds are equally effective. The ability of S-allyl cysteine (SAC) and its nonallyl analog, S-propyl cysteine, to retard, but not diallyl disulfide (DADS), dipropyl disulfide, and diallyl sulfide suggest the cysteine residue is critical the block in nitrosamine formation (134). Because the content of allyl sulfur can vary among preparations, it is probable that all garlic sources vary in the protection they provide against nitrosamine formation. Some of the most compelling evidence that gar-
lic depresses nitrosamine formation in humans comes from studies by Mei et al. (136). In their studies, consuming 5 g garlic/d completely blocked the enhanced urinary excretion of nitrosoproline caused by ingesting supplemental nitrate and proline.

The anticancer benefits attributed to garlic are also associated with the ability of its allyl sulfur compounds to suppress carcinogen bioactivation. Evidence from a variety of sources reveals that garlic is effective in blocking DNA alklylation, a primary step in nitrosamine carcinogenesis (137,138). A block in nitrosamine bioactivation may reflect changes in several enzymes. However, substantial evidence points to the involvement of CYP2E1 (139). An autocatalytic destruction of CYP2E1 may account for some of the chemoprotective effects of diallyl sulfide, and possible other allyl sulfur compounds (140).

Garlic and several of its allyl sulfur compounds can also effectively block the bioactivation and carcinogenicity of a host of nonnitrosamines. This protection, which involves a diverse array of compounds and several target tissue sites, suggests either multiple mechanisms of action or a widespread biological effect. Because metabolic activation is required for many of these carcinogens used in these studies, there is a likelihood that phase I and II enzymes are involved. Interestingly, little, if any, change in CYP1A1, 1A2, 2B1, or 3A4 activities are observed following treatment with garlic or related sulfur compounds (141–143). Thus, other enzymes involved in the bioactivation or removal of carcinogenic metabolites might be involved in the observed protection. Singh et al. (144) provided evidence that the efficacy of various organosulfides to suppress benzo(a)pyrene tumorigenesis was correlated with their ability to suppress NAD(P)H: quinone oxidoreductase, an enzyme involved with the removal of quinones associated with this carcinogen. Changes in bioactivation resulting from a block in cyclooxygenase and lipoxygenase may also partially account for the reduction in tumors following treatment with some carcinogens (145). Changes in glutathione concentration and the activity of specific glutathione-S-transferases, both factors involved in phase II detoxification, may be important in the protection provided by garlic. Feeding garlic powder to rats has been found to increase the GST activity (146). Hu et al. (147) provided evidence that the induction of glutathione (GSH) S-transferase pi (mGSTP1-1) may be particularly important.

Considerable evidence indicates DNA hypermethylation is an important mechanism for inactivation of key regulatory genes including E-cadherin, pi-class glutathione S-transferase, the tumor suppressors cyclin-dependent kinases (CDKN2) and of the phosphatase gene (PTEN), and insulin-like growth factor (IGF-2) targeted histone acetylation/deacetylation results in remodeling of chromatin structure and correlates with activation/repression of transcription (e.g., IGFBP-2 and p21). Lea et al. (148) reported that at least part of the ability of DADS to induce differentiation in DS19 mouse erythroleukemic cells might relate to its ability to increased histone acetylation. DADS caused a marked increased in the acetylation of H4 and H3 histones in DS19 and K562 human leukemic cells. Interestingly, DADS has been also been reported to inhibit the growth of H-ras oncogene transformed tumors in nude mice (149). This inhibition correlated with the inhibition of p21H-ras membrane association in the tumor tissue.

Some allyl sulfur compounds are known to block cell growth in the G2/M stage of cell division (150–152). Activation of the p34<sup>ck2</sup> kinase complex is known to modulate the progression of cells from the G2 into the M phase by promoting chromosome condensation, cytoskeletal reorganization, and nuclear envelope breakdown (153). Recent studies from our laboratory revealed that DADS caused a marked suppression in p34<sup>ck2</sup> kinase activity (152).
Rarely have a comparison of water- and oil-soluble compounds been conducted within the same study. Whereas subtle differences among garlic preparations occur, quantity rather than source appears to be a key factor that influences the degree of protection (154). Differences that do occur between preparations likely relate to the content and effectiveness of individual sulfur compounds (see Table 3). The number of sulfur atoms present in the molecule seems to influence the degree of protection with diallyl trisulfide, diallyl disulfide, diallyl sulfide (150). Likewise, the presence of the allyl group generally enhances protection over that provided by the propyl moiety (155).

6.1. Onions

Relatively few epidemiological and preclinical studies are available to truly assess the effect of onions on the cancer process. Nevertheless, an inverse relationship has been reported between lung, colorectal, and stomach cancer with the consumption of onions and leeks (156–159).

Onions contain a variety of sulfur compounds, some of which are included in Table 4. Several of these have been examined for their ability to modify one or more stages of the cancer process.

Belman (160) reported that onion oil inhibited skin tumor yield and incidence following phorbol-myristate-acetate promotion in a dose-dependent manner over the wide range when applied three times per week. Interestingly, onion oil was more effective than garlic oil in the inhibition of these tumors. More recently, Ito et al. (161) observed that diphenyl disulfide and S-methyl methanethiosulfonate significantly suppressed chromosomal aberrations induced by both aflatoxin B1 (an indirect-acting carcinogen) and methyl methanesulfonate (a direct-acting carcinogen) (161). Changes in redox status of thiols were proposed as a possible mechanism of action. Clearly, additional studies are need to examine if the protection by onions depends on the carcinogen employed. Takada et al. (162) have found that some sulfur compounds (isothiocyanic acid isobutyl ester, dipropyl trisulfide, and allyl mercaptan) promoted liver cell proliferation in rats treated with dimethylnitrosamine suggesting that, under some circumstances, the consumption of onions may increase cancer risk. It remains to be determined how important these findings are in humans especially since Fukushima et al. (163) reported oil-soluble organosulfur compounds such as methyl propyl disulfide and propylene sulfide, as well as water-soluble compounds such as S-methylcysteine and cysteine, retarded the development of GST-P positive foci in the liver of rats treated with diethylnitrosamine.

The mechanism by which onion retards carcinogenesis remains largely unexplored. As indicated alteration in selected thiols may account for part of the anticancer benefits associated with sulfur compounds. However, other constituents including flavanoids

| Table 3 |
|-----------------|-----------------|
| **Some of the Important Sulfur Compounds Found in Garlic** |
| 3,5-Diethyl-1,2,4-trithiolane | Diallyl sulfide |
| Allyl 1-propenyl disulfide | Diallyl trisulfide |
| Allyl 1-propenyl trisulfide | Methyl allyl trisulfide |
| Allyl alcohol | Methyl allyl disulfide |
may account for the observed effects of garlic on the cancer process. Regardless of the compounds responsible, extracts and essential oils of onions are known to have other physiological effects including the retarding of platelet aggregation (164). Interestingly, heating onions tends to reduce their efficacy.

Although the health benefits of onion consumption have not been extensively examined, available evidence does suggest there is little reason to reduce its use as part of a healthy diet. Nevertheless, it remains to be determined the minimum quantity of needed to reduce cancer risk and what factors might modify this efficacy.

7. TERPENES

Terpenes consisting of varying numbers of isoprene building blocks are widely dispersed in nature. D-limonene is probably the most extensively examined monoterpene for its potential carcinogenic properties. A natural constituent of citrus oils, it also is present in oils from mint, caraway, thyme, cardamom, and coriander. D-limonene is used commercially as a flavor and fragrance.

Interest in limonene stems from its ability to inhibit chemically induced tumors during experimental conditions and for its ability to cause the regression of existing tumors. Limonene and its hydroxylated derivative, perillyl alcohol, are currently undergoing clinical evaluation in phase I studies (165). Limonene is recognized to inhibit skin carcinomas induced by benzo(a)pyrene (166), mammary tumors induced by DMBA (167), and lung tumors induced by NNK (168). Thus, the potential benefits may be widespread and not limited to a specific tissue. Much of the anticarcinogenic activity brought about during the initiation phase may be mediated through the induction of the hepatic detoxification enzymes GST and UDPGT (169). Selected changes in CYP isozymes may also contribute to the observed protection (170).

One of the most impressive effects of limonene is its ability to cause tumor regression (171). Limonene and other monoterpenes are known to suppress 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity (172). An inhibition of HMG-CoA can deplete intermediates required for the posttranslational modification of proteins, a process giving proteins lipophilic anchors that bind to membranes. Consequently, nuclear lamins and ras oncproteins remain in nascent states, and cells do not proliferate. A depression in posttranslational isoprenylation of growth-controlling small G proteins may explain this antitumorigenic property. Ruch and Sigler (173) have provided evidence the response does not relate to ras. More recently, Ren and Gould (174) found perillyl alcohol suppressed small G protein isoprenylation in rat mammary glands. The greatest inhibition of small G protein isoprenylation was with RhoA by type
I geranylgeranyl protein transferase. Because *Rho* family proteins serve as guanine nucleotide-regulated binary switches controlling signaling pathways that ultimately regulate diverse cellular processes, and have been implicated as critical regulators of oncogenic, these results may be particularly important. Increased expression of the mannose-6-phosphate/IGF-2 receptor and TGF-β1 are recognized responses in cells exposed to monoterpenes. Ariazi and Gould (175) identified 42 induced and 58 repressed genes that were altered by monoterpenes. Growth depression induced by perillyl alcohol and perillic acid is associated with a fall in the proportion of cells in the S phase and an accumulation of cells in the G1 phase of the cell cycle. This block in the cell cycle is accompanied by a depression in cyclin D1 mRNA (176).

Interestingly, limonene has been reported to cause a complete regression of mammary carcinomas (171,177). Some of this loss of tumor mass is probably explained by the induction of apoptosis (178).

D-Limonene has been found to cause kidney tumors when given at high doses to male rats and is associated with the development of hyaline droplet nephropathy (179). The tumor likely arises because of the binding of a minor metabolite, d-limonene-1,2-oxide, to α 2U-g, impeding the normal process of lysosomal proteinase. Because this protein does not exist in humans, it is likely the tumor would also not occur. Thus, D-limonene may not pose any special carcinogenic or nephrotoxic risk to human beings (179).

The multiple anticarcinogenic effects of limonene influencing all phases of the cancer process suggest that related monoterpenes may also be efficacious in the chemoprevention and chemotherapy of malignancies. Another monoterpenic, D-carvone, is a major constituent of caraway seed oil. Both D-carvone and caraway seed oil have been shown to inhibit the activation of dimethylnitrosamine and decrease the induction of forestomach tumors.

8. SUMMARY AND CONCLUSIONS

Epidemiological, clinical, and laboratory investigations provide rather-convincing evidence that dietary habits can modify cancer risk. A large number of nonnutritive compounds in foods appear to protect against one or more stages of the cancer process. Scientifically, these nonessential nutrients are reported to modify the carcinogenic process by several mechanisms including: altering carcinogen formation and metabolism; curtailing tumor promotion and progression; and cellular and host defenses. Understanding the molecular events that are modified by nutrients will be key in the effect use of diet as a treatment for the prevention of cancer. Although dietary habits are not the sole determinant of cancer, they do represent a significant point for which intervention is possible. Adjustment of dietary practices to conform to generalized dietary goals may not be necessary, or even appropriate, for all segments of the population. A greater appreciation of human genetics will hopefully provide valuable insights into who might benefit or be placed at risk from exaggerated intakes of selected foods.

9. RECOMMENDATIONS

A comprehensive understanding about the precise role that specific foods or their components have on the cancer process is fundamental in improving human health. Carefully controlled and probing investigations that examine individual dietary con-
stituents in the context of the entire diet and the environmental factors to which we are exposed will be key in unraveling the true effect of diet in the cancer process. Future research must be aimed at the identification of the critical site(s) or molecular target(s) where nutrition intervention beyond basic nutriture is most appropriate. Without this information, it will be impossible to tailor recommendations to meet the specific needs of the individual. Although experimental evidence strongly links several nonessential nutrients as significant modifiers of the cancer process, it remains to be determined if humans will benefit from the intake of one or more of these nutrients when taken in isolation from other components of the food. Whereas individual supplements of nonessential nutrients may well be as effective, their safety must be thoroughly examined, not only for toxicity, but their dependence on other components of the diet. Until this information is available, it remains prudent to eat a variety of fruits and vegetables and avoid excesses.

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II

CARDIOVASCULAR DISEASE
1. INTRODUCTION

Numerous experimental and epidemiological studies have revealed the relationship between an elevated blood cholesterol value and development of atherosclerosis (1). Since the early days when the association was based on gross pathology, a plethora of investigations, including those from cell biology (2) have carefully documented the pathogenesis of the atherosclerotic process, linking thrombogenesis, the inflammatory response, and an elevated LDL/HDL ratio with atherogenesis and coronary heart disease (CHD). More recently, the possible oxidation of the LDL particle within the damaged arterial intima has developed into a promising avenue of atherogenesis research with implications for modulation by diet (2–4).

Nonetheless, the ongoing discussion of how dietary saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), or polyunsaturated fatty acids (PUFAs) modulate the concentration of plasma cholesterol continues to be among the most confusing aspects of atherogenesis, not just for consumers, but also for investigators researching the subject. Confusion persists because a metamorphosis is in progress from a simple, earlier literature that focused on total plasma cholesterol as affected by individual fats or classes of fats (SFAs, MUFAs, PUFAs) to a newer approach that attempts to coordinate modulation of specific lipoproteins by dietary fatty acids present in the combination of fats being consumed. During this process, a number of new observations are forcing reconsideration of previous concepts.

Among the earliest observations indicating that dietary fat was related to elevated blood lipids was the report by Kinsell (5), who noted that PUFAs lowered plasma cholesterol (TC) even as SFAs elevated plasma lipids. Shortly thereafter, Bronte-Stewart (6) noted that simply adding extra PUFAs to the diet (without decreasing SATs) could dramatically lower an elevated TC. Based on these and related observations, an increase in consumption of PUFAs and decrease in SATs has become the standard dogma of nutritionists and clinicians attempting to improve the plasma lipid profile and decrease the risk of CHD. Although this dietary directive, in conjunction with various other intervention procedures, has reduced CHD mortality in the United States by 40% in the past 25 yr, the detail of the dietary fat recommendation continues to be debated in terms of which specific unsaturated or SFAs are more acceptable.
Because a high intake of polyunsaturates increases the potential for in vivo lipid peroxidation and the need for vitamin E antioxidant protection, it has been suggested that the LDL particle might be more prone to peroxidative damage during high polyunsaturated fat intake, thereby reducing the efficacy of PUFAs as an antiatherogenic fat. Rather, it has been recommended that a high-MUFA oil should be substituted for the high-PUFA oil, based on the assumption (arguably incorrect) that MUFAs lower TC and LDL-C as effectively as polyunsaturates without rendering the MUFA-rich LDL as susceptible to oxidative stress (4). Although in vitro estimates of LDL oxidizability favor the latter recommendation, certain in vivo evidence questions whether LDL circulating during a high-PUFA fat diet is normally more prone to peroxidative damage than LDL circulating during high-MUFA fat intake (7) or whether LDL in atheroma are actually limited in antioxidant protection (8). Furthermore, in vivo data concerning CHD risk clearly suggests that risk is inversely related to the plasma 18:2/18:1 cholesteryl ester ratio which is directly related to 18:2 intake and inversely related to 18:1 consumption (9). In other words, the greater the 18:2-cholesteryl ester pool, the less atherosclerosis is observed in both human and animal studies (10,11). Reducing 18:2 intake below a critical level also increases the 18:1-cholesteryl ester pool, and expanding 18:1-cholesteryl esters at the expense of 18:2-cholesteryl esters is associated with more cholesterol deposition in the arterial intima, not less. Thus, the simple suggestion to “replace PUFAs with MUFAs while reducing SFAs” is not necessarily the best advice, considering that a high-polyunsaturated fat intake (7–10% total energy [en]) induces the greatest decrease in circulating LDL and is associated with the least cholesterol deposition in arterial lesions (12). Reducing the mass of LDL cholesterol while preserving or increasing HDL should remain the primary objective of diet therapy against atherosclerosis (13). Ironically, a balance between SFAs and PUFAs may produce the most favorable LDL/HDL ratio (14).

The literature on dietary fat and CHD has been substantially detailed and collated by expert committees (1) wherein the epidemiological, experimental, and clinical data are convincingly marshalled to make the point that our plasma lipids are best kept below 180 mg/dL TC with LDL-C <125 mg/dL for good cardiovascular health. However, a moderate TC value is not a full guarantee against CHD because platelet aggregation and thrombogenesis are probably more important than elevated lipids in the development of CHD. Generally speaking, both excessive thrombosis and elevated LDL-C appear to be required for advanced CHD to become clinically manifest (2).

Although most of the evidence incriminating fat with CHD risk has involved the modulation of lipoproteins, fat can also influence blood pressure and platelet aggregation through its effect on prostaglandin and leukotriene metabolism. These physiological events have a major impact on atherogenesis (2). Dietary sodium can also factor into the equation by virtue of its influence on blood volume, and indirectly, blood pressure (1,2). However, the scope of these interrelationships is beyond the focus of this chapter.

2. IMPORTANCE OF LIPOPROTEIN PROFILE

A compelling set of data currently exists that demonstrates the importance of the plasma lipoprotein profile in risk assessment for CHD (13,15,16). Furthermore, this profile can be modulated by manipulating the quality of dietary fat (fatty acids) and the amount of dietary cholesterol consumed each day. The risk is clearly associated with LDL. Although the specific objective of raising HDL to lower CHD incidence is not a proven means of therapy, certain interventions that lower CHD risk, such as increased
physical activity, cessation of smoking, and alcohol consumption, also raise HDL (17).

Furthermore, drugs that have raised HDL during intervention trials tended to reduce CHD incidence (18,19).

Most would agree that an elevated LDL/HDL ratio represents a risk for CHD and that lowering blood lipids, particularly LDL, is beneficial. In addition, LDL particle size appears to be important, with small dense LDL being more prone to oxidation and increasing the risk of CHD (20). Surprisingly, the early indication is that saturated fat, even though increasing LDL number, may induce a larger, more buoyant LDL than more unsaturated fats (21). Thus, a clear consensus has not been reached on specific recommendations for lowering lipids by dietary fat manipulation, especially pertaining to individual lipoproteins, or whether once lowered, a significant reduction in CHD will result for patients currently diagnosed with the disease. Furthermore, if CHD mortality is reduced, will overall age-adjusted morbidity and mortality be improved? These questions are especially important to patients who have already suffered a myocardial infarct and are looking to diet for corrective therapy. Significant clinical trials have shown that drug intervention (with inhibitors of cholesterol synthesis) for 5 yr reduced LDL-C by 35% and deaths by 30% without increasing non-CHD deaths (19,22). Whether long-term dietary intervention alone can mediate such a result is open to question, but the preliminary evidence is that drastic lifestyle modifications, including diet, do substantially reduce CHD risk (23).

3. DIETARY CHOLESTEROL IMPACT ON TC

Comprehending the difference between dietary cholesterol and dietary fat as they relate to plasma cholesterol regulation is often conceptually difficult for the average consumer, especially because the relative importance of dietary fat is thought to be greater than that for dietary cholesterol, at least in humans. In fact, a normal intake of dietary cholesterol does elevate plasma cholesterol, especially LDL-C, in certain species, including most humans, but not in others. Those species most responsive to cholesterol intake normally do not consume appreciable dietary cholesterol, e.g., herbivorous animals, like the rabbit, hamster, and certain monkeys, like the rhesus and cynomolgus. Highly carnivorous species, like cats and dogs, and certain omnivorous ones, like rats and mice, tend not to respond to ordinary intakes of dietary cholesterol. The omnivorous human reveals an intermediate sensitivity that tends to vary between individuals.

Reasons for species differences are several and relevant to our understanding of the physiology of cholesterol metabolism. The ability of rats to counterbalance dietary cholesterol intake reflects its proportionally great hepatic cholesterol synthesis relative to whole body synthesis (24) and its resistance to store excess dietary cholesterol in the liver. In such species, absorbed cholesterol effectively downregulates whole body synthesis by suppressing hepatic synthesis, in effect balancing the overall body pool without expanding either the hepatic or plasma pools appreciably. In species like hamsters with minimal hepatic cholesterol synthesis, the absorbed cholesterol is rapidly stored in the liver and suppresses hepatic synthetic capacity, simultaneously depressing LDLr activity while spilling stored hepatic CE into the plasma, greatly increasing LDL production and adding to the plasma LDL (and HDL) waiting to clear into the liver. In several species, dietary cholesterol represents a powerful suppressant of hepatic LDLr activity, especially if the liver becomes “laden” with this sterol. Overload is most readily documented by accumulation of hepatic cholesteryl esters in susceptible species. The exact relevance of this scenario for humans is more complicated and less than certain.
Among humans, the impact of dietary cholesterol varies with different circumstances. Individuals can be hypo- or hyper-responders to cholesterol intake, presumably for genetic and dietary reasons not completely understood (25). For example, a protein important for uptake and clearance of triglyceride-rich lipoproteins, apoprotein E exists as three major isoforms E2, E3, and E4. The apoprotein E4 phenotype seems to promote slight enhancement of cholesterol absorption, and such individuals may also have imperfect feedback inhibition of hepatic cholesterol synthesis resulting in general expansion of the whole body cholesterol pool. They are also at higher risk for atherosclerosis (26). Furthermore, effective removal of cholesterol from the liver also depends somewhat on bile acid synthesis and excretion via feces. Humans are much less capable of cholesterol removal than rats because their bile acid synthesis rate is substantially lower (27). Liver cholesterol storage and overload presumably depend, in part, on the balance between the ability to convert free cholesterol to its cholesteryl ester storage form (via the ACAT enzyme), either for resecretion in lipoproteins or hepatic deposition, or for the aforementioned excretion in the form of biliary cholesterol or bile acids. However, the relative capacity for cholesteryl ester formation in human liver is presumably minimal because little evidence exists that the human liver ever experiences appreciable cholesterol storage. Thus, the ability of dietary cholesterol to expand the plasma lipoprotein pool in humans is much less than that for species that store hepatic cholesterol, e.g., rabbits and hamsters.

The dietary cholesterol impact in humans also depends on other factors in the diet affecting absorption of both cholesterol and bile acids. Plant sterols, like sitosterol, and certain soluble dietary fibers, like psyllium, can deter bile acid and cholesterol absorption, thereby reducing the cholesterol-raising impact of any cholesterol available in the diet. For example, utilization of sitostanol esters and antibiotics to completely block cholesterol absorption in hypercholesterolemic men decreased total cholesterol and LDL-C about 35% (28). This observation clearly indicates that cholesterol absorption, both from dietary and biliary sources, impacts the lipoprotein cholesterol pool in humans. In quantitative terms, the impact of dietary cholesterol in humans is reflected mainly in LDL-C, and is nonlinear. That is, a 100 mg increment of dietary cholesterol between 0–300 mg/d intake appears to have 2–3 times the impact of 100 mg between 500–800 mg/d, increasing total TC by 4–6 mg/dL per 100 mg in the first instance, but only 2–4 mg/dL for the 100-mg increment in the second case (29). The implication is that extremely low cholesterol intake (close to zero) or extremely high intake of plant sterols (2–3 g/d) may be desirable when lipoprotein metabolism is clinically stressed, a point supported by actual experience (23,28).

4. DIETARY FAT AND LIPOPROTEIN METABOLISM

The amount and type of dietary fat have a greater influence on human plasma cholesterol and lipoproteins than dietary cholesterol, but cholesterol intake also influences the response to fatty acids by depressing LDLr activity (Fig. 1). Although it is not certain that fat (fatty acids) routinely alters the rate of bile acid synthesis, high intake of polyunsaturates (>20% en 18:2) may increase bile acid production (30). On the other hand, certain fatty acids, similar to dietary cholesterol, can modify both LDL production rate and LDLr activity to exert a major impact on plasma LDL and total plasma cholesterol pools (22,31).
4.1. Amount of Fat

The American Heart Association (AHA) and National Cholesterol Education Program (NCEP) have published comparable recommendations (15) incorporating the rationale for lowering total cholesterol and LDL-C by reducing dietary fat to 30% of total dietary energy or less, saturated fat to 10% en or less, and dietary cholesterol to 200–300 mg/d depending on the initial elevation in LDL-C above 130 mg/dL. Generally, TC will decline 10–15% in response to sustained diet changes, but a decline in excess of 20% is occasionally encountered in the best case. In one study, hypercholesterolemic lean individuals were more apt to respond to sustained restrictions in saturated fat and cholesterol intake than hypercholesterolemic obese or normcholesterolemic lean subjects (32). The association with leanness may reflect the relative absence of the 18:2 reserves in adipose of the obese. This available pool of 18:2 may ultimately affect the individual responsiveness of the host (see Subheading 4.3.).

It has been suggested that a specific decrease in saturated fatty acids (as a class) must be a major aspect of restricting fat to 30% en if TC is to decline (33), but this does not necessarily apply to 16:0-rich or 18:0-rich fats as much as to 14:0-rich fats, i.e., the primary restriction of milkfat from dairy products (34,35).

It is well established that consumption of a high-carbohydrate low-fat diet (e.g., 20% en from fat reflecting a substantial decrease in saturated fatty acids) can induce a substantial 10–20% reduction in TC, even though plasma triglycerides generally increase, at least temporarily (30,36). Even this rule may not pertain if subjects have normal lipoprotein metabolism and the same fatty acid profile (the exact saturated:monounsat-

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**Fig. 1.** Correlations between the observed TC response and the % dietary energy from 14:0 (consumed in 14:0-rich fats) reveal a linear relationship (A), whereas that with 18:2 is nonlinear (B) in monkeys fed cholesterol-free diets (from ref. 35).
urated: polyunsaturated fatty acid configuration) is maintained between the 40% en and 20% en fat exchange (37), i.e., it appears that no change in any lipoprotein parameter is induced by reducing the fat load in normolipemic subjects unless the saturated fatty acid mass and profile are decreased relative to unsaturates. Addition of any fat to a high-carbohydrate diet typically increases HDL-C and plasma cholesterol depending on the amount of carbohydrate replaced. Adding saturated fat usually increases LDL-C, as well, whereas monounsaturates increase LDL-C slightly, and polyunsaturates produce a minimal change in this pool (38). Even as a high-carbohydrate diet produces a lower LDL-C, it often increases plasma triglycerides, decreases HDL-C, and decreases LDL size (20,30,36,38).

This latter triad of changes affecting lipoproteins during a high-carbohydrate diet (especially decreased LDL size) is thought by some to represent an added risk for CHD (20,30,36). As a result, a program of limiting fat reduction to about 30% en has been advocated, whereas emphasizing a residual fat content that favors monounsaturates over polyunsaturates so that HDL will be less likely to decline and the small dense LDL less likely to form. However, polyunsaturates generally do not depress HDL unless they represent >15–20% en in the diet, and evidence has been presented that the most favorable LDL/HDL ratio (i.e., lowest LDL and highest HDL) is induced by a diet with 30% en as fat containing a combination of 16:0-rich saturated fat (to elevate HDL) and polyunsaturates (to assure removal of LDL by the liver), each representing 8–10% en (14). Thus, in addition to raising LDL, saturated fats induce the greatest rise in HDL, followed by monounsaturates, and then polyunsaturates. As inferred from the above, monounsaturates appear singularly benign in terms of their impact on lipoprotein metabolism, a conclusion in accord with the most inclusive analysis (i.e., hundreds of human comparisons) on the subject of dietary fat and plasma lipids (39).

Despite the concern (aforementioned) associated with lowering an elevated TC by feeding a high-carbohydrate diet, it is clear from epidemiologic evidence that populations that normally consume high-carbohydrate low-fat diets (<25% en as fat) are also afflicted with much less diet-related chronic disease, especially CHD, than populations that consume high-fat diets (>30% en as fat) (1,2). The point is that both long-term diet and other environmental circumstances influence those considerations involving the therapeutic manipulation of diet for altering CHD risk. Thus, a relatively lean, physically active population with a history of moderate food consumption (e.g., most developing nations) exemplifies different metabolic characteristics, including lipoproteins, than an overweight, sedentary population consuming excessive calories based on high-fat and sugar. The response to a high-carbohydrate diet by the two populations differs, but the evidence suggests that it is probably advantageous for both groups to either maintain or return to a lifestyle that ultimately includes a diet based on complex carbohydrates (including high fiber) represented by cereal grains, vegetables, and fruit (40). To re-establish a “normal” metabolic profile in an overweight population may require severe, sustained reduction in fat intake, e.g., 10% en as fat advocated by Pritikin or Ornish (23). Such a regimen has been shown to drastically reduce plasma lipids and even causes dissolution of arterial cholesterol deposits and atherosclerotic plaques. Thus, in certain situations, diets extremely high in complex carbohydrate and low in fat can be advantageous, even for those at high risk for CHD. However, a major consideration is the individual response, i.e., the concept that individuals (or even subpopulations) vary in their lipoprotein response to altered fat (fatty acid) intake.
4.2. Dietary Fat (Fatty Acid) Quality

Although the focus on fat has historically emphasized the key role of saturated fat and certain fatty acids (12:0, 14:0, and 16:0) in raising TC, it now appears evident that the TC response to these fatty acids must be considered in the context of other dietary fatty acids and cholesterol consumed simultaneously in addition to the metabolic status of the subject at the time of dietary intervention. Thus, the concurrent intake of both 18:2 and other saturated fatty acids modifies the impact of any individual saturated fatty acid, which, in turn, reflects the overall metabolism of lipoproteins at the time intervention is initiated (Table 1).

After dietary fat saturation had been identified as a major factor regulating plasma cholesterol in humans, it was not long before Keys (41) and Hegsted (42) developed equations that would predict the relative response in plasma cholesterol when switching from one dietary fat profile to another. However, their estimates, though generally applicable to world populations, now appear oversimplified because they fail to distinguish between the impact of specific fatty acids or to discriminate between the considerable variability in host response. Nonetheless, our current understanding of the plasma lipid response to dietary fat derives from the Keys-Hegsted studies (41,42) and their original regression equations that were used to predict the impact of fat (and in certain cases, fatty acids) on circulating cholesterol in populations eating Western diets. However, those equations (i.e., $\Delta TC \approx \Delta SATs - 2 \Delta POLYs + \Delta dietary cholesterol$) were based on several assumptions that do not fully account for more current information.

4.3. Nonlinear Response to Polyunsaturates

Although Keys and Hegsted pointed out the cholesterol-lowering potential of polyunsaturates (primarily 18:2), they assumed this relationship was linear. However, animal model systems where dietary fatty acids and animals can be meticulously controlled, as well as retrospective analysis of the best set of human data, indicate that the decline in plasma cholesterol associated with increasing polyunsaturated fat consumption is nonlinear (35,43,44), typically reaching a bottom or “threshold” at about 5% en as 18:2 (Fig. 2). Extra 18:2 only produces a minor further decrease in TC. This 18:2 “threshold” presumably exists for humans, and may correspond to maximum up-regulation of LDL receptors by 18:2 coupled with an 18:2-induced decrease in LDL production. Once the nonlinear nature of the plasma cholesterol response to 18:2 is appreciated, previous linear responses in human data sets can be reconciled as merely incomplete and representative of a linear portion of the overall curve, and not as contradictory evidence.

When the 18:2 threshold has been reached, it appears possible to exchange 18:1 or 18:0, and even 16:0, for any extra 18:2 (above threshold) without modulating the plasma lipids appreciably (Fig. 2). This ability to exchange other fatty acids for 18:2 without effect is evident in a number of human studies (14,45–49) and explains much of the confusion and controversy surrounding the ability of certain fatty acids, especially 16:0 and 18:1, to raise or lower plasma cholesterol, respectively, in certain circumstances, but not in others. For example, the exclusive intake of a monounsaturated oil (which is not practical) can lower plasma cholesterol effectively because it supplies enough 18:2 to satisfy the 18:2 threshold requirement in the absence of the especially cholesterolemic combination of 12:0+14:0 plus dietary cholesterol found in certain an-
imal fats (see ref. 44 for discussion of this point).

Because the TC response to 18:2 is nonlinear, small increments of 18:2 between 1%–3% en in the diet cause a major decrease in TC, whereas a shift between 15% en and 20% en as 18:2 is often without effect (Fig. 2). Whereas both LDL production and receptor activity may be modulated at low intakes of 18:2, the progressive, but small, decline in LDL-C and TC at higher 18:2 intakes may reflect the continued fall in LDL (and HDL) production associated with decreased VLDL output linked to decreasing hepatic triglyceride synthesis.

It is possible that the 18:2 threshold represents the biological requirement for a fixed mass of 18:2 (i.e., mg/kg bd wt/d), as opposed to a requirement based on percent of total daily energy as originally conceived (35). If so, it would help explain why the percent energy threshold for 18:2 seems to vary somewhat with experimental circumstances in human studies. For example, obese hypercholesterolemic subjects failed to respond to incremental increases in 18:2 from 3 to 12% energy, whereas their lean counterparts did (32). Because the obese tend to eat more food than lean individuals, even at 3% energy the mass of their 18:2 intake (mg/d) may be above “threshold” and already maximally effective in terms of reducing LDL production and enhancing LDL activity. Or possibly their excess adipose reserves have more than enough 18:2 to act as a “buffer” against any diet shortfall that might leave them below “threshold.”

### 4.4. Saturated Fatty Acids Are Not Equivalent

The dissimilarity between individual saturated fatty acids regarding their impact on TC was recognized early on by investigators (30,41,42) who consistently found that coconut oil and milkfat were more cholesterolemic than other fats. These two dietary fats, along with palm kernel oil, are the richest sources of lauric (12:0) and myristic (14:0) acids, the latter of which was originally identified by Hegsted et al. (42) as the most cholesterolemic fatty acid. They are also the fats lowest in 18:2, so they present the combined problem of high saturated fatty acids and low 18:2. It has been subsequently shown in monkeys (35,50), humans (51,52), and gerbils (43,53) that 12:0±14:0 (lumped together because they generally occur together, often on the same TG molecule) are, indeed, more cholesterolemic than 16:0 or 18:0. The 14:0-rich triglycerides consumed in their natural form are the only saturated fats that invariably raise TC, and they do so in a linear fashion (35,43) (Fig. 1). Although both 16:0 and 18:0 can be neutral in the absence of dietary cholesterol, practically speaking in Western diets the main sources of 16:0 and 18:0, i.e., milkfat, beef and chicken fat, and milk chocolate, also

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**Table 1**

Dietary Fatty Acid Impact on Plasma Cholesterol in Humans: Summary

<table>
<thead>
<tr>
<th>Dietary Fatty Acid Impact</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0+14:0 &gt; 16:0 ≥ 18:0</td>
<td>TC decrease with 18:2 appears nonlinear</td>
</tr>
<tr>
<td>16:0 ≥ 18:1 ≥ 18:2</td>
<td>16:0 x dietary cholesterol can interact to raise TC</td>
</tr>
<tr>
<td>TC increase with dietary cholesterol appears nonlinear</td>
<td></td>
</tr>
<tr>
<td>Dietary TG structure can influence fatty acid impact on TC</td>
<td></td>
</tr>
<tr>
<td>Trans elaidic (t18:1n9) raises LDL, depresses HDL</td>
<td></td>
</tr>
</tbody>
</table>

---
contain cholesterol, so that the neutrality of 16:0 may be lost because of its interaction with dietary cholesterol (see Subheading 5.) (Table 2). The 16:0-rich saturates may also raise TC depending on the metabolic status of the host (see Subheading 4.5.). The saturated medium-chain triglycerides (MCTs) composed of 8:0 and 10:0 are generally considered neutral or hypocholesterolemic in limited human studies (54).

When TC rises during saturated fat intake, the major effect is on LDL, although saturated fats containing 12:0, 14:0, and 16:0 are the only fats that consistently raise HDL as well. Stearic acid (18:0) tends not to influence either LDL or HDL at the levels normally consumed (2–5% en), but at atypically high 18:0 intake, HDL can be severely depressed along with a major decline in LDL (52,55) (Table 2). The peculiar neutrality associated with the consumption of normal amounts of stearic acid has been reviewed

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**Fig. 2.** The scheme depicts the perceived dynamics in humans between the relative importance of 18:2 (as percent dietary energy) and modulation of plasma LDL-C, which is based on the putative antagonism between 18:2 and primarily 14:0-rich fats. Only 18:2 is thought to exert a major positive influence on LDL, decreasing LDL production while enhancing removal via increased LDLr activity. By shifting the typical 18:2 intake from low (3% en) to moderate (7% en), LDL production decreases and hepatic clearance of LDL-C is increases (lowering LDL-C and plasma cholesterol) to counter the cholesterol-elevating influence of 14:0. Consumption of 16:0 exerts a negative effect (increases LDL-C) only if the 18:2 intake is too low (below threshold), probably because 16:0 increases VLDL production and LDL formation (see text). Other factors affecting cholesterol metabolism, such as estrogen and thyroid hormone, presumably affect the 18:2 threshold requirement in this model, accounting for individual and population differences in the threshold.

From a practical standpoint, the figure makes several points: (1) The 18:2 threshold (dietary 18:2 requirement for normal TC) can vary between individuals due to undisclosed metabolic variables including the endogenous 18:2 supply in adipose reserves. (2) Below the "dietary 18:2 threshold" certain other dietary fatty acids (fats) become progressively hypercholesterolemic. (3) Thus, the first clinical approach to a hypercholesterolemic individual is to ascertain that daily POLY intake exceeds threshold. Then begin to restrict fats rich in cholesterol and 14:0, 16:0, 18:1 and 18:0 in that order, i.e., most animal fats.

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**PUTATIVE RELATIONSHIP BETWEEN THE DIETARY 18:2 THRESHOLD AND LDL-C IN HUMANS**

![Diagram showing the relationship between dietary 18:2 and LDL-C in humans.](image)

**DIETARY ENERGY FROM 18:2 (%)**

---

From a practical standpoint, the figure makes several points: (1) The 18:2 threshold (dietary 18:2 requirement for normal TC) can vary between individuals due to undisclosed metabolic variables including the endogenous 18:2 supply in adipose reserves. (2) Below the "dietary 18:2 threshold" certain other dietary fatty acids (fats) become progressively hypercholesterolemic. (3) Thus, the first clinical approach to a hypercholesterolemic individual is to ascertain that daily POLY intake exceeds threshold. Then begin to restrict fats rich in cholesterol and 14:0, 16:0, 18:1 and 18:0 in that order, i.e., most animal fats.
4.5. Response to 16:0 Is Host Dependent

The TC response to 16:0-rich fats has been problematic because the response seems to vary with experimental conditions and investigative design. Although 16:0 was eventually included with 12:0 and 14:0 by Keys (41) and Hegsted (39), the most comprehensive experiments on the subject to date in humans were conducted by Hegsted et al. (42). These unique experiments included 36 diets fed to the same individuals under the same environmental circumstances over a span of years. Multiple regression analysis of the data generated under the experimental circumstances employed (middle-aged men with TC of 225 mg/dL on the basal diet), revealed that 14:0 was about four times more cholesterolemic than 16:0, which was not much more cholesterol-elevating than 18:0 or 18:1. The latter two fatty acids were considered neutral, neither raising nor lowering total cholesterol.

The “conditional” nature of the host response to 16:0, and to a lesser extent to 18:1, can be elicited if the circulating lipoproteins are artificially raised by dietary cholesterol ≥300 mg/d or if subject is already severely hypercholesterolemic.

Table 2
General Lipoprotein Response (Increase, Neutral, Decrease) to Major Dietary Fatty Acids in Humans (Relative to cis 18:1)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Typical % en</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0+14:0</td>
<td>1–4</td>
<td>Incr</td>
<td>Incr</td>
<td>Incr</td>
<td>Decr</td>
</tr>
<tr>
<td>16:0</td>
<td>8–12</td>
<td>Incr</td>
<td>Neutr-incr</td>
<td>Incr</td>
<td>Decr</td>
</tr>
<tr>
<td>18:0</td>
<td>2–4</td>
<td>Neutr</td>
<td>Neutr</td>
<td>Neutr</td>
<td>Neutr</td>
</tr>
<tr>
<td>TRANS (t18:1n9)</td>
<td>2–7</td>
<td>Incr</td>
<td>Incr</td>
<td>Decr</td>
<td>Incr</td>
</tr>
<tr>
<td>MUFAs (c18:1n9)</td>
<td>10–18</td>
<td>Neutr</td>
<td>Neutr</td>
<td>Neutr</td>
<td>Neutr</td>
</tr>
<tr>
<td>PUFAs (18:2,18:3)</td>
<td>3–8</td>
<td>Decr</td>
<td>Decr</td>
<td>Neutr-decr</td>
<td>Neutr</td>
</tr>
</tbody>
</table>

* 16:0 response is “conditional” and can increase LDL if present with saturated animal fat and dietary cholesterol ≥300 mg/d or if subject is already severely hypercholesterolemic.

b 18:0 can decrease LDL and HDL if intake is artificially elevated with modified dietary TG, i.e., unnatural fats.

c PUFA intake > 20% en typically leads to a decrease in HDL.

recently (56).

The ability of 16:0 to be truly neutral was initially demonstrated 25 yr ago by Vergroesen (57) in a direct comparison with 18:1. He found identical TC values (189,191 mg/dL) in normolipemic Benedictine monks fed liquid formulas containing all their fat (40% en) as either olive oil or palm oil. The shift between 16:0 and 18:1 intake represented a 15% en exchange between the two fatty acids. A key factor in this comparison was that the 18:2 content of the two oils was essentially identical (at 10% of fatty acids, which represented 4% total energy intake) and dietary cholesterol was restricted to <225 mg/dL. Our investigations in monkeys (58) and others in humans (49,59–61) elicited the same normocholesterolemic response when these two fatty acids were exchanged by manipulating fats in the diets of subjects with normal lipoprotein profiles.

The “conditional” nature of the host response to 16:0, and to a lesser extent to 18:1, can be elicited if the circulating lipoproteins are artificially raised by dietary cholesterol (62) or when plasma lipoproteins are spontaneously elevated by long-standing metabolic circumstances (46). The later situation was clearly demonstrated in the data of Mattson and Grundy (45) who essentially fed the Vergroesen liquid formulas of 20
yr earlier (57) to elderly men experiencing moderately severe cholesterolemia (average entry cholesterol of 263 mg/dL). In this situation, 16:0-rich palm oil lowered the average TC from entry (to 224 mg/dL), but the 16:0-rich fat was decidedly more cholesterolemic than either the 18:1-rich safflower oil (197 mg/dL) or 18:2-rich safflower oil (191 mg/dL). However, as detailed previously (58,63), the sensitivity to 16:0 was highly individualized among the 20 subjects tested. Men with the highest cholesterol values (>240 mg/dL when fed palm oil) were much more responsive to 16:0 than the several men with normal cholesterol values (<200 mg/dL) under the same challenge. In the latter subgroup, the response to 16:0, 18:1, and 18:2 was not significantly different, even though 16:0 tended to elicit the highest values and 18:2 the lowest. Essentially, the same pattern of supersensitivity to both 16:0 and 18:1 relative to 18:2 was described among subgroups of Benedictine nuns having initially distinct lipoprotein profiles (46).

The exact determinants of the 16:0-sensitivity are not understood, but it appears to reflect the absolute TC value (LDL-C and presumably LDL production and LDL_s activity as modulated by the 18:2 threshold) (Fig. 2). However, this is not the sole predictor. Responsiveness to 16:0 is probably affected by BMI, age, sex, insulin sensitivity, thyroid hormone, bile acid synthesis rate, and so on, i.e., any number of factors influencing lipoprotein metabolism and sensitive to fatty acid modulation. The easiest way to increase sensitivity to 16:0 is to impair lipoprotein metabolism by feeding excess dietary cholesterol (63), as detailed elsewhere in cebus monkeys (62) and gerbils (43). Dietschy and colleagues (24) have also applied this technique of cholesterol-feeding in an attempt to amplify and distinguish the response of the LDL receptor to various dietary fatty acids in their hamster model. When this approach is used, a constant load of 16:0-rich fat (more than other fats) becomes progressively cholesterolemic as LDL production increases and LDL receptors are downregulated by incremental increases in dietary cholesterol (43,63). In a previous report (35), the implications of this sensitivity to 16:0 was considered relative to the dietary fat intake and plasma lipids of the world population. Obviously, without knowing all the variables that dictate responsiveness to 16:0, we can only estimate at what point a “stressed” lipoprotein metabolism expresses the 16:0 sensitivity in humans. Perusal of data in the world literature suggests that the initial cholesterolemic effect of 16:0 is associated with a TC of 200–225 mg/dL for most populations (35), but most likely the response is progressive and related to the “18:2 threshold” as well as the production and clearance rates of LDL. More work is needed to refine these relationships and the implications for cardiovascular health vis a vis fatty acid intake in humans.

4.6. Trans Fatty Acids May Be Worse than Saturates

Trans fatty acids are mentioned by way of comparison with saturates, primarily because their structure, as well as their metabolic impact on cholesterol metabolism, is often thought to mimic the dietary saturated fatty acids that they were designed to replace. In fact, growing evidence reveals that consumption of trans fatty acids may be more detrimental than saturated fatty acids because of their negative impact on the LDL/HDL ratio, Lp(a) concentration, and LCAT activity (64–66) (see Table 2). Consumption of trans 18:1, especially the elaidic acid isomer (t18:1n9) in the range of 2–7% en, has been demonstrated to substantially elevate the LDL/HDL ratio because it often depresses HDL-C while raising LDL-C. By contrast, saturated fatty acids that
raise LDL-C also raise HDL-C. In addition, most \textit{trans} fat experiments with humans have described a 20–25\% increase in Lp(a), the unique lipoprotein in the LDL fraction that has been identified as an independent risk for CHD, especially in Type II diabetes where elevated triglycerides are typical (67). On that point, it is noteworthy that \textit{trans} markedly increased triglycerides in the fat-sensitive mature gerbil (53). The ability of \textit{trans} to inhibit plasma LCAT activity is significant because LCAT represents an HDL-associated enzyme with antiatherosclerotic potential, effectively deterring the oxidation of LDL particles (68).

Epidemiological studies indicate that the upper range of \textit{trans} fatty acid intake is associated with a substantial increase (as much as 90\%) in risk for CHD (69,70). The Nurses Health Study followed 80,000 subjects over 14 yr and found that each 1\% en from \textit{trans} fatty acids increased CHD risk 47\%, compared to only 3.4\% increase for a comparable intake of saturated fat, whereas the same amount of monounsaturated fat reduced risk with about half the potency (−4\%) of polyunsaturates (70). In addition, the Norwegian cohort of the nine-country EURAMIC study described a fivefold increase in CHD risk from the lowest to highest quartiles of \textit{trans} intake, predominately derived from partially hydrogenated fish oil (PHFO) margarine (71). A separate clinical study in Norwegian men (72) found that 7\% en from PHFO elevated the LDL/HDL ratio even more than butter, which had a slightly more negative effect than partially hydrogenated soybean oil (PHSBO). In a similar vein, a Malaysian study fed PHSBO and exchanged 5\% en from \textit{trans} 18:1n9 (elaidic acid) for \textit{cis} 18:1n9, or 16:0, or 12:0+14:0 by manipulating various fat blends and found that elaidic acid increased the LDL/HDL ratio relative to all other fatty acids, even including the 12:0 + 14:0-rich fat blend based on coconut oil (73).

5. FATTY ACID AND CHOLESTEROL INTERACTION

The interactive synergy between saturated fat (arguably the 16:0-rich portion) and dietary cholesterol in humans was described a decade ago by Schonfeld et al. (74) and again recently by Fielding et al. (75). Similar to the first study the latter investigators studied four groups of men fed either a modestly polyunsaturated (P/S ratio 0.8) or saturated (P/S ratio 0.3) fat diet with low (200 mg/d) or high (600 mg/d) intakes of dietary cholesterol. Two results were noteworthy. First, at low cholesterol intake (when lipoprotein metabolism would be least compromised) the rise in LDL-C from basal values for all subjects in the presence of saturates was minimal (+3 mg/dL) compared to the polyunsaturates (−2 mg/dL), but at the high cholesterol intake, the “saturated fat effect” was striking and exaggerated (+25 mg/dL) compared to the polyunsaturates (+16 mg/dL) or compared to the low-cholesterol, saturated-fat diet intake (+3 mg/dL). The point is that the saturated-fat effect (the major challenge was from relatively neutral 16:0 and 18:0) was only elicited after lipoprotein metabolism was “stressed” by dietary cholesterol. Second, the response to dietary fats was surprisingly affected by ethnic background such that Caucasians were significantly more responsive to both saturated fat and cholesterol challenges than non-Caucasians. For example, the LDL-C increase during the high-cholesterol, saturated-fat diet was +31 vs +16 mg/dL for the two ethnic groups, respectively. These combined data indicate that the lipoprotein status of the host (affected both by previous and concurrent dietary or inherent genetic factors) dictates the potential impact of the dietary saturated-fat component.

The striking synergy between 16:0 × dietary cholesterol was further elaborated in gerbils by feeding graded intakes of cholesterol in diets rich in 12:0+14:0, 16:0, 18:1,
or 18:2 (43,63). Only the 16:0-rich diet elicited a progressive interaction, presumably because 16:0 causes the greatest production of VLDL, which can divert to LDL (or HDL and HDL₂ in gerbils) as the LDL receptors are depressed by cholesterol (76).

6. LP(A) RESPONSE TO FAT CONSUMPTION

An interesting development in the fat-CHD story is the recent report that diets rich in 12:0/H₁₁₀₀1 14:0 and 16:0, but not 18:0, cause a reduction in the baseline Lp(a) level (77). Only individuals with the highest Lp(a) values were responsive, but these are the persons who appear to be at greatest risk for CHD. Lp(a) is a unique apoprotein found in the LDL fraction of certain individuals which is thought to inhibit fibrinolysis in vivo by virtue of its homology to, and thus competition with, plasminogen. The only other dietary situation known to modulate (in this case, increase) Lp(a) is consumption of trans fatty acids, particularly trans 18:1n9, elaidic acid, formed during partial hydrogenation of vegetable oils or fish oils (64,65,73). An underlying theme seems to be that fats (fatty acids) that elevate HDL also depress Lp(a) levels. No relationship has been found between LDL-C and Lp(a), but the correlation between HDL-C and Lp(a), presumably inverse, appears not to have been examined within the data set of a study designed to manipulate lipoproteins by diet. This could be quite revealing especially because HDL-C, in contrast to Lp(a), is protective against platelet aggregation and CHD.

7. RECOMMENDATIONS

The evidence concerning diet and chronic disease implies that body weight gain after age 21 should be avoided when possible, especially based on the association between BMI, increased serum lipids, and CHD. However, practical reality indicates that this is a difficult assignment in an affluent society where activity levels are reduced and sugar and fat are readily available for consumption.

Having said that, it is also apparent that only approx 50% of the population is genetically prone to experience accelerated CHD risk given the above circumstances of dietary and other environmental insults. Accordingly, one can suggest two approaches, i.e., a general public health recommendation that fits all types or a more specific recommendation for the high-risk subpopulation. In the first instance, the recommendation would be to avoid the weight gain contributed by excess caloric intake, including dietary fat in general, with special consideration to the dietary fat and cholesterol interaction outlined in Tables 2 and 3. The more susceptible subpopulation should definitely reduce saturated fat to <10% energy and cholesterol <200 mg/d. In general, the bulk of dietary energy for this subpopulation should derive from cereal grains and vegetables with a generous supply of fruit as snack-foods or "treats." Emphasis on a "low-fat diet," i.e., less than 20% en from fat, appears not to be in the best interest of the general population because the potentially adverse effects on plasma triglycerides, HDL, and LDL size. Balance in fatty acid intake is important, specifically between saturates and polyunsaturated fatty acids, and with the latter, between n6/n3 polyunsaturated fatty acids. In addition, exercise regularly and consume above average amounts of antioxidants, such as vitamin E and vitamin C, to protect vulnerable lipoprotein lipids (and apolipoproteins) from peroxidation. Also avoid trans fatty acids, and consume visible fat only in the form of salad dressing oils. No visible spreads or cheeses should be con-
Table 3
Dietary Recommendations for Cardiovascular Fitness

- Golden rule: maintain body weight (energy balance) at young adult level with BMI of 20–22 kg/m², i.e., “eat less, exercise more.”
- Adults keep fat intake <30% of total energy (<33 g/1000 kcal) and saturated fatty acids SFA's <10% en. Avoid trans fatty acid-rich foods and fats (partially hydrogenated vegetable oils).
- Derive the major portion of energy from complex carbohydrates in cereal grains, vegetables, and fruits.
- Keep dietary cholesterol <200–300 mg/d or even lower depending on total cholesterol and LDL-cholesterol concentrations.
- Restrict visible fat consumption, especially animal fat sources of saturates and cholesterol (cheese, ice cream, whole milk, fatty meats).
- Assure daily intake of 5% en as 18:2 and 1% en as 18:3 (5 g and 1 g/1000 kcal, respectively). Best accomplished with soybean oil- or canola oil-based salad dressing (1 tsp/1000 kcal/d).
- Eat fish at least once per week.
- Consume a daily supplement of vitamin E (100 mg) and vitamin C (100 mg).

suomed in the second scenario, and milk should be skim or 1% fat only.

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Iron and Heart Disease
A Review of the Epidemiologic Data

Christopher T. Sempos, Richard F. Gillum, and Anne C. Looker

1. INTRODUCTION

In 1981, Dr. Jerome Sullivan (1) proposed a new theory to explain the differences in coronary heart disease (CHD) incidence and mortality between men and women. He noticed that as men and women age, the gaps between them in heart disease incidence and in body iron stores both decrease (2,3). Lower stores of iron levels in women are caused mostly by menstrual blood loss, and with menopause the differences in iron stores decrease. As a result, he theorized that body iron stores are directly or positively related to CHD risk, i.e., the higher your body iron stores the greater your CHD risk. Until the publication of results from Finland by Salonen et al. (4), which showed that men with serum ferritin levels (a measure of body iron stores), at or above 200 μg/L had double the risk of having a heart attack, the hypothesis was largely ignored. Since then, however, there has been an intense interest in this topic. It is thought that iron might promote the atherosclerosis leading to CHD by catalyzing the oxidation of low density lipoprotein (LDL) cholesterol (5–10).

The purpose of this chapter is to examine and update the recent epidemiologic data that are directly related to Dr. Sullivan’s hypothesis (11,12). Important related topics, e.g., the relationship of antioxidant nutrients, oral contraceptives, estrogen replacement therapy, and menopause to CHD risk, and iron and myocardial reperfusion injury are not discussed.

2. SERUM MEASURES OF BODY IRON STORES

Serum ferritin is currently the best measure of body iron stores that is feasible to use in epidemiologic studies (13). It is a fairly sensitive indicator of changes in body iron stores as you move along the stages of iron status from deficient to replete to iron overload in healthy individuals, e.g., not suffering from an infection, inflammation, or cancer. Less direct and sensitive measures of body iron stores are serum iron, total iron binding capacity (TIBC), and transferrin saturation (TS), which is calculated as the ratio of serum iron to total iron binding capacity. TS is the best measure of circulating iron available to tissues and is considered to be a better measure of stores...
than serum iron or TIBC. Other common iron status measures are even less directly related to body iron stores, e.g., hemoglobin, hematocrit, and erythrocyte protoporphyrin. Hemoglobin and hematocrit are measures of the oxygen-carrying capacity of blood and viscosity.

As body iron stores increase, so do serum ferritin levels \((14,15)\). As a result, serum ferritin can be useful in detecting iron deficiency and overload. A serum ferritin level of \(<15 \mu g/L\) has been used as an indicator of iron deficiency in both men and women \((16)\). Separate upper limits have been suggested for adult men \((400 \mu g/L)\), menstruating women \((200 \mu g/L)\), and postmenopausal women \((300 \mu g/L)\) \((16)\).

TS and serum iron levels also tend to increase as stores increase over the normal range, whereas TIBC levels tend to decrease as stores increase. The opposite trends occur as body iron stores decrease. At very high levels of body iron stores, as in homozygous hemochromatosis \((TS > 60\%)\), or at depleted levels \((TS < 16\%)\), i.e., iron deficiency, TS is considered to be a good measure of body iron stores. Within the normal range of TS, i.e., 20–60\%, it is not clear how well TS reflects body iron stores. Data from the second national Health and Nutrition Examination Survey (see Table 1) show that there does appear to be a positive correlation, albeit low, between the two measurements with the result that with increasing level of TS mean levels of serum ferritin tend to rise as well, especially in women. The correlation between log transformed serum ferritin and TS for men and women 45–74 yr of age was \(r = 0.22\) overall, \(r = 0.14\) for men, and \(r = 0.25\) for women.

Serum iron status measures are also affected by inflammation, cancer, and infection. Serum ferritin levels tend to increase in response to inflammation \((14–17)\), although TS, TIBC, and serum iron levels decrease \((18)\). For example, in response to a heart attack, ferritin levels are initially raised, whereas TS, TIBC, and serum iron levels decrease \((19,20)\). In the study by van der Schouw et al. \((21)\), serum ferritin levels returned to control levels 6 wk after the heart attack, although TS and serum iron levels continued to be depressed.

### Table 1

<table>
<thead>
<tr>
<th>Transferrin saturation (%)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferritin (μg/L)</td>
<td>Ferritin (μg/L)</td>
</tr>
<tr>
<td>&lt;16%</td>
<td>74</td>
<td>38</td>
</tr>
<tr>
<td>16–19%</td>
<td>94</td>
<td>54</td>
</tr>
<tr>
<td>20–29%</td>
<td>94</td>
<td>62</td>
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<td>30–44%</td>
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<td>70</td>
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<td>45–59%</td>
<td>105</td>
<td>89</td>
</tr>
<tr>
<td>≥60%</td>
<td>231</td>
<td>285</td>
</tr>
</tbody>
</table>

\(^a\) Within each transferrin saturation group, the serum ferritin data was transformed using natural logarithms. The antilog of the mean of the log transformed distribution is the geometric mean value shown in the table.

\(^b\) U.S. men and women ages 45–74 yr of age.

\(^c\) Calculated as the ratio of serum iron (μmol/L) divided by total iron binding capacity (μmol/L).

3. BODY IRON STORES AND RISK OF HEART DISEASE:  
THE EPIDEMIOLOGIC DATA

3.1. Cohort Studies Based on Serum Ferritin

Cohort studies are referred to also as prospective, incidence, follow-up, and longitudinal studies. The question being asked in cohort studies is: Do persons with the risk factor develop or die from the disease more frequently or sooner than those who do not have the risk factor? For example, are persons who have high serum ferritin levels more likely to develop CHD in the future than are persons who do not? Because risk-factor exposure is measured prior to the onset of clinically apparent disease, cohort studies are considered to be the strongest observational study design in epidemiology (22,23). Using various measures of body iron stores, a number of researchers have attempted to assess the hypothesis that CHD risk increases with body iron stores (see Table 2).

As stated earlier, the theory was largely ignored until the publication of results from the Finnish Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) (4). The study consisted of 1931 randomly selected men who were 42, 48, 54, or 60 yr of age and who were free of clinical symptoms of CHD at baseline. During an average of 3 yr of follow-up, 46 men had either a definite or possible heart attack as defined by ECG or enzyme criteria. Five additional men who were admitted to the hospital for prolonged chest pain did not meet the criteria. The authors reported that the results were not changed substantively by including those men. Also, the published results were based on 51 heart attacks.

Salonen et al. reported finding a statistically significant linear association between serum ferritin level and the risk of heart attack ($z = 2.64, p < 0.01$) after adjusting for possible confounding. Thus, as serum ferritin levels increased, so did the risk of heart attack. The more surprising finding was, however, that men with a serum ferritin $\geq 200$ g/L had a greater than twofold higher risk of heart attack compared to those with lower serum ferritin values. The difference was statistically significant (relative risk [RR] = 2.2, 95% CI 1.2–4.0, $p < 0.01$). Again, the results were adjusted for possible confounding in a multivariate model. Additionally, they reported finding that compared to men with serum ferritin levels $<200$ g/L, men with a ferritin of 200–399 g/L had a nearly identical risk of heart attack as did men with ferritin levels $\geq 400$ g/L (4).

The study by Salonen et al. (4), although based on a small number of heart attacks, was a well-conducted study. (In a letter to the editor, they presented data to indicate that the relationship was still significant after an average of 5 yr of follow-up and 83 heart attacks—RR = 2.0, 95% CI = 1.2–3.1, $p = 0.004$ (24).) They have been criticized, however, for not having adequately adjusted for inflammation (25) and because there was a negative correlation between age and serum ferritin (26). But neither criticism appears to be entirely justified (27,28). The authors reported that they found no correlation between serum ferritin and plasma fibrinogen (an acute phase protein) in the whole sample or with C-reactive protein in a subsample (4,27,28). Moreover, the authors adjusted for blood leukocyte count in their analyses (4). More importantly, the association between serum ferritin and heart attack was not attenuated when the analysis was repeated after removing heart attacks that occurred within the first 6 mo following blood collection. As was stated earlier, serum ferritin levels go up after a heart attack, but the return to baseline levels within 6 wk after the heart attack (21).
In a subsample of their cohort, the Salonen research group also reported that the ratio of transferrin to ferritin was positively related to CHD risk (29). This is not surprising given the strong finding in the larger cohort. Moreover, because the three studies by their group were based on the same set of individuals from the KIHD cohort, we have considered them as one study supporting the Sullivan hypothesis (see Table 2).

The results from six other cohort studies (31–36) on the association between serum ferritin and CHD have been reported (see Table 2). Only one of them found an association between serum ferritin and CHD (35). In that study, Kiechl et al. (35) reported that the 5-yr progression of carotid stenosis was significantly related with serum ferritin levels. The study consisted of 826 men and women ages 40–79 yr of age who were randomly selected from the population Bruneck, Italy. Carotid atherosclerosis was assessed by repeated carotid ultrasound evaluation. The authors further reported that changes in iron stores were associated with changes in the progression of carotid atherosclerosis in that lowering of stores was associated with a decreased risk of progression, whereas increases in stores were associated with an increased risk. We can find no reports where these interesting results have been replicated.

Of the studies that found no association between serum ferritin and CHD, three were cohort studies (31–33) in the usual sense and two appear to be based on case-series (34,36). The studies by Magnusson et al. (31), Stampfer et al. (32), and Mänttäri et al. (33) all used slightly different procedures to analyze their results and address different aspects of the iron hypothesis.

### Table 2

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Sex</th>
<th>Sample size</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuopio Ischemic Heart Disease Risk Factor Study (KIHD)&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salonen (4)</td>
<td>M</td>
<td>1931</td>
<td>+</td>
</tr>
<tr>
<td>Salonen (24)</td>
<td>M</td>
<td>1931</td>
<td>+</td>
</tr>
<tr>
<td>Tuomainen (29)</td>
<td>M</td>
<td>197b</td>
<td>+</td>
</tr>
<tr>
<td>Magnusson (31)</td>
<td>M</td>
<td>990</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>1046</td>
<td>None</td>
</tr>
<tr>
<td>Stampfer (32)</td>
<td>M</td>
<td>476b</td>
<td>None</td>
</tr>
<tr>
<td>Mänttäri (33)</td>
<td>M</td>
<td>268b</td>
<td>None</td>
</tr>
<tr>
<td>Frey (34)</td>
<td>M</td>
<td>298c</td>
<td>None</td>
</tr>
<tr>
<td>Kiechl (35)</td>
<td>M/W</td>
<td>826</td>
<td>+</td>
</tr>
<tr>
<td>Aronow (36)</td>
<td>M/W</td>
<td>577</td>
<td>None</td>
</tr>
</tbody>
</table>

"All three studies are from the same cohort—Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). The difference between the two studies by Salonen et al. is that the first consisted of the results after of 3-yr follow-up and the second after a 5-yr follow-up of the same individuals. The third study is on a subset of the cohort in the first two studies looking at the transferrin/ferritin ratio and risk of heart attack.

b Nested case-control study, i.e., a case-control or retrospective study that is “nested” within a cohort or cohort study (30). Serum transferrin/ferritin ratio [Tuomainen (29)], or ferritin [Stampfer (32), Mänttäri (33)] were determined on frozen sera collected at the beginning of the study. Cases accrued during the follow-up period and controls were selected from the pool of individuals who were at risk of having CHD at the time a case was diagnosed. Because of the efficient sample design, only small sample sizes are required (30).
In the study by Magnusson et al. (31), 2036 Icelandic men and women ages 25–74 yr were followed for an average of 8.5 yr. During that time, 81 participants (63 men and 18 women) had a heart attack. In their multivariate models, Magnusson et al. included both serum ferritin, in the normal units or log transformed, and serum Total Iron Binding Capacity or TIBC as continuous variables to test if a statistically significant linear association exists between serum ferritin and risk of heart attack. Neither serum ferritin (RR = 0.999, 95% CI 0.997–1.001) or log ferritin (RR = 0.781, 95% CI 0.540–1.129) were significantly associated with risk of heart attack. In contrast with Salonen’s results, those results as indicating that body iron stores as measured by serum ferritin were not associated with the risk of having a heart attack.

The interesting finding was that TIBC was shown to have a significant negative association with risk of heart attack (RR = 0.95, 95% CI 0.92–0.98). That is, those with higher TIBC levels had a lower risk of heart attack. Their interpretation of the results for TIBC were very interesting. As we have said, TIBC levels are inversely related to body iron stores, but compared to ferritin, it is a relatively weak index of them. However, TIBC is also known to have antioxidant properties (37). Given the recent results concerning the oxidative modification of LDL cholesterol and the reported protective effects of antioxidant nutrients, Magnusson et al. postulated that “if iron increases the risk of coronary artery disease by oxidizing LDL, as has been postulated, a critical step in its pathogenic pathway would be the accumulation of free iron in the subendothelial space. The serum iron binding capacity might be a more reliable indicator of this accumulation of free iron in the vessel wall than the total iron stores” (31). Additionally, they felt that their results “support the concept that iron being an important transition metal might contribute to atherogenesis along with other classic risk factors, although arguing against the recent hypothesis that iron stores per se increase risk” (31).

Stampfer et al. (32) directly addressed the issue of a threshold at 200 μg/L. Using a nested case-control design (30), 238 men participating in the United States. Physicians Study had a heart attack during the period after the 1982 baseline. Stored serum for those men and for 238 controls matched for age and smoking status were analyzed for serum ferritin concentrations. And after adjustment for other CHD risk factors, men with serum ferritin levels ≥200 μg/L were not found to have a higher risk of heart attack (RR = 1.1, 95% CI 0.7–1.6).

Similar results were found also in another nested case-control study by Mänttäri et al. (33). The participants in this study were a subset of men from the Finnish Helsinki Heart Study—a randomized clinical trial of the lipid-lowering drug gemfibrozil. In that study, the authors looked to see if the threshold for ferritin occurs at much lower levels than the 200 μg/L cutoff used by Salonen et al. (4). Mänttäri et al. looked at the risk of developing CHD in two groups of men with serum ferritin levels of 43–84 μg/L or ≥85 μg/L compared with men with serum ferritin levels of ≤42 μg/L and found that it was not different.

In case-series studies, patients who receive a particular test or procedure are followed over time using cohort study methods in order to evaluate the association between the test result and an outcome. The information from such a study may be very useful in suggesting hypotheses or testing a current one. But because the test or procedure was ordered for a particular medical reason, which may be favorably or unfavorably related to the outcome of interest, the results from those studies may be biased and thus cannot be considered as rigorous as the data from a standard cohort study (38). The study by
Frey and Krider (34) was based on clinical case series of 298 men with serum ferritin measurements taken at some point over a 10-yr period (mean 5.2 yr) in a West Virginia medical practice. Over that follow-up period, 32 men had a heart attack. The authors reported finding no difference in mean serum ferritin levels between patients who had or did not have a heart attack. Nor was there any association between risk of heart attack and having a serum ferritin level above 200 μg/L. Unfortunately, none of the results appeared to have been adjusted for age or for other CHD risk factors. Finally, in a small study (it is not clear if it is a defined cohort or a case series) of 577 men and women ages 62 yr and older, no association was found between serum ferritin and incident CHD (56) based on 3 yr of follow-up (36).

3.2. Cohort Studies Based on TS

The association between TS (39–43), serum iron (40,42,44,45), or TIBC (31,40,42,43) and CHD risk has been investigated in seven different cohorts (see Table 3). Only the study by Morrison et al. (44) reported finding a significant positive association. In that study, 9920 men and women 35–79 yr of age were followed for approx 16 yr. During that time, 141 men and 83 women died of an acute myocardial infarction (MI) or heart attack. Persons with a serum iron level ≥175 μg/L compared to those with a value <120 μg/L were found to have a significantly higher risk of dying of an acute MI (men: RR = 2.18, 95% CI 1.01–4.74; women: RR = 5.53, 95% CI 1.69–18.12). However, there were only seven deaths among the men and three among the women for those with a serum iron in the highest category. Although the results are consistent with those from Finland, the small numbers of events in the highest category tend to make their results less certain. In contrast, Coti et al. (45) reported finding a significant inverse association between serum iron and CHD and cardiovascular death (CVD), i.e., the higher a persons serum iron level, the lower their risk of death from CHD or CVD.

The aforementioned papers that found no association between iron status and CHD have been criticized for using TS as a measure of body iron stores (24,46,47) and for using the lowest levels of TS as the comparison group. However, although criticizing the heart disease studies that have not found an association, the proponents of the iron hypothesis have cited studies finding an association between TS and risk of cancer (47,48) or serum iron and risk of CHD (48) as evidence that high body iron stores generally increase the risk of both heart disease and cancer. Clearly, if TS is an indicator of body iron stores in one setting, it must also be in the other.

Because persons with very low TS levels (<16%), as well as those with very high levels (>60%) are probably sick individuals, we agree that persons with the lowest TS levels may not be the most appropriate comparison group. To explore the effects of using the lowest TS levels as the comparison group, we have reanalyzed the relationship between TS and risk of CHD (11) using methods previously described (11,39) and where TS was been divided into six categories (<16%, 16–19%, 20–29%, 30–44%, 45–59%, and ≥60%). For those new analyses, the TS category of 20–29% was used as the reference category. A TS <16% is used to indicate iron deficiency, and a TS between 16–29% is at the low end of the normal range. The relative risk of CHD for the TS <16%, 16–19%, 30–44%, 45–59%, and the ≥60% categories was 1.09, 1.09, 1.01, 0.75, and 0.76, respectively, for men and 1.28, 1.18, 0.84, 0.97, and 0.86, respectively, for women. None of the estimates were significantly different (p > 0.05) than the risk of CHD in the 20–29% TS category. Persons with TS levels between
16–19% appeared to be at equal or higher risk of CHD, whereas persons between 30% and 60% appeared to be possibly at lower risk of CHD than those with a TS of 20–29%. As a result, changing the comparison group for TS does not appear to change the conclusion that those data do not support the hypothesis that body iron stores are positively related to CHD risk.

There have also been a few cohort studies that used TS to look at the association between iron status and risk of stroke and all causes of mortality. A U-shaped association between TS and stroke was reported for white women (49) where those with a TS < 29% and a TS > 44% had a significantly higher risk of stroke, compared to those with a TS of 30–36%. However, no association was found for white or black men. Additionally, no association has been found between TS and all causes of mortality (11,45,50).

### Table 3
Serum TS and Heart Disease: Cohort Studies

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Sex</th>
<th>Sample size</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES I Follow-up Study&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sempo (39)</td>
<td>M</td>
<td>1345</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>1750</td>
<td>None</td>
</tr>
<tr>
<td>Liao (40)</td>
<td>M</td>
<td>1827</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>2410</td>
<td>None</td>
</tr>
<tr>
<td>Baer (41)</td>
<td>M</td>
<td>15,167</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>31,765</td>
<td>None</td>
</tr>
<tr>
<td>Reunanen (42)</td>
<td>M</td>
<td>6068</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>6102</td>
<td>None</td>
</tr>
<tr>
<td>van Asperen (43)</td>
<td>M</td>
<td>129</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>131</td>
<td>None</td>
</tr>
<tr>
<td>Morrison (44)</td>
<td>M</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>(serum iron)</td>
<td>W</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Magnusson (31)</td>
<td>M</td>
<td>990</td>
<td>−</td>
</tr>
<tr>
<td>(TIBC)</td>
<td>W</td>
<td>1046</td>
<td>None</td>
</tr>
<tr>
<td>Corti (45)</td>
<td>M</td>
<td>1385</td>
<td>−</td>
</tr>
<tr>
<td>(serum iron)</td>
<td>W</td>
<td>2551</td>
<td>−</td>
</tr>
</tbody>
</table>

<sup>a</sup>The studies by Sempo et al. and Liao et al. are from the same cohort—The NHANES I Epidemiologic Follow-up study. Because cases within the first 3 yr of follow-up were deleted in the paper by Sempo et al., the sample size in their study is smaller than the sample size reported by Liao et al.

There have also been a few cohort studies that used TS to look at the association between iron status and risk of stroke and all causes of mortality. A U-shaped association between TS and stroke was reported for white women (49) where those with a TS < 29% and a TS ≥ 44% had a significantly higher risk of stroke, compared to those with a TS of 30–36%. However, no association was found for white or black men. Additionally, no association has been found between TS and all causes of mortality (11,45,50).

### 3.3. Case-Control or Cross-Sectional Studies

There have been a number of studies (51–60) that have used a case-control or cross-sectional study design (see Table 4). All of the studies used serum ferritin as the measure of body iron stores. Only one of the studies (60) reported finding a significant positive association between serum ferritin and CHD and then in only the youngest of two age groups examined, i.e., ages 40–59 yr.

Case-control and cross-sectional studies are not generally considered to be as rigorous an observational study design as cohort studies (22,23,38). The problem with both types of studies is that both disease status, e.g., heart disease, and risk factor exposure, e.g., serum ferritin, are measured at the same time so that it is impossible to tell if
risk-factor exposure preceded the disease or was a consequence of it. For example, the
disease itself may alter body iron stores or serum ferritin levels. Additionally, people
who already know they are sick may change their behavior so as to alter their levels of
body iron stores. As a result, it is difficult to establish if the exposure lead to the disease
or vice versa with those experimental designs.

Because serum ferritin levels rise in response to inflammation, infection, cancer, and
heart attack, clinically apparent CHD should increase serum ferritin levels so that more
often than not a false-positive association would be found between ferritin and CHD. The
fact that only one of the studies in this category found no association is then a somewhat
stronger argument against the hypothesis. On the other hand, including persons with
CHD in these studies who have changed their diets to lower their serum cholesterol lev-
els would tend to produce false-negative results because a reduction in the intake of meat
and animal products may also, over time, reduce their levels of body iron stores and, as
a result, their serum ferritin levels. One way to evaluate this possibility is to assess the
association between serum total and LDL cholesterol and CHD concurrently with the
serum ferritin and CHD assessments. In six of the nine studies that found no association
between serum ferritin and CHD (51–53,56,58,59) and in the one study that found a pos-
itive association (60), LDL cholesterol was positively associated with having CHD sug-
gest ing that it is less likely that changes in behavior have produced false-negative results.

### 3.4. Dietary Iron and CHD Risk

Salonen et al. (4) also reported that dietary iron intake was positively associated with
the risk of having a heart attack. Other researchers have not been able to corroborate
this finding (11,40,42,44,53,59,61–63) and to date only one other study (62) has found
a positive association between dietary iron intake and CHD risk.
Two studies have reported finding an association between heme iron intake and risk of heart attack, but not with total iron intake \cite{61,63} whereas one-third found no association \cite{42}. In any event, it is not clear what a relationship between dietary heme iron and CHD would mean in the context of a discussion of the association between body iron stores and CHD risk. Dietary intake methods do not adequately capture long-term dietary patterns, nor do they reflect the influence of growth and development or the effect of menopausal status on body iron stores, all of which are important factors in determining body iron stores. Additionally, an association between dietary iron and CHD risk may be a marker for a high-fat high-cholesterol diet or something totally unrelated to body iron stores. For example, in the study by Klipstein-Grobusch et al. \cite{63}, heme iron intake was positively correlated with hypercholesterolemia, a clear marker for a diet high in saturated fatty acids and cholesterol, as well as with hypertension, current cigarette smoking, and diabetes.

To help reduce the potential for confounding, epidemiologists use multivariate models to simulate an experiment \cite{30}, as did the authors of the papers on heme iron intake and CHD risk. By using multivariate models that adjust for possible confounding factors, it is possible to evaluate the association between the exposure of interest and the outcome variable while holding all other variables in the model constant \cite{30}. But there are limits to modeling. The sad fact is that confounding caused by correlation in intakes or metabolism simply cannot be reduced or eliminated by statistical modeling \cite{64–66}.

### 3.5. Blood Donor Studies and Iron and Oxidized LDL Cholesterol

Blood donation has been hypothesized as a way to decrease body iron stores to reduce the risk of heart disease \cite{67,68}. Clearly frequent blood donation will reduce body iron stores and serum ferritin levels \cite{69}. But although there has been no direct evidence that blood donation will lead to a reduction in CHD, two approaches have been used to address this issue.

An indirect way to test the hypothesis is to look at the risk of CHD in voluntary blood donors and nondonors in existing epidemiologic studies. There have been two such studies \cite{70–72} [Salonen et al. \cite{72} expanded the version of an earlier study by Tuomainen et al. \cite{71}, it is not a separate study], as well as a study looking at the effects of blood donation on cancer incidence \cite{73}. The results were mixed. Meyers et al. \cite{70} reported that blood donation was associated with a reduced risk of CHD in non-smoking men, but not in male smokers or in women, although Tuomainen et al. \cite{71,72} reported a significant reduction in risk of heart attack in the cohort of Finnish men from the KIHD Study that originally reported an association between serum ferritin and risk of heart attack.

There are several concerns about these interesting studies. The principal one is that volunteers for blood donation are healthier than nondonors and that any association may be a result of some unmeasured selection bias \cite{74,75}. The data from both studies do clearly indicate that the volunteers were healthier. For example, in contrast to the usual practice in cohort studies, Salonen et al. \cite{71,72} did not eliminate persons with preexisting clinical CHD at baseline from their analyses as they had done in their previous papers \cite{4}. In their study, over a quarter of the 2529 nondonors (26.3%) had preexisting disease compared to 8.5% in the 153 voluntary donors. As a result, it is impossible to determine if voluntary blood donation was influenced, the presence of heart disease and as stated earlier that bias cannot be reduced or eliminated by statistical analysis \cite{64–66}.
In what might be a more direct test of the hypothesis, Salonen et al. (48) used a Latin Squares design to look at the effects of donating 500 mL of blood three times over a 14-wk period in 14 men who were heavy smokers on measures of non-HDL (very-low density lipoprotein [VLDL] plus LDL) cholesterol oxidizability. The authors reported that serum ferritin levels were reduced by 44%, whereas the maximal oxidation velocity was decreased by 20% and the lag time to start oxidation was lengthened by 33%.

Although interesting, the results of Salonen et al., even if replicated, are uncertain (76). Currently, the LDL oxidation theory is an interesting and attractive, but yet unproved, hypothesis (9). Another problem lies in the measurement of LDL oxidation itself (5). LDL oxidizability is usually measured by using LDL and exposing it to oxidative stress. The question is whether such a marker of LDL oxidizability corresponds to the extent of oxidation in vivo and whether it predicts risk of CHD (77). The results of the few epidemiological studies that have looked at the association between LDL oxidizability and markers of atherosclerosis or CHD risk are mixed (78–80). There are also mixed results concerning the association between autoantibodies against oxidized LDL and atherosclerosis (81). “Thus the available markers for oxidized LDL cannot yet be regarded as valid predictors of CAD (coronary artery disease) risk in humans (77).”

Putting aside the possible problems with the measurement of oxidized LDL, several observational studies have looked at the association between serum ferritin and LDL oxidizability (80,82,83). No association was found in any of the studies. In fact, Craig et al. reported that serum ferritin account for about 1.6% of the variability in measures of LDL oxidizability, whereas serum copper accounted for 21% of that variability (83). Serum copper also is considered as a possible catalyst for the oxidation of LDL (12,84).

In another experiment reported only in abstract form, Derstine et al. (85) tested the oxidizability of the LDL cholesterol in the plasma samples of 77 men and women ages 20–65 yr who were participating in one of three feeding studies. No association was found between serum ferritin and the measures of LDL oxidizability.

5. IRON OVERLOAD AND CHD RISK

If excess body iron stores are related to an increased risk of CHD then you would expect to see increased rates of the disease in persons with iron overload; but this does not appear to be the case. There seems to be no evidence of higher rates of atherosclerosis or CHD in persons with hemochromatosis (86). Patients with hemochromatosis may develop cardiomyopathy, which can result in arrhythmia, bradycardia, congestive heart failure, and death (87). Liver diseases are the most common causes of death (88,89).

Another piece of evidence comes from studies of dietary iron overload in Sub-Saharan Africa. There is a high prevalence of iron overload among Sub-Saharan blacks, which appears to be related, in part, to the consumption of traditional beer, which is made in steel drums, coupled with a genetic defect that may be different from the HLA-linked trait seen in whites with hemochromatosis (90). However, to date, there have been no reports that iron overload in Africa was associated with an increased risk of CHD (14,91).

6. SUMMARY

In 1981, Dr. Jerome Sullivan (1) proposed that body iron stores are directly or positively related to CHD risk, i.e., the higher your body iron stores, the greater your CHD risk. Until the publication of results from Finland by Salonen et al. (4), showing
a positive relationship between serum ferritin levels and risk of heart attack in men, the hypothesis was largely ignored. Although a plausible hypothesis was proposed by Dr. Sullivan to define a role for iron in the development of CHD, possibly by catalyzing the free radical oxidation of LDL cholesterol, the vast majority of the epidemiologic results published since the study by Salonen et al. (4) have failed to support the original hypothesis. Whether looking at the direct relationship between serum ferritin and CHD risk, serum ferritin and measures of atherosclerosis, serum TS and CHD risk, iron intake and CHD risk, serum ferritin and measures of LDL oxidizability, or iron overload and CHD risk, the results are the same: the data do not support the hypothesis that body iron stores are a risk factor for CHD. Additionally, the effect of blood donation on serum levels of oxidized LDL remains an open question as does the measurement of oxidized LDL and the question if oxidized LDL is itself a risk factor for CHD.

7. RECOMMENDATIONS

Sound clinical guidance and public health recommendations must be based on reasonably solid evidence that what is being recommended is both safe and effective. Given the results to date concerning the iron hypothesis, there can be no doubt about the recommendations. “Further research, including basic research and large-scale epidemiologic studies, is needed to fully assess the association between iron status and the risk of CVD (cardiovascular disease) and other adverse outcomes. At present, the currently available data do not support radical changes in dietary recommendations or screening to detect high normal levels nor do they support the need for large-scale randomized trials of dietary restriction or phlebotomy as a means of lowering iron stores” (89).

Finally, it must be remembered that there are currently a number of proven CHD risk factors including high blood cholesterol, high blood pressure, cigarette smoking, obesity, and lack of exercise, for which there are proven and effective guidelines and measures for decreasing CHD risk (92–95). Until other measures are proven and established, it is our recommendation that we should focus on them rather than on unproven measures.

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1. INTRODUCTION

The interest in homocysteine as a potential modifiable risk factor for cardiovascular disease (CVD) has grown exponentially over the past 5 yr. Homocysteine is an amino acid byproduct of methionine metabolism. Historically, the finding of thrombotic complications in the rare hereditary disease of homocystinuria led to the hypothesis that the carriers for this condition, who number about 1 in 200 of the general population and have a partial metabolic defect and slightly elevated homocysteine levels, might have an increased frequency of vascular disease. Many studies were therefore done to relate elevated homocysteine blood levels—whether genetic in origin or not—to vascular disease. The majority of these investigations showed an association of hyperhomocyst(e)inemia with coronary artery disease, cerebrovascular disease, and peripheral vascular disease.

In this chapter, we review the metabolism of homocysteine, its regulation, and measurement. Next, we review the relationship between folate intake and homocysteine levels. This is followed by a summary of those studies of the association between blood folate levels and homocysteine levels, and of the additional roles of pyridoxine and cobalamin in reducing elevated homocysteine. The formal metaanalyses of the association between homocysteine and coronary heart disease (CHD) follows, with a review of the association between homocysteine and cerebrovascular disease and peripheral vascular disease. Finally, we review the evidence linking plasma folate and cardiovascular disease, and discuss the possible impact of population strategies to change folic acid intake on risk of CHD.

2. HOMOCYSTEINE

2.1. Homocysteine Metabolism

When protein is digested, methionine, an essential amino acid, is released. Homocysteine, a sulfur-containing amino acid, is a product of methionine metabolism. Under normal circumstances, methionine and homocysteine cycle metabolically, any excess homocysteine being excreted (Fig. 1).

Homocysteine can be metabolized via transsulfuration or remethylation (1,2). Vitamin B_6 is important in homocysteine transsulfuration, whereas folate and vitamin B_{12} play principal roles in homocysteine remethylation. The condensation of homocysteine and
serine to form cystathionine is catalyzed by cystathionine β-synthase, an enzyme widely distributed in mammalian tissues. This enzyme requires pyridoxal 5'-phosphate, the biologically active form of vitamin B6, as a cofactor. Remethylation of homocysteine to methionine is accomplished by two pathways in humans. One pathway is catalyzed by N-5-methyltetrahydrofolate: homocysteine methyltransferase (methionine synthase). The enzyme, widely distributed in animal tissues, requires 5-methyltetrahydrofolate (the main circulating form of folate in plasma) as the methyl donor and cobalamin (vitamin B12) as a cofactor. The enzyme methylenetetrahydrofolate reductase regenerates 5-methyltetrahydrofolate. In the second pathway, betaine acts as the methyl donor and the active enzyme is betaine: homocysteine methyltransferase. This latter reaction is confined to the liver; its quantitative significance in humans is not fully elucidated (3). Thus, the amount of circulating homocysteine is tightly regulated by three B vitamins: folate, B12, and B6.

Homocysteine exists in plasma in several forms. In normal subjects, approx 70–85% of the amino acid homocysteine is protein-bound via disulfide linkages, primarily to albumin (4,5). The remaining homocysteine in plasma rapidly oxidizes to the disulfides homocysteine (two homocysteine molecules linked together) and cysteine-homocysteine. The amount of total plasma/serum homocysteine is the sum of these three components and is referred to as homocysteine or total homocysteine (6,7). Throughout this chapter, we have abbreviated this as tHcy.

Even with the various methods for measuring tHcy correlating well ($r = 0.95$ and $r = 0.98$) with each other (8,9), values for tHcy may vary somewhat depending on the laboratory and collection methods. Some variable changes in tHcy have been observed postprandially (6), therefore, it is recommended to obtain fasting samples when possible. Fasting levels between 5 and 15 μmol/L are considered to be normal. Whereas elevated levels of tHcy are referred to as moderate, intermediate, and severe hyperhomocyst(e)inemia for concentrations between 16 and 30, between 31 and 100, and >100 μmol/L, respectively (5).

Shimakawa et al. (10) reported that among men and women in the Atherosclerosis Risk in Communities (ARIC) study, methionine and protein intake did not show any significant association with plasma tHcy. However, a single dose of methionine admin-
istered as 100 mg/kg of body weight, i.e., about four times the dietary intake of a normal Western diet ([11,12]), has been used as a diagnostic test to detect disordered homocysteine metabolism. Fasting and postmethionine-load (PML) levels of tHcy have been found to be highly interrelated ([13]). Elevated levels of fasting tHcy are believed to reflect folate- and cobalamin-dependent remethylation, whereas elevated PML is dependent on pyridoxal 5′-phosphate-dependent transulfuration ([14]).

2.2. Abnormalities of Homocysteine Regulation

The balance of methionine and homocysteine in the body can be disturbed because of inadequate intake of the three B-vitamins, or because of genetic defects leading to low or absent enzyme activity rates in the relevant metabolic pathways. That there is indeed a strong genetic influence on homocysteine levels is supported by twin studies ([15,16]), whereas familial studies point to a role of shared environment also ([17,18]). An interruption of the transulfuration or remethylation pathways of homocysteine (Fig. 1) produces hyperhomocyst(e)inemia. Severe hyperhomocyst(e)inemia (>100 μmol/L) is most often the result of homozygous cystathione β-synthase deficiency and leads to a condition called homocystinuria, characterized by an overaccumulation of tHcy, as evidenced by urinary excretion of homocystine. The frequency of this genetic disorder in the United States population has been estimated as 1 in 291,000 ([1]), with 0.5% of the population being carriers or heterozygotes for cystathione β-synthase deficiency. Several other autosomal recessive inborn errors of metabolism can cause homocystinuria. These are, once again, very rare, and include five different vitamin B12 metabolic defects (CbC, D, E, F, G) ([19]). A homozygous deficiency of the enzyme, methylene-trihydrofolate reductase (MTHFR), presents with neurological symptoms, but is extremely rare and occurs in the general population at a rate of about one-tenth that of cystathione β-synthase deficiency ([20]).

In 1988, Kang et al. ([21]) described a less-severe defect of the MTHFR enzyme. This thermolabile variant of the MTHFR enzyme was associated with raised levels of tHcy. This form occurs in neurologically normal subjects, and is inherited as an autosomal recessive trait at a frequency of 5%. This implies that 35% of the general population is heterozygous for this trait. The elevated levels of tHcy seen in individuals with MTHFR enzyme variant in the homozygous state have been found to be correctable with oral folic acid supplementation ([22,23]), suggesting that this metabolic block can be overcome with pharmacological doses of folic acid.

This heat-labile variant of MTHFR has been shown to result from a cytosine to thymine (C to T) mutation at nucleotide 677 ([24,25]). Homozygotes for the TT genotype have higher tHcy levels compared with heterozygotes (CT) and normal homozygotes (CC) ([26]). This is most pronounced in homozygotes with low serum-folate levels. The Physicians’ Health Study ([27]) has examined the hypotheses that individuals with the TT mutation might experience an increased risk of MI secondary to elevated tHcy; and that high-folate intake might compensate by lowering plasma tHcy in individuals with the mutation. During an 8-yr follow-up ([27]), the risk for nonfatal myocardial infarction (MI) and fatal CHD was not significantly different between the three genotypes, despite the (TT) genotype having significantly higher mean tHcy levels than the (CC) genotype. This difference was most marked among men with low folate levels. Thus, in this population, the MTHFR polymorphism was associated with higher tHcy levels, but not with risk of MI. In general, these conclusions are similar to the many other studies addressing the role of...
Indeed, a meta-analysis of 23 reports in the literature concluded the prevalence of the TT genotype in cardiovascular disease patients was almost identical to the prevalence in controls (11.9% and 11.7%, respectively). There have been some suggestions that the combination of CT or TT genotype and low-folate status together might increase the risk of CHD [for example, in the Physician’s Health study, there was an elevated risk of CHD in the group with CT genotype and low folate]. The impact of this common genetic trait on vascular disease and folate requirements is currently receiving intense investigation.

2.3. Total Homocysteine Concentration in the Population

The concentration of tHcy in plasma among healthy, disease-free individuals ranges between 5 and 15 μmol/L. Fig. 2 shows mean levels of tHcy for male and female adults by age group based on NHANES III data. The 95% confidence intervals (CI) are shown for two 4-yr age groups (12–15 yr and 16–19 yr) and six 10-yr age groups from 20–29 to 80+.

Plasma tHcy is dependent on age and gender, with plasma homocysteine concentrations increasing as age increases. As shown in Fig. 2, and is generally found in population-based studies, men have higher tHcy concentrations than women. In women, homocysteine levels are possibly related to menopausal status. On the other hand, large differences between pre- and postmenopausal women found when measuring homocysteine-cysteine mixed disulfide were not found when measuring tHcy.

The reasons for the higher tHcy concentrations observed in the older ages may be secondary to decreased enzyme activity, depressed renal function, and other changes associated with aging. Gartler et al. showed that cystathionine β-synthase activity in
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Healthy subjects decreased with age so that persons above 70 yr, and particularly the very old, had very low enzyme activity. Bostom et al. (35) used a rat model to document that loss of homocysteine-metabolizing capacity of the kidneys may be a determinant of elevated tHcy concentration. A decreased intake and absorption of the vitamin co-factors responsible for homocysteine metabolism might also contribute. The consistent results of higher tHcy levels in the older population generally fits these findings.

3. FOLATE AND HOMOCYSTEINE

3.1. The Influence of Folate Intake on Plasma Homocysteine

Several investigators have conducted studies examining the effect on homocysteine levels in response to changes in folic acid intake. Many of these have been included in a careful metaanalysis published by the homocysteine lowering trialists’ collaboration guided by the Clinical Trial Service Unit in Oxford, U.K. (36). The metaanalysis used individual level data contributed by the collaborating authors. The results confirmed that folic acid supplements decrease homocysteine levels at all levels of homocysteine, but that the amount of reduction was greater for higher baseline levels, and lower blood folate levels, as had been suggested previously (37,38). At average pretreatment values of homocysteine (12 μmol/L) and folate (12 nmol/L), added folic acid of at least 0.5 mg daily reduced homocysteine levels by about one-quarter (36). The addition of vitamin B12 to folic acid supplements further reduced homocysteine levels by about 7%. Supplementation with vitamin B6 had little additional effect on homocysteine levels. (The metaanalysis did not include an evaluation of PML levels of homocysteine.)

We are aware of two other studies of healthy subjects with normal concentrations of folate, vitamin B12, and vitamin B6, in which the effect of added folic acid alone for at least 3 wk could be evaluated.

Brouwer and colleagues (39) studied 144 women aged 18–40 yr, and assigned them by alternation to a placebo group, a group receiving 500 μg folic acid daily and a group receiving 500 μg folic acid every other day (equivalent to a dose of 250 μg folic acid daily). The baseline concentrations of homocysteine differed significantly between the groups, but an evaluation of the change in homocysteine levels from baseline to the end of the 4-wk intervention period found a significantly larger decrease in homocysteine in the two treatment groups compared to placebo, and a greater reduction in the group receiving 500 μg compared to the group receiving 250 μg daily.

O’Keefe and colleagues (40) studied 18 healthy nonpregnant women, with normal blood chemistry profiles, and randomized them into three groups in a metabolic study. Each group consumed 30 μg/d dietary folate with supplemental folic acid bringing the daily folate dose to 200, 300, or 400 μg/d. After 70 d, levels of tHcy in the group consuming 200 μg/d of folate were statistically significantly higher (p < 0.05) than levels in either the group consuming 300 μg/d or the group consuming 400 μg/d of folate, that is, the homocysteine lowering effect of both 300 μg daily and 400 μg daily was greater than that of 200 μg daily.

These two studies suggest a graded benefit at doses of folic acid below 0.5 g daily. If indeed, there is such a graded benefit, it may not extend above about 0.5 mg folic acid daily, because the metaanalysis (36) suggests that a plateau may be reached at about 500 μg daily of added folic acid. This existence of a plateau is supported by the result of
Ubbink and colleagues (41) in which subjects, after 6 wk of treatment with 1000 μg folic acid, 0.4 mg cobalamin, and 12.2 mg pyridoxine, had no additional lowering of tHcy when twice these amounts were given.

Many studies have shown that supplemental folic acid decreases tHcy concentrations and improves folate status, however, studies investigating the effects of natural food folate on tHcy and folate status are rare. Brouwer and colleagues (42) randomized 66 healthy subjects into three groups for a dietary controlled intervention of 14 wk. The dietary folate group received a diet high in vegetables and citrus fruit (total folate content about 560 μg), the folic acid group received a diet (about 260 μg) plus the equivalent of 250 μg of folic acid/d, and the placebo group received the same low-folate diet as the folic acid group plus a placebo tablet. Both the dietary folate group and the folic acid group experienced significant increases in plasma folate and red blood cell folate concentrations, as well as significant reductions in fasting plasma tHcy. Although the amount of folate consumed by the dietary folate group is higher than commonly consumed in the general population, this is the first study to show that folate-dense foods can significantly enhance folate status and decrease tHcy concentration. Another study by Malinow (43) of men and women with a history of CHD, examined the effects of breakfast cereals fortified with three levels of folic acid on homocysteine levels in a crossover trial. In spite of random allocation, more women than men were assigned to group A (assigned placebo or cereal with 127 μg folic acid per serving first) compared to group B (assigned placebo or cereal with 499 μg folic acid per serving first) or C (assigned placebo or 665 μg folic acid per serving first). All groups received two treatment periods of 5 wk, with a 5-wk washout period in between. The homocysteine lowering effect of 499 μg daily or 665 μg daily was similar, and significantly greater than that of 127 μg folic acid as fortified cereal. The latter treatment was not significantly different from the placebo treatment with respect to homocysteine levels. These results are consistent with the studies of folic acid supplements. It is interesting to note that the fortified cereal with 127 μg folic acid is comparable with the increased intake specified by the FDA’s enrichment policy for flour and other cereal-grain products (44). This study also suggests the plateau of homocysteine lowering attributable to folic acid has not yet been achieved with current levels of fortification.

### 3.2. Homocysteine and Folate Concentrations

Seven studies (6,8,45–49) of tHcy and folate found that tHcy levels markedly increased as plasma/serum folate levels decreased. Folate levels were determined from blood (8), plasma (46,47), or serum (6,45) samples and quantified by radioassay (45,49) or microbiological assay (46,47). Five additional studies (12,32,50–52) provide support for a strong inverse association between total tHcy levels and plasma/serum folate levels.

Kang et al. (45) measured serum protein-bound homocysteine in 239 stored samples representing extreme depletion or sufficiency of folate or vitamin B₁₂, among 1826 subjects seen in a 1-yr period at a major metropolitan medical center. The mean age of included subjects was 63.7 yr (range 16–97 yr) in this study, with 56% being female. Among subjects with subnormal serum folate (<4.5 nmol/L), 84% had homocysteine values that exceeded 2 SD above the mean for a normal group. A level of serum folate concentration above which homocysteine levels did not change significantly appeared to be 9.1 nmol/L. Because carefully conducted assessment of dietary folate intake re-
reflects biochemically measured folate status (53), these findings are consistent with an inverse association between tHcy levels and dietary folate over the low portion of the range of folate intake, with a flattening out of the relationship at the high end, as has been suggested by the studies of folate intake. The criteria used to evaluate plasma folate levels in the absence of Vitamin B_{12} deficiency are: persons with levels <6.7 nmol/L are at “high risk,” with 6.7–11.1 nmol/L are at “moderate risk,” and with levels >11.1 nmol/L are at “low risk” (53).

3.3. Pyridoxine and Vitamin B_{12}

Cobalamin alone is effective in lowering tHcy levels in cases with overt cobalamin (vitamin B_{12}) deficiency (54–57). In 20 subjects with vascular disease with elevated tHcy values (55), treatment with pyridoxine alone improved the result of the methionine loading test, whereas the inclusion of folic acid resulted in a marked decrease in basal tHcy levels, especially in subjects with hyperhomocyst(e)inemia. From this and other studies (58), it appears that pyridoxine may be effective in reducing elevated tHcy following a methionine load test, but not in reducing the elevated fasting tHcy. Dudman et al. (59) found treatment with either pyridoxine or folic acid similarly effective to those from combined treatment for subjects with vascular disease as assessed with a PML test. Folic acid, on the other hand, seems to be the key factor in reducing fasting hyperhomocyst(e)inemia.

4. HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

4.1. Homocysteine and Coronary Heart Disease

Many studies have been conducted concerning various kinds of vascular diseases and their relationship to tHcy levels and/or folate levels or folate intake. Previously (60), we searched the English language medical literature for the period 1988 to June 1994, using MEDLINE, to find descriptive, observational, and experimental human studies. Recently, we extended the previous search to include articles published through November 1999. Study designs varied from prospective through cross-sectional to case-control studies in which controls were selected from a source population different to that of the cases. These source populations of controls ranged from volunteers to hospital patients. Other studies recruited controls from screening programs or population-based registries. Some studies included cases of both fatal and nonfatal MI, whereas others included only nonfatal cases, sometimes defined by degree of angiographically confirmed occlusion of a coronary vessel. Some studies measured PML levels, whereas others used fasting or basal (casual) levels of homocysteine. Basal levels are easier to obtain in large cohort investigations than fasting levels.

The majority of epidemiological studies used fasting or basal total homocysteine, and for simplicity and homogeneity considerations, we restricted our quantitative review to these studies. In order to use the best inferential tools available, we restricted our quantitative review to population-based case-control studies or prospective studies with a nested case-control evaluation of homocysteine.

Nested case-control studies were those whose controls were sampled from the cohort from which the cases arose; blood samples were obtained (and frozen) before the disease occurred. Population-based case-control studies were retrospective studies in which cases and controls were drawn from the same defined general population. These two study types
are considered to be “high quality.” In contrast, we have excluded cross-sectional and “other” case-control studies. Cross-sectional studies used no sampling by outcome status and may have included convenience samples. “Other” case-control studies included hospital-based investigations and those whose controls were from a different population than the cases. Other researchers have decided against conducting a formal metaanalysis of these studies because of the variability in study methods and quality (61).

At present, there is no consensus on the exact level for defining elevated tHcy. The definitions commonly employed are values exceeding the 90th or 95th percentile among a group of healthy subjects or values exceeding the mean of normal controls by more than two standard deviations. The cut-off values used by investigators for classification of elevated tHcy levels in their respective studies, therefore, vary according to the distribution among their control subjects. This presents a challenge for summarizing the evidence concerning the link between hyperhomocyst(e)inemia and risk of CHD in a quantitative fashion.

All of the studies measuring tHcy provided mean levels for controls, and all but three (62–64) provided mean levels for cases also. These 21 studies are summarized in Table 1. There were 13 prospective studies (29,62,63,65–74) and 8 population-based case-control studies (13,64,75–80). A significant difference \((p < 0.05)\) between mean homocysteine levels of cases and controls was found in 12 of the 18 studies that reported such data. All studies conducted multiple logistic regression analyses, adjusting the estimated odds ratio, for CHD associated with change in homocysteine level, for other risk factors for CHD. All studies adjusted for age, and most also adjusted for cholesterol, smoking, and blood pressure, although the adjustment set was not uniform across studies, as can be seen in Table 1.

**4.2. Metaanalysis of the Graded Relationship Between Homocysteine and Coronary Heart Disease**

Although early studies examined the risk of CVD associated with elevated homocysteine, many recent studies have found evidence of a graded relationship between homocysteine and risk of CVD (67,81–84). Indeed, several studies (75,85,86) of carotid and coronary arteries indicate that the extent of quantitatively defined arterial narrowing is consistent with a graded response to increasing tHcy levels. Pooling information from studies that used disparate definitions of elevated homocysteine, as aforementioned, leads to grouping of quite disparate estimates of risk. On the other hand, each of these studies can contribute estimates of the linear association between tHcy and CHD risk. In the following metaanalysis, we excluded one small study (78) whose estimate of risk associated with change in homocysteine level was large, and whose selection of controls was inadequately described.

Some studies published risk estimates per unit change in homocysteine levels or per 5 \(\mu\)mol/L change in homocysteine levels (62–64,67,69,71,72,75,77,80), as we did in the earlier synthesis (60). We calculated odds ratio estimates for a difference of 5 \(\mu\)mol/L in fasting tHcy concentration, from each study that measured fasting levels of tHcy in cases and controls. For studies that published results only in terms of quartile or quintile cutpoints in comparison with a referent category, we estimated a representative value for each of the high category and the referent category, from which we estimated a delta \((\Delta)\). We calculated the corresponding odds ratios per 5 \(\mu\)mol/L by scaling the ln(OR) for \(\Delta\) down to 5, and taking the exponential. For one study (68), published only
as an abstract, we translated the differences in mean values between cases and controls to estimated odds ratios per \( \mu \text{mol/L} \) change in tHcy, using the linear discriminant function method \((87)\), as we did in the earlier synthesis \((60)\). Although this method is approximate, the assumption of a normal distribution for tHcy leads to a conservative estimate. We combined the odds ratio estimates using the general variance-based confidence interval method, and calculated summary estimates of the effect of tHcy on vascular disease risk assuming a fixed effects model, and using the methods of Greenland \((87)\) and Pettiti \((88)\). The resultant odds ratios estimate the effect of moving the whole population distribution of homocyst(e)ine levels down by 5 \( \mu \text{mol/L} \).

Therefore, assuming a linear relationship between tHcy levels and risk of coronary artery disease, we calculated a summary odds ratio based on a 5 \( \mu \text{mol/L} \) increment in tHcy concentration for 20 studies \((13,29,62–75,77,79,80)\) that measured fasting levels of tHcy in cases and controls. These are arranged in Figs. 3 and 4 within study type, for males and females, respectively. The combined odds ratio, adjusting for conventional risk factors for CHD as reported in the individual studies, for a 5 \( \mu \text{mol/L} \) increment in tHcy is 1.15 (95% CI:1.09, 1.21) for male prospective studies, 1.35 (95% CI:1.24, 1.48) for male population-based case-control studies, 1.42 (95% CI:1.25, 1.62) for female prospective studies and 1.37 (95% CI:1.15, 1.55) for female population-based case-control studies. It should be noted that some prospective studies were able to distinguish between short-term and longer-term risk of CHD associated with high homocysteine \((62,65,89)\). These studies suggest the association of tHcy with risk of coronary disease may be limited to the first 4 or 5 yr of follow-up. Some of the studies found evidence of an interaction effect \((90)\) with one or more of the conventional risk factors \((13)\). This was not taken into account in our metaanalysis. Adjusting for conventional risk factors and ignoring possible interaction could lead to an underestimation of risk.

### 4.3. Homocysteine and Cerebrovascular Disease

Studies of homocysteine and risk of stroke have been summarized before \((60,91)\), and these have included three prospective studies \((66,84,92)\) and one population-based case-control study \((58)\). Since then, seven studies have been published that report risk estimates of stroke alone \((13,63,69,80,93)\) or in combination with other CVD \((62,73)\). Taking these population-based studies together, 7 of the 11 found a significant association between higher homocysteine levels and risk of cerebrovascular disease. Four studies of presymptomatic carotid arteriosclerosis provide additional support for tHcy as a risk factor in cerebrovascular disease. Evidence of carotid atherosclerosis using ultrasound methods was noted in heterozygotes for cystathionine-\(\beta\)-synthase deficiency \((94,95)\). Two larger cross-sectional studies also showed an association of hyperhomocyst(e)inemia with objective evidence of carotid-artery stenosis \((85,86)\).

For the studies published before 1996, we calculated a summary odds ratio based on a change of 5 \( \mu \text{mol/L} \) in tHcy levels using these four prospective or population-based case-control studies that measured fasting levels of tHcy \((32,66,84,92)\). The combined odds ratio was 1.5 (95% CI: 1.3,1.8).

### 4.4. Homocysteine and Peripheral Vascular Disease

Three population-based case-control studies \((96–98)\) were part of our earlier synthesis of homocysteine and peripheral vascular disease \((11,12,55,91,96,97,99–101)\), and two others have been published since 1996 \((13,64)\). All six studies found homocysteine
Table 1
Studies of Homocysteine and Coronary Artery Disease

<table>
<thead>
<tr>
<th>First author</th>
<th>No. of cases/controls</th>
<th>Age, yrs range</th>
<th>Mean homocysteine levelsb</th>
<th>RR confidence</th>
<th>95% confidence interval</th>
<th>Adjustment factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfthan (66)</td>
<td>92/141 m, 40–64</td>
<td>9.8</td>
<td>9.8</td>
<td>&gt;90th centile vs remainder</td>
<td>1.06 (0.64, 1.77)</td>
<td>Age, cholesterol, smoking, blood pressure</td>
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<td></td>
<td>99/128 f, 9.4</td>
<td></td>
<td></td>
<td>*</td>
<td>1.05 (0.61, 1.80)</td>
<td>Cholesterol (total &amp; HDL), smoking, triglycerides, blood pressure, diabetes, angina history</td>
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<tr>
<td>Arnesen (67)</td>
<td>122/478 12–61</td>
<td>12.7</td>
<td>11.3**</td>
<td>Per 4 μmol/L increase</td>
<td>1.32 (1.05, 1.65)</td>
<td></td>
</tr>
<tr>
<td>A’Brook (68)</td>
<td>250/250 m, 85/85 f</td>
<td>15.2</td>
<td>13.7***</td>
<td>n.a.</td>
<td>1.05 (1.01, 1.12)</td>
<td>Age, sex, cholesterol (total and HDL), smoking, blood pressure (SBP and hypertension), diabetes, previous MI, previous stroke</td>
</tr>
<tr>
<td>Bots (69)</td>
<td>104/533 55+</td>
<td>17.3</td>
<td>15.2*</td>
<td>Per 1 μmol/L</td>
<td>1.05 (1.01, 1.12)</td>
<td></td>
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<tr>
<td>Evans (70)</td>
<td>93/186 m, 147/286 m</td>
<td>12.6 (non-fatal MI)</td>
<td>13.1</td>
<td>≥ 15 μmol/L vs ≤ 9.5 μmol/L</td>
<td>0.82 (0.55, 1.54)</td>
<td>Age, cholesterol (HDL, LDL), smoking, blood pressure, triglycerides</td>
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<tr>
<td>Study</td>
<td>n (sex)</td>
<td>Age (years)</td>
<td>Median Hcy (μmol/L)</td>
<td>Hazard Ratio (95% CI)</td>
<td>Risk Factors</td>
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<tr>
<td>Folsom (29)</td>
<td>232/527</td>
<td>45–64</td>
<td>8.86</td>
<td>8.53</td>
<td>≥ 11.5 μmol/L vs ≤ 6.28 μmol/L</td>
<td>Age, sex, race, cholesterol (total and HDL), smoking, blood pressure, diabetes, field center</td>
</tr>
<tr>
<td>Kark (62)</td>
<td>64/808 m 71/980 f</td>
<td>50+ n.a. n.a.</td>
<td>13.2 11.6</td>
<td>Per 1 unit increase in ln(Hcy)</td>
<td>1.92 (1.03, 3.56)</td>
<td>Age, cholesterol (total and HDL), smoking, blood pressure, glucose</td>
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<td>Ridker (73)</td>
<td>122/244 f</td>
<td>Post-menopausal</td>
<td>12.4</td>
<td>&gt; 13.26 μmol/L vs &lt; 9.54 μmol/L</td>
<td>2.4 (1.2, 5.0)</td>
<td>Age, hyperlipidemia, hypertension, smoking, body mass index, exercise, diabetes, and parental Hx of early MI</td>
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<tr>
<td>Stampfer (65)</td>
<td>271/271 m</td>
<td>40–84 11.1</td>
<td>10.5a</td>
<td>&gt; 15.8 μmol/L vs &lt; 14.1 μmol/L</td>
<td>3.4 (1.3, 8.8)</td>
<td>Age, smoking, cholesterol (total/HDL), diabetes, body mass index, aspirin assignment, angina</td>
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<td>Stehouwer (63)</td>
<td>98/780</td>
<td>64–84 n.a.</td>
<td>15.8</td>
<td>Per 1 μmol/L</td>
<td>1.01 (0.99, 1.03)</td>
<td>Age, cholesterol (total and HDL), smoking, blood pressure, body mass index, diabetes, body mass index, prevalent IHD</td>
</tr>
<tr>
<td>Ubbink (74)</td>
<td>154/2136 m</td>
<td>50–64 12.4</td>
<td>11.7</td>
<td>Top 5th (median 16.74 μmol/L) vs Bottom 5th (median 8.36 μmol/L)</td>
<td>1.4 (0.8, 2.3)</td>
<td>Age, HDL cholesterol, smoking, blood pressure, body mass index, diabetes, social class, prevalent IHD</td>
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*continued*
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<tr>
<th>First author</th>
<th>No. of cases/controls</th>
<th>Age, yrs range or mean</th>
<th>Homocysteine levels$^b$</th>
<th>RR estimate</th>
<th>95% confidence interval</th>
<th>Adjustment factors</th>
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<td>Wald (71)</td>
<td>229/1126 m</td>
<td>35–64</td>
<td>13.1</td>
<td>11.8***</td>
<td>1.33 (1.22, 1.59)</td>
<td>Age, blood pressure, apoB</td>
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<tr>
<td>Whincup (72)</td>
<td>386/454</td>
<td>40–59</td>
<td>14.2</td>
<td>13.5</td>
<td>1.17 (0.99, 1.38)</td>
<td>Age, cholesterol (total and HDL), smoking, blood pressure, body mass index, creatinine, social class, alcohol, exercise, town, serum urate, FEV$_1$, packed cell volume</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population case-control</td>
<td>383/800</td>
<td>43.9 yr</td>
<td>11.2</td>
<td>9.7</td>
<td>Top 5th (≥ 12 μmol/L) vs remainder</td>
<td>2.0 (1.4, 2.8)</td>
</tr>
<tr>
<td>Graham (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoogeven (64)</td>
<td>40/564</td>
<td>50–75</td>
<td>n.a.</td>
<td>11.4</td>
<td>1.25 (1.03, 1.51)</td>
<td>Age, sex, cholesterol, smoking, blood pressure, diabetes</td>
</tr>
<tr>
<td>Hopkins (80)</td>
<td>120/85 m 42/70 f</td>
<td>38–68</td>
<td>13.7</td>
<td>11.3***</td>
<td>8.1 (3.2, 20.4)</td>
<td>Age, smoking, blood pressure, diabetes, triglycerides</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.6</td>
<td>8.9***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Gender</td>
<td>Age (yr)</td>
<td>Body Mass Index</td>
<td>Mean tHcy (μmol/L)</td>
<td>95% CI</td>
<td>Clinical Parameters</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Loehrer (78)</td>
<td>56/23 m</td>
<td>25–79</td>
<td>28.2</td>
<td>8.0*** vs &lt; 5.7 μmol/L</td>
<td>10.9 (1.48, 81.3)</td>
<td>Age, sex, cholesterol, triglycerides, body mass index</td>
</tr>
<tr>
<td></td>
<td>14/22 f</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malinow (79)</td>
<td>191/171 B</td>
<td>25–64</td>
<td>26.4</td>
<td>14.7* vs ≥ 9.8 μmol/L</td>
<td>1.84 (0.8, 4.5)</td>
<td>Age, cholesterol (HDL, LDL), smoking, blood pressure, Apo B, triglycerides, body mass index, alcohol</td>
</tr>
<tr>
<td></td>
<td>229/315 F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancharuniti (75)</td>
<td>101/108 m</td>
<td>30–50</td>
<td>23.4</td>
<td>11.9*** Per quartile</td>
<td>1.4 (1.0, 2.0)</td>
<td>Age, cholesterol (HDL, LDL), smoking, blood pressure, body mass index, alcohol</td>
</tr>
<tr>
<td>Schwartz (76)</td>
<td>79/386 f</td>
<td>&lt;45 yr</td>
<td>26.4</td>
<td>11.1*** ≥ 15.6 μmol/L vs &lt; 10 μmol/L</td>
<td>2.30 (0.94, 5.64)</td>
<td>Age, smoking, diabetes, body mass index</td>
</tr>
<tr>
<td>Verhoef (77)</td>
<td>131/189</td>
<td>25–65</td>
<td>28.5</td>
<td>12.5** Per 5 μmol/L</td>
<td>1.3 (1.0, 1.6)</td>
<td>Age, sex, cholesterol, triglycerides, body mass index, alcohol, creatinine</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, *** p < 0.001 difference between cases and controls.
* tHcy = homocysteine, m = males, f = females, n.a. = not available, h = hours, Hx = History, MI = myocardial infarction, B = Belfast, F = France, FEV1 = Forced expiratory volume in 1 s, RR = relative risk: estimates are for combined genders or combined endpoints.
* Elevated tHcy levels are usually determined as above the 95th percentile for the control group or above 2 SD (standard deviations) of the control mean.
* Unless marked subjects are males and females.
* Geometric mean level of serum/plasma tHcy.
Fig. 3. Coronary heart disease risk in men per 5 μmol/L change in homocysteine: odds ratios with 95% confidence intervals on a log scale.
**Fig. 4.** Coronary heart disease risk in women per 5 μmol/L change in homocysteine: odds ratios with 95% confidence intervals on a log scale.

<table>
<thead>
<tr>
<th>Author, cases/controls</th>
<th>Females</th>
<th>Population-based case-control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prospective studies</td>
<td></td>
</tr>
<tr>
<td>Altham</td>
<td>99/128</td>
<td></td>
</tr>
<tr>
<td>Amesen</td>
<td>12/48</td>
<td></td>
</tr>
<tr>
<td>A’Brook</td>
<td>85/85</td>
<td></td>
</tr>
<tr>
<td>Bots</td>
<td>35/332</td>
<td></td>
</tr>
<tr>
<td>Folsom</td>
<td>58/130</td>
<td></td>
</tr>
<tr>
<td>Kark</td>
<td>71/80</td>
<td></td>
</tr>
<tr>
<td>Ridker</td>
<td>85/170</td>
<td></td>
</tr>
<tr>
<td><strong>Pooled OR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graham</td>
<td>61/230</td>
<td></td>
</tr>
<tr>
<td>Hoogeveen</td>
<td>40/564</td>
<td></td>
</tr>
<tr>
<td>Hopkins</td>
<td>42/70</td>
<td></td>
</tr>
<tr>
<td>Schwartz</td>
<td>79/386</td>
<td></td>
</tr>
<tr>
<td>Verhoef</td>
<td>20/62</td>
<td></td>
</tr>
<tr>
<td><strong>Pooled OR</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
to be a risk factor for peripheral vascular disease, independent of conventional risk factors. The Framingham study (102), with median follow-up of 10 yr, also found an increased risk of CVD (including peripheral vascular disease) of 1.53 (95% CI:1.16, 1.98) associated with homocysteine levels in the highest quartile compared to the other three quartiles, after adjustment for age, sex, cholesterol (total and HDL), smoking, blood pressure, and diabetes.

5. FOLATE AND CARDIOVASCULAR DISEASE

5.1. Dietary Folate and Folate Concentration and Risk of Cardiovascular Disease

Many studies of CVD and tHcy levels also measured folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>. Mean levels of plasma/serum folate among cases were reported significantly lower than among controls in four studies (75,77,97,103). On the other hand, the mean folate concentrations between cases and controls were not significantly different in five studies (55,96,104–106). When subjects in studies of vascular disease were divided according to levels of tHcy, individuals with reduced tHcy levels had significantly higher levels of blood folate (51,52) and red cell folate (12). Finally, plasma, red cell and serum folate levels were significantly and negatively correlated (−0.20 to −0.57) with tHcy levels (52,55,75,96,103,106–108) among cases with vascular disease. Studies examining the correlation between levels of tHcy and folate among controls were less consistent; three studies (52,55,96) reported insignificant results among controls, whereas the studies with significant correlation among controls (−0.16 to −0.49) had a wider range of results (75,89,103,106,108).

If tHcy is associated with CVD, and folate is associated with levels of tHcy, then also of importance would be the link between folate status and risk of CVD. Ten relevant studies examining this issue are outlined in Table 2. No one study used the same criteria for case definition, thus the end points of interest must be broadly defined as vascular disease. Most of the studies have divided the folate concentration into quartiles (75,76,86,109,110), quintiles (103), or other categories (89,105,108). However, because the classification of categories is usually based on the distribution among the controls in a study, the levels of folate differ among the group of studies. Despite these differences, the results are fairly consistent, showing that at concentrations of plasma/serum folate above 10 nmol/L, there is a significant reduction in risk for vascular disease (75,86,103,108), particularly among females (29,109), and adults 35–55 yr old (110). Point estimates from other studies (76,89,105) support these findings, but were not statistically significant. Brouwer et al. (111) have suggested this level of folate (10 nmol/L) be used as a cutoff for adequate folate, based on folate’s functional relationship with tHcy.

The NHANES Epidemiologic Follow-up Study (105) reported an elevated risk for stroke in men and women, but only the risk for black subjects with folate levels <9.2 nmol/L was significantly elevated (OR 3.6; 95% CI: 1.02–12.71). In an analysis of this same data, but using incident CHD (110), a folate concentration of ≥21.8 nmol/L was found to provide a significant protective effect among those 35–55 yr old. However, among the individuals ≥55 yr old, this same level of folate significantly elevated the risk of CHD (RR = 2.0; 95% CI: 1.25–3.33). Among the participants in the ARIC study (29), the relative risk among women for CHD was found to be significant, however,
there was not a significant interaction for gender so the final multivariate risk estimate combined males and females. Although the risk estimates declined across the quintiles for folate concentration (1.0–0.66) the confidence intervals consistently included 1.0. This study did find that vitamin B6 likely offers independent protection for CHD.

Several of the studies had available levels of tHcy, as well as concentration of folate. This allowed investigators to examine the association of folate with vascular disease while simultaneously controlling for fasting tHcy. Theoretically, if the risk associated with folate and vascular disease changes with the addition of tHcy in the model, then one can assume that folate is affecting the risk of vascular disease via the tHcy pathway. In the population-based case-control studies, reported by Pancharuniti et al. (75) and Robinson et al. (108), the addition of tHcy to their models of folate and CVD, resulted in folate being no longer significant. In the study by Schwartz et al. (76) among younger-aged women, the association with folate was weakened when tHcy was added to the model. In the prospective Physicians’ Health Study, the presence of tHcy in the model only slightly attenuated the relative risk for low levels of folate, thus it is not clear in this study whether folate is affecting the risk of MI only via the tHcy pathway, or has an independent effect.

Fewer studies have examined the relationship between intake of dietary folate and risk for vascular disease. In a subset of subjects from the Framingham Heart Study (86), stenosis was inversely related to reported intakes of folate. Intakes less than 327 µg/d showed a fivefold increase in risk compared to intakes greater than 475 µg/d. Another analysis from the Framingham Study (112) indicated that intake of fruits and vegetables was inversely associated with incidence of strokes and transient ischemic attacks (TIA). Although many factors could potentially explain this finding, one consideration is the large contribution fruits and vegetables make to total folate intake. Thus, the protective effect of fruits and vegetables for stroke and TIA is compatible with folate lowering the risk of CVD, possibly through lowering tHcy levels. Graham et al. (13) found users of vitamin supplements to have a substantially lower risk of vascular disease than nonusers, and some of this lowering was attributable to lower plasma tHcy levels. Perhaps the best evidence for diet and supplements comes from the prospective analysis of the Nurses’ Health Study by Rimm et al. (113). During a 14-yr follow-up, the risk for nonfatal MI and fatal CHD was considerably lower among women who consumed 696 µg of folate per day compared to 158 µg (RR = 0.69; 95% CI: 0.55–0.87). Risk of CHD was also reduced among women who regularly used multiple vitamins, the major source of folate and vitamin B6 (RR = 0.76; 95% CI: 0.65–0.90).

A single, but compelling, intervention to reduce carotid plaque area provides the only evidence of vitamin supplementation influencing the progression of atherosclerosis (114). This study followed 18 men and 20 women (mean age 57.9 yr) with initial tHcy concentration >14 µmol/L for 4.4 yr after treatment with 2.5 mg folic acid, 25 mg pyridoxine, and 250 µg vitamin B12. Each individual had at least two measurements of total plaque area before, and two measurements after treatment, as determined by ultrasound. Prior to treatment, plaque area had been measured as increasing. After treatment, plaque area decreased significantly and both genders and all ages responded equally. No concentration levels of vitamins or tHcy were reported.

These results would suggest that intakes of folate in excess of 400 µg of dietary folate equivalents per day, the current Dietary Reference Intake (115,116), may be important in the prevention of vascular disease.
<table>
<thead>
<tr>
<th>First author</th>
<th>No. of subjects</th>
<th>Type of study</th>
<th>End point</th>
<th>Measurement</th>
<th>Folate concentration (nmol/L) in quartiles or quintiles</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selhub (86)</td>
<td>1041</td>
<td>Cross-sectional</td>
<td>Stroke</td>
<td>Plasma OR CI</td>
<td>5.69–9.77 9.78–17.95 ≥17.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pancharaniti (75)</td>
<td>101 cases Males</td>
<td>Case</td>
<td>Stroke</td>
<td>Plasma OR CI</td>
<td>3.5 5.3 9.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Verhoef (103)</td>
<td>130 cases</td>
<td>Case-control</td>
<td>1st MI</td>
<td>Plasma OR CI</td>
<td>≤6.56 ≤8.31 ≤9.95 ≤12.0 &gt;12.0</td>
<td>&lt;12.0 &gt;12.0</td>
</tr>
<tr>
<td>Schwartz (76)</td>
<td>77 cases Females</td>
<td>Case-control</td>
<td>MI/stroke</td>
<td>Plasma OR CI</td>
<td>≤5.25 5.27–8.38 8.39–13.92 ≥13.93</td>
<td>≤12.0 ≥13.93</td>
</tr>
<tr>
<td>Folsom (29)</td>
<td>517 cases</td>
<td>Nested case-control</td>
<td>CHD</td>
<td>Plasma OR CI</td>
<td>3.1 6.4 10.6 17.9 17.9</td>
<td>0.14 0.14</td>
</tr>
<tr>
<td>Morrison (109)</td>
<td>5056</td>
<td>Retrospective cohort</td>
<td>Fatal CHD</td>
<td>Serum OR CI</td>
<td>&lt;6.8 6.8–9.1 9.1–13.6 ≥13.6</td>
<td>0.04 0.04</td>
</tr>
<tr>
<td>Giles (110)</td>
<td>1921</td>
<td>Prospective cohort</td>
<td>CHD</td>
<td>Serum OR CI</td>
<td>≤9.9 10.0 10.1 10.2 10.3</td>
<td>21.8 21.8</td>
</tr>
</tbody>
</table>

Note: OR = odds ratio, CI = confidence interval.
<table>
<thead>
<tr>
<th>First author</th>
<th>No. of subjects</th>
<th>Type of study</th>
<th>End point</th>
<th>Measurement</th>
<th>Folate concentration (nmol/L) in percentiles (pctl) or dichotomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robinson</td>
<td>750 cases</td>
<td>Case-control</td>
<td>Clinicalvascular disease</td>
<td>Red cell OR CI</td>
<td>&lt;10th pctl $\leq 513 $ $\geq 513$ 0.67 0.45–0.97</td>
</tr>
<tr>
<td>(108)</td>
<td>800 controls</td>
<td></td>
<td></td>
<td></td>
<td>1.0 0.67 0.45–0.97</td>
</tr>
<tr>
<td>Chasen-Taber</td>
<td>333 cases</td>
<td>Nested MI</td>
<td>MI</td>
<td>Plasma OR CI</td>
<td>&lt;20th pctl $\leq 4.5 $ $\geq 4.5$ 0.71 0.43–1.22</td>
</tr>
<tr>
<td>(89)</td>
<td>333 controls</td>
<td>case-control</td>
<td></td>
<td></td>
<td>1.0 0.71 0.43–1.22</td>
</tr>
<tr>
<td>Giles (105)</td>
<td>2006 Males</td>
<td>Prospective cohort</td>
<td>Ischemic stroke</td>
<td>Serum RR CI</td>
<td>$\leq 9.2$ $&gt;9.2$ 0.73 0.44–1.22</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated, numbers refer to males and females combined.

* MI = myocardial infarction, CHD = coronary heart disease.

* Risk estimates converted to same direction, i.e., lowest folate concentration being reference or risk of 1.0. Multivariately adjusted risk estimates from each study were selected for presentation in the table, although the covariates used differed between each study. OR = odds ratio, RR = relative risk, CI = 95% confidence interval, yr = years.
6. CONCLUSIONS

6.1. A Causal Relationship Between Homocysteine and Coronary Heart Disease

The evidence linking homocysteine levels with risk of CVD is strong and consistent. Both prospective and case-control studies suggest a positive association. There is also evidence for CHD that hyperhomocyst(e)inemia is a major risk factor independent of some other known major risk factors such as total cholesterol (65,75,117–120), low-density lipoprotein (LDL) or high density lipoprotein (HDL) cholesterol (65,75,107,120), body mass index (65,75), age (75), and high blood pressure (65,75,119,120).

Although the magnitude of the association is not large between tHcy and risk of CHD, it could be estimated with good precision. In our metaanalysis, the summary risk estimate for coronary artery disease for an increase of 5 μmol/L of homocysteine was between 1.2 and 1.4 after adjusting for conventional risk factors. This level of risk is similar to other well-established risk factors for CHD, and is comparable to other meta-analyses of tHcy and CHD (90,121).

There is some evidence that elevated tHcy levels precede the occurrence of coronary artery disease. There is no evidence that coronary artery disease or related diagnostic manipulations influence tHcy concentrations. In the positive prospective studies (65), (62,67–69,71,73,84), high tHcy levels clearly antedated the manifestations of either coronary artery disease or stroke.

There are biologically plausible mechanisms by which tHcy might alter risk of developing vascular disease. Atherogenic mechanisms promoted by homocysteine include endothelial cell desquamation, oxidation of LDL, and monocyte adhesion to the vessel wall (20,122). Direct toxicity of homocysteine to the endothelium has been reported in laboratory studies (123–125). Harker et al. showed endothelial desquamation in vivo in baboons infused with homocystine (126) or homocysteine (127) at the high levels typical of patients with homocystinuria. Tsai and coworkers (128) have shown that homocysteine increases DNA synthesis in vascular smooth muscle cells consistent with early arteriosclerotic lesions and induces these cells to proliferate while impeding the regeneration of endothelial cells. Homocysteine may predispose to arteriosclerosis by promoting oxidation of LDL (129) or by decreasing thrombomodulin cell-surface expression and inhibiting protein C activation, thus probably contributing to development of thrombosis (130). One study provides the first preliminary evidence that folate may have beneficial cardiovascular effects in adults with hyperhomocyst(e)inemia (131). Eighteen healthy subjects with tHcy >13 μmol/L participated in a randomized double-blind placebo-controlled crossover study of oral folic acid (5 mg daily for 6 wk) with a 6-wk washout period between treatments. Flow-mediated (endothelium-dependent) and (endothelium-independent) glyceryl trinitrate (GTN)-mediated brachial artery dilation were measured. Folate supplementation significantly enhanced endothelium-dependent responses, although endothelium-independent responses were unchanged. Thus, high-dose folate acid supplementation enhanced endothelium-dependent vascular function and lowered plasma tHcy in human subjects.

In summary, the association between hyperhomocyst(e)inemia and CHD has the required elements of strength, consistency, temporality, and biological plausibility that allow a causal inference from observational studies (132).
6.2. Limitations

In metaanalysis, results are combined from studies that may differ in methodology and include noncomparable populations. Publication bias, i.e., not publishing small negative studies, is possible. Although those negative studies that were published were included in the summary risk ratios, there may have been other negative studies performed whose lack of inclusion may have inflated the summary risk ratios calculated by us. Some authors (121) have conducted metaanalyses on a set of studies overlapping with those analyzed here, but others (61) concluded that heterogeneity between studies precluded a metaanalysis. A metaanalysis using individual data from collaborating research groups, whose published studies we have reviewed, is expected soon (R. Clarke, personal communication).

The relationship between homocysteine and CHD risk is strong, but there have been no randomized controlled trials published that have investigated the effects of homocysteine lowering on CVD incidence or mortality (133,134). Several are now under way and we await their results with interest. Many investigations demonstrate that folic acid reduces tHcy levels. Whereas various combinations of folic acid, vitamin B12, and vitamin B6 were often administered, folic acid appeared to be the effective agent, because it reduced tHcy levels even when given alone. However, as the interactions of folic acid, vitamin B12, and vitamin B6 are not fully known, further studies of all three of these vitamins will be useful.

Whether nutritional inadequacy of folic acid alone will raise tHcy levels, or whether this response is limited to individuals predisposed genetically to develop hyperhomocyst(e)inemia needs further study. The existence of several different genetic traits (including the common MTHFR polymorphism aforementioned) predisposing to high tHcy levels suggests that genetic variability in response to folic acid intake might be expected. At least until such differences are fully elucidated, a population approach that treats the different genetic and environmental origins of the homocysteine-folic acid relationship in the same way appears appropriate.

6.3. The Broader Health Context

Are there negative implications of widespread supplementation or fortification with folic acid? It has been known for a long time that folic acid may mask the hematological manifestation of unrecognized pernicious anemia (cobalamin deficiency) although its neurological manifestations, which may be severe and include spinal cord damage, may progress (135). However, such effects were mainly observed with high pharmacological doses of folic acid, i.e., 5000 μg or more. It is not clear whether such high doses precipitate or exacerbate the neuropathy of cobalamin deficiency. The effect on the neuropathy of vitamin B12 deficiency of lower doses of folic acid, such as might be expected to occur were grain to be fortified or dietary supplements to be taken on a widespread basis, remains poorly defined (135). Recent studies have estimated among the elderly a 5% prevalence of serum vitamin B12 deficiency associated with methylmalonic acidemia (49,57), unrelated to pernicious anemia. This deficiency could be corrected by low doses of cobalamin. If the oral dose of vitamin B12 were large enough, even persons with pernicious anemia (estimated to be 1/5000) (136) would derive some benefit, because 1–3% of vitamin B12 can be absorbed by simple diffusion (137,138). In any of the obvious strategies to increase folic acid intake, namely increasing intake of foods naturally high in folate (such as fruits and vegetables), food fortification, or taking a
daily dietary supplement containing folic acid, the highest consumers of folic acid
would be those taking vitamin supplements containing 400 μg of folic acid. Were 1 mg
cobalamin to be added to vitamin supplements containing 400 μg folic acid, most vita-
mint B₁₂ deficiencies would be corrected, and concern about the possible masking ef-
facts of folic acid would be considerably lessened (135).

What are other implications of a nutritional policy to increase folic acid intake?
Approximately 2500 cases of neural tube defects are currently born per year in the
United States, and approx 1500 fetuses with this condition are aborted following detec-
tion by prenatal diagnosis (139). Women who had children with neural tube defects
have slightly elevated tHcy levels. In some women, this is because of low folate intake
or poor absorption and, in some, this is presumably caused by yet undescribed genetic
defects or variants predisposing to hyperhomocyst(e)inemia (140). There is excellent
evidence from a variety of studies that at least 50% of neural tube defects can be pre-
vented if 400 μg of folic acid is taken daily before and during the first 4 wk of preg-
nancy (139). Because many women do not realize when they are in the very early stages
of pregnancy, folic acid supplementation and dietary counseling have not been suc-
cessful in increasing folic acid intake in the population during this critical time (141).
The FDA has mandated adding folic acid to the food supply by fortification of enriched
grains (44). This policy is expected to prevent many neural tube defects and, in the light
of our metaanalysis, at the same time promises to have the much larger effect of reduc-
ing vascular disease in many thousands of older men and women.

7. RECOMMENDATIONS

Although the cause and mechanism of homocysteine-associated cardiovascular dis-
ease have not yet been firmly established, the American Heart Association (AHA)
deemed the association compelling enough to issue a Science Advisory in early 1999
(142). At the present time, widespread screening of tHcy levels is not recommended.
However, determining fasting tHcy levels in high-risk patients, i.e., individuals with
strong family history for premature atherosclerosis or occlusive vascular disease in the
absence of other “traditional” risk factors, is considered a reasonable approach. Once
high tHcy concentration has been established, determination of blood levels of folate,
B₆, and B₁₂ is important because of the inverse relationship of these vitamins to tHcy
levels discussed earlier in this chapter. Currently, no firm protocols exist for recom-
mending therapeutic targets for tHcy levels. On the other hand, a recent report of the
Food and Nutrition Board of the National Academy of Sciences, Institute of Medicine
(115) includes Dietary Reference Intakes (DRI) of 400 μg of dietary folate equivalents,
1.7 mg vitamin B₆, and 2.4 μg vitamin B₁₂ as an average daily intake. Because a large
proportion of the population does not currently meet these dietary recommendations, a
reasonable population approach is to promote an increase in the intake of foods con-
taining these vitamins.

On a population basis, fortification of grains with enough folic acid to deliver 400 μg
to the majority of women of childbearing age would be the most logical step to prevent
a large fraction of neural tube defects. The strength of the evidence suggesting increased
folic acid as a public health measure to lower homocysteine might benefit large num-
ers of older people in the prevention of vascular disease, far outweighs the available
evidence concerning possible risks for mild neurological impairment with undiagnosed
vitamin B₁₂ deficiency. Clinical trials to assess the role of folic acid for vascular disease
prevention, with careful attention to its possible neurological effects in vitamin B12 deficient individuals, have been initiated. Meanwhile, action on a population basis should not wait. A combined strategy to increase both folic acid and vitamin B12 intake appears prudent at this stage (135), and would likely prevent much arteriosclerotic vascular disease, as well as neural tube defects. We recommend a policy of: (1) fortification of grains with folic acid at 350 μg/100 g (higher than the current level of 140 μg/100g) and (2) the mandatory addition of 1 mg vitamin B12 to all vitamin supplements containing 400 μg folic acid.

On an individual basis, we recommend taking a dietary supplement of 400 μg/d folic acid to prevent vascular disease. This recommendation has particular potential benefit for middle-aged and older people, both men and women. Such a dose is found in most multivitamins of the one-a-day type.

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1. INTRODUCTION

Fish and fish oils contain the very long-chained and highly polyunsaturated n-3 fatty acids, which are derived from phytoplankton, the base of the food chain in the oceans, lakes, and rivers (1). Phytoplankton synthesize the n-3 fatty acids, eicosapentaenoic (20:5) (EPA), and docosahexaenoic (22:6) (DHA), which are subsequently incorporated into fish, shellfish, and sea mammals. The plants synthesize an n-3 fatty acid, linolenic acid (18:3) that can be converted by the body to EPA and more slowly to DHA (2,3). The n-3 fatty acids have profound biological and biochemical effects in the body. Despite a wealth of scientific information (a review listed over 120 references about cardiovascular effects alone [4]), clinical interest in n-3 fatty acids has not been high in the United States despite considerable attention to their use in Europe and Japan. This chapter will focus upon the considerable and underappreciated potential benefits of the n-3 fatty acids in cardiovascular disease.

In the 1950s, it was discovered that polyunsaturated vegetable oils containing the n-6 linoleic acid had a pronounced plasma cholesterol-lowering effect, yet the mechanism of this action has remained obscure (1). In those early days, it was noted that fish oil, which was also polyunsaturated, had a similar hypocholesterolemic effect. No mention was made of the fact that fish oil contained very-long-chain n-3 fatty acids (C20:5 and C22:6) and that these might act differently than the n-6 fatty acid of vegetable oils, such as linoleic acid (C18:2). These early data about fish oil lay fallow until the pioneering observations of Dyerberg and Bang focused special attention upon the n-3 fatty acids, eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) found in marine oils (5). They observed a lower coronary mortality among the Greenland Eskimos whose diet was especially rich in marine oils compared to Danish people eating a high saturated-fat diet (6). Later it was found that not only were these n-3 fatty acids cholesterol-lowering, but, in addition, they had a profound plasma triglyceride-lowering effect, especially in hypertriglyceridemic patients (7–9). Over two decades of

*In this chapter, the terms n-3 and Ω-3 are used interchangeably.

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research in humans, animals, perfused organs, and tissue cultures have firmly documented the mechanisms of the hypolipidemic actions of these n-3 fatty acids from fish and, furthermore, have demonstrated that these fatty acids have many other beneficial effects in cardiovascular disease (CVD).

This chapter will focus upon seven different areas of research that will help to answer the question about the potential benefits of n-3 fatty acids from fish oil on primary and secondary prevention of CVD. These are listed below and will be discussed in detail subsequently. We first will discuss how n-3 fatty acids will prevent further events in those patients who already have coronary heart disease (CHD) (secondary prevention). This is especially relevant to staving off fatal arrhythmias of the heart and thrombosis. Then we will discuss how n-3 fatty acids might prevent coronary disease in healthy individuals, especially in those with certain risk factors.

Secondary Prevention of CHD:
1. Antiarrhythmic actions.
2. Thrombosis.

Primary Prevention of CHD:
1. Experimental animal studies to inhibit the growth of atherosclerotic plaques.
2. Lipid and lipoprotein disorders.
3. Diabetes mellitus.
4. Hypertension.

2. ANTIARRHYTHMIC ACTIONS

2.1. Animal Studies

Sudden death from ventricular arrhythmias is a much-dreaded complication in patients with CHD. Several experimental studies have addressed this problem with the use of n-3 fatty acids from fish oil. McLennan et al. used coronary artery ligation in the rat to produce an in vivo model of ventricular fibrillation and myocardial infarction (MI) (10). They found that the number of ventricular ectopic beats and duration of tachycardia or fibrillation was increased when the rats were fed sheep kidney fat (a saturated fat) when compared to rats fed tuna fish oil, a rich source of n-3 fatty acids. The rats fed tuna fish oil had a significantly reduced incidence and severity of arrhythmias. In another animal study, ventricular fibrillation was prevented by fish oil during both the occlusion of the coronary artery and during reperfusion (11).

In other experiments, Hallaq et al. (12) have used isolated neonatal cardiac myocytes (from hearts of 1-d-old rats) as a model for the study of cardiac arrhythmogenic factors that are modified by n-3 fatty acids. They incubated isolated myocytes (for 3–5 d) in a culture medium enriched with arachidonic acid (AA) or EPA (20:5 n-3). The AA-enriched myocytes developed a toxic cytosolic calcium concentration on exposure to ouabain, whereas EPA-enriched myocytes preserved physiologic calcium levels. An increase of EPA in the membrane phospholipids was demonstrated with a small reduction in arachidonic acid in myocytes fed EPA. A second study by the same researchers further indicated the mechanism of action of the fish oil fatty acids in preventing the arrhythmias of these isolated myocytes (13). It was found that n-3 fatty acids prevented a calcium-depleted state in the myocytes caused by the L-type calcium channel blocker nifedipine. The protective effects of the n-3 fatty acids appeared to result from their
modulatory effects on nifedipine-sensitive L-type calcium channels. In a recent study, dogs were given intravenously pure EPA, DHA, or alpha-linolenic acid (14). Tests were performed in a dog model of sudden death. With infusion of EPA, 5 of 7 dogs did not have a fatal ventricular arrhythmia ($p < 0.02$); with DHA and alpha-linolenic acid, 6 of 8 dogs in each group were protected ($p < 0.004$ for each). These studies indicated a definite beneficial effect of dietary n-3 fatty acids upon the heart; both marine and plant sources of these fatty acids prevented cardiac arrhythmias in animals.

2.2. Population Studies

The epidemiological data about dietary n-3 fatty acids and coronary disease are extensive and go back to the initial observations of Dyerberg and Bang who found a much lower rate of CHD in the Greenland Eskimos compared with Danes (6). They deduced by means of extensive studies that it was the n-3 fatty acid content of the Eskimo diet that inhibited the atherosclerotic disease despite the fact that the Eskimo diet was a high-cholesterol high-fat diet (5). Their dietary fat, instead of being pathogenic, was protective because it was derived from the seas (fish, seal, and so on) and contained the n-3 fatty acids. Furthermore, autopsy studies revealed that atherosclerosis in Alaskan Eskimos was much less than atherosclerosis found in Caucasians living in Alaska (15).

A number of studies correlating fish consumption (providing n-3 fatty acids) and the mortality from CHD have been carried out (4). In Dutch men, the mortality from CHD was more than 50% lower among those who consumed at least 30 g of fish/d than among those who did not eat fish (16). In the MRFIT trial, n-3 fatty acid consumption correlated inversely with all-cause mortality and coronary mortality (17). Even in the Harvard Health Professionals Follow-up Study of 51,529 men, the consumption of one to two servings of fish per week was associated with a lower incidence of CHD (18). Some of these studies will be described in greater detail later.

More recently, fatty fish consumption was associated with prevention of cardiac arrest from ventricular fibrillation in coronary patients. Ventricular fibrillation is the cause of death in most patients with CHD and it accounts for the 20–30% of people whose first indication of coronary disease is cardiac arrest. A study from the University of Washington compared the effects of eating fish with the incidence of cardiac arrest (19). There was a 50% reduction in the risk of cardiac arrest in people who consumed at least one fatty fish meal per week. A typical fatty fish would be salmon. Other fatty fish include sardines, mackerel, and Chilean sea bass. Even those who consumed a less fatty fish, such as tuna, had benefit because all fish and shellfish contain the beneficial n-3 fatty acids.

This protection against cardiac arrest occurred from the n-3 fatty acids (EPA and DHA) in the fat of the fish. The effects of eating fish were reflected biochemically in the fatty acids of the red blood cells. If the red blood cells had a relatively low level of the n-3 fatty acids, 3.3% of total fatty acids, there was a much greater risk of cardiac arrest than in those individuals whose red blood cell n-3 fatty acids were 5% or more of the total fatty acids. In other words, there was a 70% reduction in the risk of cardiac arrest in those people with the higher red blood cell n-3 fatty acid content (19). Likewise, the 20,551 men aged 40–84 yr in the United States Physicians Health Study had a 52% reduction in the risk of sudden cardiac death in men who consumed fish at least once a week (20).
Data from 76,283 women in the Nurses’ Health Study showed that a daily intake of 1.1 g or more of alpha-linolenic acid (18:3 n-3) protected against fatal ischemic heart disease and that this protection probably resulted from an antiarrhythmic effect of alpha-linolenic acid (21). However, the protective effect of alpha-linolenic acid did not extend to nonfatal MI, for which there was a nonsignificant trend for an effect.

2.3. Clinical Trials

These epidemiological data are buttressed by a randomized controlled clinical trial in 2333 men who had recovered from MI and who were then asked to increase their intake of fatty fish or take fish oil (22). There was a 29% reduction in the 2-yr all-cause mortality in subjects advised to eat fatty fish and there was also a reduction in deaths from ischemic heart disease, but no reduction in nonfatal MI. This was the first intervention trial in which all-cause mortality was reduced in a coronary intervention program. One likely reason for the reduction in coronary mortality was the decrease in cardiac arrest as documented by Siscovick et al. (19). Men who ate fatty fish at least once a week had a 50% reduction in cardiac arrest which probably resulted from the antiarrhythmic action of the n-3 fatty acids aforementioned.

In a second clinical trial, 223 patients with angiographically proven coronary artery disease received about 1.5 g/d n-3 fatty acids from fish oil concentrate for 2 yr (23). The progression of coronary disease was significantly, but modestly, decreased compared to the control group. Thus, the evidence becomes stronger and stronger that even some fish (or n-3 fatty acid consumption from fish oil) on a consistent basis (at least one serving a week) may prevent many deaths from CHD.

However, the most recent and largest test of fish oil in coronary patients occurred in Italy as the GISSI-Prevenzione Trial in 11,324 patients who had survived a recent MI (24). These patients were divided into four groups and were randomly assigned daily supplements of 1 g n-3 polyunsaturated fatty acids and 300 mg vitamin E or no supplements (control). The amount of fish oil utilized provided approx 0.85 g of EPA and DHA in ethyl esters in a ratio of 1:2. There was no placebo control group. The duration of the supplementation was 3.5 yr. In this massive trial, treatment with the fish oil n-3 polyunsaturated fatty acids, but not vitamin E, significantly lowered the risk of death (14–20% less) and of cardiovascular death (17–30% less, dependent on the type of statistical analyses). The deaths and cardiovascular events included nonfatal MI and stroke. Plasma triglycerides were lowered in the n-3 polyunsaturated fatty acid treated patients.

Several conclusions may be drawn from this study. Fish oil was given over the relatively long time period of 3.5 yr and it was safe. The authors expected a greater reduction in deaths as occurred in the DART study in Wales (29%). They attributed their less positive, but still positive, results to a number of coexisting factors. One problem was the relatively low death rate in the control group. To be noted is the fact that all of the patients were consuming the Mediterranean diet that in itself is associated with a lowered death rate from CHD, perhaps in part from more antioxidant consumption and more fish. A second possible problem was the ratio of EPA to DHA, which in most other studies was 1.5–2.0, but in this study was 0.5. EPA may be the more active of the two n-3 polyunsaturated fatty acids in its clinical effects; i.e., a direct antagonist of the cyclooxygenase enzyme that produces thromboxane A2 in platelets. It was felt that the reduction in death rate occurred largely because of the prevention of sudden death from
cardiac arrhythmias. This is the first large-scale trial proving that a low dose of fish oil (n-3 polyunsaturated fatty acids) over a 3.5 yr period of time saved lives. The evidence is steadily mounting about the cardioprotective effects of fish oil fatty acids.

The Lyon Diet Heart Study, a randomized secondary prevention trial (following a first MI), showed a remarkable 76% reduction in risk of cardiac death and nonfatal heart attack after 4 yr (25,26). Subjects followed a diet high in canola oil and canola oil margarine (high in the plant n-3 fatty acid alpha-linolenic acid). The diet was also low in fat and high in complex carbohydrate and fiber. Very recent data indicate that alpha-linolenic acid may have a direct effect on cardiac arrhythmias, whereas other cardiovascular effects are likely mediated through the synthesis of EPA and DHA (2). Conversion of alpha-linolenic acid to eicosapentaenoic acid is fairly rapid and measurable within a few days after the dietary ingestion of linolenic acid and consists of desaturation, elongation, and further desaturation, with the rate-limiting enzyme step being the first desaturation step brought about by \( \Delta^6 \)-desaturase.

3. THROMBOSIS

N-3 fatty acids from fish oil have invariably had an antithrombotic effect, particularly a diminution in thromboxane A\(_2\) that produces platelet aggregation and vasoconstriction (1,27,28). Platelet reactivity and adhesion were, therefore, considerably reduced after fish oil ingestion (29). There have been reductions also of PAI-1, fibrinogen, TPA, and increases in platelet survival and bleeding time (4). Enhanced fibrinolysis has also been observed (4). Perhaps even more significant was a study in baboons showing that n-3 fatty acids eliminated both vascular thrombus formation and vascular lesions after vascular injury (30). The baboons treated with fish oil showed decreases in thrombus formation at sites of surgical carotid endarterectomy. The intake of alpha-linolenic acid either has no effect or leads to decreased platelet aggregation when compared with linoleic acid (31,32). The function of the endothelium, important in both thrombosis and atherosclerosis, is affected by n-3 fatty acids (27). The production of prostacyclin is enhanced and endothelial-derived relaxation factor (EDRF), or nitric oxide, which is depressed in atherosclerotic disease, was greatly increased by the n-3 fatty acids of fish oil (33–35). Eight patients with coronary artery disease received 1.8 g/d EPA for 6 wk (36). EPA had beneficial effects on both nitric oxide-dependent and nondependent forearm vasodilitation. The production of prostacyclin was increased in rats fed 2.5% and 5% linseed oil (37). Fish oil supplementation has improved arterial compliance in diabetic subjects (38). Fish oil supplementation also inhibits norepinephrine-mediated vasoconstriction that also increases arterial compliance (39). Fifteen obese people with insulin resistance were fed four diets of 4 wk each. The 20 g/d alpha-linolenic acid (flaxseed oil) diet (40) caused a marked rise in arterial compliance; however, insulin sensitivity and HDL cholesterol decreased and LDL oxidizability increased.

4. EXPERIMENTAL ATHEROSCLEROSIS AND FISH OIL

When menhaden oil (a fish oil product) was incorporated in atherogenic diets fed to rhesus monkeys, aortic plaques and their cholesterol content were much less than in the nonfish-oil fed groups (41). Because the plasma lipid levels were roughly similar in control groups, the inhibition of atherosclerosis may have involved other mechanisms
operative in the vessel wall itself. Carotid atherosclerosis was likewise inhibited. Pigs fed an atherogenic diet had much less coronary atherosclerosis when given cod liver oil containing the n-3 fatty acids (42). There is good evidence that EPA and DHA from fish oil are even incorporated into advanced human atherosclerotic plaques (43). They are present in complicated plaques as components of cholesterol esters and phospholipids. The incorporation of EPA and DHA from the plasma lipoproteins into the plaques is detectable within a week of fish oil feeding. Perhaps the inhibition of atherosclerosis occurs because EPA and DHA inhibit cellular growth in the arterial wall (44). Atherosclerosis cannot develop even after injury and the influx of low-density lipoprotein (LDL) cholesterol and cholesterol ester unless there is also a cellular reaction. Two important cells in atherosclerosis are smooth muscle cells and macrophages. Because of the suppression of cellular growth factors by n-3 fatty acids, proliferation of smooth muscle cells was inhibited (45). Likewise, macrophage infiltration into the vessel wall was lessened by n-3 fatty acids (41). Even the initial lesion of atherosclerosis—the fatty streak—develops less under the influence of dietary n-3 fatty acids (41).

5. EFFECTS UPON THE PLASMA LIPIDS AND LIPOPROTEINS

A major effect of dietary n-3 fatty acids from fish oil is upon the plasma levels of lipids and lipoproteins (8,46). As will be shown, the science in this area is very clear: n-3 fatty acids in practical doses (<7 g/d) lower the plasma very low-density lipoprotein (VLDL) and triglyceride levels through depression of synthesis of triglyceride in the liver. n-3 fatty acids from fish oil also suppress postprandial lipemia, the chylomicron remnants of which are considered atherogenic. As VLDL concentrations decrease, LDL cholesterol rises, possible transiently. HDL cholesterol does not change. Like the drug gemfibrozil, n-3 fatty acids may cause an increase in LDL as they lower the plasma triglyceride concentration in some hyperlipidemic states such as familial combined hyperlipidemia as will be discussed in detail later. It is unclear whether n-3 fatty acids from plants (alpha-linolenic acid) have these actions. Alpha-linolenic acid from flaxseed oil has not been shown to lower the plasma triglyceride levels except in very large amounts (38 g/d) (47).

Theoretically, the ideal nutritional program to reduce the plasma lipid and lipoprotein concentrations maximally would be a very low cholesterol and saturated fat diet, which would upregulate the LDL receptor and reduce LDL plasma concentrations, combined with a diet containing fish oil, which would suppress VLDL production and lower plasma triglyceride concentrations. This point of view is buttressed by a well-controlled dietary study of fish oil and saturated fat fed as isolated variables. Saturated fat raised the plasma LDL cholesterol levels and fish oil lowered the plasma triglyceride levels (48).

5.1. Effects of Fish Oil in Normal Subjects

Several recent reviews have documented that n-3 fatty acids from fish have a great effect upon plasma lipids and lipoproteins, even in normal subjects (1,8,46). The principal action is upon the plasma triglyceride and VLDL concentrations. This hypolipidemic action is well illustrated in a study of 12 healthy adults (six men and six women) who were given three different diets fed in random order for 4 wk each: a saturated control diet, a salmon diet containing considerable amounts of n-3 fatty acids, and a vegetable oil diet high in n-6 fatty acids (49). Both the salmon diet and the vegetable oil
diet decreased the plasma cholesterol similarly, from 188 to 162 mg/dL. Both diets reduced LDL, from 128 to 108 mg/dL. HDL cholesterol levels were not changed by the salmon oil diet. The salmon diet decreased VLDL cholesterol levels and the changes in plasma triglyceride were most striking, from 76 to 50 mg/dL. The polyunsaturated vegetable oils did not lower VLDL and triglyceride levels.

5.2. Studies in Hyperlipidemic Patients

Because of the hypolipidemic effect of n-3 fatty acids from fish oil in normal subjects, it seemed most reasonable to test their effects in hyperlipidemic patients (9). The two groups of hyperlipidemic patients selected for study were characterized by hypertriglyceridemia because the depression of the plasma triglyceride and VLDL appeared to be a unique effect of n-3 fatty acids from fish oil.

Twenty hypertriglyceridemic patients volunteered for the study (eight men and twelve women). Ten of the patients presented with increased levels of both VLDL and LDL, consistent with the type II-b phenotype. Their mean plasma lipid levels at time of entry were 337 mg/dL for cholesterol and 355 mg/dL for triglyceride. Clinically, many of these patients had familial combined hyperlipidemia, a disorder characterized by a strong disposition to the development of coronary heart disease and by overproduction of lipoproteins, particularly VLDL.

The other 10 patients had apparent type-V hyperlipidemia, as characterized by increased chylomicrons and greatly increased VLDL levels in the fasting state. Their mean plasma lipid levels at entry were 514 mg/dL for cholesterol and 2874 mg/dL for triglyceride. Four of the type-V patients had concomitant noninsulin-dependent diabetes mellitus, and two had adult-onset insulin-dependent diabetes mellitus. Their insulin doses and diabetic control remained constant throughout the study despite the salmon oil.

Both overproduction of VLDL and impaired clearance of the remnants of chylomicron and VLDL metabolism characterize the type-V phenotype. Clinically, type-V patients have the “chylomicronemia” syndrome, which is characterized by episodes of abdominal pain from enlargement of abdominal viscera (hepatomegaly and splenomegaly) and by episodes of acute pancreatitis. These patients also suffer from eruptive xanthomata, neuropathy, and lipemia retinalis. Although LDL levels are low in patients with fasting chylomicronemia (type V), the presence of the atherogenic remnant particles predisposes them to the development of atherosclerotic complications, including CHD.

Special care was taken to make certain that the patients were in steady-state conditions before entry. Steady state was defined as a constancy of body weight and diet, and an absence of any residual hypolipidemic drug effect. Most of the patients had not been receiving any hypolipidemic drugs just prior to the study. In the patients previously given drugs, these were discontinued, and plasma lipid levels were monitored until pre-drug levels were attained.

Two different control diets were used for the two groups of hypertriglyceridemic patients, depending upon the phenotype of hyperlipidemia. Patients with combined hyperlipidemia (type II-B) received their usual low-cholesterol (100 mg/d) low-fat (20–30% of total calories) diet. Subsequent dietary periods for these patients consisted of a fish oil diet for 4 wk, followed, in some patients, by a 4-wk period of a diet high in a vegetable oil containing a predominance of n-6 fatty acids. Both of these diets were balanced for cholesterol content (approx 250 mg/d) and contained 30% of calories as fat. The diets in all periods were eucaloric, such that the subjects neither gained nor lost
weight.

For patients with fasting chylomicronemia (type V), the control diet consisted of a very-low-fat diet (5%), in order to lower plasma triglyceride levels maximally. The next dietary interval contained fish oil at 20% or 30% of total calories. Finally, a high n-6 vegetable oil diet was also provided, which contained 20%–30% of total calories as fat, and 200–300 mg of cholesterol per day. Both the fish oil and the vegetable oil diets were initially used cautiously in the patients with fasting chylomicronemia (type V) in order to minimize the risk of hepatosplenomegaly, abdominal pain, and acute pancreatitis.

The salmon oil diet provided about 20 g/d of n-3 fatty acids for a 2600-kcal intake, with 30% of total calories as fat. On the other hand, the vegetable oil diet provided about 47 g of the n-6 polyunsaturated fatty acid, linoleic acid. Thus, the fish oil diets actually provided 43–64% less total polyunsaturated fatty acids than the vegetable oil diet, gram for gram.

The fish oil diet decreased the plasma LDL-cholesterol levels in the patients with combined hyperlipidemia (type II-B) by 26 mg/dL. Of individual lipoprotein cholesterol changes, the decline of VLDL cholesterol was most striking; but LDL and HDL cholesterol also decreased. The plasma triglyceride changes were even greater than the cholesterol changes with the fish oil diet. The plasma triglyceride level decreased from 334 to 118 mg/dL. This occurred largely because of the change in VLDL triglyceride, which was lowered from 216 to 55 mg/dL.

The highly polyunsaturated n-6 vegetable oil diet had a much weaker effect upon VLDL cholesterol and triglyceride. LDL values were similar; but in contrast, HDL cholesterol was higher after the vegetable oil diet. Plasma apolipoprotein changes reflected the lipoprotein lipid changes. In the type II-b patients, there were significant reductions in apo B and C-III levels in the fish oil period, which paralleled the declines in LDL and VLDL levels.

In the type-V patients, effects of the fish oil diet were even more striking (Figs. 1 and 2). With consumption of the very-low-fat control diet, their initial plasma lipid levels declined considerably but still remained greatly elevated. Many of these patients still had milky-appearing plasma, with chylomicrons present in the fasting state. The first change to occur in these patients after the fish oil diet was the virtual disappearance of fasting chylomicronemia, which had been present in five of the patients. During the fish oil diet, total plasma triglyceride decreased from a control value of 1353 to 281 mg/dL, a drop of 79% (see Fig. 1). VLDL triglyceride decreased similarly, from 1087 to 167 mg/dL. Plasma cholesterol levels declined into the normal range after the fish diet, from 373 to 207 mg/dL (Fig. 2). Most of this total plasma-cholesterol decrease occurred as the result of marked changes in the amount of VLDL cholesterol, which decreased from 270 to 70 mg/dL. Of interest was the 48% concomitant rise of LDL cholesterol, from the low value of 84–125 mg/dL. Apolipoprotein levels changed to reflect the altered lipoprotein lipid levels. Apo A-1 levels did not change, whereas apo B, C-III, and E all decreased significantly.

When the n-6-rich vegetable oil replaced the fish oil in the diets of eight patients, all patients with fasting chylomicronemia (type V) had increases in plasma triglyceride levels within 3 to 4 d. After 10–14 d of the n-6 vegetable oil feeding, the mean plasma triglyceride values rose 198%, and VLDL triglyceride increased from 171 to 350 mg/dL. Plasma cholesterol also increased, from 195 to 264 mg/dL. LDL-cholesterol levels, on the contrary, were decreased 28% by the vegetable oil diet: another indication
that the metabolic abnormality of the type-V phenotype was worsening. Because of enhanced hypertriglyceridemia and the risk of development of abdominal pain typical of this type-V disorder, the vegetable oil feeding period was discontinued prematurely in all type V patients (9).

5.3. Implications of the Fish Oil Studies in Hypertriglyceridemic Patients

In the 20 hypertriglyceridemic patients, fish oil incorporated in the diet led to an even more profound hypolipidemic effect than had been observed in normal subjects. The plasma triglyceride levels decreased in each of the 20 patients, a 79% decrease in the type-V patients and a 64% decrease in the patients with combined hyperlipidemia (type II-B); plasma-cholesterol levels decreased 45% and 27%, respectively. In the 12 normal subjects previously investigated (49), decreases were less for plasma triglyceride (38%) and much less for plasma cholesterol (14%). Apparently, the greater the hypertriglyceridemia, the greater the reductions brought about by dietary fish oil—in plasma lipids, and especially in VLDL.

These results may have considerable therapeutic importance for patients with severe and moderate hypertriglyceridemia. The only dietary treatment to date for severely hypertriglyceridemic patients with fasting chylomicronemia (type V) has been the very severe, and therapeutically difficult restriction of dietary fat to between 5% and 10% of
total calories in an effort to approach normal plasma triglyceride levels. Americans find this possible to do on a short-term basis, but very difficult on a long-term basis because they are accustomed to eating higher quantities of fat; i.e., approx 40% of total calories. Therefore, all fatty foods have been contraindicated in patients with fasting chylomicronemia (type V). The findings of this study suggest that some fatty, and even high-cholesterol, foods (i.e., fish or shellfish) containing marine n-3 fatty acids are quite appropriate for ingestion and may produce further triglyceride lowering over and above that which results from the very low-fat diet.

Other studies in familial-combined hyperlipidemia and in type-IV hyperlipidemia have shown increases in LDL and apo B, whereas plasma VLDL and triglyceride values were declining \((8,50,51)\). Such LDL increases have also occurred in type IV patients given the drug gemfibrozil. Perhaps this is an expected physiological action when hypertriglyceridemia is being corrected. Should the LDL levels become abnormally high after either drugs or fish oil, then further therapy of the LDL specifically is warranted (i.e., bile acid-binding resins or one of the statins such as lovastatin). Fish oil has also produced plasma cholesterol and triglyceride lowering in type-III patients and in familial hypercholesterolemia \((52)\).

5.4. Reduction of Postprandial Lipemia After Fatty Meals

It has been observed that fish oils markedly decreased the usual chylomicronemia that follows fatty meals \((53,54)\). In other words, fat tolerance was greatly improved (see Fig.
3). This improvement could result from diminished absorption, slower synthesis, and slower entry of chylomicrons into the circulation or, alternatively, from a more rapid removal of the chylomicrons that do appear in the circulation. There is no evidence for diminished absorption, and fat balance studies have not shown increased fat excretion in stools after dietary periods enriched with fish oil. Whether reduced chylomicron production or enhanced removal of chylomicrons is responsible has not yet been completely clarified. Fish oil feeding produces smaller VLDL particle size in animals compared to vegetable oil feeding. Smaller VLDL would have, then, an enhanced catabolism. Perhaps, after a background diet of fish oil, chylomicrons are smaller in size and hence more rapidly catabolized, the result being a much flatter fat tolerance curve (53).

5.5. The Mechanism of the Hypolipidemic Effects of Fish Oil

How n-3 fatty acids exert their effects to decrease the levels of plasma triglyceride and cholesterol has been tested in humans in two different sets of experiments: (1) the inhibition by fish oil of the usual hypertriglyceridemia that inevitably results when a high-carbohydrate (CHO) diet is suddenly fed to humans; and (2) the effects of fish oil upon apo B, VLDL, and LDL production rates and turnovers.

5.6. Fish Oil and the Inhibition of Carbohydrate-Induced Hypertriglyceridemia

The well-known phenomenon of carbohydrate-induced hypertriglyceridemia is a physiologic response. In this model, VLDL triglyceride synthesis is stimulated as the dietary CHO intake abruptly increases. The increased VLDL synthesis leads to hypertriglyceridemia, which may persist for many weeks. If n-3 fatty acids do inhibit VLDL synthesis, then the usual CHO-induced hypertriglyceridemia should not occur when fish oil is incorporated into the high-CHO diet.

Seven mildly hypertriglyceridemic, but otherwise healthy subjects, (ages 22–54 yr) were fed three different experimental diets (55). Each was composed of a liquid formula plus three bran muffins per day to supply fiber. The baseline diet contained 45% of calo-
The high-CHO diets were then divided into control and fish groups; both containing 15%, 10%, and 75% of calories as fat, protein, and CHO, respectively. In the baseline and high-CHO control diets, a blend of peanut oil and cocoa butter provided the fat, which was replaced by fish oil, in the form of a commercially available marine lipid concentrate, in the high-CHO fish oil diet. The total amount of fish oil consumed per day was 50 g (in a 3000-kcal diet), equivalent to approx 3.3 tablespoons of oil. This amount provided 8.5 g of EPA and 5.5 g of DHA.

The three experimental diets were fed in three different sequences in the Clinical Research Center (Fig. 4). In the first sequence, the high-CHO control diet preceded the high-CHO fish oil diet (Fig. 4A). In the second sequence, the high-CHO diet was given for 20 d instead of 10 d in order to demonstrate that the hypertriglyceridemia did not spontaneously resolve after the first 10 d. It was then followed by the fish oil diet (Fig. 4B). In the third sequence, the fish oil was fed first with the high CHO diet for 25 d and then removed to permit the effects of the high CHO to be manifest for the next 15 d (Fig. 4C). Three subjects were studied with the first sequence, and two subjects each were studied with the second and third sequences.

In all seven subjects, the high-CHO control diet increased the plasma triglyceride levels over the baseline diet: from 105 to 194 mg/dL (55). The magnitude of the CHO-induced hypertriglyceridemia correlated significantly with each individual’s baseline triglyceride levels. The rise in plasma triglyceride levels was complete by d 5 and resulted almost entirely from an increase in the VLDL triglyceride fraction, which more than doubled during the control diet: from 69 to 156 mg/dL (Fig. 4). Although the total plasma cholesterol levels did not change, VLDL cholesterol levels approximately doubled: from 18 to 34 mg/dL; and HDL cholesterol was reduced: from 49 to 41 mg/dL.

When the fat of the high-CHO control diet was replaced isocalorically with fish oil, the elevated plasma triglyceride concentration was reduced from 194 to 75 mg/dL, a decrease of 61%. This decrease usually occurred within 3 d (Fig. 4A). Once again, changes in VLDL triglyceride levels were largely responsible for this effect (156 to 34 mg/dL) (Fig. 5). Total cholesterol levels decreased insignificantly during the high-CHO fish oil diet—from 172 to 153 mg/dL—primarily because of the drop in VLDL cholesterol levels (34 to 12 mg/dL) (55).

The hypertriglyceridemia persisted even when the period of CHO induction was prolonged from 10 to 20 d and did not significantly decrease until fish oil was incorporated into the diet (Fig. 4B). When the high-CHO fish oil diet followed the baseline diet, the plasma triglyceride level did not rise, but the level increased when the high-CHO control diet was fed subsequently (Fig. 4C). The high-CHO control diet decreased the levels of apo B and increased apo C-III concentrations; apo A-1 and E levels did not change. The high-CHO fish oil diet decreased apo A-1 and apo C-III levels; apo B and E concentrations did not change.

The incorporation of corn oil in place of fish oil into the high-CHO regimen failed to prevent the induced hypertriglyceridemia. For the three subjects who participated in this study, the average triglyceride levels were as follows: baseline, 93 ± 23 mg/dL; high-CHO control, 196 ± 58 mg/dL; high-CHO corn oil, 215 ± 90 mg/dL; and high-CHO fish oil, 86 ± 10 mg/dL.

Dietary fish oil not only prevented, but also rapidly reversed, the dietary, CHO-induced elevations in plasma triglyceride and VLDL levels, whereas the n-6 fatty acid-rich corn oil had no effect at all. Because the primary difference between corn oil and...
Chapter 9 / n-3 Fatty Acids from Fish

the commercial fish oil preparation is the type of polyunsaturated fatty acids present (corn oil, 57% 18:2 n-6; linoleic acid; the commercially available fish oil preparation, 32% n-3 fatty acids), the difference in effect was caused by to the n-3 fatty acids in the fish oil. This finding implied a probable inhibitory effect of n-3 fatty acids on hepatic VLDL production.

5.7. Fish Oil and the Synthesis and Turnover of Apo B, VLDL, and LDL

The hypothesis that n-3 fatty acids probably reduced VLDL levels by inhibiting VLDL synthesis was supported by studies designed to elucidate further mechanisms of the hypotriglyceridemic effect of n-3 fatty acids. Dietary fish oil either affected the synthesis or the removal of VLDL. The rates of flux and turnover of VLDL triglyceride...
were measured after injection of $^3$H-glycerol into people studied under two dietary protocols, one containing fish oil and the other containing fats typical of the American diet (56). This technique permitted the calculation of both synthetic and removal rates of VLDL.

Ten male subjects were selected on the basis of having a wide range of fasting plasma triglyceride concentrations, from 34 to 4180 mg/dL, so that the hypothesis about the mechanism of action of dietary fish oils could be tested in subjects with greatly different pool sizes of plasma triglyceride. Liquid formula diets containing 15–20% fat, 65–75% CHO, and 10–15% protein were fed during both the control and the fish oil dietary periods. The two diets differed only in the type of fat they contained. In the control diet, a blend of cocoa butter and peanut oil (1:2) was incorporated into the formulas. The fish oil diet containing the commercial preparation was taken in three divided doses daily and was not mixed into the formulas. The principal difference between the two diets was the higher content of linoleic acid (18:2 n-6) in the control diet, and the presence of n-3 fatty acids in the fish oil diet. The former diet contained virtually no n-3 fatty acids, whereas the latter provided about 17 g/d of these highly polyunsaturated fatty acids.

The experimental diets were consumed for a period of 3–5 wk before the actual VLDL turnover procedure was conducted. This time was needed for the plasma triglyceride levels to stabilize, particularly in the subjects whose triglyceride levels were above normal. Seven subjects consumed the control diet first, followed by the fish oil diet; in the remaining three, the order was reversed. The order in which the diets were admin-
The isocaloric substitution of fish oil for the control vegetable fat produced the expected significant reductions in the total and lipoprotein lipid levels in all 10 subjects. Total cholesterol levels for all 10 subjects fell from 195 to 144 mg/dL, a reduction of 22%. Decreases in VLDL levels accounted for most of the drop in plasma cholesterol (83 to 21 mg/dL). LDL cholesterol levels did not change significantly, whereas HDL cholesterol concentrations fell from 31 to 24 mg/dL. All of these changes were evident in both the normal and the hypertriglyceridermic groups.

After the administration of \(^3\)H-glycerol and its incorporation into the triglyceride of VLDL, the decay curves were analyzed by computer-models, so that VLDL synthesis and turnover could be calculated. The incorporation of n-3 fatty acids into the diet caused a 72% decrease in the VLDL triglyceride pool size (11.4 to 3.2 g; \(p < 0.025\)). The decreased pool size was associated with a 45% reduction in the VLDL triglyceride synthetic rate (23 to 12.6 mg/h/IW; \(p < 0.005\)) and a 45% decrease in the residence time of VLDL triglyceride in the plasma (5.8 to 3.2 h; \(p < 0.005\)). The reciprocal of the residence time is the fractional catabolic rate (FCR), which was increased by 65% (0.23 to 0.38 h\(^{-1}\); \(p < 0.005\)). There was a significant rise in the cholesterol/triglyceride ratio in VLDL during the fish oil interval (0.18 to 0.25; \(p < 0.05\)). Finally, the ratio of the fast to the slow synthetic pathways did not change with fish oil feeding. The same trends were seen in both normal and hypertriglyceridermic patients. Similar results have also been found by a slightly different dietary plan and with the labeling of VLDL apo B with \(^{125}\)I (57). There was a striking reduction of VLDL synthesis and enhanced turnover.

Direct evidence that the hepatic synthesis of triglyceride and VLDL is suppressed by n-3 fatty acids from fish oil has been seen in three in vitro studies of the perfused rat liver and in studies of liver cells from rats and rabbits in primary culture (58–60). In all of these studies, triglyceride synthesis was reduced. In one, enhanced ketone body production resulted; in the others there was a diversion of n-3 fatty acids from triglyceride synthesis into phospholipid synthesis (60). When the net results of the human and animal studies are taken together, the evidence is very strong that suppression of VLDL and triglyceride synthesis is a primary mechanism for the hypolipidemic effects of n-3 fatty acids, coupled with an increased fractional catabolic rate of VLDL.

### 5.8. Fish Oil and LDL Turnover

Labeled LDL turnover studies have been carried out in normal subjects given fish oil. It was demonstrated that there was a decreased synthesis of LDL and a tendency for an increased fractional catabolic rate (61). Spady and colleagues have shown enhancement of LDL receptor activity after the administration of fish oil in the rat (62). This latter result fits in very well with the increased fractional catabolic rate observed in the normal human subjects. It seems very clear that the n-3 fatty acids from fish oil have major effects upon all of the major lipoproteins with the exception of HDL.

### 5.9. Summary and Conclusions:

#### Fish Oil Effects Upon Plasma Lipids and Lipoproteins

The n-3 fatty acids from fish oil and fish have been shown to have a remarkable effect upon the synthesis and clearance of triglyceride-rich lipoproteins, especially VLDL and chylomicrons. Even LDL synthesis and clearance have been affected. Because of these significant effects upon lipoprotein synthesis and clearance, beneficial effects of
fish oil have been demonstrated in a variety of hyperlipidemic states, especially those with conditions such as hypertriglyceridemia and chylomicronemia. Therapeutic implications for fish oil are especially positive in type V, type IV, and type III hyperlipidemia. A similar effectiveness has been shown in hypertriglyceridemic diabetic patients without affecting glucose homeostasis (63).

Difficulty in interpreting the effects of fish oil in various hyperlipidemic patients has occurred because of vastly different experimental conditions (8). In some studies, fish oil was simply added as a supplement to the usual diet in doses of 8–16 g/d. In the control period, a placebo oil such as olive oil or safflower oil was not always utilized. In other studies, there was the customary diet plus the use of an appropriate placebo oil. Furthermore, various kinds of fish oils have been utilized, some containing a considerable amount of cholesterol and saturated fat. Newer fish oils have less-saturated fatty acids, have higher concentrations of n-3 fatty acids, and a lower cholesterol content.

Some conclusions have emerged from the wide variety of studies, most of which have not been controlled for caloric and body weight stability. Fish oil is most effective when the diet is well controlled. In these studies, LDL lowering has usually occurred, as well as profound VLDL and triglyceride lowering in normal subjects and in a wide variety of hyperlipidemic states. In our experience this lowering of plasma cholesterol levels has occurred in patients with elevated plasma triglyceride concentrations (types V, IIa, IIb, III, and IV hyperlipidemia), with the most dramatic results occurring in the patients with fasting chylomicronemia (type V) who do not tolerate any other kind of dietary fat (8,9,46,64). In the literature and in our experience, HDL levels have not been greatly affected by fish oil. Clearly the use of fish oil in hyperlipidemia must be individualized as to both use and dosage. Lower doses of fish oil (8–15 g/d) or 2.4–4.5 g of n-3 fatty acids (EPA and DHA) particularly lower the plasma triglyceride levels (8).

Why the plasma LDL and apo B have, at times, increased after fish oil administration when, at the same time, the plasma VLDL and triglyceride have decreased is a most challenging question and may relate to fundamental aspects of VLDL-LDL metabolism. Normally, LDL is derived from two sources: conversion from VLDL and direct synthesis from the liver. The catabolism of VLDL is likewise in two directions through intermediate density lipoprotein (IDL). This lipoprotein may be removed by the apo E receptor in the liver or converted to LDL. The animal experiments of Huff and Telford suggest why, in some instances, fish oil might increase LDL (65). Turnover studies in the miniature pig revealed that fish oil feeding increased the proportion of VLDL being converted to LDL. Apparently, the n-3 fatty acids of fish oil produce a smaller VLDL particle, which is more likely to be converted to LDL. In this pig study, LDL concentrations, however, did not increase because the direct synthesis of LDL was reduced more by fish oil than the increase in LDL from VLDL. These pig studies await confirmation in humans. They do explain why LDL may increase in some humans fed fish oil: more VLDL is converted to LDL and direct LDL synthesis does not decrease, thus adding up to more LDL. LDL turnover studies have shown decreased production of LDL in normal humans given large amounts of salmon oil vs vegetable oil (61). In this study, the plasma LDL decreased after n-3 fatty acids.

6. FISH OIL IN DIABETIC PATIENTS

In diabetic patients, there is enhanced risk for vascular disease, so that the use of fish oil might be particularly desirable if glucose control is not disturbed. The literature
is controversial regarding the effects of n-3 fatty acids in diabetic patients (66,67). In type-I, insulin-dependent diabetics, there is universal agreement that glucose control is not hampered with fish oil supplementation and that the beneficial effects upon the plasma lipids and lipoproteins have been demonstrated. In type-II adult-onset noninsulin-dependent diabetic patients, the results have been somewhat conflicting, possibly because such patients are very susceptible to the caloric load imposed. In most studies, the plasma triglyceride and VLDL concentrations have declined, but some studies have shown the deterioration in glucose homeostasis. This literature has recently been reviewed by Heine and colleagues (66). Most of the studies have been short term and, in some, caloric control has been somewhat distorted by the administration of calorie-dense fish oil without there being a suitable placebo. The addition of fish oil to the usual diet would be hypercaloric, thereby disturbing glucose control. When there is attention to the caloric content of the supplement, then there are beneficial effects on the plasma triglyceride and VLDL without disturbing glucose homeostasis as illustrated in the following experiments.

These problems and objections were considered in an experimental design of a study in 16 adult-onset diabetics who were randomized to a double-blind placebo-controlled crossover study (63). The subjects of the study were overweight and most were receiving hypoglycemic agents. There was a 3-mo stabilization baseline period in which they were given a eucaloric, lower fat (30% of the calories from fat), high-complex CHO diet with 55% from carbohydrate. This was followed by two 6-mo intervention periods in which the subjects continued on the same diet and received a supplement of 15 g/d of either olive oil or fish oil. The fish oil contained 6 g/d of n-3 fatty acids. The end points of the study were plasma lipid and lipoprotein concentrations and glucose homeostasis. The plasma triglyceride concentrations were much lower with the fish oil preparation vs olive oil (260 vs 449 mg/dL). VLDL cholesterol was lower and VLDL triglyceride as well. The total plasma cholesterol was unchanged. There was a significant increase in LDL cholesterol, as has been aforementioned, when hypertriglyceridemic individuals are given fish oil, from 117 to 145 mg/dL. HDL cholesterol did not change.

However, the effects of fish oil upon glucose homeostasis revealed no difference from the 6-mo period of olive oil administration. Body weights were unchanged. Fasting glucose levels were 172 and 178 mg/dL, olive oil vs fish oil. Another measure of diabetic control, hemoglobin A-1 C, revealed no difference as did also the 24-h urinary glucose excretion, the plasma C-peptide, and the 24-h urinary C-peptide.

In view of the extremely high mortality from CHD in adult-onset diabetic patients, this hypolipidemic action of fish oil was of interest because diabetic control did not deteriorate and there were significantly beneficial plasma lipid-lipoprotein effects. The other actions of the n-3 fatty acids from fish oil in inhibiting the development of atherosclerosis, in preventing thromboxane A2 formation, in increasing endothelial-derived relaxing factor and in inhibiting platelet-derived growth factor would all be additional reasons for postulating a therapeutic benefit from the use of fish oil in diabetic patients.

7. HYPERTENSION

n-3 fatty acids in 11 studies have been uniformly associated with a mild decrease in systolic blood pressure uniformly and, at times, a decrease in diastolic blood pressure, particularly in the upright position (4). This has especially occurred in mild hyperten-
A randomized controlled trial was conducted to study the effects of fish intake and weight loss on blood pressure in medication-treated overweight hypertensives. Combining a daily fish meal with weight loss resulted in additive decreases in ambulatory blood pressure and decreased heart rate. The suggested mechanism has been an attenuation in the responses of forearm vascular resistance and blood flow to angiotensin; i.e., less vascular reactivity. Because the decreases in both systolic and diastolic pressures are not great, even in the best studies (4.6 and 3.0 mm Hg), fish oil cannot be regarded as a single treatment modality for hypertension. However, when used for other purposes, the mild blood pressure-lowering effect of n-3 fatty acids would certainly provide an added benefit.

8. RECOMMENDATIONS

The intake of n-3 fatty acids from fish and plants should definitely be increased to prevent CHD. This could be best in the form of two to three fish meals per week in the context of a low-fat diet. Fish, of course, could be substituted for meat in the diet. An intake of alpha-linolenic acid of 2 g/d or 1% of energy has been suggested. At the same time, the diet should be reduced in fat content to 20% of the total calories with a high-carbohydrate high-fiber intake. The intake of cholesterol should be limited to 100 mg/d.

Table 1 provides the fat content and n-3 fatty acid content for a wide variety of fish and shellfish. All fish and shellfish contain the n-3 fatty acids, even when the fat content is rather low, as it is in shellfish. The lower the fat content, the higher the percentage of n-3 fatty acids that are present in a given fish or shellfish. The goal of this recommendation is to produce an increased content of the n-3 fatty acids, EPA and DHA, in the blood and tissues of the body. This will occur if there is regular consumption of fish and shellfish (at least to the extent of 200–300 g/wk), such that these n-3 fatty acids will be present in the diet.

With wild fish stocks being depleted, consumers will more and more find only farmed fish on the markets, especially salmon and catfish. An important question is the EPA and DHA content of farmed fish. Preliminary data from our laboratory indicate that EPA and DHA is 50 to 75% lower in farmed catfish. Farmed and wild salmon have similar amounts of EPA and DHA. Additional analyses are needed to confirm these data.

Table 2 provides the n-3 fatty acid (alpha-linolenic acid) content from various oils and foods. Daily consumption of 15 g (1 tablespoon) canola oil or canola oil margarine provides as much as 1 g alpha-linolenic acid/d. Other rich dietary sources of alpha-linolenic acid include English walnuts and flaxseed oil. Alpha-linolenic acid is also a prominent fatty acid of green leafy vegetables; however, because these vegetables have such a low fat content, the net amount of alpha-linolenic acid ingested from these sources is small. Human milk, but not cow’s milk, is a good source of the n-3 fatty acids including alpha-linolenic acid, EPA, and DHA. The amounts in human milk (2.2% of total fatty acids) provide a good basis for considering similar amounts in the diets of children and adults. In recognition of the importance of n-3 fatty acids in human nutrition, the producers of infant formulas are including soy oil, an excellent source of alpha-linolenic acid, as one of the fat ingredients instead of corn and coconut oils, which are poor sources. Likewise, intravenous fat preparations use soy oil instead of safflower oil which is poor in n-3 fatty acids.

Should it be desired to ascertain what the long-term intake of fish and shellfish has been in the past, there are excellent markers to document this point. The markers would be the measurement of the n-3 fatty acids in the plasma, which would reflect a more immediate intake, their measurement in red blood cells which, because of the greater half-
### Table 1
Fat and n-3 Fatty Acid (Eicosapentaenoic Acid and Docosahexaenoic Acid) Content of Seafood and Fish Oils

<table>
<thead>
<tr>
<th>Seafood (100 g, edible portion, raw)</th>
<th>Fat (g)</th>
<th>n-3 fatty acids (EPA &amp; DHA) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anchovy, European</td>
<td>4.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Bass, striped</td>
<td>2.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Bluefish</td>
<td>6.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Carp</td>
<td>5.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Catfish, channel</td>
<td>4.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Cod, Atlantic</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Cod, Pacific</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Flounder, unspecified</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Haddock</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Halibut, Pacific</td>
<td>2.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Herring, Atlantic</td>
<td>9.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Herring, Pacific</td>
<td>13.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Mackerel, Atlantic</td>
<td>13.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Mullet, unspecified</td>
<td>4.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Ocean perch</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Pike, Walleye</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Pompano, Florida</td>
<td>9.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Sablefish</td>
<td>15.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Salmon, Atlantic</td>
<td>5.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Salmon, Chinook</td>
<td>10.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Salmon, pink</td>
<td>3.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Salmon, Sockey</td>
<td>8.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Sardines, in sardine oil*</td>
<td>15.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Snapper, red</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Sole</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>3.3</td>
<td>0.3</td>
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<tr>
<td>Swordfish</td>
<td>2.1</td>
<td>0.2</td>
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<tr>
<td>Trout, brook</td>
<td>2.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Trout, lake</td>
<td>9.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Trout, rainbow</td>
<td>3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Tuna</td>
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<td>0.5</td>
</tr>
<tr>
<td>Crustaceans</td>
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<td></td>
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<tr>
<td>Crab, Alaska King</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Crab, Dungeness</td>
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<td>0.3</td>
</tr>
<tr>
<td>Crayfish, unspecified</td>
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<td>0.1</td>
</tr>
<tr>
<td>Lobster, Northern</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Shrimp, unspecified</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Mollusks</td>
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<td></td>
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<tr>
<td>Abalone, New Zealand</td>
<td>1.0</td>
<td>Trace</td>
</tr>
<tr>
<td>Clam, Hardshell</td>
<td>0.6</td>
<td>Trace</td>
</tr>
<tr>
<td>Clam, Littleneck</td>
<td>0.8</td>
<td>Trace</td>
</tr>
<tr>
<td>Mussel, blue</td>
<td>2.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Octopus, common</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Oyster, Pacific</td>
<td>2.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Scallop, unspecified</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Squid, unspecified</td>
<td>1.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Analysis by the Atherosclerosis Research Laboratory, Portland, OR.
life of these cells, would reflect the intake over a longer period of time, and finally, biopsies of the adipose tissue whose fatty acids would reflect the intake over many months and years (73).

For the intensive treatment of various forms of hyperlipidemia, as well as the production of an antithrombotic state, fish oils would need to be utilized, in addition to the consumption of fish. The dose of fish oil might well be from 6–15 g/d, titrated according to the end point desired.

For people who are unable to consume fish or shellfish, the use of fish oil would again be advisable. For primary prevention, 2–3 g/d would be desirable. Higher doses, as aforementioned, should be used for secondary prevention and the attainment of discrete end points of plasma lipid and lipoprotein levels and platelet function.

9. CONCLUSIONS/RECOMMENDATIONS

The n-3 fatty acids from fish, fish oil, and plants greatly inhibit the atherosclerotic process, coronary thrombosis, and cardiac arrhythmias by a variety of actions and should be considered as an important therapeutic modality in patients with already established coronary heart disease and to prevent coronary disease in highly susceptible people.

REFERENCES

III PREVENTION OF MAJOR DISABILITIES: IMPROVEMENT IN HEALTH OUTCOMES
The Relationship Between Nutritional Factors and Age-Related Macular Degeneration

Shirley Hung and Johanna Seddon

1. INTRODUCTION

Age-related macular degeneration (AMD) is the primary cause of incurable blindness in the United States (1). Among people aged 65 yr and older, approx 25% have signs of age-related maculopathy, which include large or confluent drusen, retinal pigmentary changes, geographic atrophy, and exudative disease (2). About 7% of persons 75 yr of age or older have advanced AMD with visual loss (2). We expect to see 1.9 million cases of advanced AMD with visual loss in this age group by the year 2025 (2,3). It is also estimated that 25.7 million people age 45 and older will have signs of either early or late AMD in the year 2025, 23.1 million with signs of early AMD, and 2.6 million with late AMD (2,3). Given the large public health impact of this disease, research focused on causes of this disease is essential.

Treatment for AMD is essentially limited to laser photocoagulation (4–6). This method of treatment is effective for only a small portion of patients. The causes of AMD have not been identified, although the possible role of low intake of antioxidant vitamins and minerals has recently received increasing attention (7). The biologic plausibility of this diet-health hypothesis focuses on the control of oxidative byproducts, in particular the reactive oxygen species (ROS). Current theories on oxidative damage estimate that the body is exposed to $10^{10}$ ROS mols/d under normal, metabolic conditions resulting from endogenous sources alone (8). Oxidative damage from environmental factors, such as exposure to sunlight, is also thought to increase ROS (9). It is plausible that through dietary intervention, increasing the intake of antioxidant nutrients could have a major impact on the control of excessive ROS damage.

This chapter reviews current theories on the pathogenesis of AMD and the potential role of specific dietary nutrients in its development or progression. Current epidemiologic and animal experimental evidence regarding nutrition and its relationship with AMD, as well as a review of ongoing studies, recommendations for future studies and possible nutritional interventions to reduce damage as a result of photooxidative insults are discussed.

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2. DIET-RELATED THEORIES ASSOCIATED WITH THE DEVELOPMENT OF AMD

2.1. Antioxidant Theory

Because normal physiological processes produce free radicals or ROS (8), controlling the damaging effects of ROS is essential for maintaining good health. According to their functions, two types of antioxidants are most relevant to AMD. Protective antioxidants act by reducing the formation of radicals and reactive oxygen species by decomposition of hydrogen peroxide without generating radicals (glutathione peroxidase, glutathione S-transferase, peroxidase, catalase), or by quenching active oxygen (carotenoids, superoxide dismutase) (9). Radical-scavenging antioxidants can either inhibit chain initiation or break chain propagation by trapping a radical before it reaches its cellular target (vitamin C, vitamin E, carotenoids) (9).

The retina may be prone to oxidative damage because of the abundance of long-chain lipids (10,11). The macula, in particular, may be the most prone to oxidative damage because of its increased exposure to blue light and higher concentrations of highly unsaturated long-chain fatty acids (12). The electromagnetic energy carried in a photon of visible blue light (450–500 nm/s), is higher than the energy of a photon of visible light in the red spectrum (650–700 nm/s). In the presence of a photosensitizer (natural cell constituents that absorb light, such as porphyrins, flavins, such as riboflavin (13), ketones, quinone, aromatic molecules, and other compounds) and blue light, molecular oxygen in its ground state—known as triplet oxygen—is converted into its high energy form, singlet oxygen (1O2). Singlet oxygen has paired antiparallel electrons, whereas other free-radical ROS produced by regular oxidative processes have an unpaired electron (molecular oxygen contains two electrons with parallel spin states). The flipping of one of the electrons in singlet oxygen results in a higher energy potential, and thus makes it an oxidative agent capable of causing damage to DNA, proteins, lipids, and carbohydrates in the eye.

Several nutrients with antioxidant capacities can quench different types of ROS (see Table 1). Carotenoids have been shown to be ideally suited to quench singlet oxygen (8) whereas vitamin E is uniquely suited to stop nonenzymatic peroxidation of lipids (15). It seems reasonable to infer that if dietary intake of several nutrients with antioxidant capacities—carotenoids, vitamin C, vitamin E, zinc, selenium, copper—is less than optimal, the body’s ability to regulate ROS may be compromised (8,15). Just as it has been suggested that antioxidants can inhibit oxidative modification of LDL thereby preventing or slowing the progression of atherosclerosis (8), we speculate that a similar mechanism may also exist for antioxidants in the eye. Inadequate levels of antioxidants may lead to excess oxidized byproducts accumulating in the retinal pigment epithelium (RPE) (16). It is unknown at what point the process may become irreversible.

2.2. Atherosclerotic Theory

Although the pathogenesis of AMD is still not well understood, an alternative theory suggests that the dietary risk factors associated with atherosclerosis (17) are also common to AMD. Vascular circulation to the retina may be compromised through the narrowing of the choriocapillaris possibly because of the formation of atherosclerotic-like plaques. This may result in a decreased supply of oxygen and nutrients to the retina, as well as impairing heat dissipation when light strikes the RPE (18,19).
Another possible mechanism is based on susceptibility of lipids to peroxidation by ROS as a result of insufficient levels of antioxidants. The presence of two compounds, lipofuscin (an age-related pigment found in all older animals) and malondialdehyde (product of autoxidized polyunsaturated fatty acids and a possible intermediate compound of lipofuscin), found in atherosclerotic plaques and in RPE deposits, are indigestible by cellular enzymes and are thought to be products of lipid oxidation. The accumulation of these deposits in healthy tissue can result in necrosis or death of functioning tissue. The limitation of this theory is the lack of evidence linking autoxidation and lipofuscin accumulation in the RPE. The key to the understanding of the pathogenesis of AMD may be the inability to effectively prevent or remove nondigestible compounds from healthy eye tissue.

In the literature, only two studies have found an association between cardiovascular disease and AMD. Cardiovascular disease (CVD) risk factors, smoking, hypertension, and elevated blood cholesterol, have been reported to be risk factors for AMD also. Postmenopausal hormone use is reported to be inversely associated with exudative AMD. The relationship between dietary risk factors for CVD, such as saturated fat, trans-fatty acids, and cholesterol and AMD is not yet clear. The role of long-chain omega-3 fatty acids, such as docosahexaenoic acid (DHA), may be directly related to AMD development because high concentrations of these types of fat are found in photoreceptor tissue. Studies investigating these specific types of fat are in progress.

3. PROPERTIES OF RELEVANT NUTRIENTS AND THEIR FUNCTIONS

Micronutrient deficiencies have been shown to play critical roles in the etiology of many diseases. This may also be the case for AMD. Although genetic susceptibility may be an important risk factor, low intake of key nutrients during the critical period of disease initiation may have just as much influence in the development and progression of the disease. This has yet to be determined.

3.1. Nutrients Involved in the Antioxidant Theory

3.1.1. Nutrient Dependent Enzymatic Complexes

As aforementioned, antioxidants fall into two classes: preventive antioxidants and chain-breaking antioxidants. Oxidoreductases are major antioxidant regulators and comprise a broad class of enzymes that include: oxidases, dehydrogenases, hydroperoxi-
A major class of oxygenases are enzymes in the cytochrome P450 system, whose primary function is to detoxify substances such as drugs, pollutants, and chemical carcinogens (xenobiotics) adding an oxygen atom to the substrate to inactivate them (15). There are three oxidoreductase enzymes that may be relevant to AMD.

3.1.1.1. Catalase. Catalase is an enzyme that has four heme groups and is found in the cytosol (15). It plays a major role in catalytic splitting of hydrogen peroxide into water and molecular oxygen (15). Catalase activity in human RPE has been shown to be six times greater than in other ocular tissues (28), suggesting that it is an important component of the RPE antioxidant system (28). Catalase activity also has been observed to decrease with age as degeneration in both macular and peripheral RPE increases (28).

3.1.1.2. Superoxide Dismutase. Superoxide dismutase is a metalloenzyme found in all aerobic cells. It quenches superoxide anion free radicals produced by cellular respiration (15). Superoxide dismutase generates hydrogen peroxide using two molecules of superoxide and requires both zinc and copper for catalytic function (15). The hydrogen peroxide can then be converted into water and molecular oxygen by catalase or glutathione peroxidase (15). Superoxide dismutase activity has not been shown to decrease with age (28).

3.1.1.3. Glutathione Peroxidase. Glutathione peroxidase, also found in the cytosol, provides a second line of defense against hydroperoxides, which can damage membranes and other cell parts (14). Selenium is an essential part of this enzyme (see Subheading 3.1.4.2.). Selenium, as part of glutathione peroxidase, appears to act synergistically with tocopherol, vitamin E, in the regulation of lipid peroxidation (14). In tandem with catalase, glutathione peroxidase degrades hydrogen peroxide to water via glutathione reductase and flavin adenine dehydrogenase (FAD) in the pentose phosphate shunt (14). One recent cross-sectional study found a positive association between late AMD and higher levels of plasma glutathione peroxidase in multivariate models. The association was statistically significant only for the middle vs lowest quintile of plasma glutathione peroxidase (RR = 9.9, 95% CI = 1.3–73.9), but not the highest quintile (RR = 6.7, 95% CI = 0.8–55.4) (28a).

3.1.2. Vitamin C. Vitamin C, or ascorbic acid, has antioxidant ability that is well known (29–31). Ascorbate is capable of reacting with a broad category of reactive oxygen species, making it an excellent first line of defense against oxidative damage (15). Ascorbate, as an endogenous antioxidant in plasma, can protect against peroxidative damage caused by aqueous peroxyl radicals and the oxidants released from activated neutrophils (32). Ascorbate in the cytosol has been shown to regenerate oxidized vitamin E found primarily in cellular lipid membranes (33). One study has demonstrated that levels of ascorbic acids can be increased in the lens, aqueous humor, and plasma through dietary supplementation (34). Vitamin C supplements and/or vitamin C-rich foods have been shown to increase the concentrations of ascorbic acid found in the lens, aqueous humor, and plasma (35). Although a protective dose–response relationship against retinal injury was found in animals (36–38) exposed to intense light, it is not supported in human epidemiologic studies to date (23,39–42).

3.1.2.2. Vitamin E. Vitamin E is the generic name for a class of fat-soluble compounds called tocopherols (30,43). There are four different homologs, namely, α, β, γ, and δ-tocopherol, of which only α-tocopherol is the most effective against heart disease. Major dietary sources of vitamin E are wheat germ, corn, soy bean, sunflower, and olive oils (15,30). The requirement for vitamin E increases with increasing polyunsaturated...
fat intake (15,30,44,45). Vitamin E exists in both the macular and peripheral regions of the retina and RPE, but is the most highly concentrated in the RPE (46). It reaches a local maximum in the fovea and a minimum near the foveal crest (47).

The tocopherols act as chain-breaking antioxidants by transferring a phenolic hydroxyl group to a peroxyl free radical of a peroxidized polyunsaturated fatty acid (43). Vitamin E is particularly effective in breaking chain reactions involving nonenzymatic lipid peroxidation (15). The phenoxy free radicals of tocopherol formed after reacting with a peroxyl radical may react with vitamin C to regenerate tocopherol (33). Alternatively, it may react with other peroxyl free radicals resulting in oxidation of the chromane ring and side chain producing a nonfree-radical product (15,30). This oxidation product is then conjugated with glucuronic acid via the 2-hydroxyl group and excreted in the bile (43).

Vitamin E protects against tissue oxidation (48); epidemiologic research shows that vitamin E reduces the risk of CVD (49). It has been suggested that a cell's antioxidant ability may also be dependent on the ratio of glutathione peroxidase to vitamin E (50). As glutathione concentrations become lower, vitamin E is rapidly depleted from the cells (31).

The function of vitamin E as an antioxidant in the retina may be of particular importance because of the increased levels of highly unsaturated, long-chain fatty acids. Animal studies report that vitamin E deficiency produces retinal degeneration and lipofuscin deposits similar to AMD and drusen found in humans (10). However, human epidemiologic studies have not conclusively confirmed the animal findings based on this hypothesis.

3.1.2.3. Vitamin A. Vitamin A encompasses a large group of compounds, which include alcohol (retinol), aldehyde (retinal), acid (retinoic acid). These are the active forms of vitamin A. Retinol is an essential precursor of 11-cis-retinaldehyde, a fundamental component of rhodopsin, the primary visual pigment in the retinal rod cells (51). Active forms of vitamin A are found only in animal sources, although carotenoid precursors such as β-carotene, obtained mainly from plant sources, can be converted in the body to form the active compound (15,43). The necessity of vitamin A for ocular health has long been established (30). Deficiencies manifest themselves in the form of xerophthalmia and other degenerative eye diseases (30). Vitamin A itself (retinol, retinal, or retinoic acid), however, has not been shown to be related to AMD.

3.1.3. CAROTENOIDS

There are approx 600 carotenoid pigments currently documented (52). Of these, more than 50 can be metabolized to active vitamin A in a variety of animal species (53), although carotenoids with pro-vitamin A activity are not the only ones that are biologically important. Carotenoids are the primary pigments found in red-, yellow-, and orange-colored plant and animal foods (30). Primary dietary sources of these carotenoids are fruits, vegetables, (30,31,54), and egg yolks (55).

Carotenoids can function directly by reacting with ROS (53). They show both antioxidant and prooxidant activities under certain conditions (56). Four carotenoids, grouped as those with or without pro-vitamin A activity, may play important roles in AMD development.

3.1.3.1. Carotenoids with Pro-Vitamin A Activity. β-carotene is a precursor of the active form of vitamin A, retinol; cleavage between the 9–10 carbons results in the formation of two molecules of retinal (15). Although β-carotene can quench singlet oxygen and prevent lipid peroxidation, it is not as effective an antioxidant in vivo as other carotenoids, such as lycopene (57). α-carotene, when cleaved, results in half the amount of active retinol as β-carotene. α-carotene has a similar antioxidant capacity as β-carotene (57).
3.1.3.2. Carotenoids with No Pro-Vitamin A Activity. Lutein and zeaxanthin are two carotenoids that have recently sparked growing interest in the field of nutritional AMD research. Recent studies suggest that consumption of foods rich in lutein and zeaxanthin may be protective against AMD (40). Lutein and zeaxanthin have no provitamin A activity (53), although they are similar in chemical structure to \( \alpha \)- and \( \beta \)-carotene (alpha carotene and lutein differ by two hydroxyl groups on the ring structures; the same is true for \( \beta \)-carotene and zeaxanthin).

Both lutein and zeaxanthin are preferentially deposited in the retina. The concentration of zeaxanthin is almost twice that of lutein in the inner macula (58). The ratio of lutein to zeaxanthin is approx 2:1 and 3:1 at distances exceeding 6 nm from the fovea, which corresponds with an increasing rod-to-cone ratio (58). The yellow pigment absorbs in the light spectrum 450–500 nm, and thus may prevent excessive oxidative damage that could be caused by blue light (9,59). The high concentration of these two carotenoids in the macula suggests an important antioxidant or other protective function for these two compounds.

Independent levels of lutein and zeaxanthin are still difficult to obtain. In general, lutein is far more abundant in vegetables and fruits than zeaxanthin. The USDA-NCI carotenoid database developed by Chug-Ahuja et al. and Mangels et al. (60,61), which includes more than 2400 different fruits, vegetables, and combination dishes containing fruits and vegetables, has been applied to validated food frequency questionnaires (FFQ), such as the Block and Willett FFQs. From one study using these data, raw and cooked spinach contributed the most to the plasma lutein of nonsmoking men and women, followed by zucchini and corn (62). In another study, the highest amount of both lutein and zeaxanthin were found in egg yolk and corn (55).

It is still believed that both lutein and zeaxanthin are essential nutrients, meaning that their only source is from diet. Studies show that increased consumption of foods containing lutein and zeaxanthin increase macular pigment density (63,64). Although zeaxanthin is the predominant carotenoid in the macular region, there is much less of it in food than lutein. Lutein is also present in much higher concentrations in the plasma than zeaxanthin (62,65). One might wonder whether dietary sources of zeaxanthin are sufficient to supply the amounts found in the eye.

One possible explanation for how the body compensates for the limited supply of zeaxanthin from dietary sources could be that a form of zeaxanthin is made from lutein in the retina. Mesozeaxanthin has been found in considerable quantities in the macula (58,66). This form of zeaxanthin is not found in plants, thus endogenous conversion is probable (58,66,67).

3.1.4. MINERALS AS METALLOENZYMES

3.1.4.1. Zinc. Zinc is an essential trace element of the metalloenzyme retinal dehydrogenase, which is involved in the metabolism of vitamin A containing visual pigments (15). Zinc is also necessary for the synthesis of retinol-binding protein, the primary transport protein of vitamin A throughout the body (15,43). Zinc is an essential cofactor in more than 100 enzymes including carbonic anhydrase, superoxide dismutase, alkaline phosphatase, catalase, and retinol dehydrogenase (31). It is the second most abundant trace element in the body and high concentrations can be found in the RPE and choroid (30). Zinc-binding capacity in the RPE may also be dependent on a low-mol-wt protein called metallothionein (68). Catalase activity in human RPE was six times higher than in other other ocular tis-
sues. Zinc concentrations have been reported to decrease with age or AMD (28,69). Although zinc is known to be necessary for the normal metabolism of the retinochoroidal complex, its exact role in the maintenance of normal retinal function remains undefined (70). Zinc is involved in the synthesis of extracellular matrix molecules and is essential for the stability of cellular membranes, as well as for normal immune function (31). It is possible that zinc deficiency may indirectly affect the RPE through its coenzyme functions (27).

3.1.4.2. Selenium. Selenium is an essential component of glutathione peroxidase, a key enzyme in the regulation of ROS (15). Selenium is required for normal pancreatic function, which is necessary for the digestion and absorption of lipids, including vitamin E (15). Selenium’s proposed relationship to AMD is in the function of glutathione peroxidase as an antioxidant. Deficiencies in selenium may decrease enzyme activity and may lead to increased lipid peroxidation and accumulation of oxidative byproducts in the RPE (71).

3.1.4.3. Other Minerals. Copper is a metal cofactor in copper-dependent superoxide dismutase. Manganese is another required cofactor in the enzyme superoxide dismutase. Manganese is concentrated in the photoreceptor outer-segment membrane of the retina where it acts as a free-radical scavenger (43).

3.2. Atherosclerotic Theory

Cardiovascular and AMD pathogenesis seem similar, but are far from identical. Although cardiovascular risk factors, such as smoking, hypertension, and elevated cholesterol level, have also been reported as AMD risk factors, the relationship between dietary fats and AMD may not be as similar.

The proposed relationship between lipids and AMD involves not only their susceptibility to oxidation by ROS, but also their net effect on serum cholesterol levels. Three studies report that lipids in the retina form hydroperoxide byproducts that accumulate with constant exposure to light (11). It has been generally accepted that dietary intake of polyunsaturated fatty acids is associated with lipofuscin accumulation (20). Dietary supplementation of vegetable oils rich in linoleic acid increase both n-6 and n-3 polyunsaturated fatty acids in the retinas of piglets (72).

3.2.1. Lipids

In 1929, Burr et al. reported that certain types of fat may be essential to our diet. Two long-chain fatty acids, linoleic and linolenic fatty acids with unsaturated bonds at the ω-6 position, are polyunsaturated fats that cannot be made in the body. Major sources of these fats are from vegetable oils, such as soybean, and canola oil. In recent years, it has become clear that ω-3 fatty acids, very long-chain polyunsaturated fatty acids, 22 carbons or longer, found mostly in deep sea fish, are also essential fats and must be consumed from foods (30).

In the eye, fats are packaged as phospholipids, specifically, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidyl serine (73), and are the major complex lipids found in the retina, RPE, and Bruch’s membrane or choroid. Phosphatidyl choline and phosphatidylethanolamine also contain high concentrations of (DHA). Other major fatty acids found in the retina and the macular area are palmitic (16:0), stearic (18:0), and oleic (18:1) fats (73).

Because photoreceptors concentrate n-3 fatty acids, especially DHA, dietary n-3 fatty acids may have other roles in retinal function beside their antithrombotic and hypolipidemic effects on serum cholesterol (26). Rod outer segments absorb light, which
makes them highly susceptible to light damage, and because they also contain high levels of DHA, lipid peroxidation is a potential problem. As photoreceptor outer segments are constantly being renewed, a steady supply of DHA may be needed to replace what was lost. It will be important to determine whether suboptimal levels of Ω-3 may be contributing to AMD development.

Early- and late-stage AMD may not have the same associations with dietary fat (74). Saturated fat, cholesterol, and trans-fatty acids have been positively associated with heart disease, and polyunsaturated fats, particularly vegetable oils and fish oils, have been inversely associated with CVD. The literature suggests that only Ω-3 fats, such as DHA, may be inversely associated with AMD (25, 74). Other types of fat including animal fats, which are thought be adverse, and vegetable fats, which are thought to be beneficial, may be positively related to both dry and wet AMD (25, 74).

One important point of discussion that needs to be addressed when reporting associations between dietary fat and AMD is relative vs absolute amount of fat. It is much harder to change the absolute percentage of energy intake contributed by fat than it is to change the relative composition. People can modify their relative composition of fat intake by including different types of fat that may reduce the risk of AMD or excluding types of fat that increase risk easier than they can change the total amount of fat consumed. However, it is still too early to form any definitive conclusions about the relationship between specific types of fat from diet or serum and any type of AMD.

4. REVIEW OF ANIMAL STUDIES ON DIET AND AMD

4.1. Antioxidant Theory Animal Studies

The animal models supporting the role of antioxidant nutrients in the prevention of retinal tissue degeneration are relatively consistent (see Table 2). The nutrients that have been studied include vitamin C, vitamin E, β-carotene, lutein, and zeaxanthin, and a few trace minerals.

Many studies have investigated the protective effect of ascorbate against retinal injury caused by excessive exposure to blue light and excessive oxygen (36–38,75,76). In addition to vitamin C, β-carotene protected the RPE from damage caused by excessive light in primates (76).

Vitamin E deficiency in primates produced changes in the macula similar to the drusen seen in humans within 3 yr of dietary depletion (10). Similar studies in rats also produced central retinal changes and loss of photoreceptor cells, especially in groups deficient in vitamin E and sulfur-containing amino acids (71). One study that used primates showed dietary depletion of zeaxanthin and lutein inhibited the development of the yellow pigment usually seen in the macula (77). Among one group of free-living primates, aging was associated with retinal changes similar to those seen in early age-related maculopathy (78,79).

5. REVIEW OF EPIDEMIOLOGIC STUDIES ON DIET AND AMD

In the past few years, more data have accumulated about the relationship between dietary nutrients and AMD. Although the reports still tend to be mixed for each nutrient, it is becoming clearer that nutrients related to dry AMD may or may not be related to wet AMD in the same way and vice versa (see Table 3).
<table>
<thead>
<tr>
<th>Principal author, ref.</th>
<th>Animal</th>
<th>Primary exposure</th>
<th>Outcome</th>
<th>Nutrient(s) studied</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayes (10)</td>
<td>Monkeys</td>
<td>Deficient diets</td>
<td>Retinal changes</td>
<td>Vitamin E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Deficient diet produced AMD within 2 yr, specifically—ceroid accumulation, retinal degeneration and photoreceptor layer disruption restricted to macula.</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Vitamin A&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Katz (71)</td>
<td>Male rats</td>
<td>Antioxidant</td>
<td>Effects on RPE and retina</td>
<td>Vitamin E, Selenium (Se), Chromium (Cr), Methionine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>More accumulation of autofluorescent pigment&lt;sup&gt;d&lt;/sup&gt; in RPE of deficient rats than in rats supplemented with methionine and Cr. Very little autofluorescence found in rats receiving all four antioxidants.</td>
</tr>
<tr>
<td>Tripathi (75)</td>
<td>Rabbits</td>
<td>Oxygen</td>
<td>Hyperoxic injury to immature retinal vascular cells</td>
<td>Vitamin E&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Retinal vascular degeneration retarded in groups supplemented with vitamin E and GABA. Immature retinal vascular cells are rapidly and adversely affected by hypoxia compared to mature cells under same conditions; this suggests protective free-radical scavenging enzymes develop with cell maturity.</td>
</tr>
<tr>
<td>Tso (36)</td>
<td>Baboons</td>
<td>Light (30 min) on macula</td>
<td>Distribution of vitamin C in retinas with and without photic injury</td>
<td>Vitamin C</td>
<td>Aqueous vitamin C higher in light-exposed eye. Total vitamin C concentration in RPE, choroid decreased significantly ($p &lt; 0.05$) in light-exposed eyes.</td>
</tr>
<tr>
<td>Ham (76)</td>
<td>Macaque monkeys</td>
<td>Blue light, oxygen</td>
<td>Lesions of macaque retina</td>
<td>β-carotene (d)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Animals supplemented with β-carotene have increased threshold for oxygen and light to produce the same minimal lesion in macaque retina. This suggests that β-carotene is protective against free radicals produced by blue light and oxygen.</td>
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</tbody>
</table>

continued
<table>
<thead>
<tr>
<th>Principal author, ref.</th>
<th>Animal</th>
<th>Primary exposure</th>
<th>Outcome</th>
<th>Nutrient(s) studied</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson (11)</td>
<td>Albino rats frogs</td>
<td>Constant light FeSO₄, NaSO₄</td>
<td>Retinal changes</td>
<td>Polyunsaturated fatty acids (PUFA)</td>
<td>Treatments with light and FeSO₄ resulted in loss of long chain PUFA and accumulation of lipid hydroperoxides in isolated outer-rod segments. Decrease in 22:6 n-3 in photoreceptor ROS membrane, but concentration of palmitic acid was unchanged.</td>
</tr>
<tr>
<td>Li (37)</td>
<td>Rats</td>
<td>Diet</td>
<td>Photoreceptor damage in RPE</td>
<td>Vitamin C</td>
<td>6–13 d after 24-h exposure to light, supplemented rats had significantly less outer-segment photoreceptor cells than rats on normal diets.</td>
</tr>
<tr>
<td>Organisciak (38)</td>
<td>Rats</td>
<td>Light-dark cycles</td>
<td>Rhodopsin, photoreceptor cell nuclei</td>
<td>Vitamin C, docosahexaenoic acid (22:6 n-3)</td>
<td>Rats supplemented with vitamin C had higher concentrations of rhodopsin and 22:6 n-3 than unsupplemented light exposed rats.</td>
</tr>
<tr>
<td>Whitley (91) (abstract)</td>
<td>Pigs</td>
<td>Diet</td>
<td>Photoreceptor cells, RPE edema,</td>
<td>Zinc</td>
<td>Abnormal migration of photoreceptor cells from outer nuclear layer toward the RPE in animals with zinc-deficient diets. RPE exhibited signs of edema and occasional opaque bodies seen in zinc-deficient rats, suggesting chronic zinc deficiency may be important in AMD.</td>
</tr>
<tr>
<td>Feeney-Burns (92)</td>
<td>Monkeys</td>
<td>Semipurified diet chow (control)</td>
<td>Macular changes</td>
<td>Protein</td>
<td>No macular pigment was observed in animals fed semipurified diets; virtually no plasma carotene or xanthophyll was found. Serial histopathologic sections revealed punctate window defects to be sites of vacuolated RPE cells, not drusen. No drusen were found in any animal.</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Diet</td>
<td>Retinal Fatty Acid Composition</td>
<td>RPE Changes</td>
<td>Notes</td>
</tr>
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<tr>
<td>Samuelson (93)</td>
<td>Sheep</td>
<td>Age</td>
<td>RPE, progression of ovine lipofuscinosis (OCL)</td>
<td>Zinc, iron, copper, phosphate, sulfur, manganese, chromium</td>
<td>Age-related changes in Zn, Fe, Cu associated with lipopigment accumulation in RPE. Decrease in levels of phosphate, S, Mn as photoreceptor cells and their outer segments are lost in the disease process. Levels of metals observed in retina of 1-yr-old sheep with OCL the same as levels in the RPE of 10+ yr-old normal sheep.</td>
</tr>
<tr>
<td>Hroboticky (72)</td>
<td>Piglets</td>
<td>Diet</td>
<td>Retinal polyunsaturated fatty acid (PUFA) composition</td>
<td>Linoleic acid (18:2 n-6 or 18:2 w-6), linolenic acid (18:3 n-3)</td>
<td>Normal accretion of 22:6 n-3 compromised in retinas of piglets deficient in long chain PUFAs but sufficient in 18:2 n-6, 18:2 n-3, 18:3 n-3.</td>
</tr>
<tr>
<td>Hope (78)</td>
<td>Monkeys</td>
<td>Free-living diet</td>
<td>Drusen: natural progression</td>
<td>Free-living diet</td>
<td>The prevalence and severity of drusen are linearly related to increasing age and significantly higher in specific maternal lineage. Percentage of female animals with drusen was twice that of male animals.</td>
</tr>
</tbody>
</table>

* Plasma vitamin E < 100 μg/dL in deficient group compared with >600 μg/dL in control group.
* Plasma vitamin A <5 μg/dL in deficient group compared with >15–20 μg/dL in control group.
* Nutrient concentrations determined from liver homogenates.
* Autofluorescence pigment in rat RPE is similar to lipofuscin deposits found in human RPE.
* d indicates 64-d dietary source of β-carotene in form of Solatene mixture prepared by Roche. Plasma β-carotene levels >360 μg/dL.
* Vitamin C injection given peritoneally 24 h 0.5 g/kg.
<table>
<thead>
<tr>
<th>Principal author, ref.</th>
<th>Design</th>
<th>Subjects, n</th>
<th>AMD outcome</th>
<th>Nutrient(s) studied</th>
<th>RR for multivariate model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blumenkranz (79a)</td>
<td>Case-control</td>
<td>26 cases 23 controls (spouses)</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt; NA 26</td>
<td>Vitamins A, C, E</td>
<td>Not significant</td>
</tr>
<tr>
<td>Newsome (80)</td>
<td>Prospective randomized trial</td>
<td>80 treatment (T) 71 placebo (P)</td>
<td>15 (T) 65 80 8 (P) 63 71</td>
<td>Zinc supplement</td>
<td>Less drusen in supplement group and decreased vision loss</td>
</tr>
<tr>
<td>Goldberg (23) (NHANES1)</td>
<td>Cross-sectional</td>
<td>178 cases</td>
<td>NA NA 178</td>
<td>Vitamin A (s)&lt;sup&gt;b&lt;/sup&gt; Vitamin C (s)</td>
<td>0.59 (0.37, 0.99) 0.98 (0.79–1.23)</td>
</tr>
<tr>
<td>Eye Disease Case Control study (39)</td>
<td>Case-control</td>
<td>421 cases 615 controls</td>
<td>421 0 421</td>
<td>Vitamin C (s) Vitamin E (s) Selenium (s) Carotenoids (s)&lt;sup&gt;c&lt;/sup&gt; Antioxidant index&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.70 (0.5–1.2) 0.60 (0.4–1.04) 1.30 (0.8, 2.1) 0.30 (0.2, 0.6) 0.40 (0.2, 0.6)</td>
</tr>
<tr>
<td>Sanders (81)</td>
<td>Matched case-control</td>
<td>65 cases 65 controls</td>
<td>9 56 65</td>
<td>Vitamin A (s)&lt;sup&gt;b&lt;/sup&gt; Vitamin E (s) β-carotene (s) α-carotene (s) Lutein (s) Lycopene (s) β-cryptoxanthin (s) Antioxidant index&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.20 (0.67, 2.14) 0.85 (0.48, 1.50) 0.50 (0.20, 1.20) 0.85 (0.48, 1.50) 1.37 (0.57, 3.38) 1.00 (0.40, 2.57) 1.35 (0.58, 3.28)</td>
</tr>
<tr>
<td>West (41) (Baltimore Longitudinal Study on Aging)</td>
<td>Cross-sectional</td>
<td>226 cases</td>
<td>11 215 226</td>
<td>Vitamin A (s)&lt;sup&gt;b&lt;/sup&gt; Vitamin C (s) Vitamin E (s) β-carotene (s) Antioxidant index&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.01 (0.6–1.8) 0.70 (0.5, 1.2) 0.45 (0.3–0.7) 0.62 (0.4–1.1) 0.43 (0.3, 0.7)</td>
</tr>
<tr>
<td>Seddon (40)</td>
<td>Case-control</td>
<td>356 cases 520 controls</td>
<td>356 0 356</td>
<td>Carotenoids (d)&lt;sup&gt;e&lt;/sup&gt; Lutein/zeaxanthin (d) Vitamin A (d)&lt;sup&gt;f&lt;/sup&gt; Vitamin C (d) Vitamin E (d)</td>
<td>0.57(0.4–0.9) 0.44 (0.2, 0.8)&lt;sup&gt;f&lt;/sup&gt; 0.57 (0.4–0.9) 0.83 (0.5–1.3) 1.46 (0.9–2.4)</td>
</tr>
<tr>
<td>Study</td>
<td>Design Type</td>
<td>Cases/Sizes</td>
<td>Controls</td>
<td>α-carotene (s)</td>
<td>β-carotene (s)</td>
</tr>
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<tr>
<td>Mares-Perlman</td>
<td>Nested case</td>
<td>167 cases</td>
<td>167</td>
<td>1.2 (0.7–2.3)</td>
<td>0.8 (0.4–1.5)</td>
</tr>
<tr>
<td>(82)</td>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mares-Perlman</td>
<td>Retrospective</td>
<td>314 cases</td>
<td>314</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(42)</td>
<td>cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith (24)</td>
<td>Case-control</td>
<td>156 cases</td>
<td>156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanden-Langenburg</td>
<td>Prospective</td>
<td>177 cases</td>
<td>177</td>
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</tbody>
</table>

* NA = number of cases not specified.
* s Nutrients derived from serum.
* Serum carotenoids included lutein/zeaxanthin, β-carotene, α-carotene, cryptoxanthin, lycopene.
* Antioxidant index includes nutrients listed singly.
* d Nutrients obtained from a semiquantitative food frequency questionnaire (SFFQ).
* e Energy-adjusted, two-nutrient multivariate model comparing highest to lowest quintiles.
* f Preformed vitamin A or retinol estimated from SFFQ.
* g Combined dry and wet AMD.
* h Dry AMD characterized by 5-yr incidence of large drusen, relative risk and 95% CI for the highest vs lowest quintile of past dietary intake.
5.1. Antioxidant Theory

The epidemiologic studies on zinc and AMD have been mixed. Newsome et al. undertook the first prospective, randomized, double-masked placebo-controlled trial involving zinc supplementation of persons with AMD (80). This small pilot study compared photos taken at the last study visit and at the baseline visit and found that eyes in the zinc-treated group were more stable or had less accumulation of visible drusen than subjects who did not receive zinc. The 1992 EDCCS study found nonsignificant increased risk with increasing zinc levels and neovascular AMD after adjusting for age, sex, and clinic. Beaver Dam found higher dietary intake of zinc, the highest quintile of zinc intake compared to the lowest quintile of intake, to be inversely associated with early AMD (RR = 0.6, 95% CI = 0.4–1.0) but not wet AMD. Stur et al. conducted a clinical trial following Newsome’s protocol in 1996. They failed to find any beneficial effect of zinc supplementation on patients developing AMD in the second eye. VandenLangenberg et al. found no association between dietary zinc intake and 5-yr incidence of dry AMD.

All reported associations between serum or dietary vitamin C and dry or wet AMD are nonsignificant. They are consistent in that they are all inversely associated. The strongest inverse association between serum vitamin C and dry AMD is a 40% reduction in risk. The strongest association for serum vitamin C and wet AMD is a 30% reduction in risk. The strongest association for dietary vitamin C and either dry or wet AMD is approx a 20% reduction in risk. Goldberg found no association in his cross-sectional study among those who consumed fruits and vegetables rich in vitamin C more than seven times a week vs those who ate them less than once a week. The 1993 EDCCS study examined plasma vitamin C levels and exudative AMD (RR = 0.7, 95% CI = 0.5–1.2). Seddon studied dietary vitamin C and exudative AMD (RR = 0.8, 95% CI = 0.5–1.3). West et al. investigated plasma vitamin C and dry AMD cases only (RR = 0.6, 95% CI = 0.3–1.1). Mares-Perlman ’96 evaluated dietary vitamin C and early AMD (RR = 0.8, 95% CI = 0.5–1.2). VandenLangenberg et al. investigated dietary vitamin C with dietary supplements and early AMD, specifically dry AMD characterized by large drusen (RR = 0.83, 95% CI = 0.4–1.6) or pigmentary abnormalities (RR = 0.77, 95% CI = 0.3–2.0).

As new research emerges, the relationship between plasma vitamin E, more specifically α-tocopherol, and AMD may exist, although past findings have been mixed. In a recent cross-sectional study, the POLA group reported a significant inverse association between serum α-tocopherol and 1841 cases of early ARM (RR = 0.72, 95% CI = 0.53–0.98), after controlling for multiple covariates including age, smoking, and serum cholesterol level (80a). West et al. also reported a significant inverse relationship between plasma α-tocopherol and early ARM (RR = 0.43, 95% CI = 0.25–0.73), however, their finding may be confounded by total serum lipid concentration. EDCCS ’93 reported a nonsignificant inverse relationship between plasma vitamin E and late AMD (RR = 0.6, 95% CI = 0.4–1.04). Sanders et al. also reported nonsignificant findings for plasma α-tocopherol and mostly dry AMD (RR = 0.9, 95% CI = 0.5–1.5). Since most serum assays use only α-tocopherol for vitamin E, diet may be a better measure because it includes the three other tocopherol homologs (beta, gamma, and delta), giving a more complete picture of vitamin E intake. The studies for dietary vitamin E with and without supplements is as fol-
lows: VandenLangenberg et al. observed an inverse association between the highest vs
lowest quintile of dietary vitamin E without supplements and dry AMD with large drusen
(RR = 0.40, 95% CI = 0.2–0.9, p for trend = 0.04). Seddon et al. found a nonsignificant
increased risk between higher dietary vitamin E intake without supplements and wet
AMD (RR = 1.5, 95% CI = 0.6–2.4). Additional research is currently underway (80a).

Studies investigating the relationship between plasma or dietary carotenoids and AMD
are the most compelling to date, but the findings are also mixed. In EDCCS ’93 study, higher
serum carotenoid levels were inversely associated with advanced AMD (OR = 0.3, 95%
CI 0.2–0.6). Specific serum carotenoids, α-carotene, β-carotene, lutein-zeaxanthin, and β-
cryptoxyanthin were also significant and inversely associated with wet AMD. Seddon et al.
found that the highest quintile of dietary carotenoid intake compared to the lowest quintile
was associated with a 40% reduction in risk of wet AMD (RR = 0.6, 95% CI = 0.4–0.96)
(40). Among specific carotenoids, lutein and zeaxanthin, commonly found primarily in dark
green leafy vegetables, were most strongly protective (40). Higher consumption of spinach,
or collard greens specifically, was associated with a strong protective effect against AMD
(OR for trend <0.001) (40). VandenLangenberg found significant associations for the highest
vs lowest quintile of dietary α-carotene (RR = 0.52, 95% CI = 0.3–1.0) and pro-vitamin A
carotenoids (RR = 0.53, 95% CI = 0.3–1.0) and dry AMD with large drusen; they were also
significant for trend (p < 0.05). The three other studies that investigate either plasma or di-
etary lutein-zeaxanthin and AMD have relative risks which range from 0.7 to 1.4, all of
which have 95% confidence intervals that include the null (42,81,82).

When all serum values for carotenoids, vitamins C, E, and selenium were combined into
an antioxidant index, the association was protective (OR = 0.3, 95% CI 0.1–0.7) (39).
Using an antioxidant index for fasting serum levels of ascorbate, α-tocopherol, and β-
carotene, they also found a protective effect on AMD (OR = 0.43, 95% CI 0.26–0.70) (41).

5.2. Similar Risk Factors for Atherosclerosis and AMD Epidemiology

Verhoeff et al. were possibly the first to propose the relationship between athero-
sclerotic risk factors and AMD (19). There are several epidemiologic studies that have
examined this relationship (see Table 4).

Hyman et al. reported an increased prevalence of AMD among those with a history
of CVD (22). This matched case-control study of 162 cases and 175 controls investigated
history of one or more cardiovascular diseases, smoking, and other variables, such as
chemical exposures, family history of macular disease, blue- or medium-pigmented
eyes, decreased hand grip strength, and hyperopia. The risk of AMD was significant for
those with a history of one or more CVDs, OR = 1.7 (95% CI 1.1–2.7) and among male
smokers, OR = 2.6 (95% CI 1.15–5.75).

Sanders et al. also conducted a matched case-control study of 65 cases of AMD (9 wet
and 59 dry). They investigated the relationship between plasma retinol, α-tocopherol,
carotenoids, such as lutein, β-cryptoxyanthin, α- and β-carotene, cholesterol concentration,
polysaturated fatty acid content of plasma, and erythrocyte phospholipids and AMD
(81). Overall, they found no significant associations. An interesting point to note is that
when plasma carotenoid concentrations between smoking and nonsmoking subjects were
compared, after adjusting for age and sex, nonsmokers had significantly higher carotenoid
levels than smokers. Lutein was significantly lower for smokers than nonsmokers at p <
0.001 level. β-carotene and β-cryptoxyanthin, another nonpro-vitamin A carotenoid, were
significantly lower for smokers than nonsmokers at the p < 0.05 level.
<table>
<thead>
<tr>
<th>Principal author, ref.</th>
<th>Design</th>
<th>Subjects, n</th>
<th>AMD outcome</th>
<th>Main exposure(s) studied</th>
<th>OR for multivariate model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyman (22)</td>
<td>Matched&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162 cases 175 controls</td>
<td>NA NA 162</td>
<td>≥ 1 CVD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7 (1.1, 2.7)</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td></td>
<td></td>
<td>Smoke, men</td>
<td>2.6 (1.15, 5.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Smoke, women</td>
<td>0.84 (0.48, 1.47)</td>
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<tr>
<td></td>
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<td></td>
<td>Total cholesterol</td>
<td>4.1 (2.5, 7.3)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Smoking</td>
<td>2.2 (1.3, 3.5)</td>
</tr>
<tr>
<td>EDCCS&lt;sup&gt;d&lt;/sup&gt; (83)</td>
<td>Case-control</td>
<td>421 cases 0 421</td>
<td></td>
<td>Estrogen use</td>
<td>0.3 (0.1, 0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>Cholesterol, women</td>
<td>0.89 (0.80, 0.98)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Cholesterol, men</td>
<td>0.89 (0.84, 0.96)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Hematocrit&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.09 (1.00, 1.19)</td>
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<td></td>
<td></td>
<td></td>
<td>Leukocyte&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.10 (1.00, 1.19)</td>
</tr>
<tr>
<td>Klein (84)</td>
<td>Cross-sectional</td>
<td>803 cases 59 744 803</td>
<td></td>
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<td></td>
<td></td>
<td>Cholesterol, women&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.89 (0.80, 0.98)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Cholesterol, men&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.89 (0.84, 0.96)</td>
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<td></td>
<td></td>
<td>Hematocrit&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.09 (1.00, 1.19)</td>
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<td></td>
<td></td>
<td></td>
<td>Leukocyte&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.10 (1.00, 1.19)</td>
</tr>
<tr>
<td>Sanders (81)</td>
<td>Matched&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65 cases 65 controls</td>
<td>9 56 65</td>
<td>Plasma cholesterol</td>
<td>1.00 (0.40, 2.48)</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td></td>
<td></td>
<td>18:2n-6 plasma&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.62 (0.26, 1.46)</td>
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<td></td>
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<td></td>
<td></td>
<td>18:2n-6 erythrocyte&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.9 (0.37, 2.18)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>22:6n-3 plasma&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.82 (0.35, 1.93)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>22:6n-3 erythrocyte&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92 (0.37, 2.32)</td>
</tr>
<tr>
<td>Klein (85)</td>
<td>Cross-sectional</td>
<td>871 cases 41 W&lt;sup&gt;h&lt;/sup&gt; 422 W 463 W 56 M&lt;sup&gt;h&lt;/sup&gt; 352 M 408 M</td>
<td>Smoke (female, early AMD)</td>
<td>1.29 (0.98, 1.70)&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>Smoke (male, early AMD)</td>
<td>1.02 (0.81, 1.29)&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td>Smoke (female, wet AMD)</td>
<td>2.06 (1.03, 4.10)</td>
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<td>Smoke (male, wet AMD)</td>
<td>2.50 (1.01, 6.20)</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Cases</td>
<td>Controls</td>
<td>Odds Ratio (95% CI)</td>
<td></td>
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<tr>
<td>Seddon (25)</td>
<td>Case control</td>
<td>356</td>
<td>520</td>
<td>Total fat 1.71 (1.0–2.8)</td>
<td></td>
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<td></td>
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<td>Vegetable fat 1.99 (1.2–3.3)</td>
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<td>Animal fat 0.84 (0.5–1.9)</td>
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<td>Ω-3 fatty acids 0.59 (0.5–0.9)</td>
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<td>Smoke 1–14 cigarettes 1.05 (0.56, 1.94)</td>
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<td>Smoke 15–24 cigarettes 1.38 (0.81, 2.25)</td>
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<td></td>
<td>Smoke ≥ 25 cigarettes 1.99 (1.18, 1.71)</td>
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<tr>
<td>Vingerling (95)</td>
<td>Cross–sectional</td>
<td>104</td>
<td></td>
<td>Plaques in carotid bifurcation 4.7(1.8, 12.2)</td>
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<td></td>
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<td></td>
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<td>Plaques carotid artery 2.5 (1.4, 4.5)</td>
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<tr>
<td>Mares-Perlman (82)</td>
<td>Case-control</td>
<td>167</td>
<td>167</td>
<td>Total fat (%kJ) 1.30 (0.90, 1.90)</td>
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<td>Saturated fat 1.80 (1.20, 2.70)</td>
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<td></td>
<td></td>
<td>18:2 oleate 1.20 (0.80, 1.80)</td>
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<td></td>
<td></td>
<td>18:3 linoleate 1.10 (1.10, 2.40)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cholesterol (mg/4200 kJ) 1.60 (1.10, 2.40)</td>
<td></td>
</tr>
</tbody>
</table>

- Matched on age and sex.
- Cardiovascular disease includes myocardial infarction, angina, other heart problems, arteriosclerosis, hypertension, stroke, circulatory problems, and/or transient ischemic attacks.
- Phospholipids.
- Eye Disease Case-Control Study Group.
- Cholesterol in mmol/L; OR using early AMD as dichotomous outcome.
- OR using exudative AMD as dichotomous outcome.
- Ever smokers compared to never smokers measured in pack years (total number cigarettes smoked per day divided by 20).
- W denotes women 43–86 yr of age, M denotes men 43–86 yr of age.
- Adjusted odds ratios for early AMD only; odds ratios for late AMD were in similar directions, but all nonsignificant at p < 0.10 level.
- AMD with visual loss ≥ 20/30.
- Current vs never smokers with a significant dose-dependent test for trend (p < 0.05).
The EDCCS study found that current use of postmenopausal exogenous estrogens among women was inversely associated with AMD, OR = 0.3 (95% CI 0.1–0.6) (83). Cigarette smoking and higher serum cholesterol levels (83) were associated with increased risk for neovascular AMD. These observations are consistent with the hypothesis linking risk factors for CVD with AMD (83).

Klein et al. also examined the relationship between AMD and CVD and smoking (84,85) in a cross-sectional study. This population-based prevalence study of 492 persons aged 43–86 yr in Beaver Dam, WI, showed an inverse association between serum lipids and early AMD. Higher hematocrit levels and leukocyte counts were also associated with exudative AMD (84). Although no association between smoking status and early AMD was found, exudative AMD was more common in current smokers compared to those who never smoked (85).

Seddon et al. conducted the first prospective analysis to examine the relationship between smoking and AMD in a cohort of women from the Nurses’ Health Study. There were 223 incident cases of AMD confirmed by medical records. An independent review of fundus photographs was also performed on a sample of cases. They observed that both current and past smokers had a higher risk of developing AMD with visual loss of 20/30 or worse, compared to those who had never smoked. These multivariate models controlled for age, body mass index (BMI), history of hypertension, alcohol consumption, and energy-adjusted carotene intake (86).

Using the EDCCS case-control study group to examine the relationship between different types of dietary fat and AMD among 356 cases of wet AMD (25). Seddon et al. found that total fat and vegetable fats were significantly and adversely associated with risk for AMD. Animal fats were not significantly associated with AMD. There was a small nonsignificant inverse association with fats found in fish.

Finally, Mares-Perlman et al. also looked at the relationship between dietary fat and AMD among residents participating in the Beaver Dam eye study. There were 314 cases of early AMD and 30 cases of wet AMD (82). Saturated fat, linoleic fatty acid, and cholesterol (reported in mg/4200 kJ) were associated with increased risk for early AMD. Total fat, as % kJ, and oleic acid were not significantly associated with risk for early AMD.

6. CONCLUSIONS

Animal research suggests that higher levels of vitamin C, vitamin E, and β-carotene are protective against the damaging effects of excess oxygen and light exposure on the retina. Primate models show that dietary carotenoids, lutein and zeaxanthin, which are components of the yellow pigment seen in the macula, may prevent the development of drusen-like bodies. No animal studies to date have directly examined the relationship between cardiovascular risk factors and AMD. A limitation of animal studies is that the retinal changes observed do not include all of the signs of AMD found in humans including neovascularization and atrophy. There are currently no animal models for AMD which completely reflect the human disease.

Epidemiologic studies of AMD in humans have also provided evidence that dietary antioxidant vitamins, zinc, and fat, may be related to AMD either through direct mechanisms related to retinal maintenance or through the development of vascular disease (27). Supplementation of individual vitamins or minerals is not warranted at this time because the danger of toxicity is possible with high doses of zinc or vitamin E. Excess dietary zinc aggravates signs of low copper status (30,87). Also, decreased HDL levels (87) have been observed among people consuming large amounts of zinc. Until long-
Nutrition and AMD

Several epidemiologic studies that evaluated cardiovascular risk factors and AMD indicate that higher serum-cholesterol levels (83,84), cigarette smoking (both past and current) (83,86), and higher hematocrit and leukocyte levels are positively associated with AMD (84). Current use of postmenopausal estrogens (compared to never and past users) among women was inversely associated with neovascular AMD (83). Because \( \Omega-3 \) fats, commonly found in dark meat and oily fish, is beneficial for cardiovascular health, increased intake of foods containing this fat is recommended even though conclusive evidence of its beneficial effect on AMD is still being investigated.

Randomized clinical trials would provide the most conclusive data about the effect of antioxidant nutrients in the eye (7). More prospective studies and randomized clinical trials are needed to clarify the association between diet and AMD, particularly for vitamin E, vitamin C, selenium, and the carotenoids—lutein, zeaxanthin, \( \beta \)-carotene, and dietary lipids. Further studies are also needed to further clarify the roles of nutrients such as selenium, manganese, zinc, copper, and riboflavin. Additional basic research is needed to define further the mechanism of oxidation and its effect on the eye, and the extent to which oxidative effects occur in vivo. Better markers of oxidative stress may be helpful to identify people at higher risk who may possibly benefit from antioxidants.

6.3. Current Research in Progress

Ongoing large-scale randomized trials should provide valuable data on which to base public health recommendations. The Age-Related Eye Disease Study (AREDS) is a multicenter randomized clinical trial sponsored by the National Eye Institute. The study has approx. 5000 patients enrolled at 11 centers across the United States to examine the effects of supplemental vitamin C, vitamin E, \( \beta \)-carotene, zinc, and copper. Patients at lower risk for developing visual loss as a result of AMD have been randomized into two groups: high-dose antioxidant vitamins or placebo. Patients at higher risk of developing visual loss because of AMD have been randomized into four groups in a factorial design: (1) antioxidant vitamins; (2) zinc and copper; (3) antioxidant vitamins, zinc, and copper; or (4) placebo. Other large randomized trials that focus on cancer and heart disease outcomes may also provide additional information.

Several prospective observational studies are also in progress. These data are valuable because they include dietary information (estimated from validated food frequency questionnaires or serum), cardiovascular risk factors, and other environmental risk factors related to AMD development. Prospective dietary data from women in the Nurses’ Health Study (NHS) are being evaluated to examine the relationship between dietary lutein, zeaxanthin, zinc, alcohol, fat, and other nutrients and AMD. The Health Professional’s Follow-up Study, having similar data as the NHS, is investigating dietary nutrients and AMD among men also. The Beaver Dam eye study, a cohort of men and women in Wisconsin, is also ongoing. All of these studies will provide valuable information regarding the potential role of diet and supplements in the prevention of AMD or slowing its progression. Large sample sizes in these studies will permit investigators to address these and other questions that may be relevant to AMD development.

6.4. Dietary Recommendations

The data at present are insufficient to support a clinical recommendation for vitamin
and mineral supplement use for AMD prevention because effectiveness, appropriate dose, and duration are still unknown. The 1989 Recommended Dietary Allowances (RDA) can be used as the minimum level to be consumed by all individuals, regardless of AMD status (31). The allowances may not, however, represent optimal intakes to prevent retinal disease (31) and may be lower than what is needed for optimal ocular health. Nutrient-dense foods containing lutein and/or zeaxanthin like corn, dark green leafy vegetables such as spinach, kale, collard greens, zucchini, squash and lettuce, should be encouraged, since the most consistent epidemiologic evidence to date supports a possible inverse association between carotenoids and AMD. Prospective studies are needed to evaluate this further. Even though it is biologically plausible that the intake of antioxidant-rich foods is protective, the benefit may be caused by other compounds in these foods and not the antioxidant nutrients themselves. Clinical trials evaluating the effectiveness of specific antioxidant supplements will shed light on this subject.

Recommendations regarding cholesterol and dietary-fat intake should follow current guidelines for decreasing cardiovascular risk. These data are insufficient to support recommendations to change intake of specific dietary fats, although increased intake of \( \Omega-3 \) fatty acids and decreased intake of saturated fat, and trans-fatty acids is consistent with heart-healthy recommendations by the American Heart Association.

In summary, following the current National Cancer Institute and National Academy of Sciences’ recommendations to eat at least five servings of fruit and/or vegetables every day to reduce the risk for developing heart disease and some types of cancers may yield additional benefits to patients at risk for developing AMD.

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Osteoporosis

Minerals, Vitamins, and Other Micronutrients

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1. INTRODUCTION

1.1. Nutrition in the Osteoporotic Fracture Context

Osteoporosis is a condition of skeletal fragility caused by decreased bone mass and by microarchitectural deterioration of bone tissue, with consequent increased risk of fracture. The condition is multifactorial in pathogenesis. Nutrition affects bone health in two distinct ways. First, bone tissue deposition, maintenance, and repair are the result of cellular processes, which are as dependent upon nutrition as are the corresponding processes of any other tissue. The production of bone matrix, for example, requires the synthesis and posttranslational modification of collagen and an array of other proteins. Nutrients involved in these cellular activities include vitamins C, D, and K, and the minerals phosphorus, copper, manganese, and zinc. Additionally, the regulation of calcium homeostasis requires normal magnesium nutrition. Second, the skeleton serves as a very large nutrient reserve for two minerals, calcium and phosphorus, and the size of that reserve (in other words, the strength of the skeletal structures) will be dependent in part upon the daily balance between absorbed intake and excretory loss of these two minerals.

Strength in bone, as in most engineering structures, is dependent not only upon its mass density, but also on the arrangement of its material in space, and on the intrinsic strength of its component material (particularly, in bone, as that strength is influenced over long periods of use by the accumulation of unrepaired fatigue damage). All three factors play a role in most low trauma fractures, and it is not possible to say which may be the most important in any given case.

Bone mass and density are themselves influenced by many factors. The three most commonly found to be limiting in industrialized nations are physical activity, gonadal hormones, and nutrition. In adults of these nations, the nutrients most apt to be in short supply are calcium and vitamin D. Calcium intake, specifically, may be inadequate for the straightforward reason that it is low; however, even when statistically “normal,” it may still be inadequate because of subnormal absorption (1) or greater-than-normal excretory loss (2,3).

Other nutrients, such as protein and the trace minerals, are also essential for building a healthy skeleton, but, except for calcium, their effects are usually seen most clearly during growth.
Much of the following discussion will focus on calcium, and it is necessary to stress at the outset that calcium is a nutrient, not a drug. Hence, its beneficial effects will be confined to individuals whose intake of calcium is insufficient. Also, calcium is not an isolated nutrient; it occurs in foods along with other nutrients; and it has been shown that diets low in calcium tend also to be nutritionally poor in other respects as well (4). Thus, although it is necessary to deal with nutrients one at a time in an analysis such as this, it is useful to bear in mind that the disorders in our patients are likely to be more complex.

2. CALCIUM

2.1. The Requirement for Calcium

The primitive function of the skeleton is to serve as a source and as a sink for calcium and phosphorus, that is, as a reserve to offset shortages and as a place for safely storing dietary surpluses, at least after periods of depletion. We see this reserve feature of skeletal function expressed, for example, in laboratory animals such as cats, rats, and dogs, which, when placed on low calcium intakes, will reduce bone mass as needed to maintain near constancy of calcium levels in the extracellular fluid (5). This activity is mediated by parathyroid hormone (PTH) (6) and involves actual bone destruction, not leaching of calcium from bone.

Reserves, of their nature, are designed to tide us over external shortages. When intake is inadequate, the reserve is the first component to be depleted. With most nutrients, this has no detectable impact upon the health or functioning of the organism. Only after the reserve is exhausted and the metabolic pool begins to be depleted, does clinical disease express itself. For some nutrients (e.g., vitamin A, energy), the reserve can be quite large, and the latent period may last many months. But for others (e.g., the water-soluble vitamins), the reserve may be very small and detectable dysfunction develops quickly when intake drops.

Calcium is a unique nutrient in that the calcium reserve in the higher vertebrates acquired a second role, namely internal stiffening and rigidity—what is today the most apparent feature of the skeleton. Calcium is the only nutrient with a reserve that possesses such a secondary function, and the size of the reserve is unusually large, relative to the cellular and extracellular metabolic pools of calcium. As a result, dietary insufficiency virtually never impairs tissue functions that are dependent upon calcium, at least in ways we now recognize. However, because bone strength is a function of bone mass, it follows inexorably that any decrease whatsoever in the size of the calcium reserve—any decrease in bone mass—will produce a corresponding decrease in bone strength. We literally walk about on our calcium reserve. It is this unique feature of calcium nutriture that is the basis for the linkage of calcium and bone status.

2.2. Ascertaining the Requirement for Calcium

Calcium functions as a threshold nutrient, much as does iron. This means that below some critical value the nutrient effect (bone mass for calcium and hemoglobin mass for iron) will be limited by available supplies, whereas above that value, i.e., the “threshold,” no further benefit will accrue from additional increases in intake. This biphasic relationship is illustrated in Fig. 1, in which the intake–effect relationship is depicted first schematically (Fig. 1A), and then (Fig. 1B) as exemplified by bone calcium data derived from a growing
animal model. The minimum requirement can be defined as the intake at which the curve first becomes flat. In Fig. 1B, the effect of the nutrient is expressed directly as the amount of bone calcium an animal is able to accumulate from any given intake.

But the same basic relationship holds for all life stages, even when bone may be undergoing some degree of involution. The threshold concept is generalized to all life situations in Fig. 2A, which shows schematically what the intake/retention curves look like during growth, maturity, and involution. In brief, the plateau occurs at a positive value during growth, at zero retention in the mature individual, and sometimes at a negative value in the elderly. (Available evidence suggests that the plateau during involution is at a negative value in the first 3–5 yr after menopause, rises to zero for the next 10–15 yr, and then becomes increasingly negative with age in the elderly.) At all life stages, the best representation of the minimum requirement is the intake value just at or above the effect threshold of Figs. 1 and 2.

In Fig. 2B, which shows only the involutional curve, there are two points located along the curve, one below (B) and one above (A) the threshold. At A, calcium retention is negative for reasons intrinsic to the skeleton, whereas at B, involutional effects are compounded by inadequate intake, which makes the balance more negative than it needs to be. Point B is probably where most older adults in the industrialized nations would be situated today. The goal of calcium nutrition in this life stage is to move them to point A and thereby to make certain that insufficient calcium intake is not aggravating any underlying bone loss.

There has been much uncertainty and confusion in recent years about what that intake may be for various ages and physiological states. With the 1994 Consensus Development Conference on Optimal Calcium Intake (8), and the DRIs released by the Food and Nutrition Board of the Institute of Medicine (9), the bulk of that confusion has been resolved. The ev-

Fig. 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., J Nutr 1979; 109:1652–1660 (7).] (Copyright Robert P. Heaney, 1992. Reproduced with permission.)
idence for the intakes recommended by these expert panels is summarized both in their respective reports and in recent reviews of the relationship of nutrition and osteoporosis (10,11), and only the highlights will be mentioned in ensuing sections of this chapter.

It is worth noting, however, that the recommendations of both panels, although expressed in quantitative terms, are basically qualitative: Contemporary calcium intakes in the United States, by both men and women, are too low for optimal bone health. The most persuasive of the evidence leading to this conclusion came in the form of several randomized controlled trials showing both reduction in age-related bone loss and reduction in fractures following augmentation of prevailing calcium intakes (12–19). For technical reasons relating to bone remodeling biology (20), randomized controlled trials are not well suited to dose ranging. Hence, although the evidence was persuasive that prevailing intakes were too low, recommended levels in several cases involve ranges, and are clearly prudential judgements, centered of necessity on intakes employed in the trials concerned. The consensus panel’s recommendations, the DRIs of the Institute of Medicine, and the corresponding 1989 RDAs (21) are set forth in Table 1.

2.3. Primary Prevention: The Acquisition of Genetically Programmed Bone Mass

The human skeleton contains, at birth, approx 25–30 g calcium and, at maturity in women, 1000–1200 g. This difference must come by way of diet. Further, unlike other structural nutrients such as protein, the amount of calcium retained is always substantially less than the amount ingested. This is because absorption efficiency is relatively low even during growth, and also because calcium is lost daily through shed skin, nails, hair, and sweat, as well as in urine and nonreabsorbed digestive secretions. Only about 4–8% of ingested calcium is retained. This inefficient retention is not so much because ability to build bone is limited, rather because the primitive calcium intake to which our physiologies are adapted was high. An absorptive barrier is a protection against calcium surfeit, and inefficient retention reflects primitive environmental abundance.
When ingested calcium is less than optimal, the balance between formation and resorption, normally positive during growth, falls toward zero. This occurs because PTH augments bone resorption at the endosteal-trabecular surface of growing bones in order to sustain the level of ionized calcium in the extracellular fluid. When the demands of mineralization at the periosteum and growth plates exceed the amount of calcium absorbed from the diet and released from growth-related bone modeling, more PTH is secreted, and resorption increases still further, until balance becomes zero or even negative. Growth in bone size continues, however, and a limited quantity of mineral now has to be redistributed over an expanding structural volume.

Net bone accumulation will be greater as calcium intake increases, but only to the point where endosteal-trabecular resorption is solely caused by the genetic program governing the shaping of bone during growth, and is not being driven by body needs for calcium. Above that level, as depicted in Figs. 1 and 2, further increases in calcium intake will produce no further bony accumulation. The intake required to achieve the full genetic program, and thus to assure peak bone mass, is the intake that corresponds to the beginning of the plateau region in Fig. 1. This value will be different for different stages of growth, in part because growth rates are not constant and also because, as body size increases, obligatory calcium losses through skin and excreta increase as well.

When the many published reports of calcium-balance studies during growth are combined, it is possible to make out in humans the pattern of plateau behavior found in laboratory animals and then, from the aggregated data, to estimate the intake values that correspond to the threshold (22,23). Fig. 3 represents one example of the relationship between intake and retention, combining the results of many published studies of calcium balance, derived from a subset of the adolescents whose balances were assembled by Matkovic (24). It clearly shows the plateau type of behavior that both animal studies and theoretical considerations predict. It also shows that, at intakes less than the plateau threshold, daily storage is less than optimal, i.e., accumulation of bone is limited by intake. Any such limiting intake must therefore be considered inadequate.

As can be seen, the threshold value for adolescents is about 1500–1600 mg. Best available estimates for the value of this threshold at other stages of growth are 1400 mg in children and 1000 mg in young adults out to age 30.
These values, based on retrospective analysis of balance data, are buttressed by several randomized controlled trials of calcium supplementation in children and adolescents \((15,16,25,26)\) and by a longitudinal observational study in young adults \((27)\). The controlled trials demonstrated that bone gain during growth was greater when intake was elevated above the 1989 RDA. Some of the gain seen within the first 6–12 mo of augmented calcium intake represents a phenomenon known as the remodeling transient \((28)\), which, although it confers improved bone strength in its own right, cannot be used to estimate the requirement because it reflects mainly the transition between two bone-remodeling steady states. Nevertheless, computer modeling of the transient in these trials indicates that not all of the additional gain can be explained solely as a transient. Thus, these data suggest that the 1989 RDAs lie on the ascending portion of the threshold curves of Fig. 2, rather than on the plateau, as they should. This explains the upward revisions reflected in the recommendations of the NIH Optimal Intake panel and of the Food and Nutrition Board.

The longitudinal study of young adults \((27)\) showed prospectively that bone augmentation continues into the third decade. Bone mass gains in this study ranged from 0.5%/yr for the forearm to 1.25%/yr for total body bone mineral. The single most important correlate of the rate of bone accumulation was calcium intake. This study, although it lacked the inferential power of a randomized controlled trial, nevertheless had an advantage over such trials in that it studied individuals on their self-selected intakes, i.e., at a steady state for bone remodeling, and thus avoided the confounding effect of the remodeling transient. The rate of bone accumulation in this study was inversely proportional to age, with the best estimate of the age at which the rate reached zero being approx 29–30 yr. Thus the window of opportunity to achieve the full genetic program appears to remain at least partly open until about age 30.

### 2.4. Secondary Prevention: The Conservation of Acquired Bone Mass

Studies of calcium requirement in mature, but still premenopausal women, have, in general, yielded results compatible with the newer recommendations of Table 1. Welten et al. \((29)\), in a metaanalysis of studies in this age group, concluded that calcium intake
was positively associated with bone mass. Heaney et al. (30), in a study of estrogen-replete women ingesting their habitual calcium intakes, found zero calcium balance at a mean intake slightly under 1000 mg/d, and Nordin et al. (2), a figure closer to 600 mg/d. Recker et al. (31) in a prospective study of bone mass in premenopausal women found no detectable bone loss over a 2-yr period on an estimated mean calcium intake of 651 mg. (Corresponding RDAs, meeting the requirement of 95% of all individuals, would be between approx 800 mg/d and approx 1200.)

In conclusion, although there may be other health reasons for maintaining an even higher calcium intake during the reproductive years, bone health seems to be supported adequately by an intake in the range of 800–1200 mg/d; lower intakes may lead to premenopausal bone loss or failure to achieve peak mass, or both.

2.5. Menopause

Estrogen has a bewildering variety of actions. In bone, it seems to adjust the bending setpoint of the mechanical feedback loop that regulates bone mass. Accordingly, whenever women lose ovarian hormones, either naturally at menopause or earlier as a result of anorexia nervosa or athletic amenorrhea, the skeleton appears to sense that it has more bone than it needs, and hence allows resorption to carry away more bone than formation replaces. (Precisely the same change occurs when men lose gonadal hormones for any reason.) This amounts to raising the setpoint of the feedback loop, which functions to maintain bone bending under load within safe limits. Although varying somewhat from site to site across the skeleton, the downward adjustment in bone mass as a result of gonadal hormone lack amounts to approx 15% of the bone a woman had prior to menopause (32).

The importance of this phenomenon in a discussion of nutrient effects is to distinguish menopausal bone loss from nutrient deficiency loss and to stress that menopausal loss, which is mainly caused by an absence of gonadal hormones, not to nutrient deficiency, cannot be substantially influenced by diet. Almost all of the published studies of calcium supplementation within 5 yr following menopause failed to prevent bone loss. Even Elders et al., who employed a calcium intake in excess of 3100 mg/d, succeeded only in slowing menopausal loss, not in preventing it (33). Only a few reports, such as the study of Aloia (19), contain clear evidence for a benefit of a high calcium intake at this life stage, and even here, estrogen produced a greater effect. Nevertheless, one can find in many of the published reports evidence of small calcium effects at even this life stage, and it may be that, in any group of early menopausal women, there are some whose calcium intake is so inadequate that they are losing bone for two reasons (estrogen lack plus calcium insufficiency).

Important as menopausal bone loss is, it is only a one-time downward adjustment, and, if nutrition is adequate, the loss continues for only a few years, after which the skeleton comes into a new steady state (although at a 15% lower bone mass). It is in this context that the importance of achieving a high peak-skeletal mass during growth becomes apparent. One standard deviation for lumbar spine bone-mineral content in normal women is about 12–15% of the young adult mean, and for total body bone mineral, about 10–12%. Hence, a woman at least one standard deviation above the mean can sustain the 15% menopausal loss 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 premenopausally drops to two standard deviations below the mean as she crosses menopause and is therefore,
As noted, the menopausal bone-mass adjustment theoretically stops with a loss of about 15%, but this is true only so long as calcium intake is adequate. In this regard, it is important to note that estrogen has nonskeletal effects as well, i.e., it improves intestinal calcium absorption and renal calcium conservation (32,35,36). As a result, an estrogen-deficient woman has a higher calcium requirement, and unless she raises her calcium intake after menopause, she will continue to lose bone after the estrogen-dependent quantum has been lost, even if the same diet would have been adequate to maintain her skeleton before menopause. In other words, early in the menopausal period, her bone loss is mainly (or entirely) because of estrogen withdrawal, whereas later it will be because of inadequate calcium intake. Fig. 4 assembles, schematically, the set of factors contributing to bone loss in the postmenopausal period. The figure shows both the self-limiting character of the loss as a result of estrogen deficiency and the usually slower, but progressive loss caused by nutritional deficiency (if present). Unlike the estrogen-related loss, which mostly plays itself out in 3–6 yr, an ongoing calcium deficiency loss will continue to deplete the skeleton indefinitely for the remainder of a woman’s life, that is, unless calcium intake is raised to a level sufficient to stop it. Furthermore, because both absorption efficiency (35) and calcium intake (37) decline with age, the degree of calcium shortfall actually tends to worsen with age.

Thus, it is important for a woman to increase her calcium intake after menopause. Both the 1984 NIH consensus conference on osteoporosis, and the 1994 Consensus Conference on Optimal Calcium Intake (8) recommended intakes of 1500 mg/d for estrogen-deprived postmenopausal women. The “Adequate Intakes” of the Food and Nutrition Board (9) for everyone over age 50, when translated into RDA format, are nearly identical (1450 mg/d). It may be that the optimal intake is somewhat higher still, but median intakes in the United States for women of this age are in the range of 500–600 mg/d (37,38), and if the bulk of them could be raised even to 1500 mg/d, the impact on skeletal health would be considerable.

2.6. Senescence

Age-related bone loss occurs in both sexes, regardless of gonadal hormone levels, generally starting about age 50. However, it is obscured in the years immediately following menopause in women by the substantially larger effect of estrogen withdrawal (see Fig. 4). It probably occurs, however, even in estrogen-treated women, at about the same rate as in men. This rate is generally reported to be on the order of 0.5–1.0%/yr during the sixth and seventh decades, and then accelerates with advancing age. For example, loss from the hip in the control subjects of the study by Chapuy et al. (13), at an average age of 84, was 3%/yr. Age-related loss involves both cortical and trabecular bone and can come about by several mechanisms: disuse, remodeling errors, and nutritional deficiency, summarized in Fig. 4.

Whereas nutrient deficiency is clearly only a part of the total problem, nevertheless it is common. That the 3% loss in the control subjects of Chapuy et al. was related to their nutritional status is indicated by the fact that this loss was completely obliterated in the calcium and vitamin D supplemented women. Intestinal calcium absorption efficiency declines with age (35), at the same time as nutrient intake itself generally declines (37); the result is that the diet of aging individuals becomes more and more
inadequate. McKane et al. (39) have recently shown that the high PTH levels and abnormal PTH secretory dynamics typically found in elderly women are caused by calcium deficiency, and that PTH function can be entirely normalized by calcium intakes of 2400 mg/d.

It is in this age group also that the most dramatic and persuasive evidence for fracture prevention by high calcium intakes has been produced in recent years. This is partly because most fragility fractures rise in frequency with age, and hence the opportunity to see a fracture benefit (if one exists) is then greater. Chapuy et al. (13) showed a reduction in hip fracture risk of 43% by 18 mo after starting supplementation with calcium plus vitamin D, and a 32% reduction in other extremity fractures. Chevalley et al. (17) in another study in elderly women, showed that, even when vitamin D was given to both groups, extra calcium-reduced femoral bone loss and vertebral fracture incidence. More recently Recker et al. (40) in a 4-yr, randomized controlled trial in elderly women (mean age 73), demonstrated that a calcium supplement reduced both age-related bone loss and incident vertebral fractures. Their subjects had all received a multivitamin supplement containing 400 IU of vitamin D, as had the subjects of Chevalley et al.; hence, the effect in the calcium-supplemented groups of both studies can be attributed to the extra calcium alone.

These findings do not mean that vitamin D is unimportant in this age group. It is likely that intakes of both calcium and vitamin D are inadequate in the elderly, and the unrecognized prevalence of combined deficiency has made it difficult to study the actual requirements of either nutrient in this age group.

The calcium intake achieved in the Chapuy study was about 1700 mg/d, 1400 mg/d in the Chevalley study, and about 1600 mg/d in the Recker study. These values are in the range of the intake earlier found by Heaney et al. (30) to be the mean requirement for healthy estrogen-deprived older women (1500–1700 mg/d). All of these studies are, therefore, congruent with the newer recommendations of 1400–1500 mg/d (Table 1).

An important feature of these controlled trials in already elderly individuals was that bone mass was low in both treated and control groups at the start of the study, and al-
though a significant difference in fracture rate was produced by calcium supplemen-
tation, even the supplemented groups would have to be considered as having an unac-
ceptably high fracture rate. What these studies do not establish is how much lower the
fracture rate might have been if a high calcium intake had been provided for the pre-
ceding 20–30 yr of these women’s lives. The studies of Matkovic et al. (41) and
Holbrook et al. (42), although not randomized trials, strongly suggest that the effect
may be larger than has been found with treatment started in the eighth and ninth decades
of life. Both of these observational studies reported a hip fracture rate that was roughly
60% lower in elderly whose habitual calcium intakes had been high. Although findings
from observational studies such as these had not been considered persuasive in the ab-
sence of proof from controlled trials, the now large number of controlled trials showing
a skeletal benefit of added calcium have removed that uncertainty.

Together the aggregate of available studies underscores the importance of achieving
at least the 1500-mg target figure for the elderly. At the same time, it must be stressed,
one again, that osteoporosis is a multifactorial condition and that removing one of the
pathogenic factors (i.e., ensuring an adequate calcium intake) cannot be expected to
eradicate all osteoporotic fractures.

2.7. Nutrient–Nutrient Interactions: Factors that Influence the Requirement

There are several nutritional factors that influence or have been proposed to influence
the calcium requirement. The principal interacting nutrients are sodium, protein, caf-
eine, and fiber. Fiber and caffeine influence calcium absorption (43–45) and typically
exert relatively minor effects, while sodium and protein influence urinary excretion of
calcium (45,46), and can be of much greater significance for the calcium economy. The
effects of phosphorus and fat in humans are minor to nonexistent.

The basis for the differing importance of these nutritional factors on the calcium
 economy is illustrated in Fig. 5, which partitions the variance in calcium balance ob-
served in 560 balances in healthy middle-aged women consuming typical intakes, and
studied in the author’s laboratory. As Fig. 5 shows, only 11% of the variance in balance
among these women is explained by differences in their actual calcium intakes. By con-
trast, absorption efficiency explains about 15%, whereas urinary losses explain more
than half.

2.7.1. Influences on Intestinal Absorption of Calcium

2.7.1.1. Fiber. The effect of fiber is variable, and generally small. Many kinds of fiber
have no influence at all on absorption, such as the fiber in green, leafy vegetables (10).
The fiber in wheat bran, by contrast, reduces absorption of coingested calcium, although
except for extremes of fiber intake (47), the overall effect is generally relatively small.
Often lumped together with fiber are associated plant food constituents, such as phytate
and oxalate. Both can reduce the availability of any calcium contained in the same food,
but, unlike bran, generally do not affect coingested calcium from other foods. For ex-
ample, for equal ingested loads, the calcium of beans is only about half as available as
the calcium of milk (48), although the calcium of spinach and rhubarb is nearly totally
unavailable (4). For spinach and rhubarb, the inhibition is mostly because of oxalate. For
common beans, phytate is responsible for about half the interference, and oxalate, the
other half. Even so, the effects of phytate and oxalate are highly variable from food to
food. There is a sufficient quantity of both antiabsorbers in beans to complex all the cal-

2.7.1.2. Caffeine. Often considered to have a deleterious effect on the calcium economy, caffeine actually has the smallest effect of the known interacting nutrients. A single cup of brewed coffee causes deterioration in calcium balance of approx 3 mg (44,45,50), mainly by reducing absorption of calcium (44). The effect is probably on active transport, although this is not known for certain. This small effect is more than adequately offset by a tablespoon or two of milk (44,50).

2.7.2. Influences on Renal Conservation of Calcium

2.7.2.1. Protein and Sodium. As noted, the effects of protein and of sodium can be substantial (2,3,45). Both nutrients increase urinary calcium loss across the full range of their own intakes, from very low to very high—so it is not a question of harmful effects of an excess of these nutrients. Sodium and calcium share the same transport system in the proximal tubule, and every 2300 mg sodium excreted by the kidney pulls 20–60 mg of calcium out with it. And every gram of typical protein (whether from animal or vegetable sources) metabolized in adults causes an increment in urine calcium loss of about 1 mg. This latter effect is probably a result of excretion of the sulfate load produced in the metabolism of sulfur-containing amino acids (and is thus a kind of endogenous analog of the acid-rain problem).

At low sodium and protein intakes, the minimum calcium requirement for an adult female may be as little as 450 mg/d (46), whereas if her intake of both nutrients is high, she may require as much as 2000 mg/d to maintain calcium balance. A forceful illustration of the importance of sodium intake is provided by the report of Matkovic et al. (51) that urine calcium remains high in adolescent girls on calcium intakes too low to permit bone gain. The principal determinant of urinary calcium in such young women is sodium intake (52), not calcium intake.

Differences in protein and sodium intake from one national group to another are part of the explanation why studies in different countries have shown sometimes strikingly different calcium requirements. At the same time, one usually finds a positive correlation between calcium intake and bone mass within the national range of intakes (53). Hence, although sodium (and protein) intake differences between cultures obscure the calcium effect, they do not obliterate it.

For diets high in calcium, as would have been the case for our hunter-gatherer ancestors, high protein and possibly high sodium intakes could have been handled by the body per-
fectly well. This is because an individual’s absorptive performance is close to maximal at the low intakes that prevail today. Augmented loss from increased sodium or protein intake cannot be offset by increasing extraction from the diet, both because there is less there to extract, and because extraction efficiency is already at the upper end of its possible range. By contrast, at intake levels typical of those that prevailed during hominid evolution, intestinal absorption is predominantly passive, and the full range of absorptive adaptation is available to offset increased urinary losses. In brief, these nutrients create problems for the calcium economy of contemporary adult humans mainly because we typically have calcium intakes that are low relative to those of preagricultural humans, and sodium intakes that are high.

### 2.7.2.2. Acid Ash Residue

The acid/alkaline ash characteristic of the diet is also important, although the quantitative relationship of this diet feature to the calcium requirement has been less fully explored to date. Nevertheless, it has clearly been shown that substitution of metabolizable anions (e.g., bicarbonate or acetate) for fixed anions (e.g., chloride) in various test diets will lower obligatory urinary calcium loss substantially (54,55). This suggests that primarily vegetarian diets create a lower calcium requirement, and provides a further explanation for the seemingly lower requirement in many nonindustrialized populations. However, it is not yet clear whether, within a population, vegetarians have higher bone mass values than omnivores, and some data suggest they may actually have lower bone mass, possibly because of the often very low calcium levels of such diets (56,57).

### 2.7.2.3. Phosphorus

Phosphorus is commonly believed to reduce calcium absorption, but the evidence for that effect is scant to nonexistent, and there is much contrary evidence. Spencer has shown no effect of even large increments in phosphate intake on overall calcium balance at low, normal, and high intakes of calcium (57). In adults, Ca:P ratios ranging from 0.2 to above 2.0 are without effect on calcium balance, at least so long as adjustments are made for calcium intake (45). What phosphorus does is depress urinary calcium loss and elevate digestive juice secretion of calcium, by approximately equal amounts, with little or no net effect on balance (58). Although it is true that stoichiometric excesses of phosphate will tend to form complexes with calcium in the chyme, various calcium phosphate salts have been shown to exhibit absorbability similar to other calcium salts, and phosphate is, of course, a principal anion of the major food source of calcium (dairy products). In any case, phosphate itself is more readily absorbed than calcium (by a factor of at least 2×), and at intakes of both nutrients in the range of their respective RDAs, absorption will leave a stoichiometric excess of calcium in the ileum, not the other way about. This explains the seeming paradox that high calcium intakes can block phosphate absorption (as in management of end-stage renal disease), whereas achievable high phosphate intakes have little or no effect on calcium absorption.

### 2.7.2.4. Aluminum

Although not in any proper sense a nutrient, aluminum, in the form of Al-containing antacids, also exerts significant effects on obligatory calcium loss in the urine (59). By binding phosphate in the gut, these substances reduce phosphate absorption, lower integrated 24-h serum phosphate levels, and thereby elevate urinary calcium loss. (This is the opposite of the more familiar hypocalcuiric effect of oral phosphate supplements.) Therapeutic doses of Al-containing antacids can elevate urine calcium by 50 mg/d or more.

### 2.8. Calcium Sources

The best calcium sources are, of course, foods. In a modern, Western diet, food items
that provide more than 100 mg of calcium per serving are limited to dairy products (with the exception of cottage cheese), greens of the mustard family (collards, kale, mustard), calcium-set tofu, sardines, and a few nuts (especially hazelnuts and almonds). Smaller amounts of calcium are ubiquitous in many leafy vegetables, but with the exception of shellfish, calcium levels are low in most meats, poultry, or fish. As noted earlier, the calcium of beans is only about half as available as the calcium of milk, and the calcium of high oxalate vegetables (such as spinach and rhubarb) is almost completely unavailable. Fig. 6 displays the available calcium in a variety of foods. “Available” represents the product of the fractional absorbability of the calcium in a food and its total calcium content. It is thus the actual amount of calcium a particular food delivers into the blood of the absorbing subject.

In general, most diets without dairy products have gross calcium nutrient densities under 20 mg Ca/100 kCal, and available calcium densities lower still. Because total energy intake for adult American women is in the range of 1800 kCal/d, it follows that most diets low in dairy products will be low in calcium—probably 300–400 mg Ca/d or less—far short of levels currently considered optimal.

Partly as a response to this dilemma, the Surgeon General, in his 1988 report on Nutrition and Health (60), recommended judicious, low-level calcium fortification of many items in the food chain. An increasing number of fortified foods is becoming available each year—ranging from fruit juice, to bread, to breakfast cereals, to potato chips, to rice. Where bioavailability of the calcium in these foods has been ensured, these foods should be useful adjuncts in the attempt to improve calcium intake at the population level.

The principal supplement on the United States market is calcium carbonate, available as such, or as oyster shell or dolomite. When the tablet is competently formulated, so that it disintegrates in gastric juice, or when the supplement is chewed (as with TUMS™ or Os-Cal™ chewables), the carbonate is quite well absorbed and generally very well tolerated. There is no requirement for gastric acid so long as the carbonate salt is taken with meals. Calcium citrate and calcium citrate malate are also good sources,
but they tend to be more expensive. In the rare case in which the carbonate seems not well tolerated, these other sources provide useful alternatives.

Divided doses enhance absorption from both supplements and foods, because absorption fraction is an inverse function of load size. All calcium sources (including food) interfere with iron absorption when the two nutrients are ingested at the same meal. However, single-meal studies miss the body’s upregulation of iron absorption in the face of need, and chronic feeding studies have revealed no deterioration of iron status in subjects consuming high calcium diets. Perhaps of greater relevance, Matkovic et al. have convincingly shown that adolescent girls are able to increase total body iron stores fully normally in the presence of 1600 mg calcium diets (61). This is a particularly reassuring finding because this age group is the one most vulnerable to iron deficiency in the United States today. However, if an adult is iron-deficient (e.g., as a result of severe blood loss) and is taking an iron supplement, it may be best if the meal at which the iron is taken not contain a large amount of calcium (food or supplement).

3. VITAMIN D

Vitamin D facilitates active transport of calcium across the intestinal mucosa, at least partly by inducing the formation of a calcium-binding transport protein in intestinal mucosal cells. This function is particularly important for adaptation to low calcium intakes. Absorption also occurs passively, probably mainly by way of paracellular diffusion. This route is not dependent upon vitamin D, and is not as well studied. The proportion of absorption by the two mechanisms varies with intake and is not well characterized in humans; at high calcium intakes (above 2000 mg/d) absorption fraction approaches 10–15% of intake. Under these circumstances, it is likely that active transport contributes relatively little to the total absorbed load. Nevertheless, it is generally considered that vitamin D status influences absorptive performance and that it thereby influences the calcium requirement.

A principal storage form of the vitamin is 25-hydroxyvitamin D [25(OH)D], and its plasma level is generally regarded as the best clinical indicator of vitamin D status. Although usually considered to be about three orders of magnitude less potent than calcitriol in promoting active transport in animal receptor assays, there is growing evidence that it may possess physiological functions in its own right (62), and in the only human dose-response studies performed to date, 25(OH)D was found to have a molar potency in the range of 1/100 to 1/125 that of 1,25(OH)2D3 (63,64), not the 1/2000 figure usually considered to reflect relative 25(OH)D activity.

Vitamin D status commonly deteriorates in the elderly, whose plasma 25(OH)D levels are generally lower than in young adults (65). This difference is partly a result of decreased solar exposure, decreased efficiency of skin vitamin D synthesis, and decreased intake of milk, the principal dietary source of the vitamin. Moreover, the elderly exhibit other abnormalities of the vitamin D endocrine system that may further impair their ability to adapt to reduced calcium intake. These include decreased responsiveness of the renal 1-α-hydroxylase to parathyroid hormone (66) and decreased mucosal responsiveness to calcitriol (67).

For all these reasons, there is a growing consensus that the requirement for vitamin D rises with age (9,68), and a body of data that strongly suggests relative vitamin D deficiency plays a role in several components of the osteoporosis syndrome. Whereas a trial by Lips et al. (68) of vitamin D supplementation noted no fracture reduction, oth-
ers have demonstrated clear benefits from supplementing vitamin D in the elderly. One example is the finding by Heikinheimo et al. (69), in a randomized controlled trial of significant reduction in all fractures in an elderly Finnish population given a single annual injection of 150,000–300,000 IU vitamin D each fall (equivalent to approx 400–800 IU/d). The impressive fracture reductions noted in the trials of Chapuy et al. (13) and Dawson-Hughes et al. (70) may also have been partly because the vitamin D supplementation that was a component of both trials.

The foregoing studies [as well as others (71,72)] strongly suggest that vitamin D insufficiency is prevalent in the middle-aged and elderly of Northern Europe and North America. Moreover, in virtually none of these studies was frank osteomalacia a significant feature of the problem. This old criterion for true vitamin D deficiency is much too insensitive to be clinically useful today. How the vitamin D requirement ought to be defined is another matter. Holick has shown that it takes an intake of at least 600 IU/d, from all sources, to sustain serum 25(OH)D levels (73), and the doses of vitamin D used in the studies summarized above also suggest that an intake in the range of 500–800 IU/d is required for full expression of the known effects of vitamin D in adults. This is substantially above the 1989 RDA of only 200 IU for adults (21). The new DRIs for vitamin D included an increase from 200 to 400 IU for adults aged 50–70, and to 600 IU for those over age 70. Vieth presents evidence that the requirement may be higher still (74).

This field is complicated by the mixed input of dermal and dietary vitamin D. Because dermal production is largely unknown, it is difficult to estimate how much must be provided orally in those who are housebound or excluded from skin exposure to solar radiation.

4. VITAMIN K

The chemistry and physiology of Vitamin K have been extensively reviewed elsewhere (75,76). In brief, vitamin K is necessary for the \( \gamma \)-carboxylation of glutamic acid residues in a large number of proteins. Most familiar are those related to coagulation, in which seven vitamin K-dependent proteins are involved in one way or another. The \( \gamma \)-carboxyglutamic acid residues in the peptide chain bind calcium, either free or on the surface layers of crystals, and have been thought to function in varying ways including catalysis of the coagulation cascade, inhibition of mineralization (as in urine), and generation of osteoclast chemotactic signals.

Three vitamin K-dependent proteins are found in bone matrix: osteocalcin (bone gla protein [BGP]), matrix gla-protein, and protein S. Only BGP is unique to bone. There is also a kidney gla protein (nephrocalcin), which may be involved in renal reabsorption of calcium. BGP binds avidly to hydroxyapatite and is chemotactic for bone-resorbing cells. Roughly 30% of the synthesized BGP is not incorporated into matrix, but is released instead into the circulation, where, like alkaline phosphatase, it can be measured and used as an indicator of bone turnover. In vitamin K deficiency, such as would occur with coumarin anticoagulants, serum BGP levels decline, and the degree of carboxylation of the circulating BGP falls dramatically. Although it would seem, therefore, that vitamin K deficiency would have detectable skeletal effects, they have been very hard to find. Rats reared and sustained to adult life under near total suppression of BGP \( \gamma \)-carboxylation show only minor skeletal defects, mostly related to abnormalities in the growth apparatus (75). In aging humans, the problem of detecting skeletal abnormalities is compounded by the fact that the bulk of the skeleton was formed prior to the
onset of any deficiency, and thus bone tends to be an insensitive indicator of current nutritional stresses.

Various vitamin K-related abnormalities have been described in association with osteoporosis, but their significance to skeletal status remains unclear. Circulating vitamin K and menaquinone levels are low in hip fracture patients (77). BGP is undercarboxylated in osteoporotics, and this defect responds to relatively small doses of vitamin K. Finally, urine calcium has been reported to be high in osteoporotics and to fall on administering vitamin K (78).

Whether or not vitamin K is important for bone health, serum vitamin K levels are indicators of general nutritional status, and it may simply be that the observation of low vitamin K levels in osteoporotics, especially in those with hip fracture, is mainly a reflection of the often poor nutrition of these individuals.

5. MAGNESIUM

The adult female RDA for magnesium was 280 mg/d in the 1989 RDAs (21) and revised upward to 320 mg/d in the 1997 DRIs (9). Only about 25% of adult United States females achieve this level of intake. Average intakes tend to be in the range of 70–80% of the RDA. Whereas severe magnesium deficiency is a well-described syndrome (79), interfering both with PTH secretion and PTH action on bone, it is uncertain whether mild departures from the RDA have any adverse effect, or even whether the RDA needs to be as high as it is now set. There is, as well, a widespread popular belief that magnesium is necessary for optimal calcium absorption. However, the many studies establishing the benefit of supplemental calcium described earlier achieved their effect without adding magnesium to the diets of their subjects. Furthermore, Spencer, in a series of careful metabolic studies, showed that major increases in magnesium intake had no significant effect on absorption efficiency for calcium (80). Thus, there is no known justification for supplemental magnesium in prevention or treatment of osteoporosis. Moreover, magnesium salts, when used as a component of a combined supplement tablet (e.g., as in dolomite), displace calcium and make it more difficult (i.e., more pills are required) to get sufficient calcium by this route.

However, a small proportion of patients with osteoporosis have silent celiac disease as a contributory factor in their disease. These individuals commonly have some degree of magnesium deficiency. Because the underlying problem in such cases is asymptomatic, it is usually unrecognized, and hence, untreated. For that reason, there may well be a small group of osteoporotic patients who would benefit from supplemental magnesium (as well as from calcium and vitamin D).

6. TRACE MINERALS

Several trace minerals, notably zinc, manganese, and copper, are essential metallic cofactors for enzymes involved in synthesis of various bone matrix constituents. Ascorbic acid (along with zinc) is needed for collagen crosslinkage. In growing animals, diets deficient in these nutrients produce definite skeletal abnormalities (81). Additionally, zinc deficiency is well known to produce growth retardation and other abnormalities in humans. But it is not known whether significant deficiencies of these elements develop in previously healthy adults, or at least, if they do, whether such
deficiencies contribute detectably to the osteoporosis problem. Copper deficiency is reported to be associated with osteoporotic lesions in sheep, cattle, and rats (82). Copper has not been much studied in connection with human osteoporosis, but in one study in which serum copper was measured, levels were negatively correlated with lumbar spine BMD, even after adjusting for body weight and dietary calcium intake (83).

Copper-deficient animals develop reduced collagen crosslinks, a factor that is known to weaken bone strength. Oxlund has reported reduced extractable crosslinks in the bone of osteoporotic patients (84), but it is not known whether copper deficiency was the cause.

In one four-way, randomized trial, copper, as a part of a trace mineral cocktail including also zinc and manganese, slowed bone mineral loss in postmenopausal women, when given either with or without supplemental calcium (85). There appeared to be a small additional benefit from the extra trace minerals; however, the only statistically significant effect in this study was associated with the calcium supplement. This could mean that trace mineral deficiency plays no role in osteoporosis, but it could also mean that not all of the women treated suffered from such deficiency. In fact, because both osteoporotic and age-related bone loss are multifactorial, and because there is no known way to select subjects for inclusion on the basis of presumed trace mineral need, one would presume that only some of the subjects in such a study might be deficient. Thus, the suggestive findings of this study have to be considered grounds for further exploration of this issue.

7. NUTRITION AND HIP FRACTURE

Nutrition enters into the hip fracture problem in two ways: in predisposing to fracture and in recovery from the assault of the injury and its repair. Fractures in the elderly, and particularly hip fractures, are concentrated in institutionalized persons with multiple disabilities. The osteoporotic elderly, generally, are known to have depleted lean body mass and fat mass, and, when studied, have been found to have low circulating values for several key nutritional indicator variables, from serum albumin to ferritin and vitamin A (86). Survival two years after injury is four times higher in patients with serum albumin values above 3.5 g/dL than in patients with values below 3.0 g (86). Additionally, patients with hip fracture often have low calcium intakes, and in the majority of studies evaluating the matter, dietary calcium earlier in life is inversely associated with hip fracture risk. In brief, hip fracture is a problem concentrated in multiply compromised individuals, and the prospect of successfully intervening to reduce risk has proved daunting even to contemplate.

However, one aspect of the problem is partly amenable to control. The relative malnutrition of patients suffering hip fracture and coming to hospital for repair contributes significantly to the often unsatisfactory outcomes for this common fracture (i.e., 15–20% excess mortality; 50% institutionalization of the survivors). Delmi et al. (86), in a randomized trial of a protein-based nutrient supplement given to patients newly hospitalized for hip fracture, found that only 26% of unsupplemented individuals had outcomes classified as good at 6 mo after injury, whereas nearly 60% of supplemented individuals had good outcomes. The investigators noted that the hospital diets offered the unsupplemented individuals were nutritionally adequate, but were frequently unconsumed, whereas the investigators ensured the ingestion of the supplement. This is not an isolated observation; others (87) had earlier found qualitatively similar benefit from nutritional supplementation in such patients, and thus the consistency of these
findings constitutes a challenge to the health professions to apply these basic nutritional principles in the management of their patients.

8. RECOMMENDATION

Calcium intake should be high throughout life: 1500 mg/d during growth and at least that much in the elderly. Foods are the best sources, but given caloric restriction, that means fat-free milk and yogurt, for the most part, as well as the widespread availability of calcium fortified foods. Supplements are convenient and often necessary, but should not be a substitute for a national nutritional policy or for a good diet. The elderly are commonly vitamin D deficient, as well as calcium-deprived. Conscious efforts must be made to ensure a daily intake of 600–800 IU/d. The elderly often suffer some degree of global undernutrition in addition to their specific deficiencies of calcium and vitamin D. Given the common isolation of elderly living alone, this is not an easy problem to solve. At least we can mount an effort to feed them when they develop fractures.

REFERENCES


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1. INTRODUCTION

Certain high-risk occupations, adverse socioeconomic and sociocultural circumstances, and unhealthy, avoidable aspects of lifestyle may, individually or collectively, result in heightened levels of oxidative stress, predisposing the individual to future development of oxidant-mediated organ dysfunction and disease (1,2). Outdated and/or poorly regulated industrial practices, unacceptably high levels of vehicle exhaust emissions, overpopulation associated with overcrowded, poorly ventilated, nonelectrified dwellings, and poor dispersal of atmospheric pollutants caused by unfavorable climatic conditions and/or topography are problems commonly encountered in, but not limited to, many developing countries (3,4). In these circumstances, chronic exposure to excessive levels of atmospheric pollution in the workplace, environment, and home is accompanied by increased oxidative stress. Unhealthy lifestyles, such as poor dietary habits, especially low intake of fresh fruits and vegetables (5), cigarette smoking (2), and in some cases, excessive exposure to ultraviolet radiation (6) also accelerate the onset of those degenerative diseases (cataracts, cardiovascular diseases, cancer, pulmonary dysfunction, and emphysema), which have a suspected oxidant-mediated etiology (1,2).

Cigarette smoking is the most common and eminently avoidable cause of lifestyle-related oxidative stress associated with accelerated onset of degenerative disease and premature death. Moreover, the toxicology of cigarette smoke inhalation is probably broadly similar to that of many other inhaled, prooxidative irritants. For these reasons I have focused primarily on cigarette smoking to review the relationships that exist between atmospheric pollution, nutrition, and oxidant-mediated diseases.

2. CIGARETTE SMOKING

The magnitude of the ongoing, and apparently increasing, threat posed to public health by cigarette smoking is emphasized by the following recently published comment (7):

Smoking represents a great failure in public health; more than 40 years after the hazards were first established, cigarettes are still responsible for 30% of deaths in middle age in Britain and the United States, and worldwide sales are increasing (7).
This statement is based on data derived from an epidemiological study designed to investigate mortality in relation to smoking over a 40-yr period in male British doctors (8). Alarmingly, the results of this study demonstrate that the hazards of long-term use of tobacco were substantially underestimated in previous studies conducted over shorter periods; a revised estimate is that about half of all regular cigarette smokers will eventually be killed by their habit (8). The average decrease in life expectancy of smokers relative to nonsmokers is 8 yr (8). If current smoking trends persist, it is predicted that smoking will be one of the largest causes of premature death in the world (7).

2.1. Origins of Oxidants During Smoke Exposure

There are two major sources of oxidative stress in smoke-exposed individuals. Firstly, the gas and tar phases of cigarette smoke contain extremely high levels of organic radicals, the respective, approximate concentrations being $10^{15}$/puff and $10^{17}$/g. Second, inhalation of cigarette smoke has a profoundly irritant effect on the phagocytic cells of the immune system, causing an increase in both the numbers and oxidant-generating activities of these cells (9–12). Consequently, chronic exposure of the smoker to high levels of reactive oxidants is accompanied by an increased risk of oxidant-mediated diseases. These events are summarized in Fig. 1.

2.2. Proinflammatory Effects of Cigarette Smoking

Cigarette smoking causes an acute localized inflammatory reaction that is characterized by the accumulation of phagocytes (neutrophils and macrophages) in the membranous bronchioles and alveoli of the lungs, leading to destruction of the peribronchiolar alveolar attachments and pulmonary dysfunction (13,14). These inflammatory events are not confined to the lung, however, and systemic, proinflammatory effects of cigarette smoking have also been demonstrated in numerous studies. Compared to nonsmokers, cigarette smokers have significantly increased circulating leukocyte counts, which are inversely correlated with the degree of airflow limitation (9,15). In addition, the decline in forced expiratory flow in one second (FEV$_1$), an important measure of pulmonary function, is inversely correlated with both the initial peripheral leukocyte count (16) and the change in leukocyte count over time, independently of the smoking habit (17). The normal tempo of neutrophil production in the bone marrow is about $0.5–1.5 \times 10^9$/kg of body weight daily. These short-lived phagocytes are released into the circulation and account for up to 70% of the circulating leukocyte population. The normal range for circulating leukocyte counts in apparently healthy adult humans is $4–11 \times 10^9$/mL of blood, which is an underestimate because approx 50% of the circulating neutrophil pool is adherent to vascular endothelium. On average, cigarette smoking increases the numbers of circulating leukocytes (mainly neutrophils and monocytes) by 20–30%, but in some cases, the increase may exceed 100%. Cigarette smoking not only increases the numbers of circulating and pulmonary phagocytes, but also enhances the prooxidative and adhesive properties of these cells. Phagocytes from cigarette smokers are sensitized for increased production of reactive oxidants (11,12,18), which is attributable, at least in part, to increased content of the prooxidative enzyme myeloperoxidase (MPO) and is closely correlated with the degree of pulmonary dysfunction (19). Cigarette smoke-mediated activation of neutrophils in vivo causes delayed transit of these cells through the pulmonary microcirculation (21,22), which is probably a result of oxidant-mediated adhesion of these cells to vascular endothelium.
(23,24), altered expression of cellular adhesion molecules (25), intravascular aggregation of neutrophils and platelets (23,26), and increased release of MPO and elastase (22,27).

3. HARMFUL ACTIVITIES OF OXIDANTS

Phagocyte-derived reactive oxidants, as well as those present in cigarette smoke, have a range of harmful activities. These oxidants have been reported to be cytotoxic for a wide variety of eukaryotic cells (28,29) and are also immunosuppressive, carcinogenic, proteolytic, proadhesive, and proatherogenic. The biochemical mechanisms involved in the etiology of oxidant-mediated disorders are summarized in Table 1. Whereas the relative contributions of oxidants derived directly from cigarette smoke and those from smoke-activated phagocytes to the oxidative events involved in the pathogenesis of smoking-related diseases are unknown, the combined assault from these two sources is unremitting and difficult to counteract.

3.1. Oxidants as Carcinogens

Given the complexity and range of reactive oxidants that it contains, it is hardly surprising that several pro-oxidative mechanisms have been proposed to account for the observed direct DNA-damaging effects of cigarette smoke (2,29,30). Polyhydroxy aromatic compounds present in tar have been proposed to bind to the genetic material, leading to generation of hydrogen peroxide (H$_2$O$_2$), which in turn causes DNA strand breaks by a hydroxyl radical (·OH)-dependent mechanism (30).

The association between chronic inflammation, phagocyte-derived oxidants, and development of epithelial cancers is well recognized (31). Activated phagocytes have been identified as potential carcinogens because they oxidatively damage DNA and promote malignant transformation in bystander cells in tissue culture (22). Hydrogen peroxide is the oxidant primarily responsible for phagocyte-mediated DNA damage to neighboring cells. This permeant oxidant interacts with intracellular transition metals to generate ·OH in close proximity to DNA, leading to oxidative damage to adenine, guanine, thymine, and cytosine, and DNA strand breaks (31). Although DNA damage in living cells is subject to cellular repair, it may occasionally escape repair, or repair may be incorrect. In such cases, unrepaired or misrepaired DNA could have deleterious consequences, leading to

![Diagram](image-url) Fig. 1. Events leading to pulmonary dysfunction in smokers.
gene modifications that may ultimately promote cellular transformation (32). Ominously, it has also been reported that the permeant, phagocyte-derived reactive oxidant, hypochlorous acid (HOCl), oxidatively inactivates the DNA repair enzyme poly(ADP-ribose)polymerase in bystander cells exposed to activated neutrophils (33), indicating that phagocyte-derived oxidants not only damage DNA, but also compromise DNA repair mechanisms.

3.2. Proteolytic Activities of Oxidants

Reactive oxidants present in cigarette smoke, as well as those released by activated phagocytes, have been reported to potentiate the activity of neutrophil-derived proteolytic enzymes. Neutrophil granules contain a large family of over 20 enzymes, but four proteolytic enzymes, the neutral serine proteases, elastase and proteinase 3, and the two metalloproteinases, collagenase and gelatinase, seem to have the greatest potential to act as mediators of tissue injury (34). These proteases are released extracellularly by activated neutrophils (27), and each cleaves key components of the extracellular matrix, which is composed of a complex mix of collagens, elastin, proteoglycans, and glycoproteins that lies under epithelia and surrounds connective tissue cells (34). Extracellular release of proteases and generation of reactive oxidants by activated neutrophils are concomitant and interrelated events. Reactive oxidants, especially phagocyte-derived HOCl (34), as well as poorly defined oxidants present in cigarette smoke (2), dramatically potentiate the proteolytic activity of these neutrophil proteases by direct and indirect mechanisms. In the case of elastase and proteinase 3, HOCl and cigarette smoke promote the oxidative inactivation of α-1-protease inhibitor (API), the major plasma and tissue inhibitor of these enzymes. Collagenase and gelatinase, on the other hand, are secreted in a latent form by neutrophils and undergo oxidative activation on exposure to HOCl (34,35). When oxidatively activated, collagenase and gelatinase potentiate the activity of elastase and proteinase 3 by cleaving API within its active site loop, causing irreversible inactivation of this protease inhibitor (34,35). The consequence is uncontrolled elastolysis in the lungs of cigarette smokers, leading to pulmonary dysfunction and emphysema.

3.3. Proatherogenic Properties of Oxidants

Reactive oxidants are thought to be intimately involved in the pathogenesis of atherosclerosis by promoting oxidative modification of low-density lipoproteins (LDL),

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pro-Oxidative mechanism</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>Oxidative damage to DNA</td>
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<td></td>
<td>Inactivation of DNA repair enzymes</td>
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<tr>
<td>Pulmonary emphysema and tissue damage in autoimmune diseases such as rheumatoid arthritis</td>
<td>Potentiation of the proteolytic activity of phagocyte-derived proteases</td>
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<tr>
<td>Cardiovascular disease</td>
<td>Oxidative modification of LDL</td>
</tr>
<tr>
<td>Acquired immunosuppression</td>
<td>Oxidative inactivation of the protective activities of B- and T-lymphocytes, as well as NK cells.</td>
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which then accumulate in the arterial intima and appear to be the major contributors to
the formation of the atherosclerotic lesion (36). Oxidized LDL are selectively chemotactic for monocytes/macrophages and, unlike native LDL, are taken up by these cells,
resulting in the formation of cytokine-producing, foam cells (36). Interestingly, oxid-
ized LDL has recently been described to be immunogenic, initiating the formation of
autoantibodies against neo-epitopes on the oxidatively modified molecule (36,37). The
nature of the involvement (i.e., primary or secondary) of these autoantibodies in the eti-
ology and progression of atherosclerosis remains to be established.

There are several possible mechanisms by which inhalation of cigarette smoke may
promote oxidative modification of LDL, including direct oxidation of this molecule
by smoke-derived oxidants. Because plasma contains high concentrations of antioxi-
dants, however, it seems improbable that meaningful oxidation of LDL would occur
in the circulation. Alternatively, cigarette smoking may predispose to atherosclerosis
as a secondary consequence of accelerated consumption of circulating nutritional an-
tioxidants, such as ascorbate and β-carotene, rendering LDL vulnerable to phagocyte-
and endothelial cell-derived reactive oxidants as it passes through the artery wall
(36,38).

3.4. Proadhesive Activity of Oxidants

Exposure of vascular endothelium to superoxide (O$_2^-$) or H$_2$O$_2$ in vitro induces pro-
longed expression of the adhesion molecules P- and E-selectins on endothelial cells
with resultant adherence of neutrophils (23,24). Oxidant-mediated activation of neut-
rophil adhesion to vascular endothelium is probably intimately involved in the patho-
genesis of posts ischemic vascular injury. In this setting, however, the proadhesive
oxidants originate primarily from the endothelial cells. Ischemia and hypoxia promote
the proteolytic conversion of xanthine dehydrogenase to the superoxide-generating en-
zyme xanthine oxidase in endothelial cells. Subsequent activation of this enzyme dur-
ing reperfusion/reoxygenation leads to generation of O$_2^-$ and H$_2$O$_2$:

\[
\text{xanthine} \xrightarrow{\text{xanthine oxidase}} \text{Urate} + \text{O}_2^-. 
\]

These oxidants (O$_2^-$ and H$_2$O$_2$) not only cause direct vascular injury, but also upregu-
late expression of P- and E-selectins on vascular endothelium, leading to adherence and
activation of neutrophils, which contribute to vascular damage by prooxidative mecha-
nisms. These events may explain the involvement of neutrophils in the exacerbation of
myocardial damage that accompanies restoration of circulation and reoxygenation during
the postinfarct period.

An additional mechanism of oxidant-mediated proadhesive activity has recently been
described in smoke-exposed rodents. Using a dorsal skinfold chamber combined with
intravital fluorescence microscopy to study the microcirculation in fine-striped skin
muscle, Lehr et al. have demonstrated that exposure of hamsters to cigarette smoke re-
results in rapid adhesion of leukocytes to the endothelium of postcapillary venules and
arterioles, as well as the formation of intravascular aggregates of leukocytes and platelets
(23). Although the exact mechanisms of these smoke-induced adhesive interactions be-
tween circulating leukocytes and vascular endothelium were not established, it was sug-
gested that reactive oxidants present in cigarette smoke may upregulate expression of
P-selectin on endothelial cells (23). These proadhesive events are probably mechanisti-
cally involved in the etiology of smoking-related pulmonary and cardiovascular diseases.

3.5. Immunosuppressive Properties of Oxidants

Reactive oxidants are also potent antiproliferative agents. Permeant oxidants, such as H$_2$O$_2$ and HOCl inhibit the proliferative activity and functions of B-lymphocytes, T-lymphocytes, and natural killer (NK) cells in vitro, probably by interfering with the activity of several enzymes involved in cellular energy metabolism (39,40). Smoking-related pro-oxidative events may compromise pulmonary defense mechanisms because smokers have decreased antibody responses to inhaled microbial antigens (41), as well as abnormal NK function (42). However, the precise mechanisms and relative contributions of smoke- and phagocyte-derived oxidants to smoking-related immunosuppression remain to be established.

4. ANTIOXIDANT NUTRIENTS AND SMOKING-RELATED DISEASES

It is abundantly clear that radical and nonradical reactive oxidants are intimately involved in the etiology of smoking-related pulmonary dysfunction, bronchial carcinoma, and cardiovascular disorders. The rate at which these diseases develop in individual smokers probably involves a dynamic interplay between oxidants and other toxins present in cigarette smoke, the numbers and pro-oxidative activities of circulating and resident pulmonary phagocytes, and the efficiency of the smoker’s antioxidant defenses.

Smoking-induced oxidative stress is associated with increased turnover of the antioxidant nutrients vitamin C (43), β-carotene (44), and vitamin E (45) in the circulation and lungs, and with activation of genes encoding antioxidant enzymes in the lungs of cigarette smokers (46). Oral administration of combinations of vitamin C, vitamin E, and β carotene to cigarette smokers preempts the activation of genes coding for the antioxidant enzymes glutathione peroxidase and superoxide dismutase in bronchoalveolar lavage cells, suggesting that inadequate intake of these nutrients may be a determinant of susceptibility to development of smoking-related diseases.

4.1. Vitamin C

The negative impact of cigarette smoking on plasma vitamin C levels is well recognized (43) and caused, at least in part, by increased turnover of the vitamin, which explains the observed dose–response relationship between cigarettes smoked per day and the decline in plasma vitamin C (46,47). Neutralization of oxidants present in cigarette smoke (43), as well as those released by smoke-activated phagocytes (48) are the probable mechanisms of accelerated consumption of the vitamin. Because vitamin C has been demonstrated to protect α-1-protease inhibitor against oxidative inactivation by both cigarette smoke (49) and activated phagocytes (50) in vitro, and to prevent activation of latent metalloproteinases (51), as well as cigarette smoke-mediated adhesion of leukocytes to vascular endothelium in hamsters (23), this vitamin is probably critically involved in protecting the lungs against oxidant-inflicted damage and dysfunction. Indeed, data from a recently published epidemiological study have highlighted a clear and significant positive correlation between dietary intake of vitamin C and pulmonary competence (52). Interestingly and importantly, the association between vitamin C and pulmonary function persisted after adjustment for cigarette smok-
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4.2. Vitamin E and β-Carotene

Vitamin E has also been reported to regulate both the production and reactivity of ROS by activated neutrophils in vitro and ex vivo (53–55), whereas β-carotene is a scavenger of phagocyte-derived singlet oxygen (56) and HOCl (54). Neither of these antioxidants however, appears to be an efficient scavenger of smoke-derived oxidants (38). Nevertheless, dietary intake of vitamin E and β-carotene, like that of vitamin C, may be a determinant of pulmonary competence in cigarette smokers by protecting the lungs against the destructive effects of smoke-activated phagocytes. This contention is supported by data from a recently conducted study that described significant positive correlations between plasma levels of β-carotene and several spirometric parameters in asymptomatic, male cigarette smokers, but not in nonsmokers (57). Distinct relationships between plasma levels of vitamin E and pulmonary function have also been reported in both smokers and nonsmokers (58). In nonsmoking males, plasma vitamin E was positively and significantly correlated with pulmonary competence, whereas, somewhat surprisingly, inverse correlations were observed between these parameters in a matched group of cigarette smokers (58). It was proposed that in the physiological setting, typified by nonsmokers, maintenance of vitamin E homeostasis is probably adequate to protect the lungs against routine, environmental, and endogenous oxidative stress, accounting for the positive relationship with pulmonary function. However, sustained and excessive oxidative stress may necessitate mobilization of tissue stores of the vitamin and diversion to the lungs, which would explain the apparent inverse association between plasma vitamin E and pulmonary competence in smokers (58). This type of mobilization of vitamin E to the lungs has been described in rats experimentally exposed to cigarette smoke (59) and ozone (60).

4.3. Antioxidant Nutrients and Cardiovascular Disease

An association between adequate dietary intake of β-carotene and the antioxidant vitamins, particularly vitamin E, and reduced incidence of, and mortality from cardiovascular disease, has been demonstrated in a number of large epidemiological studies (61–64). Although smoking has been identified as a major risk for development of cardiovascular disease, these associations persisted after adjustment for smoking status, demonstrating the predisposing role of additional factors, especially low dietary intake of these antioxidant nutrients. Moreover, physiologic levels of vitamin C have been reported to inhibit the oxidative modification of LDL in vitro (65), whereas short-term oral administration of relatively high doses of this vitamin or of vitamin E prevent (66) or retard (36) peroxidative alteration of LDL ex vivo. Identification of autoantibodies to oxidized LDL in individuals with atherosclerosis and the possible involvement of these in the etiology of this condition (67) suggests that antioxidant nutrient status may determine the susceptibility of LDL to oxidative, autoantigenic modification. In support of this, we have recently detected increased levels of circulating autoantibodies to both oxidized LDL and cardiolipin in asymptomatic cigarette smokers aged more than 30 yr. The levels of these autoantibodies were significantly and inversely correlated with plasma levels of vitamin C, indicating that intake of this antioxidant nutrient may be a determinant of development of atherosclerosis in smokers (68).

4.4. Antioxidant Nutrients and Cancer
Decreased dietary intake and/or accelerated consumption of the antioxidant vitamins and β-carotene during sustained oxidative stress is probably a common cause of cancer (69). Cigarette smoking is the primary cause of lung cancer and is also associated with the development of cancer at several other sites (mouth, larynx, esophagus, bladder, pancreas) (7). Significant inverse correlations between the dietary intake and/or plasma levels of the antioxidant nutrients and development of cancer at various sites have been reported in numerous epidemiological studies. The consistency of these reports in conjunction with corroborative laboratory studies, has prompted calls for the implementation of nutrient-based preventive public health strategies (5,69). These laboratory and epidemiological studies have not, however, been supported by data from some of the recently completed antioxidant nutrient intervention studies (70,71). Although aspects of the design of these intervention studies may have inadvertently obscured the possible preventive effects of vitamin C, vitamin E, and β-carotene, it is clear that definitive answers about the anticancer properties of these agents await the outcome of large-scale ongoing studies.

5. PREDICTORS OF OXIDANT-MEDIATED DISEASE

Although cigarette smoking has been used in this chapter as the prototype cause of lifestyle-related oxidative stress, the mechanisms of smoking-associated oxidant-mediated tissue damage and disease are probably broadly operative in other settings, including environmental and occupational exposure to excessive levels of atmospheric pollution. Even in these settings, however, active (72) and passive (73) exposure to cigarette smoke may often be the primary offender.

Interestingly, the inverse relationships between dietary intake and/or plasma levels of the antioxidant nutrients vitamin C, vitamin E, and β-carotene and future development of degenerative disease and cancer described in major epidemiological studies remain after adjustment for smoking history. There must, therefore, be a common mechanism operative in both smokers and nonsmokers, but clearly exacerbated by cigarette smoking, which determines susceptibility to oxidant-mediated disease. This mechanism may involve a dynamic interplay between dietary intake of antioxidant nutrients and the numbers and reactivities of the abundant, highly aggressive, oxidant-generating phagocytic cells of the immune system. Although critically involved in host defense against microbial pathogens, the sheer numbers of these cells and the undiscerning nature of their arsenal of toxic antimicrobial oxidants, constitutes an unrelenting threat to other host cells and tissues. Coexistence between the host and the phagocytic cells of his or her immune system is probably fragile, and minor imbalances may compromise the containment (damage limitation) functions of vitamin C, vitamin E, and β-carotene, creating the potential hazard of phagocyte-inflicted oxidative damage.

Such imbalances may be caused by several common, often avoidable, aspects of lifestyle that disrupt oxidant/antioxidant homeostasis. Poor dietary habits may impair antioxidant defenses through decreased intake of the antioxidant vitamins and β-carotene, whereas cigarette smoking, occupational and environmental atmospheric pollution, as well as excessive exposure to ultraviolet radiation (6) may cause a futile, potentially harmful increase in the numbers and pro-oxidative activities of resident and circulating phagocytes.

5.2. Phagocytes, Antioxidants, and Degenerative Disorders
The health threat posed by sustained, relatively moderate increases in the numbers of circulating phagocytes is emphasized by evidence from numerous epidemiological studies that have consistently shown that the circulating leukocyte count, and the neutrophil count, in particular, measured well before the onset of manifest clinical disease is an independent predictor of decline in pulmonary function (17), development of several cardiovascular conditions including myocardial infarction, sudden cardiac death, all coronary heart disease combined, stroke, and essential hypertension (74–76), as well as lung cancer incidence and mortality, possibly cancer at all sites (77,78), and death from all causes (79). For each decrease in the circulating leukocyte count of 1000/mL blood, the risk of coronary heart disease death decreased by 14% (74), although the relative odds for a 2000/mL difference in leukocyte count for development of lung cancer ranged from 1.20–1.58 in three different populations (77). The circulating leukocyte count was found to be superior to systolic blood pressure, cholesterol levels, and smoking history, and second only to age as a predictor of mortality (79). It must be emphasized that the increments in circulating leukocyte counts that seemingly predispose to degenerative disease and cancer are relatively modest, being within the normal range for apparently healthy adults.

It is interesting, and probably not coincidental, that clinical disorders for which elevated circulating leukocyte counts are predictive are essentially the same as those that have been demonstrated in epidemiological studies to be associated with decreased dietary intake and/or plasma levels of the antioxidant nutrients. This implied mechanistic relationship by which sustained, albeit modest, increases in the numbers and prooxidative activities of circulating leukocytes cause depletion of nutritional antioxidants and accelerated onset of degenerative diseases and cancer, as well as decline in pulmonary function remains to be established. Nevertheless, such a relationship is supported by recent observations that plasma levels of vitamin C are inversely related to circulating total leukocyte and neutrophil counts in smokers and nonsmokers, and positively correlated with plasma vitamin E (48). Plasma levels of β-carotene have been reported to correlate inversely with circulating neutrophil counts in young, asymptomatic cigarette smokers, but not in nonsmokers (80).

In the setting of sustained increases in the circulating leukocyte count, chronic, excessive production of reactive oxidants by phagocytes may be linked to pulmonary and cardiovascular damage, as well as carcinogenesis. Increased destruction of antioxidants as a result of elevated numbers and activities of leukocytes may also increase the vulnerability of LDL and DNA to oxidative damage by other cell types simply by depleting blood and tissues of these protective agents. The circulating leukocyte count, which has surprising predictive power for future development of degenerative diseases and cancer, may, therefore, be a primary determinant of optimum intake of antioxidant nutrients.

6. CIRCULATING LEUKOCYTE COUNTS AND RECOMMENDED DAILY INTAKE OF VITAMINS C AND E AND β-CAROTENE

The proposed dynamic, inverse association between the levels of the antioxidant nutrients and the circulating leukocyte count remains to be conclusively established. However, if the relationship is substantiated, it may be possible to use the circulating leukocyte count to formulate optimum intakes of vitamin C, vitamin E, and β-carotene.
We have used data from a recently completed study conducted on asymptomatic, young male cigarette smokers to calculate “optimum” daily intake of these antioxidant nutrients based on the numbers and prooxidative activities of circulating leukocytes. The average increase in the circulating leukocyte count of smokers in our study was $2 \times 10^9$/mL (6.2 $\times 10^9$/mL and 8.2 $\times 10^9$/mL for 85 male nonsmokers and 100 age- and sex-matched cigarette smokers, respectively). The average increase in the smoking-related phagocyte-mediated oxidant burden was calculated to be 71% (because of increased numbers and pro-oxidative activities of circulating leukocytes). Based on this increase in the oxidant burden and on RDAs of 60 mg/d and 15 mg/d for vitamin C and vitamin E, respectively, and a proposed intake of 6 mg/d for β-carotene, the estimated increases in the daily intakes (above the RDA levels) for each increment of $1 \times 10^9$/mL in the circulating leukocyte count above 6.2 $\times 10^9$/mL are 20 mg vitamin C, 5 mg vitamin E, and 2 mg β-carotene. Given that the upper limit of the normal range for circulating leukocyte counts for apparently healthy adult humans is approx $11 \times 10^9$/mL, the estimated daily intakes of the antioxidant nutrients required to protect all individuals with leukocyte counts less than or equal to this value are 160 mg, 40 mg, and 16 mg for vitamin C, vitamin E, and β-carotene, respectively. Admittedly, there are several flaws in the calculation, such as: (1) it is based on RDAs that may or may not be optimal; (2) in vitro activation of phagocytes may not be representative of the in vivo situation; and (3) it may not be necessary to increase the daily intake of all three antioxidants. This may be particularly important in the case of β-carotene, for which a daily intake in excess of 6 mg is probably not justified and may even be hazardous for cigarette smokers (71). In spite of these limitations, however, it is noteworthy that these values are in good agreement with intake estimates based on data from metabolic (81), experimental (82), and epidemiological studies (43). Modest, daily supplementation with 60 mg vitamin C, 30 mg vitamin E, and 3 mg β-carotene in combination with zinc, selenium, and copper for 60 d has recently been reported to protect nonsmokers against the harmful, pro-oxidative effects (DNA damage and lipid peroxidation) of exposure to environmental tobacco smoke in the workplace (83).

7. CONCLUSIONS

Lipoproteins and extracellular matrix proteins, such as elastin, and many cellular molecules including DNA, membrane lipids, and enzymes involved in DNA repair and energy production are extremely vulnerable to oxidant-mediated damage. Certain avoidable and unavoidable aspects of lifestyle, because they are associated with accelerated destruction and/or decreased intake of antioxidant nutrients, may, therefore, predispose to development of acquired immune dysfunction, cancer, and degenerative diseases, such as atherosclerosis, cataracts, and pulmonary emphysema.

8. RECOMMENDATIONS

Avoidance of unhealthy aspects of lifestyle, such as low intake of fresh fruits and vegetables, cigarette smoking, and excessive exposure to sunlight are essential for maintenance of antioxidant homeostasis and prevention of oxidant-mediated diseases. Because even in affluent societies, some of these objectives are difficult to achieve (84),
modest supplementation with antioxidant nutrients represents a potentially efficient and inexpensive strategy to counteract the potential threat of oxidant-inflicted diseases. Daily intakes of about 200 mg, 40 mg, and 6–16 mg of vitamin C, vitamin E, and β-carotene, respectively, would seem appropriate.

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13 Micronutrients and Immunity in Older People

John D. Bogden and Donald B. Louria

1. INTRODUCTION

Aging has been described as group of processes that promote vulnerability to challenges, thereby increasing the likelihood of death. Because there is evidence that depressed immunity can increase the risk of death, it is likely that changes in immunity with age are a key factor in the aging process.

There are a considerable number of theories of aging. These 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 that occur in all species that have been studied, including man, and on observations from cross-sectional studies that demonstrate an association between maintenance of good immune function and longevity (1,3). A limitation of this theory is that it lacks the universality of other theories, such as the free-radical theory of aging, because 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, recent evidence demonstrates that antioxidant nutrients that reduce free-radical damage can improve immunity in older people (4), suggesting that the free-radical and immunologic theories may overlap.

2. IMMUNITY/AGING RELATIONSHIPS

2.1. General Changes in Immunity with Aging

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 (5–7). Regarding 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.
The above general changes in immunity are based on data from numerous studies of specific aspects or measures of immunity that change with age, including altered lymphocyte subpopulation percentages, thymic involution and decreased thymic hormone concentrations, decreased suppressor activity of T-cells, reduced interleukin-2 (IL-2) secretion, impaired delayed-hypersensitivity responses, decreased in vitro lymphocyte-proliferative responses to mitogens, reduced antibody titers after vaccination, increased serum autoantibodies, and increased soluble-serum IL-2 receptors (sIL–2R) (9).

2.2. Specific Changes in Immunity with Aging

2.2.1. Involution of the Thymus

The most striking changes in immunity with increasing age are inhibited T-cell functions (see Table 1). These are likely related to the 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 are 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 causes it to be undetectable in people after puberty. Because 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, because memory T-cells have a long life-span (20 yr 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 becomes longer.

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 because it reduces the danger of autoimmune reactions (11). According to this theory, the increased risk of cancer or infectious diseases as a result of 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 “tradeoffs” 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 pharmacologic 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.

2.2.2. T-Lymphocyte Functions

Changes in T-lymphocytes with aging include a shift in relative percentages of subpopulations, and qualitative changes in cell surface receptors of individual cells (13). In comparison to T-cells from younger people, cells of the elderly are deficient in vitro
production of certain T-cell growth factors, such as 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 responses (DHST) 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 DHST 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 by Wayne et al. (20) and Roberts-Thomson et al. (21) for initially healthy people aged 60 yr or older.

In their investigation, Christou et al. (19) studied the relationship between presurgery DHST responses and postsurgical sepsis-related death in 245 subjects with a median age of 67 yr and a range of 24–98 yr. Initially anergic subjects experienced significantly more postsurgical mortality than those who were reactive. Because 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 (failure to respond to any of four skin test antigens) and the risk of dying postoperatively. Thus, initial disease severity could explain the apparent strong relationship between preoperative DHST responses and postsurgical mortality. However, the study of Wayne et al. (20) did not have this confounder because they looked prospectively at healthy adults over a 10-yr time period. In this investigation, the authors followed 273 initially healthy subjects age 60 yr or older with no history of serious medical problems. DHST responses were measured at enrollment. Anergy at enrollment in the study was associated with a significantly increased risk of dying in the 10-yr follow-up period (Fig. 1). For example, at the end of 10 yr, 89% of the initially reactive subjects were still alive, but 22% of the anergic subjects had died. The study demonstrates that anergy to skin test antigens, even when present in healthy older people, is associated with subsequent increased all-cause mortality. The authors also found a 2.5-fold increase in cancer mortality in the initially anergic group in comparison to the reactive group. However, this was not statistically significant 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 delayed-hypersensitivity 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 LPR 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 who are 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 a result of 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/\(\mu\)L 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 caused by increased numbers of a particular subgroup of cells vs enhanced activity by the same number of cells. An example of the latter is 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 role that changes in antigen-binding capacity per cell may play in declining immunity with age is largely unexplored.

2.2.3. 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 demonstrate reduced antibody production, as well as a greater percentage of nonresponders. 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 pyrogenic bacteria. This is consistent with studies of Nagel et al. (30), Shoham-Kesare and Gershon (31), and Corberand et al. (32) that suggest compromised in vitro activity of neutrophils from older people.

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 most 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 vs 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, local urinary tract, respiratory tract, and neurologic 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 IL-2, because its production is decreased in older people (9). Interestingly, soluble 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, because 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 lower in physically active than in inactive older people 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 but found that increased soluble IL-2R concentrations are not significantly associated with lower DHST responses (unpublished data).

There may be a “survivor” aspect to the relationship between advanced age and immune capacity. The oldest people, including centenarians studied by Sansoni et al. (38), tend to have well-preserved immune functions, such as natural killer (NK) cell activity, that are often better than those of 50–80-yr olds. In addition, those above age 90 tend to have lower serum autoantibody concentrations than those in the 60–80-yr range (14,39).
Thus, enhanced immunity and reduced autoimmunity appear to be associated with the ability to live to age 90 and beyond.

3. MICRONUTRIENT NUTRITION AND IMMUNITY

3.1. 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 β-carotene; pyridoxine; folic acid; pantothenate; vitamins A, B₁₂, C, D, and E; the trace elements iron, zinc, and copper, and magnesium. In general, cell-mediated and nonspecific immune functions are more sensitive to nutrient 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 caused, at least in part, by nutritional deficiencies and/or increased requirements. Another possibility is that micronutrient supplementation might improve immunity even in the absence of an underlying deficiency. However, relatively few studies have simultaneously investigated nutrition and immune functions in older people.

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 DHST 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 either 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 DHST responses are a consistent result of dietary inadequacies of protein, pyridoxine, iron, zinc, and vitamins A and C.

The classic review by Beisel (46) extensively examined the 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. Recent studies have shown that vitamin D is also an important immune modulator (47). Trace metals that exert substantial influences on immune functions are iron, zinc, selenium, and copper (15, 46).

Because 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. Beisel (48) has noted that individual studies of immunity in humans have not been systematic or comprehensive. This 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.

3.2. 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 (Recommended Dietary Allowance) levels (5 × RDA or greater) of micronutrients to similar subjects 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 DHST responses or in vitro LPR to mitogens. The authors suggested that the immune-enhancing properties of high doses of vitamins may 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 DHST responses, in vitro LPR to phytohemagglutinin (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.

The aforementioned 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 because 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 double-blind clinical trials.

3.3. Clinical Trials of Single Nutrients

Several clinical trials have been conducted in recent years. 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. (51) studied the effects of moderate 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/d, whereas repletion was achieved with doses of 60 to 250 mg/d. Although LPR to mitogens were not affected by ascorbate depletion/repletion, DHST responses to a panel of seven recall antigens were markedly depressed by ascorbate depletion. Repletion for 28 d at either 60 or 250 mg/d did not restore the mean antigen score to the predepletion level, though there was some improvement in induration in three of the eight men studied. These results suggest that DHST 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 DHST to baseline levels and/or the repletion doses were not large enough. The latter possibility is supported by another study (52) that demonstrates that higher doses of ascorbate can enhance LPR to mitogens. The inconsistency in the results for mitogen responses vs DHST may reflect the different cellular populations involved in these processes, the greater sensitivity of DHST to nutritional factors, the inherent variability of mitogen assays, or artifacts of the in vitro mitogen tests that do not adequately represent the in vivo situation.

Fuller et al. (53) studied the effect of β-carotene supplementation on the UV-radiation induced photosuppression of DHST in 24 young adult males, aged 19–39 yr. They found that exposure to a UV-A/B light source over a 16-d period significantly reduced DHST responses in a control (placebo) group to 39% of the initial values, but did not induce significant reductions in a group given 30 mg β-carotene per day. Because young men were studied, it was not known if these results would occur in young women or in older men and women. Recently, this group repeated this study in an elderly population and found similar effects, though there was more variability in DHST responses in the older people compared to young adults (54).

Watson et al. (55) investigated the effects of β-carotene on lymphocyte subpopulations in male and female subjects with a mean age of 56 yr. β-carotene was given at doses of 15, 30, 45, or 60 mg/d for 2 mo. Using monoclonal antibodies to identify lymphocyte subsets, they found that the percentages of T-helper and NK cells, as well as cells with IL-2 and transferrin receptors, were increased in a dose-related fashion. There were no significant effects of β-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. However, Ringer (56) and coworkers found that administration of high doses of β-carotene, up to 300 mg/d, to younger adults for 1 mo did not significantly affect LPR to mitogens or IL-2 production. The contrasting results of these studies may be a result of the younger age of the subjects in Ringer’s study, or the shorter duration of supplementation. The use of different laboratory tests may also be a factor because Watson et al. assessed T-cell subpopulations and Ringer et al. performed functional assays. In addition, augmentation of cellular immunity may not be readily achieved nor be desired in younger subjects with well-functioning immune systems.

More recently, Herriaiz et al. (57) reported that β-carotene (30 mg daily for 28 d) supplementation reduced the suppressive effect of UV light exposure on DHST responses in older men. Santos et al. (58) found that men participating in the Physician’s Health Study who consumed 50 mg of β-carotene on alternate days for an average of 12 yr had significantly greater NK cell activity than controls given placebos. Surveillance by NK cells is considered to be protective against the development of cancer. However, two large intervention trials have found an association between high doses of β-carotene and the development of lung cancer in cigarette smokers (59,60). The role of the immune
system in leading to the development of lung cancer in these studies is not known, but
the results suggest that there are risks associated with the long-term use of high doses
of β-carotene supplements.

Talbott et al. (61) in a pilot study investigated the impact of pyridoxine supplementation
on lymphocyte responses in 15 older (aged 65–81 yr) mostly female subjects and
found that administration of 50 mg/d of pyridoxine hydrochloride significantly in-
creased in vitro LPR to phytohemagglutin, pokewood mitogen, and Staphylococcus
aureus.

Meydani et al. (62) have reported that vitamin B₆ deficiency impairs IL-2 production
and lymphocyte proliferation in older adults. Each of these measurements was reduced
by about 50% by depletion, whereas repletion with near RDA levels of B₆ eventually
increased values to about the baseline levels. Although only eight subjects were stud-
iied, this well-designed investigation supports a number of other studies that suggest that
vitamin B₆ may play a key role in immune responses (63).

In another study Meydani et al. (64) gave older people 50, 200, or 800 mg of vitamin
E daily for 4–5 mo. This resulted in improved antibody titers to hepatitis B vaccine and
enhanced DHST responses, especially in the group consuming 200 mg of vitamin E/d.
This suggests 200 mg as a recommended dose, although lower doses may be equally ef-
f ective when administered for longer periods of time. In a more recent study, Pallast et
al. (65) investigated the effects of 6 mo of supplementation of healthy older men (aged
65–80 yr) with vitamin E at doses of 50 and 100 mg daily for 6 mo. There was a dose-
related trend of increased DHST 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 func-
tions 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 immu-
nity and lead to the development of life-threatening opportunistic infections (66). There
are also reports of significant associations between plasma or cellular zinc concentrations
and immune functions such as DHST responses in older people (8,67). However, studies
of the impact of zinc supplementation on immunity in older people have not been en-
couraging. They have either demonstrated no beneficial effect of zinc supplements on im-
munity, or an adverse effect even when the supplements contained modest doses of zinc
in the range of 15–25 mg/d (68,69). In the absence of an underlying deficiency, use of zinc
supplements by older people, especially at doses that exceed the current RDA of 15 mg/d,
are more likely to adversely affect immunity than improve it.

Doherty et al. (70) studied the effect of low (1.5 mg/kg) vs high (6.0 mg/kg) dose
zinc supplementation on mortality in 141 young children in Bangladesh with protein-
energy malnutrition. Mortality was significantly greater in the high-dose group, with
sepsis a frequent contributing factor. The results suggest that high-dose zinc supple-
cmentation, especially in the presence of sepsis, may contribute to increased mortality in
severely malnourished children. Although this study involved only very young children,
aged 6 mo to 3 yr, it suggests caution in the use of high-dose zinc supplements by any
age group.

3.4. Clinical Trials of Combinations of Micronutrients

The aforementioned studies focused on the effects of relatively large doses of indi-
individual micronutrients on immune functions. There have been only 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 (71). All subjects received a low-dose multivitamin/mineral supplement that contained all the essential micronutrients except zinc. In addition, subjects received 15 mg or 100 mg of zinc, or a placebo. Daily consumption of the multivitamin/mineral supplement for 1 yr was associated with enhanced DHST 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 studies that indicate that zinc supplements in healthy older people either do not improve immunity or adversely affect it (68,69).

The second is the study of Chandra (72), who reported the results of 12 mo of daily supplementation of a group of healthy subjects aged 65 or older with a micronutrient formulation containing relatively low doses of nine vitamins and five trace elements and higher levels of the antioxidants vitamin C, vitamin E, and β-carotene. Chandra found that, in comparison to a placebo group, the micronutrient group had higher numbers of some T-cell subsets and NK cells, enhanced LPR to mitogens, increased in vitro IL-2 production, higher antibody responses to influenza vaccine, and greater NK cell activity. In addition, supplemented subjects experienced significantly fewer days (23 ± 7 d) of illness per year because of infectious diseases than subjects in the placebo group (48 ± 7 d). These recent results are consistent with the results presented in our paper published in 1990 (71) that a low-dose micronutrient supplement could improve immune functions in older people. The results of Chandra further suggest that there may be beneficial clinical effects, i.e., a reduced prevalence of infectious diseases, as a result of micronutrient supplementation.

Presupplementation plasma concentrations of retinol, β-carotene, vitamin C, and vitamin B₆ were low in some subjects in Chandra’s study, with the percentage of subjects with initially low concentrations of each between 12.5 and 22.9%. Most of the low concentrations were corrected by supplementation, so that the percent of subjects with low values of the above concentrations decreased to 0–4.4%, and this was accompanied by the enhanced immune functions that he observed. However, this observation does not prove that the decrease in the percent of subjects with low concentrations was responsible for the improved immune functions found.

Limitations of the study of Chandra include the fact that there was no assessment of dietary micronutrients from food and immune functions were assessed only at baseline and after 1 yr of supplementation. In addition, the occurrence of infectious diseases was reported as the number of days for which subjects were infected per year. The latter is the product of the incidence of infectious diseases and their duration. Thus, a single infection persisting for 30 d is equivalent to six infections of 5 d duration each. It is important to know the effects of micronutrient supplementation on the incidence of new infections, as well as the nature and duration of each type of infection.

Penn et al. (73) studied the effects on immune functions of a supplement containing vitamin C (100 mg), vitamin A (8000 IU), and vitamin E (50 mg); it was given for 28 d to half of the 30 elderly subjects studied. All were patients who had been hospitalized for at
least 3 mo. 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 a result of 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. (74) enrolled 218 subjects aged 60 or older who were living independently and had not used any vitamin supplements for at least the prior 3 mo. They were given a low-dose multivitamin or placebo for 4 mo. 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 caused by the short duration of supplementation. This is consistent with our results and those of Chandra (72), which suggest that periods of supplementation of about 12 mo are required before improvements in immune functions occur in older people.

More recently, we conducted a randomized, placebo-controlled, double-blind trial of the effects of low-dose micronutrient supplementation on plasma vitamin and trace metal concentrations and immune functions in independently living healthy older subjects (75). This study will be described in some detail. The over-the-counter micronutrient supplement used in the study was Theragran M, which contains low to moderate doses of each of the essential micronutrients.

Of the 65 subjects enrolled, 56 (86%) completed the 1-yr study. About two-thirds were female. 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 mo for plasma concentrations of ascorbate, β-carotene, folate, vitamin B6, and α-tocopherol. These data verify that supplementation with low doses of the latter micronutrients can increase their plasma concentrations in older people.

Table 2 contains the data on DHST for all study subjects combined and for males and females separately. For induration in the placebo group, there were no statistically significant differences between the 0- and 6-mo results, 0- and 12-mo results, or 6- and 12-mo 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 mo. However, there was a statistically significant difference between the 0- and 12-mo induration results ($p = 0.005$). There was an increase in induration between 6 and 12 mo, 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
Table 2
Delayed-Hypersensitivity Skin-Test Responses of Placebo and Micronutrient Groups

| Subgroup and response type | Placebo group | | Micronutrient group | | |
|---------------------------|---------------|-------------|---------------------|-------------|
|                           | 0 mo          | 6 mo        | 12 mo               | 0 mo        | 6 mo        | 12 mo       |
| 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$^{bc}$ | 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$^{bc}$ | 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$^{bc}$ | 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$^{bc}$ | 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$^c$ |

$^a$Mean ± SE; $n = 26$ for placebo group (7 males, 19 females), $n = 29$ for micronutrient group (11 males, 18 females). 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 superscripts are significantly different, $p < 0.05$ (Wilcoxon signed-rank test).
group increased by only 4.8% between 0 and 12 mo, and induration by 8.0%. In con-
trast, in the micronutrient supplement group, the mean number of positive responses in-
creased by 64% and induration by 61% between 0 and 12 mo. These data provide strong
evidence for the enhancement of DHST after 1 yr of micronutrient supplementation.

The results also suggest that some enhancement of DHST responses occurred sooner
(at 6 mo) in the male subjects than in the females (Table 2). The male subjects had sig-
nificantly greater DHST responses than the females at enrollment; this is consistent with
previous data that suggest that DHST responses in males may differ from those in females
(76). The diets of the male subjects differed from the females, being higher in energy in-
take, as well as intake of individual micronutrients, and it is possible that this factor may
have interacted with micronutrient supplementation to influence DHST responses.

There was an increase between 0 and 12 mo in the number of subjects in the placebo
group with low blood concentrations of some of the micronutrients measured, specifi-
cally \(\beta\)-carotene, retinol, folate, and vitamin \(B_6\). This trend differed significantly from
the micronutrient group, for which the number of low values changed very little be-
tween 0 and 12 mo. Thus, the improvement in skin test responses in the micronutrient
group is not caused by 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 val-
ues in the placebo group suggests that older people who do not take vitamin supple-
ments for a year may have an increased risk of developing one or more low
concentrations, particularly for vitamin \(B_6\), folate, and \(\beta\)-carotene.

There were no significant correlations between DHST responses and serum vitamin
concentrations, consistent with the observations of Goodwin and Garry in their cross-
sectional studies (50). There were, however, statistically significant correlations between
the changes in skin test responses between 0 and 12 mo and the corresponding changes in
blood micronutrient concentrations for four serum micronutrients: ascorbate, \(\beta\)-carotene,
\(\alpha\)-tocopherol, and folate \((r = 0.27–0.33)\). These data suggest that future studies of mi-
cronutrient nutrition and immunity could focus on the above four micronutrients. The data
further suggest that the absence of associations prior to supplementation between DHST
responses and serum vitamin concentrations does not preclude the finding of such associ-
atations between changes in DHST responses and serum concentrations after supplemen-
tation. Of course, such associations are not proof of a causal relationship.

Our data and that of Chandra (72) suggest that enhancement of immune functions in
older subjects by low-dose micronutrient supplementation takes approx 1 yr. These re-
results also suggest that the diets of older people are inadequate in one or more micronu-
trients and/or that the current RDAs for one or more micronutrients may be too low to
support optimal immunity in older adults.

It could be argued that a 60% increase in DHST responses over a 1-yr period is only
a mean increase of about 5%/mo. However, this increase far exceeds the decline in
DHST responses per yr that occurs with aging, and thus may completely prevent it.
These results suggest that older subjects who take a “one-a-day”-type multivitamin sup-
plement faithfully for at least 6–12 mo, may experience a substantial improvement in
measures of cellular immunity such as DHST responses. It is possible that more rapid
and/or larger increases in DHST responses would occur if higher doses of micronutri-
ents were used.

More recently, Giordon et al. (77) 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 yr a placebo, a trace-element supplement containing 20 mg zinc and 100 μg selenium, a vitamin supplement with 120 mg vitamin C, 6 mg β-carotene, and 15 mg of vitamin E, or both the vitamin and trace-element supplements. DHST responses were not 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 lower in these groups. The vitamin and trace-element supplements also reduced the prevalence of underlying deficiencies of these nutrients. Because these were institutionalized subjects with a high frequency of low blood micronutrient concentrations, the applicability of these results to healthy independently living older people is uncertain. Nevertheless, this relatively large study provides the first evidence that selenium may be a key nutrient in the maintenance of immunity in older people.

### 3.5. Need for Additional Investigations of Micronutrient/Immunity Relationships

It could be argued that the aforementioned studies that focused on the effects of multivitamins on immune functions, in combination with the short-term higher-dose single-nutrient studies, such as those of Meydani \((62,64)\), Watson \((55)\), and Talbott \((61)\), provide sufficient information about the relationships among micronutrient supplementation, immunity, and the occurrence of infectious diseases, and that no new studies are needed. However, 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 yr 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 \((78,79)\). 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. None of these studies has 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. \((74)\) was of only 4-mo 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 \((73)\) was of only 1-mo duration and included only older people who had been hospitalized for at least 3 mo.
3. Our studies \((71,75)\) assessed DHST responses, LPR to mitogens and NK cell activity, but we could not examine other measures of immunity or clinical outcomes and the period of supplementation was limited to 1 yr.
4. The study of Chandra \((72)\) was also of only 1-yr duration, did not include assessment of dietary micronutrients, did not distinguish between the incidence and duration of infectious illnesses, and assessed selected immune functions only once after initiating supplementation.
5. The 2-yr study of Giordon et al. (77) included only institutionalized subjects. Thus, additional clinical studies of micronutrient/immunity relationships are warranted.

4. FACTORS THAT CAN INFLUENCE MICRONUTRIENT/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 recent study (75) of the effects of low-dose micronutrient supplements on immunity in older people, improvements in DHST 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. Thus, this effect may be a consequence of the generally greater energy and micronutrient intakes of males.

There are a considerable number of reports that psychological and physiological stress in experimental animals and people can depress cellular immune functions (80,81), though it is beyond the scope of this chapter to assess these studies in any detail. As an example, death of a spouse has been associated with depressed immune functions (81). However, virtually all studies of relationships between stress and immunity have not adequately assessed nutritional factors that may be altered by stress, and in many 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 (82–86). 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 to experience respiratory infections in the 1–2-wk period following the race (85,86). Chronic overtraining has also been associated with depressed immunity (84). In contrast, regular moderate exercise appears to enhance immune functions (84). 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 (87). In addition, alterations in cytokine levels as a result of regular exercise may also be a factor (88,89).

Stallone (90) has recently 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 that suggest 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 (91).

The well-established importance of some micronutrients 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.

Goodwin (92) has suggested that the relationship between depressed cellular immune function and subsequently increased mortality may be a result of compromised immunity being a marker for clinically latent diseases or poor overall physiologic function.
Impaired immunity may also contribute to a reduced ability to defend against infections, cancers, and perhaps cardiovascular heart disease. Each of these possibilities may contribute to the increased risk of morbidity and mortality associated with depressed immunity in healthy older people.

5. RESEARCH NEEDED ON MICRONUTRIENT NUTRITION AND IMMUNITY

Several cross-sectional studies that assess relationships between micronutrient nutrition and immunity have been done in the past 17 yr (49,50) as aforementioned. In general, significant associations between serum micronutrient concentrations or use of micronutrient supplements and various measures of immunity were not found. 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 a result of 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, because optimal immune function was not a factor in establishment of the current RDAs and DRIs (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 RDAs and DRIs and intakes that may prevent chronic diseases. For example, the current RDA for vitamin E (15 mg α-tocopherol equivalents for adult females and males, respectively) is likely to be substantially less than amounts that optimize immune functions or prevent cardiovascular heart disease (64,65,93,94). Similarly, the current RDA for vitamin C is adequate to prevent development of scurvy, but appears to be less than the intake that could optimize immunity or reduce cataract formation (4,95). 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.

There is considerable evidence that patterns of physical activity and exercise can influence immunity both acutely and chronically, but no 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, a sound diet that emphasizes fruits, vegetables, whole grains, and other sources of micronutrients and limits the intake of saturated fats should be encouraged. However, it is likely that beneficial intakes of some nutrients such as vitamin E may not be possible from a good diet alone in the absence of supplement use.

The promising results of studies done to date suggest continued research on micronutrient nutrition and immunity, especially in older people. Such efforts should include:
1. A focus on long-term placebo-controlled double-blind clinical trials and prospective epidemiologic studies.
2. Study of interactions among physical activity/exercise patterns, immunity, and micronutrient nutrition.
3. Evaluation of effects of micronutrient nutrition on both humoral (e.g., antibody responses to vaccination) and cellular (e.g., DHST 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 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 subsequent to 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, smoking) that influence micronutrient/immunity interactions and the basis for these effects.
9. Identification of the molecular mechanisms that determine the effects of micronutrients on immunity.

About 100 million Americans (approx 40%) of the population 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 urgently needed and should include evaluation of effects on the immune system.

6. SUMMARY

We still have much to learn about the effects of micronutrients on immunity. Nevertheless, the results of the above and other studies suggest that:

1. Placebo-controlled clinical trials are the best approach for studying effects of micronutrients on immunity;
2. 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;
3. Some micronutrients may interfere with the beneficial effects of other micronutrients on immunity; this effect will depend on relative doses;
4. Low- to moderate-dose multivitamin/mineral supplements may require considerable time (6 mo to 1 yr 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; and
5. High- and even low-dose micronutrient supplements may enhance immunity even in the absence of evidence of underlying deficiencies.

7. RECOMMENDATIONS

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. Higher, but safe, daily doses of the antioxidant micronutrients vitamin C (200–500 mg) or vitamin E (200–400 IU) may also be appropriate for some older people. Taking high doses of other micronutrients that can adversely affect immunity at excessive intakes, for example, zinc should be persuasively discouraged. High doses of β-carotene are not recommended for current and former smokers because of their association with the development of lung cancer and may be unwise for other people.

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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Impact of Vitamin A on Immunity and Infection in Developing Countries

Richard D. Semba

1. INTRODUCTION

The prevention of vitamin A deficiency has emerged as one of the most important public health efforts of the past century. The observation that vitamin A supplementation reduces child morbidity and mortality in developing countries (1) has led to programs and new directions of research. Vitamin A capsules have entered the basic armamentarium for child survival, along with vaccines, oral rehydration, and the promotion of breast feeding. Vitamin A plays an important role in immune function and resistance to disease. Improving vitamin A intake, whether through improved diet, fortification of foods, or periodic supplementation, is expected to reduce morbidity and mortality for millions of children (2), and there may be many other therapeutic applications for vitamin A that have not yet been realized. The purpose of this chapter is to provide health professionals with a concise review of epidemiological, immunological, and clinical studies of vitamin A, and to present the current recommendations regarding prevention of vitamin A deficiency in populations in developing countries.

2. METABOLISM OF VITAMIN A

2.1. Absorption of Vitamin A

Vitamin A is a fat-soluble substance found in animal foods and dairy products. Vitamin A is available as preformed vitamin A, contained in high amounts in such foods as liver, cod liver oil, or eggs, or as pro-vitamin A carotenoids, as found in dark green leafy vegetables, carrots, papaya, and mangos. Retinol is esterified in the intestinal mucosa, packaged into chylomicra, and carried to the liver via the lymphatic circulation (3). Pro-vitamin A carotenoids such as ß-carotene may be converted to retinaldehyde through cleavage by carotenoid-15,15'-dioxygenase, or by an eccentric cleavage pathway. Approximately 50 of over 600 carotenoids found in nature may be converted to vitamin A (4). The bioavailability of pro-vitamin A carotenoids is much less that of preformed vitamin A as a result of a variety of factors, including differences in efficacy of absorption and biochemical conversion. Vitamin A is a less potent antioxidant than ß-carotene. Although carotenoids, such as ß-carotene, and vitamin A are often
popularly regarded to be equivalent, there may be large differences in biological functions of these two nutrients, especially regarding antioxidant properties, because vitamin A is a less-potent antioxidant than β-carotene.

2.2. Storage and Transport of Vitamin A

Approximately 90% of vitamin A in the body is stored in the liver as retinyl esters (5). The liver has the capacity to store enough vitamin A to last for several months, with longer storage capacity among adults than children. Periodic high doses of vitamin A are largely effective in preventing vitamin A deficiency for long periods because of the liver’s ability to store vitamin A (6). Retinol is released from the liver in combination with plasma retinol binding protein (RBP) and transthyretin (TTR). Retinol is poorly soluble in water and is carried in the blood sequestered inside the carrier proteins, RBP and TTR. Retinol seems to enter cells via specific receptors, although it is unclear whether all cells contain these receptors (7).

2.3. The Retinoid Receptors

Vitamin A exerts its effects through retinoid receptors that are found in the nucleus of the cell. These receptors resemble steroid and thyroid hormone receptors, and support the idea that vitamin A acts much like a hormone (8). In the cell, retinol is converted to its active metabolite, all-trans-retinoic acid. Retinoic acid influences gene activation through specific receptors that belong to the superfamily of thyroid and steroid receptors (9,10). Retinoic acid receptors act as transcriptional activators for specific target genes. The retinoic acid receptor (RAR) is expressed as several isoforms, referred to as RAR α, β, and γ, and retinoid-x receptor (RXR) is also expressed as several isoforms, referred to as RXR α, β, and γ (11). All-trans retinoic acid is a ligand for RARs, whereas 9-cis retinoic acid is a ligand for both RARs and RXRs. 9-cis-retinoic acid seems to be functionally distinct from all-trans-retinoic acid, and interconversion may exist between the two isomers. Each RAR and RXR has a specific DNA-binding domain by which these nuclear receptors may effect transcriptional activity. The DNA sequences required for action by RAR and RXR are known as retinoic acid response elements (RAREs). RAR and RXR receptors form heterodimers that bind to DNA and control gene activity. In addition, RXR receptors also can form heterodimers with the thyroid hormone receptor, vitamin D3 receptor, peroxisome proliferator activator receptors, and a number of newly described “orphan receptors.” Most RAREs seem to occur in the regulatory region of genes. In the presence of 9-cis retinoic acid, RXR/RXR homodimers may form and recognize a subset of RAREs or inhibit the formation of certain heterodimers. Orphan receptors, such as chicken ovalbumin up-stream promoter transcription factor (COUP-TF) (12), ARP-1, TAK1 (13), RVR (14), RZR (15), and thymus orphan receptor (TOR) (16) may repress or modulate the induction of genes by retinoic acid. Thus, RARs and RXRs may interact with multiple transcriptional mediators and/or corepressors, adding an enormous level of complexity to regulation of retinoic acid responses. The metabolic pathway of vitamin A is shown in Fig. 1. The number of genes regulated by retinoids that have been described in the literature is growing rapidly. Other vitamin A metabolites in the retroretinoid family may support biological functions via a pathway that is distinct from the retinoic acid pathway. 14-hydroxy-4,14-retro retinol supports, whereas anhydroretinol inhibits cell growth (17–19). In addition, the oxoretinoids may play a role as retinoic acid receptor ligands (20).
3. EFFECTS OF VITAMIN A DEFICIENCY

3.1. Immunity

3.1.1. Infectious Diseases

Clinical vitamin A deficiency and/or low-circulating vitamin A levels consistent with vitamin A deficiency have been described in a variety of infectious diseases, including chicken pox (21), diarrhea (22), endometritis (23), HIV infection (24), leprosy (25), malaria (26), measles (27), meningococcal disease (28), otitis media (29), pneumonia (30), respiratory syncytial virus infection (31), rheumatic fever (32), tuberculosis (33), schistosomiasis (34), and whooping cough (35). Serum retinol levels may drop during infection because of decreased intake and absorption of dietary vitamin A caused by diarrhea or intestinal pathogens, decreased mobilization of hepatic reserves of retinol, accelerated utilization by target tissues, and increased urinary losses of vitamin A during the acute phase response (21, 36, 37). Overall, episodes of infection seem to hasten the depletion of vitamin A stores (21, 38, 39). Low serum vitamin A levels during infection may have detrimental effects on the immune response, given the close relationship between immune effector cell function and the availability of vitamin A.

Low serum vitamin A levels have been associated with night blindness and increased infectious disease morbidity (40). Individuals with deficient vitamin A levels have increased risk of morbidity and mortality during measles (27), HIV infection (24, 41), res-
piratory syncytial virus infection (31), and meningococcal disease (28). Impairment of
immune responses during infections may be related to the inability of T- and B-cells to
function without adequate levels of retinol (42) (see Table 1).

3.1.2. INCREASED MORTALITY

The biological consequences of vitamin A deficiency during infection include increased
morbidity and mortality (43). Longitudinal studies in Indonesia showed that the mortality
rate for children with mild xerophthalmia was four times greater than age-matched controls.
The higher mortality was apparent even after accounting for differences in concurrent ill-
nesses and protein-energy status. In addition, children with preexisting xerophthalmia had
higher rates of subsequent diarrhea and respiratory disease, although children who de
toped diarrhea and respiratory disease were at increased risk of subsequently developing xe-
rophthalmia (44, 45). This has led to the idea that vitamin A status and infection are related
in a vicious cycle (46). Other studies soon confirmed the association between mild vitamin
A deficiency and increased morbidity in children (47, 48). It is now becoming apparent that
even subclinical vitamin A deficiency is associated with higher morbidity and mortality.

3.1.3. MUCOSAL IMMUNITY

Vitamin A deficiency may cause a breakdown in mucosal immunity through patho-
logical changes in the epithelia of respiratory, gastrointestinal, and urinary systems.
This includes keratinizing metaplasia and loss of goblet cells and mucus (42, 49). Loss
of intestinal brush border and goblet cells and squamous metaplasia and destruction of
ciliated epithelia in the respiratory system has been reported in vitamin A-deficient
animals. Decreased levels of secretory IgA in saliva have been reported in vitamin
A-deficient children. Mucosal immunity, the “front line” of the body’s immune defenses,
may be compromised during vitamin A deficiency.

3.1.4. CELLULAR IMMUNITY

Atrophy of the thymus, spleen, and lymphoid tissues has been observed in children
who died with vitamin A deficiency (42, 49). Mild vitamin A deficiency is associated
with underlying alterations in circulating T-cell subpopulations, such as decreased
CD4+CD45RA+ T-cells, or “naive” CD4 T-cells, and decreased CD4/CD8 ratios.
During HIV-1 infection, vitamin A-deficient adults had lower circulating numbers of
CD4 T-cells compared to adults with normal vitamin A levels. Lower plasma vitamin A
levels are also associated with alterations of T-cell subpopulations during acute
meningococcal infection in children and during HIV-1 infection in adults (28, 50).

Vitamin A and its metabolites are essential for immune effector cell function, in-
cluding T-cells of the thymus, lymphocytes in lymphoid tissue, and peripheral blood
lymphocytes (51). Retinoic acid is involved in the expression of IL-2 receptor on
T-cells. Retinol is essential for growth and differentiation of B-cells and the production
of antibodies (52, 53). Retinoic acid may enhance immunoglobulin synthesis by cord
blood mononuclear cells through increased T-cell help, i.e., modulation of cytokines,
which induce B cells to differentiate into greater numbers of immunoglobulin-secreting
cells (54). In vitro studies suggest that vitamin A modulates growth and function of
T- and B-cells, either directly or through its active metabolite, all-trans retinoic acid.

Vitamin A and β-carotene supplementation are associated with changes in lympho-
cyte numbers and T-cell subpopulations. The depression in circulating lymphocytes fol-
lowing surgery can be reversed by administration of high-dose vitamin A to adults (42).
Children with clinical and subclinical vitamin A deficiency who received vitamin A had increases in circulating CD4 T-cells, especially CD4CD45RA, or “naive” T-cells, and an increase in CD4/CD8 ratio 5 wk after dosing. Measles infection is characterized by immune suppression, leukopenia, and decreased circulating CD4 T-cells. Administration of high-dose vitamin A to children with measles has been shown to increase total circulating lymphocytes, as well as enhance IgG responses to measles (55). Vitamin A and related metabolites are potent enhancers of cellular differentiation, and a possible mechanism for the increase in CD4 T-cells may be vitamin A-related differentiation of lymphocytes.

3.1.5. ANTIBODY RESPONSES

A hallmark of vitamin A deficiency is the impaired ability to mount an antibody response against T-cell dependent protein antigens (42, 50, 56) Other types of immune responses may be unaffected by vitamin A deficiency (56, 57). The antibody response to immunization with tetanus toxoid or other antigens has been used to examine immune competence. Vitamin A-deficient children in Indonesia had reduced antibody responses to tetanus toxoid (58). Supplementation with high-dose vitamin A, 200,000 IU, (60-mg retinol equivalent) was associated with more than twofold enhancement of both primary and secondary IgG responses to tetanus toxoid, compared to children who received placebo. Vitamin A potentiated IgG1 subclass responses to tetanus toxoid, which is the subclass usually involved in the protective antibody response to tetanus (42). These findings suggest that the ability to mount an IgG response to T-cell dependent antigens is improved by administration of vitamin A or related retinoids.

3.2. Growth

Vitamin A deficiency is characterized by both stunting and wasting (59). Such a relationship might be expected because the gene for growth hormone is activated by retinoic acid (60). However, the reported effects of vitamin A supplementation on child growth are not consistent (59). A randomized-controled clinical trial involving more
than 2000 preschool children in Indonesia demonstrated that vitamin A supplementation had a selective effect on ponderal growth in boys but not girls (61). Improved linear growth among both boys and girls was noted in a controlled trial of vitamin A-fortified monosodium glutamate involving more than 1600 children (62). A recent trial of vitamin A supplementation showed no effect on growth in 592 preschool children, but this small sample size may have lacked sufficient power to examine the impact of vitamin A upon growth (63).

3.3. Reproduction and Pregnancy

Vitamin A is essential for normal reproduction (64). Animals deficient in vitamin A are unable to produce sperm, and vitamin A deficiency may affect fertility in the female. In both humans and animal models, vitamin A deficiency is associated with increased placental infections. Low serum vitamin A levels in HIV-infected pregnant women are associated with higher mother-to-child transmission of HIV (65). Pregnancy increases the risk of vitamin A deficiency for both mother and newborn. Epidemics of nightblindness among pregnant women were well known in Europe in the early part of the twentieth century (66), and nightblindness is so common in some cultures in developing countries that it has been considered to be a normal associate of pregnancy, as with morning sickness. Maternal vitamin A status may be important to birth outcome, as increased mortality and low birthweight have been reported among HIV-infected pregnant women who have low serum vitamin A levels during pregnancy (67). Low serum vitamin A levels in pregnant women (HIV-negative) have also been associated with increased infant mortality (68).

3.4. Hematopoiesis

Hematopoietic stem cells are retinoid-dependent, and impaired hematopoiesis, or anemia, is a common associate of vitamin A deficiency in children (69). Anemia may be caused, in part, by impaired ability to mobilize iron stores from the liver during vitamin A deficiency. Low plasma vitamin A levels are associated with low hemoglobin in HIV-infected injection drug users (41). Vitamin A supplementation or fortification has been shown to improve iron status and reduce anemia among children and pregnant women (70). The combination of vitamin A and iron in a single supplement seems to reduce anemia more effectively than either iron or vitamin A alone (70).

3.5. Vision

Vitamin A deficiency is the leading cause of blindness among children worldwide. Xerophthalmia is used to describe the wide spectrum of eye disease associated with vitamin A deficiency, and keratomalacia refers to the most severe stage in which the cornea undergoes ulceration and often results in blindness (71). Vitamin A is essential for generation of rhodopsin, a visual pigment necessary for vision. The earliest clinical manifestation of vitamin A deficiency is nightblindness (71). Vitamin A deficiency in general has an effect on mucosal epithelia, including the conjunctiva and cornea. Mild vitamin A deficiency causes squamous metaplasia of the conjunctiva, which may be detectable microscopically, or may form a Bitot’s spot, a well-demarcated area of keratinizing squamous metaplasia on the temporal or nasal bulbar conjunctiva. Bitot’s spots are considered to be pathognomonic for mild vitamin A deficiency. Severe xerophthalmia is characterized by classic, punched-out, full-thickness corneal ulceration, and
these ulcers may be sterile or may rapidly become secondarily infected with melting of the corneal stroma. The ocular manifestations of vitamin A deficiency are described in detail elsewhere (22, 37, 71). It was previously estimated that 500,000 new cases of xerophthalmia, half of which lead to blindness, occur each year in India, Bangladesh, and the Philippines combined (72). New data suggest that vitamin A-capsule distribution programs may be reducing the prevalence of xerophthalmia (73, 74), and such estimates may need revision.

4. DIAGNOSIS OF VITAMIN A DEFICIENCY

4.1. Eye Signs and Symptoms

Although vitamin A deficiency has a wide spectrum of clinical manifestations (see Table 2), the clinical diagnosis in children is most commonly made through recognition of ocular signs and symptoms, including nightblindness, Bitot’s spots, corneal xerosis, or corneal ulceration/keratomalacia (71). The detection of nightblindness and/or Bitot’s spots is often useful for determining the prevalence of mild xerophthalmia among children in developing countries. Among pregnant women in developing countries, assessment of nightblindness is an important screening test for vitamin A deficiency.

4.2. Serum Retinol Levels

Serum vitamin A levels remain the most widely used indicator of vitamin A status (75) and are especially useful in large epidemiologic studies. Vitamin A levels less than 0.7 μmol/L in children, and less than 1.05 μmol/L in adults are considered to be consistent with vitamin A deficiency. Factors contributing to low serum vitamin A levels include poor intake and absorption, inadequate liver stores, liver disease, increased utilization, and the acute phase response. During the acute phase response, RBP may dissociate from the RBP-transthyretin-retinol complex in circulating blood, allowing abnormal vitamin A losses in the urine and decreased circulating vitamin A levels (36). High urinary losses of vitamin A have been reported in individuals with pneumonia, nephritis, tuberculosis, sepsis, and malaria (36). Biological function may be compromised when serum vitamin A levels fall below 1.05 μmol/L (75, 76).

The measurement of acute-phase proteins, such as C-reactive protein and α1-acid glycoprotein, have been advocated to improve the interpretation of serum vitamin A levels in populations with a high prevalence of infection (77). Elevated acute-phase proteins and infections are more common in pregnant women with nightblindness (40) and in preschool children with nightblindness and/or Bitot’s spots (78). These studies show that vitamin A deficiency, infections, and low serum vitamin A levels are closely associated and that acute-phase proteins do not give any mutually exclusive information about low serum vitamin A levels.

4.3. Other Methods of Diagnosis

Vitamin A status can be measured by other techniques that measure end organ effects of vitamin A (conjunctival impression cytology, dark adaptation) and liver reserves of vitamin A (relative dose response, modified relative dose response). Conjunctival impression cytology is based upon cytological examination of cells removed from the conjunctiva of the eye (79). Dark adaptation has been recognized for decades to be useful, in various forms, for measuring vitamin A deficiency (80). Relative dose response
(RDR) (81) and modified relative dose response (MRDR) (82) are indirect measures of hepatic vitamin A stores. In general, abnormal RDR and MRDR responses correlate well with deficient serum vitamin A levels. MRDR may have limited sensitivity in individuals with malnutrition (83). A new technique that involves the measurement of pupillary diameter has advantages of being simple, rapid, noninvasive, and early results with use of the technique for assessment of vitamin A status are promising (84, 85).

5. VITAMIN A AS A PUBLIC HEALTH INTERVENTION

5.1. Brief History

The use of liver extracts as a cure for nightblindness has been described in antiquity. In the nineteenth century, cod-liver oil, a potent source of vitamins A and D, was empirically recognized to have therapeutic value in the treatment of anemia, tuberculosis, and other disorders. In the eighteenth and nineteenth century, there were numerous reports associating nightblindness with increased morbidity and mortality. The existence of vitamin A was demonstrated through a long incremental process that included contributions by Magendie (86), Lunin (87), Socin (88), Pekelharing (89), Stepp (90), Hopkins (91), Osborne and Mendel (92, 93), McCollum, Davis, Simmonds, Becker, and Shipley (94, 95), Karrer (96), Holmes and Corbett (97), and others. Different investigators have claimed to have “discovered” vitamin A, but the development of scientific thought occurred only through many cumulative steps and separate contributions by many individuals (98). Nutritional deprivation experiments suggested that vitamin A deficiency increased the risk of infections, and vitamin A was termed the “anti-infective” vitamin in 1928 (99). In the 1920s and 1930s, at least 30 trials were conducted to determine whether vitamin A could reduce the morbidity and mortality from respiratory infections, measles, puerperal sepsis, tuberculosis, and other infections (100). These early trials did not provide definitive information regarding the use of vitamin A for infections. Scientific interest in use of vitamin A as “anti-infective” therapy appeared to wane with the advent of the sulfa antibiotics and improvements in diet in industrialized countries in the late 1930s.

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td>Nightblindness, Bitot’s spots, corneal xerosis, corneal ulcers, fundus xerophthalmicus, keratomalacia, blindness</td>
</tr>
<tr>
<td>Immune System</td>
<td>Atrophy of thymus, lymph nodes, spleen; depressed antibody and cell-mediated responses, T-cell subset alterations, increased morbidity and mortality</td>
</tr>
<tr>
<td>Hematologic Lungs</td>
<td>Anemia, impaired lymphopoiesis</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Loss of ciliated epithelium, squamous metaplasia, loss of goblet cells and mucus; increased pulmonary infections</td>
</tr>
<tr>
<td>Genitourinary Reproductive Growth</td>
<td>Squamous metaplasia of bladder epithelium, increased infections</td>
</tr>
<tr>
<td></td>
<td>Reproductive failure, low birthweight, placental infections</td>
</tr>
<tr>
<td></td>
<td>Stunting, wasting</td>
</tr>
</tbody>
</table>

Table 2
Clinical Features of Vitamin A Deficiency
5.2. Recent Clinical Trials of Vitamin A for Reducing Infections and Mortality

The association of xerophthalmia with mortality was noted among preschool children in Indonesia (43). This observation led to the first large community-based study that demonstrated that regular vitamin A supplementation reduced childhood mortality by 34% in Indonesia (101). The Indonesian study was followed by a large series of clinical trials in Indonesia, India, Nepal, Sudan, and Ghana (62, 102–108). These studies (see Table 3) demonstrated that periodic vitamin A supplementation or fortification reduced child mortality, primarily from diarrheal disease. The sole exception was the study conducted in the Sudan, which reported that high-dose vitamin A capsules had no impact on either child mortality or vitamin A deficiency itself (107).

The impact of vitamin A supplementation on morbidity and mortality during measles is striking. Worldwide, measles affects approx 70 million children per year and up to 2 million children may die annually from measles and associated complications. In developing countries, case-fatality rates of 10–20% are not uncommon, and a measles episode may result in diarrhea, pneumonia, encephalitis, blindness, and delayed morbidity and mortality after an attack. High-dose vitamin A supplementation to children with acute, complicated measles reduces mortality by approx 50% (Table 3) (109–111). Similarly, the morbidity of diarrhea and pneumonia associated with measles is also reduced by vitamin A supplementation (112, 113). One study using a lower total dose of vitamin A did not detect any impact of vitamin A supplementation on measles (114). It is notable in these studies that high-dose vitamin A supplementation was shown to reduce morbidity and mortality in children without any clinical signs of vitamin A deficiency.

Vitamin A supplementation has a dramatic impact on the severity of infectious disease morbidity and childhood mortality, but it seems to have less effect upon mild to moderate morbidity. Community-based trials of vitamin A supplementation have not demonstrated a consistent effect on the incidence of mild respiratory disease and diarrhea. Morbidity from respiratory infection, fever, and diarrhea, as measured by a history obtained from the mother or father, tend to be subjective and imprecise. Studies that have utilized weekly or even more frequent monitoring of morbidity are unable to address the issue of severe morbidity, because children who are ill have usually been treated. Preschool children in developing countries are constantly being exposed to new pathogens and have high rates of respiratory disease and diarrhea in the first year of life. The dramatic reductions in severe morbidity and mortality noted in hospital and community-based studies of vitamin A may have led to the inflated expectation that vitamin A might reduce the incidence of respiratory disease and diarrhea among children. On an immunological basis, there is little evidence that vitamin A would prevent infection by a pathogen that is new to the host, and thus have a measurable impact on incidence of infection. However, vitamin A-related immune enhancement may result in a vigorous host response, shorter duration of infection, and less complications. This is born out by clinical trials that show that vitamin A supplementation reduces the severity of infectious disease morbidity, as well as mortality (1).

Vitamin A supplementation has also been evaluated as disease-targeted therapy for other types of infections besides measles. Several clinical trials have been conducted to determine whether vitamin A supplementation reduces the severity and duration of diarrhea (115–121). In general, these studies show that high-dose vitamin A suppleme-
tation will reduce the duration of diarrhea, and there is a consensus that vitamin A supplementation reduces diarrheal morbidity. Trials of high-dose vitamin A supplementation suggest that there is little to no effect upon the duration or severity of acute lower-respiratory infections in children (122–126) or upon respiratory syncytial virus infection in infants and children (127–129). Analysis of data from the large community-based studies suggests that vitamin A supplementation has no impact on acute lower-respiratory infections (130). A small study suggests that high-dose vitamin A supplementation has no impact upon pulmonary tuberculosis in children (131). During HIV infection, vitamin A supplementation has been shown to reduce diarrheal morbidity in infants (132) and a stratified analysis from a clinical trial suggests that vitamin A supplementation reduced mortality among HIV-infected children with pneumonia (133). Antenatal supplementation with multivitamins reduced fetal deaths and low birthweight among HIV-infected pregnant women, but no significant effect was noted with vitamin A alone (134).

The infrastructure of childhood immunization programs, such as the Expanded Programme on Immunization (EPI), has been suggested for the delivery of vitamin A supplements to infants. Concern was raised when it was shown that high-dose vitamin A supplementation interfered with seroconversion to standard titer live measles vaccine in 6-mo-old infants who had high titers of maternally acquired antibodies to measles (135); however, subsequent studies showed that high-dose vitamin A supplementation did not interfere with seroconversion in 9-mo-old infants (136, 137). A multicenter trial showed that vitamin A supplementation given through the EPI had no effect upon morbidity and mortality (138). Vitamin A supplementation given simultaneously with live trivalent oral poliovirus vaccine did not interfere with seroconversion to any of the three poliovirus types (139). The available data suggest that integration of vitamin A supplementation with childhood immunization programs at 6, 10, 14 wk, and 9 mo will improve vitamin A status of infants and will not have any deleterious effects upon response to immunization.

Vitamin A supplementation at the time of birth reduced infant mortality in Indonesia (140), and further studies are in progress in India and Zimbabwe to confirm these findings. Another study showed that vitamin A supplementation to mother and infant had no effect on infant morbidity (141). It is currently unclear whether vitamin A supplementation has an effect upon the morbidity or mortality of infants, especially under 6 mo of

Table 3

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample size</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia</td>
<td>1986</td>
<td>29,236</td>
<td>34% mortality</td>
<td>(62)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>1988</td>
<td>11,220</td>
<td>46% mortality</td>
<td>(101)</td>
</tr>
<tr>
<td>India</td>
<td>1990</td>
<td>15,775</td>
<td>6% mortality</td>
<td>(102)</td>
</tr>
<tr>
<td>India</td>
<td>1990</td>
<td>15,419</td>
<td>54% mortality</td>
<td>(103)</td>
</tr>
<tr>
<td>Nepal</td>
<td>1991</td>
<td>24,805</td>
<td>30% mortality</td>
<td>(104)</td>
</tr>
<tr>
<td>Nepal</td>
<td>1992</td>
<td>7197</td>
<td>29% mortality</td>
<td>(106)</td>
</tr>
<tr>
<td>Sudan</td>
<td>1992</td>
<td>29,615</td>
<td>No impact on mortality or xerophthalmia</td>
<td>(107)</td>
</tr>
<tr>
<td>Ghana</td>
<td>1993</td>
<td>21,906</td>
<td>19% mortality</td>
<td>(108)</td>
</tr>
</tbody>
</table>
A large community-based trial in lowland Nepal showed that weekly vitamin A or β-carotene supplementation to women of child-bearing age could reduce maternal mortality by about 30–50% (143). This trial raises the possibility that improving micronutrient status of women during pregnancy may reduce maternal mortality.

A recent trial conducted in Papua New Guinea shows a novel approach toward the immense morbidity and mortality of *P. falciparum* malaria. Periodic high-dose vitamin A supplementation in a malaria-endemic region was shown to significantly reduce malaria-related morbidity in preschool children (144). Most efforts directed toward malaria have focused upon insecticide-impregnated bednets and malaria vaccines, and vitamin A supplementation—an extremely inexpensive and simple intervention—may have comparable effects at a much lower price. The presumed basis by which vitamin A reduces malaria morbidity is by accelerating the acquisition of immunity to malaria, and further studies of the effects of vitamin A on immunity to malaria are currently in progress.

### 5.3. Current Indications for Use of Vitamin A Supplements in Developing Countries

High-dose oral vitamin A supplements are recommended for children in three general situations: in the treatment of xerophthalmia, in the treatment of acute measles, and as periodic supplementation for the prevention of vitamin A deficiency (145, 146). For the treatment of xerophthalmia or acute measles, 200,000 IU (60 mg retinol equivalent) of vitamin A is given upon diagnosis, the following day, and 1 mo later for children over 1 yr of age, adolescents, and adults (except women of reproductive age). For children under 1 yr of age or children of any age who weigh < 8 kg, the aforementioned guidelines are followed using 100,000 IU (30-mg retinol equivalent) capsules. Many developing countries have instituted periodic high-dose vitamin A-capsule distribution, and these programs generally use 200,000 IU capsules for children 1–6 yr of age, every 3–6 mo.

For women of reproductive age, pregnant or not pregnant, the WHO/UNICEF/IVACG recommendations are a daily dose of 10,000 IU for 2 wk. Although this is a general recommendation, this dose is often unavailable in many developing countries. The DV for vitamin A in antenatal vitamins in the United States is 8000 IU/d, and many commercial vitamins contain this dose, as vitamin A, β-carotene, or a combination of both. In general, high-dose vitamin A capsules should not be used for pregnant women or women of reproductive age who are not using reliable contraception, because of concerns regarding the theoretical teratogenicity of vitamin A. It is also important to recognize that there are well-defined therapeutic indications in which vitamin A has been demonstrated to be efficacious, and indiscriminate use of vitamin A supplements should be discouraged.

### 5.4. Prevention of Vitamin A Deficiency

Approaches for the prevention of vitamin A deficiency include food fortification, nutrition education, home gardening, and preservation of vitamin A-containing fruits and vegetables. Fortification of sugar in Guatemala and monosodium glutamate in Indonesia has shown some success, and some countries, such as the Cook Islands, have specifically required that certain imported foodstuffs be fortified (147). One question that is often raised is why vitamin A deficiency is common among children in locations
with a wealth of plant-food sources of vitamin A. A common explanation is that young children may not like the bitter taste in many dark green leafy vegetables. Papaya and mango, which may be more palatable to children, are only available seasonally. It remains to be determined whether nutrition education and home gardening will have a significant impact on the vitamin A status of children in developing countries. Another debate has arisen regarding the bioavailability of pro-vitamin A carotenoids in plants sources of food, as recent studies suggest that these carotenoids may not be as bioavailable as previously believed (148–150).

6. SUMMARY AND RECOMMENDATIONS

Vitamin A is a potent immune enhancer that has shown efficacy in reducing morbidity and mortality from infectious diseases among children in developing countries. Improved vitamin A nutriture is expected to prevent 1.3–2.5 million deaths annually worldwide, and periodic high-dose vitamin A supplementation is now widely practiced in many developing countries where vitamin A deficiency is endemic (2). High-dose vitamin A supplementation for children with acute, complicated measles may also be expected to contribute to a reduction in child mortality, given the estimated two million deaths from measles each year. A variety of approaches, such as food fortification, supplementation, nutrition education, and home gardening may be utilized in developing countries to combat vitamin A deficiency and increase resistance to infectious diseases.

As disease-targeted therapy, vitamin A supplementation has been shown to enhance immunity and reduce the morbidity of measles and diarrheal disease, and there are other potential applications for vitamin A, such as adjunct therapy for tuberculosis. A recent clinical trial suggests that enhancement of immunity to malaria by high-dose vitamin A may be a potential strategy to reduce morbidity and mortality by P. falciparum malaria. In situations where daily supplements could be delivered, the use of multivitamins including vitamin A, is being evaluated for improvement of birth outcomes and reduction of maternal mortality.

7. ACKNOWLEDGMENTS

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IV  OPTIMAL PREGNANCY/
INFANCY OUTCOMES
15
Folic Acid-Containing Multivitamins and Primary Prevention of Birth Defects

Andrew E. Czeizel

1. INTRODUCTION

The deficiency or overdosage of certain nutrients may have a role in the origin of birth defects. First, in 1932 Hale (1) demonstrated that a vitamin A-free diet during early pregnancy of sows resulted in offspring without eyeballs. Some of the pigs also had other defects, such as oral clefts, accessory ears, malposition of kidney and defects of hind legs. Hale’s conclusion was “the condition is illustrative of the marked effect that a deficiency may have in the disturbance of the internal factors that control the mechanism of development” (1). Further development of experimental teratology became possible when small rodents were introduced for this purpose. Joseph Warkany (1902–1992) (2), one of the founders of teratology, recognized the importance of purified diets and used these to test various vitamin deficiencies for their teratogenic effects. He found that maternal dietary deficiency can induce structural birth defects, i.e., congenital abnormalities (CAs) (3). Marjorie M. Nelson (4) used antimetabolites, which made possible conversion of long-term nutritional experiments into short-term chemical testing. First, antimetabolites of folic acid were used and folic acid deficiency was proved highly teratogenic in pregnant rats (5–7). Later, it was confirmed in humans (8–10) as well. This research approach also had strong support from the French investigator, Giroud (11,12). These findings highlighted the developmental importance of folate-folic acid (13,14). However, this first phase of history of malnutritional teratology including folate-folic acid deficiency was followed by a longer silent period. The second phase was related to the primary prevention of neural-tube defects (NTDs) in the 1980s.

2. INTERVENTION STUDIES FOR REDUCTION OF NEURAL-TUBE DEFECTS

Anencephaly and spina bifida (aperta or cystica) are the major classes of NTDs. Although the genetic (polygenic) background of nonsyndromic, i.e., the so-called isolated NTDs (92% of all cases) is obvious because recurrence in first-degree relatives is 10 times higher than their occurrence (15), this group of CAs is very sensitive
to environmental factors. The latter is indicated by the very wide range (0.5–12/1000) of NTD incidences in different populations, rapid secular changes in their occurrence, seasonal variation of births with NTD, and mainly by their very obvious socioeconomic status dependence \((16)\): The risk of NTDs was found to increase from a low risk in the highest class to an above-average risk in the lowest class in the United Kingdom and some other countries.

The documented start of the second phase of the folate-folic acid story dates back to 1976. Smithells hypothesized that among triggering environmental factors in the origin of NTDs, undernutrition could be the common denominator, and his group tested this hypothesis: A lower concentration of red-cell folate and vitamin C was found during the first trimester of pregnancy in women who later gave birth to an infant with an NTD than in matched controls \((17)\). These findings prompted Smithells and his group to organize the first intervention study to check the efficacy of a folic acid-containing multivitamin for the reduction of recurrent NTD \((18)\). In general, NTDs occur between postconceptional d 15 and 28 in humans, i.e., at the critical period of NTDs, most women are unaware of their pregnancy. Thus, periconceptional supplementation of multivitamins or folic acid should commence at least 28 d prior to conception and continue to the date of the second missed menstrual period. The final results of the Smithells et al. study were published separately for the Yorkshire region of the United Kingdom \((19)\) and Northern Ireland \((20)\). However, the 91% and 83% reduction in NTD recurrence \(\text{(see Table 1)}\), respectively, were not accepted by some experts because of possible selection bias. Two ethical committees refused to give permission for the original protocol of the study, i.e., for a randomized clinical trial; thus, the control group was made up of women who had had one or more previous infants with NTDs and were already pregnant when referred to the study centers or declined to take part in the trial. The study in Wales performed by Laurence et al. \((21)\) was a randomized, double-blind trial; however, the difference between study and placebo groups was not significant as a result of the small number of women \(\text{(Table 1)}\). In the early 1980s, the Medical Research Council (MRC) \((22)\) in the United Kingdom decided to organize a multicenter \(\text{(43% of participants came from Hungary)}\) double-blind randomized study. This trial indicated that a pharmacological dose \(\text{(4 mg)}\) of folic acid supplementation alone can reduce NTD recurrence significantly \(\text{(by 71%)}\) \(\text{(Table 1)}\). Based upon these results, the Centers for Disease Control (CDC) \((23)\) recommended daily supplementation with 4 mg of folic acid under medical supervision in the periconception period for women at high risk \(\text{(i.e., who had one or more previous offspring with NTD)}\) for the reduction of NTD recurrence.

However, there were two major questions following the publications of the “recurrence” studies. The Hungarian randomized, double-blind controlled trial attempted to provide data to answer these questions. The first question was: “Does folic acid containing-multivitamin supplementation also reduce the risk of first occurrence of NTD?” About 95% of women who deliver infants with NTD have no previous NTD pregnancies \((15)\). Thus, one of the critical goals of the Hungarian trial was to determine the efficacy of this new primary preventive method in the reduction of first occurrence of NTD. The second question was connected with the dose. The pharmacological dose \(\text{(e.g., 4 mg)}\) of folic acid may have some adverse effects; thus, it cannot be recommended for the population at large and/or without medical supervision. The Hungarian intervention trial, therefore, tested the preventive effect of a physiological dose \(\text{(0.8 mg)}\) of folic acid as one component of a periconceptional multivitamin supplement. The
<table>
<thead>
<tr>
<th>Type</th>
<th>Method</th>
<th>Country</th>
<th>Supplement</th>
<th>With supplement</th>
<th>Without supplement</th>
<th>Risk reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recurrence</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nonrandomized</td>
<td>Yorkshire (19)</td>
<td></td>
<td>Multivitamin(^a)</td>
<td>1/187 0.5</td>
<td>18/320 5.6</td>
<td>91</td>
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<tr>
<td>Randomized</td>
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<td>Folic acid (4 mg)</td>
<td>4/511 0.8</td>
<td>17/353 4.8</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Wales (21)</td>
<td></td>
<td>Folic acid (4 mg)</td>
<td>2/60 3.3</td>
<td>4/51 7.8</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Multicenter MRC (22)</td>
<td></td>
<td>Folic acid + other vitamins</td>
<td>2/298 0.7</td>
<td>13/300 4.3</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>4/295 1.4</td>
<td>21/602(^c) 3.5</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other vitamins</td>
<td>6/593 1.0(^e)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8/302 2.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Randomized</td>
<td>Hungary (24)</td>
<td>Multivitamin(^b)</td>
<td>0/2471 0.00</td>
<td>6/2391 0.25</td>
<td>100</td>
</tr>
<tr>
<td>Nonrandomized</td>
<td>Hungary (28)</td>
<td></td>
<td>Multivitamin(^b)</td>
<td>1/3019 0.03</td>
<td>8/3432 0.23</td>
<td>92</td>
</tr>
</tbody>
</table>

\(^a\) Included 0.36 mg folic acid.
\(^b\) Included 0.8 mg folic acid.
\(^c\) Including cases supplemented with “other” vitamins.
Hungarian trial was launched on February 1, 1984 and the intervention was completed on April 30, 1992. Pregnancy outcomes were evaluated until the end of April 1993 and the postnatal follow-up continued until the end of April 1994 (24). As can be seen in Table 1, no NTD case was found in the multivitamin group, whereas six NTD cases occurred in the placebo-like trace-element group \((p = 0.01)\). Thus, the Hungarian trial demonstrated that a multivitamin containing 0.8 mg of folic acid prevented the first occurrences of NTD.

Several observational studies also indicated the efficacy of periconceptional folic acid-containing multivitamin supplementation and folate-containing foods in the prevention of NTDs (25). For example, the study of Werler et al. (26) suggested that daily periconception intake of 0.4 mg of folic acid (the dose most commonly contained in over-the-counter multivitamin preparations) reduced the risk of occurring NTDs by approx 60%. Based upon the Hungarian intervention (24) and other observational studies (25,26) the CDC (27) in September 1992 and the United States Public Health Service recommended that “all women of childbearing age who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other neural tube defects.” This recommendation was subsequently followed by several countries.

The spectrum of estimated efficacy of periconceptional folic acid-containing multivitamin or folic acid alone supplementation, however, was too wide (50–90%), therefore, it was necessary to achieve a more accurate estimate for the reduction of first NTD. Obviously, the Hungarian randomized double-blind trial could not be continued for ethical reasons, thus a prospective case-control controlled study was organized in Hungary between 1993 and 1996 (28). Supplemented women with confirmed pregnancy were recruited from the participants of the periconceptional service. They used the same multivitamin (Elevit pronatal®) than the participants of previous randomized double-blind trial in the periconceptional period. Unsupplemented matched women were invited to take part in the study after the first visit between the eighth and twelfth week of gestation in the antenatal care. Of 3019 supplemented informative offspring, one had NTD, whereas of 3432 unsupplemented informative offspring, eight were affected with NTD (adjusted odds ratio (OR) with 95% confidence interval (CI): 0.08; 0.01–0.41). Thus, now we can estimate more accurately the efficacy of periconceptional folic acid-containing multivitamin supplementation for the reduction of first occurrence of NTD after the combination of results of the two Hungarian studies. There was one NTD among 5490 supplemented informative offspring, while there were 14 NTDs among 5823 unsupplemented offspring, i.e., more than 90% of first NTD occurrence can be prevented by the help of periconceptional folic acid-containing multivitamin supplementation.

The database of the Hungarian Case-Control Surveillance of Congenital Abnormalities showed a significant, but less-effective reduction (about 70%) in the rate of NTD after the periconceptional high dose (3–6 mg) folic acid supplementation (29).

3. THE POSSIBLE MECHANISM OF NTD PREVENTION

The theoretical basis of primary prevention of NTD by folic acid or folic acid-containing multivitamin is connected with the reduction of hyperhomocysteinemia in the major group of hyperhomocysteinemia-related NTDs. There are four groups of evidence for the confirmation of this hypothesis.
1. Hyperhomocysteinemia and/or lack of methionine can induce NTD in animal experiments (30,31).
2. Mothers who gave birth to a child with an NTD have higher blood (32) and amniotic fluid (33) levels of homocysteine.
3. The causes of hyperhomocysteinemia need a longer explanation. When meat, fish, or plant proteins are digested, amino acids, among others, methionine, are released. During the conversion of methionine to cysteine, homocysteine, a toxic metabolite is formed, and humans neutralize it normally as soon as possible. On the one hand, homocysteine can be metabolized via the transsulfuration pathway to form cystathionine caused by the condensation with serine and catalyzed by cystathionine β-synthase. This enzyme requires pyridoxal 5'-phosphate, the biologically active form of vitamin B₆ as a cofactor. On the other hand, remethylation of homocysteine to methionine is catalyzed by methionine-synthase. This enzyme requires vitamin B₁₂ as a cofactor and 5-methyl-tetrahydrofolate as the methyl donor. The latter explains the importance of folate-folic acid deficiency in the origin of NTD. Recently, the introduction of a new terminology of folate-folic acid as vitamin B₁₁ has been suggested in Europe based on three arguments:
   a. This vitamin was discovered by Lucy Wills (34) in 1931, and she stressed the similarity of this “curative agent” with vitamin B₁₂, as a “twin” vitamin.
   b. The generic term folate describes the many different naturally occurring polyglutamate forms of the vitamin, whereas folic acid is the synthesized monoglutamate form, chemically designated as pteroylglutamic acid. The different names of two forms of the same vitamin is disturbing and are confused frequently, thus their joint name as vitamin B₁₁ seems to be reasonable. (The term vitamin B₁₁ has been used in some countries, e.g., the Netherlands).
   c. Finally, the term of folic “acid” sounds harmful for several prospective mothers.

Humans cannot synthesize vitamin B₁₁. The major dietary sources of folates are fresh and frozen green leafy vegetables, citrus fruits and juices, liver, wheat bread and legumes. Food folates are converted to monoglutamates by conjugases in the upper part of the small intestine. Monoglutamate folic acid, however, can be absorbed directly. After the active and passive absorption of monoglutamates, they are converted to dihydrofolate and then to tetrahydrofolate (THF) by reductase enzymes. THF is the parent compound of all biological active vitamin B₁₁. The most important cause of hyperhomocysteinemia and/or lack of methionine is the polymorphism of methylene-THF-reductase (MTHFR) gene (35,36). A 677C → T mutation has been identified in the MTHFR gene and it results in thermolabile variant (Ala225Val) of MTHFR (37) with 30–50% activity of the allelic form. This enzyme variant cannot catalyze appropriately the pathway of 5,10-methylene-THF to 5,methyl-THF, i.e., the methyl donor for methionine-synthase and it may have a role in the origin of NTD (37–40). The MTHFR gene polymorphism is so common (5–20%) in different populations (41) that it needs some explanation. The highly significant excess of C/T heterozygosity in male first-degree relatives of patients with NTD may demonstrate C/T heterozygote advantage in the context of their genetic background (42). Thus, the thermolabile variant of MTHFR and the polymorphism of MTHFR gene is an obvious risk factor for NTD. However, other common mutations of MTHFR gene may also have a role in the origin of NTD (43). In addition, the gene encoding methionine synthase was cloned (44–46) and the first mutation were identified (45).
4. Folic acid-containing multivitamins and folic acid alone are able to reduce hyperhomocysteinemia (Chapter 8) consequently the hyperhomocysteinemia-related NTDs (19,20,22,24).
Thus, the available findings suggest that the cause of a major part of NTD is not a primary lack of folate in the diet, but the addition of an inborn error of vitamin B_{12}-homocysteine metabolism. An interaction between genetic predisposition and nutrition, therefore, has causal role in the origin of NTDs, i.e., a dietary deficiency can trigger the genetic predisposition (e.g., it may explain social class gradient) and folic acid-containing multivitamins or folic acid alone can neutralize the failure of local metabolite supply. The genetic–nutrient interaction through \textit{MTHFR} polymorphism and low-folate status is associated with a greater risk for NTDs than either variable alone (47). In addition, vitamin B_{12} is an independent risk factor in the origin of NTD, thus its preventive effect is also reasonable (48,49).

At present, there are three possibilities to provide appropriate multivitamin/folic acid consumption for women of childbearing age to reduce NTD.

1. \textit{Consumption of folate-rich and other vitamin-rich diet}. The study of McPartlin et al. (50) suggested that the optimal daily intake of folate in the early postconceptional period is about 0.66 mg. However, the usual daily intake of folate is about 0.18–0.20 mg (51) and it is difficult to imagine a 3.3–3.7-fold increase in folate intake every day in anticipation of conception, which would need about 15 servings of broccoli or Brussels sprouts. In addition, an extreme increase in the consumption of extra folate from natural food is relatively ineffective at increasing folate status (52).

2. \textit{Periconceptional supplementation}. It would be a simple and useful approach, however, about 50% of pregnancies are unplanned in the United States, Hungary, and many other industrialized countries. (Only the Netherlands have a 90% proportion of planned and/or wanted pregnancies.) In addition, this type of primary prevention has not been overly successful to date in planned pregnancies either (53). Pre- or periconceptional care is very useful for the practical delivery of periconceptional multivitamin/folic acid supplementation (54,55) (see Subheading 4).

3. \textit{Food fortification}, which was introduced in United States and Hungary (see Subheading 9).

All the three possibilities should be pursued in parallel to provide options for women who are capable of becoming pregnant.

\section*{4. PERICONCEPTION CARE: PLANNING FOR PREGNANCY FOR BOTH PARTNERS}

The provision of preconception care was included in the National Health Promotion and Disease Prevention Objectives for the year 2000 in the United States (56). In Hungary, we prefer to use the term periconception instead of preconception because prenatal care usually begins in about the 8–12th wk of pregnancy; thus, the early postconceptional period was previously omitted from medical health service and the fetuses were uncared for at their most sensitive early period (from the third postconceptional wk until the eighth wk). Thus, the Hungarian Optimal Family Planning Service (HOFPS) (54,55) begins 3 mo before pregnancy and continues for 2–3 mo in the postconceptional period. The HOFPS was launched in 1984 and includes information-counseling, examinations, and interventions performed or supervised by qualified nurses. The main goal is to provide information and care for prospective parents to protect the health of the mother, to reduce untoward pregnancy outcomes, birth defects, and developmental disabilities, and to increase the potential for infants to be born as healthy as possible.
In the 1990s, the network of the HOFPS included 32 centers under the coordination of our Family Planning Clinic. Here, the 15-yr experience of our coordinating center is summarized. The HOFPS consists of three steps:

1. **Checkup of reproductive health.** A medical checkup as a “preconceptional screening” is an essential component of the HOFPS for risk identification and assessment (see Table 2), and referral of couples or persons to appropriate secondary care. We have an extra effort to incorporate males into the HOFPS to help in the development of responsible fatherhood that seems to be successful: Of 14,540 females, 11,999 (82.5%) were accompanied by their husbands or partners in life.

2. **3-mo preparation for conception** (Table 2). The preparation for conception is an appropriate period to stop smoking, alcohol drinking, and unnecessary drugs with hazards to germ cells and later the fetus. This 3-mo preparation period is an optimal time for the launch of periconceptional multivitamin supplementation. All women were supplied with a folic acid-containing multivitamin (Elevit pronatal®).

3. **Better protection of early pregnancy.** In the past, pregnancies were diagnosed after the second missed menstrual period; thus, the most sensitive period of early fetal development was not recognized and protected. In the HOFPS, after the 3-mo preparation for conception, females are asked to continue the intake of the multivitamin, to achieve conception on the optimal day (the first day prior to ovulation) and to visit the Family Planning Clinic immediately after the first missed menstrual period. Thus, the goal of the next visit is pregnancy confirmation by a serum or urine pregnancy test. At that time, an ultrasound scanning is suggested 2 wk later (nearly all ectopic pregnancies were diagnosed as a result of this examination, such an early phase assessment allowed for conservative treatment instead of surgery). Women with known occupational hazards were exempted and they were informed how to avoid other risks (e.g., heat bathing, exposure to infected cats), and pregnant women were asked to continue multivitamin supplementation and were referred to the prenatal care clinic with the discharge summary of the HOFPS.

The periconception care is appropriate for the detection of infertile couples and to treat them sooner; to provide a more effective flow of couples with positive family history, case history, and/or STDs to the appropriate secondary care. The HOFPS is optimal for the introduction of periconceptional multivitamin/folic acid supplementation, to improve the diet, and to reduce smoking and alcohol consumption before conception.Obviously, proper preparation for conception is the earliest and probably the most important method of health promotion in general, and particularly for the prevention of birth defects.

5. **OTHER REPRODUCTIVE EFFECTS OF PERICONCEPTIONAL MULTIVITAMIN SUPPLEMENTATION**

   The Hungarian trial within the HOFPS was appropriate to study possible other effects of periconceptional folic acid-containing multivitamin supplementation. The great majority of the women in the study were healthy and not malnourished (51,55).

1. During the preconceptual multivitamin supplementation the female cycle became more regular, i.e., the variance was lower (57). Thus, multivitamin supplementation may have a beneficial effect for women with irregular menstrual cycles.
2. There was no difference in the sexual activity (measured by the rate of weekly sexual intercourse) of couples between the multivitamin and the placebo-like trace-element groups in the preconceptional period (58). However, only women were supplemented and sexual activity is often determined by males.

3. A 7% higher rate of conception occurred in women who were treated with periconceptional multivitamin supplementation compared with those who were given trace-element only. The time taken to become pregnant was slightly, but significantly, shorter in the multivitamin group (59).

4. The rate of multiple births, namely twins, was about 40% higher after periconceptional multivitamin supplementation (60) most likely because of the improved fertility. The higher rate of twin conceptions could not be explained by maternal factors (age, parity) or by a higher rate of infertility drug use (61). In a recent United States study, four of five datasets showed a 30–60% greater prevalence of periconceptional folic acid-containing multivitamin supplementation among mothers of multiple births (62).

5. A significantly lower rate of treated morning sickness (nausea and vomiting) occurred in early pregnancy after periconceptional multivitamin supplementation (3 vs 6.6% in the trace-element group) (63).

Table 2
Three Steps and Different Items of the HOFPS

1. Checkup of reproductive health
   a. Family history of females and males
   b. Case history of females, e.g., epilepsy, diabetes, and so forth
   c. Vaginal and cervical smear examination for the screening of sexually transmitted infections/disorders
   d. Sperm analysis for the detection of subfertility and pyosperm
   e. Psychosexual exploration of couples
   f. Blood examination for the revealing of rubella seronegative women (who are then vaccinated), anemia, and HIV positivity

2. The 3-mo preparation for conception
   a. Necessary further examinations and/or treatments in the disorders of couples detected at the checkup examination
   b. Protection of germ cells: avoidance of smoking, alcohol, and unnecessary drugs
   c. Discontinuation of contraceptive pills to restore the internal hormonal balance of females (condoms are provided)
   d. Occupational history to reveal occupational hazards (to contact occupational doctor)
   e. Measurement of basal body temperature for detection of hormonal dysfunction (and treatment, if necessary) and determination of the so-called optimal day of conception
   f. Preconceptional multivitamin supplementation
   g. Guidelines for healthy diet
   h. Suggestion to check dental status
   i. Guidelines for physical exercise

3. The better protection of early pregnancy
   a. Achievement conception on the optimal day
   b. Early pregnancy confirmation
   c. Ultrasound scanning
   d. Postconceptional multivitamin supplementation
   e. Avoidance of teratogenic and other risks
   f. Referral of pregnant women to prenatal care clinics
6. There was no difference in maternal weight gain between the multivitamin and the trace-element groups before and during pregnancy (64).

7. All possible side effects were controlled continuously. \textit{Pernicious anemia} did not occur in the 2793 case and 2660 control pregnant women of the randomized double-blind controlled intervention trial. Moreover, the number of female participants in the Coordinating Centre of the HOFPS was 14,540 until January 1999, but patients with pernicious anemia have not been recorded among these reproductive aged women. Among female participants, 66 (0.46\%) were epileptic and 60 wanted to apply multivitamin supplementation. There was no case with multivitamin-related side effects of epilepsy during the periconception period (65). However, a 22-yr-old epileptic woman was treated continuously by carbamazepine and another folic acid (1 mg)-containing multivitamin from the 20th week of gestation. She had status epilepticus and later symptoms of systemic lupus erythematoses (65). Thus, autoimmune disease of epileptic pregnant women could damage the blood-brain barrier and the pharmacological dose (\geq 1 mg) of folic acid may trigger a cluster of seizures. Of 14,540 female participants, four (0.03\%) had severe allergic exanthema, and of these four, three discontinued the use of multivitamin (all of them had a history of drug-induced allergic diseases). Among all other possible side effects, constipation (1.8 vs 0.8\%) and diarrhea (1.4 vs 0.4\%) showed a somewhat, but not significantly, higher rate after multivitamin supplementation in the preconceptional period.

8. There was no significant difference in pregnancy outcomes of singletons including four types of fetal deaths (chemical pregnancies, ectopic pregnancies, miscarriages, including the so-called missed abortions or blighted ova, stillbirths) and three variables of liveborn infants (sex ratio, birthweight, including low birthweight, and gestation age, including preterm birth) (66). However, the rates of four kinds of fetal death were somewhat, but not significantly, higher in the multivitamin group. In addition, the ratio of boys: girls showed a slight girl excess in the multivitamin group, whereas the well-known boy predominance was seen in the trace-element group. Thus, a small change in the pattern of prenatal selection cannot be excluded because of the treated subfertility and the increase of twin pregnancies (67).

9. Postnatal somatic (body weight, body length, head circumference) and mental (measured by three tests) development did not show any significant differences between the multivitamin and trace-element groups (68,69). Thus, the previously found higher rate of worrying, fussiness, and fearfulness in girls born after periconceptional multivitamin supplementation (70) was not confirmed.

10. The rate of some other major CAs showed a significant decrease after the periconceptional folic acid-containing multivitamin supplementation. These data will be shown in Subheading 6.

6. PERICONCEPTIONAL MULTIVITAMIN SUPPLEMENTATION PREVENTS OTHER MAJOR STRUCTURAL BIRTH DEFECTS

Structural birth defects, i.e., CAs, were differentiated on the basis of three time windows of diagnosis:

1. During pregnancy, in the second and third trimesters;
2. At birth; and
3. Later, until the end of the first postnatal year in the follow-up examination of infants in the final database of the Hungarian trial (66). In addition, isolated and multiple CAs were separated (71,72).
The total rate of cases with CAs was 56.3/1000 in the multivitamin and 73.2/1000 in the trace-element group \((p = 0.018)\) in the final database \((72)\). The dropout was only 0.9% at the evaluation of cases at birth; thus, the first and second time windows with nearly complete ascertainment were evaluated together. The rate of CAs diagnosed during pregnancy and at birth was 11.3/1000 in the multivitamin and 22.2/1000 in the trace-element group \((p = 0.004)\). After the exclusion of six cases with NTD, the difference remained significant between the two study groups \((p = 0.019)\). Finally, mild CAs (mainly deformations) were excluded in another analysis \((see\ Table\ 3)\). The rate of major CAs was 20.6/1000 in the multivitamin and 40.6/1000 in the trace-element group \((p = 0.0001)\) \((72)\). After the exclusion, six NTD cases and three familial cases of other CAs, the difference in the rate of major CAs between the two study groups was very highly significant \((p < 0.0001)\) with a relative risk of 0.48 \(95\%\ CI: 0.34, 0.68\). In conclusion, periconceptional multivitamin supplementation reduced not only the occurrence of NTD but also the rate of other major CAs \((see\ Fig.\ 1)\).

The final database of the Hungarian trial indicated a significant reduction in two CA groups \((72,73)\) in addition to NTD. Nine cases with CAs of the urinary tract were found in the trace-element group and only two in the multivitamin group \((\chi^2 = 4.70; p = 0.03)\). The difference was most obvious in the obstructive CAs of the urinary tract, in addition, there were two offspring with renal agenesis in the trace-element group \((Table\ 3)\). The combined rate of the obstructive CA groups of the urinary tract and renal agenesis \((1 vs 8)\) showed a more obvious significant difference between the two study groups \((\chi^2 = 5.69; p = 0.02)\). Renal agenesis and utero-pelvic junction obstruction may have a common pathogenesis \((74)\). In the 1950s, Monie et al. \((75,76)\) were able to produce CAs of the urinary tract in rat embryos by folic acid deficiency. In a recent human study, Li et al. \((77)\) also found a highly significant reduction in the rate of urinary tract defects after the first trimester multivitamin use \((0.15 \[CI\ 95\%: 0.05, 0.43\])\).

There were 10 infants with cardiovascular CAs in the multivitamin group and 20 in the trace-element group \((Table\ 3)\). The difference was near the level of significance \((\chi^2 = 3.69; p = 0.05)\). The presence of cardiovascular CAs was confirmed before 1 yr of age by cardiological consultation (including echocardiography or cardiac catheterization), surgery, or autopsy \((72,73)\). One case with aortic stenosis was familial in the multivitamin group, after the exclusion of this case the difference was more obvious \((\chi^2 = 4.57; p = 0.03)\). The difference of cardiovascular CAs is mainly explained by two cases of ventricular septal defect in the multivitamin group and eight cases in the trace-element group \((\chi^2 = 3.81; p = 0.05)\). The difference in the rate of conotruncal CAs (in the multivitamin group: 2 ventricular septal defect, 1 double-outlet right ventricle; in the trace-element group: 8 ventricular septal defect, 1 tetralogy of Fallot, 1 complete transposition) was significant \((\chi^2 = 4.01; p = 0.045)\). Cardiovascular CAs were induced by pteroylglutamic acid deficiency during gestation in rat fetuses \((78,79)\). Shaw et al. \((80)\) examined whether a woman’s reported use of a folic acid-containing vitamin was associated with a reduced risk for conotruncal cardiovascular CAs. Data were derived from a population-based case-control study of fetuses and babies among a 1987–1988 cohort of 341,839 births ascertained by the California Birth Defects Monitoring Program. Telephone interviews were conducted with mothers of 239 conotruncal cases \((84.2\%)\) and 481 nonmalformed control cases \((76.2\%)\). The unadjusted odds ratios for any folic acid-containing vitamin use from
1 mo before conception through 2 mo postconception were 0.65 (CI 95%: 0.44–0.96) for conotruncal cardiovascular CAs. Botto et al. (81) also studied the role of periconceptional use of multivitamin in the prevention of conotruncal cardiovascular CAs (transposition of the great arteries, tetralogy of Fallot, and truncus arteriosus) in the population-based Atlanta Birth Defects Case-Control Study. One hundred and fifty eight case-infants and 3026 unaffected control-infants born from 1968–1980 were compared. Their results suggested that periconceptional use of multivitamin was associated with a 43% reduction in risk for conotruncal cardiovascular CAs. The effect was strongest for isolated conotruncal CAs (0.41; CI 95%: 0.20, 0.84). Among anatomic subgroups of conotruncal CAs, transposition of the great arteries showed the greatest reduction in risk (0.36; CI 95%: 0.15, 0.89). The protective effect of postconceptional supplementation of pharmacological dose (3–6 mg) of folic acid for cardiovascular CAs was also seen in the database of the Hungarian Case-Control Surveillance of Congenital Anomalies (29).

In conclusion, the available evidence indicates that the rates of three groups of CAs: NTD, certain CA-groups of urinary tract, and cardiovascular CAs, can be reduced significantly by periconceptional multivitamin supplementation.

There are three other groups of CAs that may also have been reduced by periconceptional multivitamin supplementation. In an animal experimental study in 1955, Nelson et al. (7) were able to obtain cleft lip in more than 90% of the rat offspring subjected to a transitory folic acid deficiency as a result of an antimetabolite during day 9–11 of gestation. In the early 1980s, Tolarova (82) reported a protective effect of a multivitamin and high dose (10 mg) including folic acid for the recurrence of cleft lips. This database was evaluated recently with similar results by Tolarova and Harris (83).

### Table 3
The Number of Cases with Major CAs in the Two Study Groups and the Relative Risk of Some Major CA Groups After Periconceptional Multivitamin Supplementation

<table>
<thead>
<tr>
<th>CA-groups</th>
<th>Multivitamin (N = 2471) no.</th>
<th>Trace element (N = 2391) no.</th>
<th>Relative risk (with 95% confidence limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neural-tube defects</td>
<td>0</td>
<td>6</td>
<td>0.06 (0.00, 0.63)</td>
</tr>
<tr>
<td>Urinary tract CAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructive and renal agenesis</td>
<td>1</td>
<td>8</td>
<td>0.12 (0.02, 0.69)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>9</td>
<td>0.21 (0.05, 0.99)</td>
</tr>
<tr>
<td>Cardiovascular CAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conotruncal</td>
<td>3</td>
<td>10</td>
<td>0.29 (0.09, 0.97)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>20</td>
<td>0.42 (0.19, 0.98)</td>
</tr>
<tr>
<td>Cleft palate</td>
<td>0</td>
<td>2</td>
<td>0.19 (0.01, 4.03)</td>
</tr>
<tr>
<td>Cleft lip ± palate</td>
<td>4</td>
<td>3</td>
<td>0.29 (0.32, 5.22)</td>
</tr>
<tr>
<td>Pyloric stenosis</td>
<td>2</td>
<td>8</td>
<td>0.24 (0.05, 1.14)</td>
</tr>
<tr>
<td>Reduction CAs of limb</td>
<td>1</td>
<td>5</td>
<td>0.19 (0.03, 1.18)</td>
</tr>
<tr>
<td>Other major CAs</td>
<td>23</td>
<td>34</td>
<td>0.68 (0.38, 1.09)</td>
</tr>
<tr>
<td>Multiple CAs</td>
<td>10</td>
<td>12</td>
<td>0.81 (0.36, 0.81)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td>97</td>
<td>0.53 (0.35, 0.70)</td>
</tr>
</tbody>
</table>
This finding was not confirmed in our Hungarian trial (84) (Table 3) and in the recent prospective case-control controlled study (28). However, in the California Birth Defects Monitoring Program, 1987–1988, Shaw et al. (85) investigated whether folic acid-containing vitamin use in the periconceptional period was associated with a reduced risk of oral clefts. Telephone interviews were conducted with mothers of 731 cleft cases (84.7%), and 734 nonmalformed control cases (78.2%). The unadjusted odds ratio for

<table>
<thead>
<tr>
<th>Condition</th>
<th>Study</th>
<th>Folic Acid Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTD</td>
<td></td>
<td></td>
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<td>(mainly conotruncal)</td>
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Fig. 1. Summary of “occurrence” studies concerning the candidates of folic acid (FA)—multivitamin (MV) preventable CAs in one randomized trial (24,72) (■), in one prospective case-control study (28) (◆) and observational studies (●). The relative risk estimate (supplemented vs nonsupplemented or users vs nonusers) is shown for each study together with its 95% confidence interval. The dose of folic acid is also shown if data are available.
isolated (0.50; CI 95%: 0.36–0.68) and for nonisolated (syndromic) (0.61; CI 95%: 0.35–1.1) cleft lip ± cleft palate, and for isolated (0.73; CI 95%: 0.46–1.2) and nonisolated (0.64; CI 95%: 0.35–1.2) cleft palate was significant in the group of isolated cleft lip ± cleft palate. In conclusion, women who used multivitamins containing folic acid periconceptionally had a 25–50% reduction in risk for offspring with orofacial clefts compared to women who did not use such vitamins. The database of the Hungarian Case-Control Surveillance of Congenital Anomalies also showed a significant reduction in the rate of both isolated cleft lip ± cleft palate and cleft palate after the first and second postconceptional month supplementation by folic acid (28,29). The explanation for discrepancies in different studies may be the dose. Tolarova (82) used a multivitamin that included an extremely high dose of folic acid (10 mg/d). The Hungarian Case-Control Surveillance evaluated the daily use of 3–6 mg of folic acid. In contrast, the Hungarian trial (24) and prospective case-control study (28) used a multivitamin including 0.8 mg of folic acid. Thus, it is possible that only pharmacological dose of folic acid has protective effect on orofacial clefts.

In the final database of the Hungarian intervention trial there was one case with limb deficiency (terminal transverse) in the multivitamin group and five cases (2 terminal transverse, 2 femur-fibula-ulna complex, 1 split hand and foot) in the trace-element group (86,72). The difference was not significant ($\chi^2_1 = 2.8; p = 0.09$ or Fisher $p = 0.12$). However, the teratogenic effect of folic acid deficiency because of folic acid antagonists, such as aminopterin and methotrexate caused, among others, limb deficiencies in human embryos (8–10,87). In addition, Shaw et al. (80) examined whether a woman’s reported use of a folic acid-containing multivitamins was associated with a reduction in the rate of limb deficiencies. Cases were ascertained from the California Birth Defects Monitoring Program, 1987–1988. Telephone interviews were conducted with mothers of 179 cases with limb deficiency and 481 nonmalformed controls. The unadjusted odds ratio for any folic acid-containing multivitamin use from one mo before conception through two mo postconception were 0.65 (CI 95%: 0.43, 0.99) for limb deficiencies. Finally, a significant reduction in the birth prevalence of limb deficiencies was also observed in a recent United States study (88).

In the final database of the Hungarian trial (72,73), the number of clinical diagnoses of congenital hypertrophic pyloric stenosis (which is not a typical CA) indicated also some difference (2 vs 8) between the study groups ($\chi^2_1 = 3.81; p = 0.05$). However, one case had no surgery in the trace-element group, thus it was necessary to exclude this case from the analysis according to our criteria of diagnosis ($\chi^2_1 = 2.95; p = 0.09$).

Obviously, these findings could have major implications in the use of periconceptional folic acid-containing multivitamin supplementation to prevent CAs in general. It is strange, that, although the data concerning the prevention of NTD were accepted with enthusiasm in the international scientific literature in the early 1990s and prompted fast new recommendations for their practical application, the new data concerning the prevention of other CAs have been received with some reservation and without any further recommendations. It may be well to remember that it has taken a long time for the association of folic acid and NTD to be accepted. However, it is worth reminding that in the first phase of folic acid history, deficiency resulted in a *general* teratogenic effect in both animal (2–7,11,12,75,76,78,79) and human studies (8–10,13,14,87). In 1964, Hibbard (13) reported a higher rate of CAs (3%) in the infants of folate-deficient mothers than in controls (1.6%). It was confirmed by Hibbard and Smithells (14) in 1965,
who showed a relationship between human embryopathies and a deficiency of folic acid metabolism. Thus, the first human publications also indicated a general teratogenic effect of folate - folic acid deficiency.

On the other hand, we know that different CAs have different etiologies; thus, it is difficult to understand that so many CAs can be prevented by such a simple primary preventive method as periconceptional folic acid-containing multivitamin supplementation. The aforementioned different CAs have different critical periods (see Fig. 2) and the latter may explain that postconceptional multivitamin supplementation also seems to be protective for some CAs. In addition, the urinary tract and cardiovascular CAs have a heterogeneous origin; thus, the different subtypes may have different etiological background. However, this is the case in NTDs as well. Finally, the preventive effect of this method is less efficient in these CAs than in NTDs. Thus, it is an important task to understand the different biological mechanisms of the preventive effect for CAs achieved by periconceptional multivitamin supplementation.

Vitamin B₁₁ (i.e., folic acid-folate) may be a key factor in the prevention of some other major CAs as well because THFs act as a cofactor for enzymes involved in DNA and RNA biosynthesis. Some metabolites of THF provide one-carbon units for the de novo synthesis of DNA bases (guanine, adenine, and thymine) because THFs accept single carbons from a variety of donors (mainly serine). On the one hand, there is an increased requirement for vitamin B₁₁ during pregnancy because of decreased absorption, accelerated breakdown of folate to p-aminobenzoylglutamate and its acetylated derivative p-acetamidobenzoylglutamate, increased urinary loss, and fetal transfer (50). The calculated total fetal and placental THF content is 0.8 mg/100 g at term (89), thus, fetal blood has a higher THF level than maternal blood, indicating active placental transfer (90). On the other hand, the intensity (i.e., “speed”) of development including the activity of mitoses is extremely high in the preconceptional life particularly in the first 8 wk. The weight of fertilized egg, an embryo at the 8th wk, a liveborn infant, and a 20-yr-old person is 0.001, 4, 3200, and 65 000 gr, respectively. The postnatal increase is only 20 times, whereas it is 800 (26.7/wk) times between the 8th and 38th wk of fetal development. However, weight increases 4000 (500/wk) times during organogenesis when the different organs develop. Thus, the requirement of bricks for body building and the mode of genetic regulations (e.g., growth factors) are significantly different between pre- and postnatal life. In addition, cell division is exceptionally rapid at critical stages of specific developmental fields in the embryo (see Fig. 2). The cell’s ability to increase the synthesis of nucleic acids and to methylate important compounds (e.g., a need for L-methionine) would be compromised by deficiency of folate and other vitamins, resulting in impaired cell function, mitosis and subsequently CAs.

7. BIRTH DEFECTS ARE PREVENTABLE

At present, 20–30% of infant mortality is caused by CAs in industrialized countries. However, CAs are also among the leading causes of death, with a high number of life years lost and impaired life (91). Another important feature of CAs is that they represent a defect condition, because it is difficult to achieve a complete recovery. Thus, prevention is considered the only optimal solution.

In the late 1980s, we attempted to estimate the preventable proportion of CAs (92) and we were able to prove that the widespread pessimistic view concerning the low chance of CA prevention was incorrect. A considerable part (about 60%) of CAs were
preventable (Table 4). However, our analysis showed that primary preventive methods played only a small part in the prevention of CAs, whereas the efficacy of secondary prevention is growing and considerable. This is explained by the increasing effectiveness of prenatal examinations as a result of the improvement of diagnostic methods (e.g., ultrasonography) and new approaches (e.g., DNA probes). Thus, we have to do our best to develop and to introduce primary preventive methods instead of the so-called secondary one, i.e., selective abortions. The history of NTD offers a good example to demonstrate the feasibility of this concept.

At present, both primary and secondary preventive methods are available for NTD. The prenatal screening for the detection of NTD fetuses is based on the measurement of the maternal serum α-fetoprotein (MS-AFP) and/or ultrasonography. The MS-AFP is measured between 16 and 18 wk of gestation after the determination of gestational age by ultrasound examination. The latter is appropriate to immediately diagnose anencephalic fetuses. Values of MS-AFP greater than 2.0–2.5 MoM (multiples of median) for the gestational age are considered suspect. The proportion of abnormal high MS-AFP, which can be confirmed by ultrasonographic examinations, varied between 1.2 and 3.9% of pregnant women (93). If it is negative, generally it is recommended to repeat MS-AFP. If the second result is also abnormal and a high-resolution ultrasonographic examination cannot detect NTD or other causes of a high MS-AFP (multiple fetuses, fetal death, other CAs, maternal diabetes mellitus), amniocentesis is offered to women because amniotic-AFP and amniotic fluid acetylcholinesterase are confirmatory for the diagnosis of NTD. Fetal death as a result of amniocentesis is about 0.5–1.0%. In general (i.e., low-risk) populations the detection rate (i.e., sensitivity) of MS-AFP testing for open NTD varies from 72%–91% and the specificity from 96.2%–98.7% (93).

Periconceptional folic acid-containing multivitamin supplementation as a primary preventive method offers an appropriate alternative with the same or better efficacy. The evaluation of adverse effects shows obviously a better picture for multivitamin supplementation. Very rare adverse effects may occur only after the use of pharmacological dose of folic acid; such dose is not recommended. The cost is much lower in the pri-
primary prevention compared to secondary one. However, the main advantage is the lack of pregnancy termination in primary prevention. Thus, obviously primary prevention is better than secondary prevention.

If we consider the results of the Hungarian trial, periconceptional multivitamin supplementation resulted in 30.8% reduction in the rate of CAs. The candidates of preventable CAs by this primary preventive method and periconceptional care therefore can be redistributed in Table 4. In this case, 68.8% of CAs are preventable and there is a significant increase in the proportion of primary prevention (1.2% → 12.9%) because of periconceptional folic acid-containing multivitamin supplementation.

8. SUGGESTIONS FOR CHANGE OF RECOMMENDED FOLIC ACID USE TO REDUCE NTD AND OTHER CA GROUPS

Periconceptional supplementation of multivitamin capsules including folic acid and other vitamins seems to be an optimal preventive method because the absorption of folic acid in the gastrointestinal tract is quick and easy. However, there are some theoretical and practical problems with the use of periconceptional vitamin supplementation.

The aforementioned recommendation for reduced NTD risk included only the supple-

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### Table 4

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<th>Type of prevention method</th>
<th>Preventable CAs, %</th>
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<tr>
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<td>Before introduction of periconception care</td>
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<td>Primary prevention</td>
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<tr>
<td>Genetic counseling (and reduction of conception)</td>
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<tr>
<td>Care of affected mothers</td>
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<tr>
<td>Avoidance of teratogens</td>
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<tr>
<td>Multivitamin supplementation</td>
<td>—</td>
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<tr>
<td>Subtotal</td>
<td>1.2</td>
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<tr>
<td>Secondary</td>
<td></td>
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<tr>
<td>Prenatal screening (chromosomal, maternal AFP and triple test, ultrasonography)</td>
<td>8.3</td>
</tr>
<tr>
<td>Neonatal blood screening (PKU, galactosemia, hypothyroidism)</td>
<td>0.6</td>
</tr>
<tr>
<td>Neonatal orthopedic screening</td>
<td>23.6</td>
</tr>
<tr>
<td>Specific postnatal treatment (in undescended testis and patent ductus arteriosus)</td>
<td>2.2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>34.7</td>
</tr>
<tr>
<td>Tertiary: early pediatric surgery</td>
<td>24.0</td>
</tr>
<tr>
<td>Total</td>
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mentation of 0.4 mg folic acid. This recommendation was difficult to accept in 1992 because there was no direct evidence from a randomized controlled study that 0.4 mg of folic acid alone can reduce the first occurrence of NTDs.* The Hungarian trial used 0.8 mg of folic acid in a multivitamin (24). Recently, the US National Academy of Sciences (95) recommended that all adult men and women need 0.4 mg of folate daily, but it is crucial that women aged 19–50 yr ingest a further 0.4 mg of folic acid to reduce the risk of having a baby with an NTD. However, it is difficult to achieve the consumption of 0.4 mg of folate by diet, thus, it is worth increasing the dose of folic acid to 0.6 mg via supplement.

On the other hand, it is well known that both vitamin B₁₁ and B₁₂ are involved in DNA synthesis and reduction of hyperhomocysteinemia related to methionine synthase deficiency. As it was mentioned previously, vitamin B₁₂ is an independent risk factor in the origin of NTD (48) and it was recently confirmed (96). Vitamin B₁₂ completely abolished the embryotoxicity of L-homocysteine in rat embryos, which was shown to be mediated by catalysis of the spontaneous oxidation of L-homocysteine to the less-toxic L-homocystine (31). Thus, a hypothesis was developed that L-homocysteine embryotoxicity is explained by the inhibition of transmethylation reactions by increased embryonic 5-adenosylhomocysteine level.

Three intervention studies (19,20,24,28) indicated a high efficacy (about 90%) of a multivitamin including a physiological dose (0.4–0.8 mg) of folic acid for the prevention of NTD. The preventive effect of pharmacological dose (4–6 mg) folic acid alone was lower (about 70%) (22,29), thus, a higher efficacy cannot be expected after the use of 0.4 mg of folic acid alone. Finally, a multivitamin contains folic acid and at least vitamin B₁₂ seems to be the optimal for the prevention not only of NTD but other CAs as well. In addition, vitamin B₆ and C would be also useful. Vitamin C may have a role to prevent the oxidation of THF, thus, helping to keep vitamin B₁₁ metabolic pool complete (97). Vitamin B₆ is important to reduce the other pathway of hyperhomocysteinemia. In conclusion, a multivitamin including a physiological dose (0.6–0.8 mg) of folic acid and vitamin B₁₂ seems to be more effective for the reduction of first occurrence NTD and some other major CAs than folic acid (0.4–4 mg) alone.

Another recommendation concerning the reduction of NTD recurrence is based on the use of the pharmacological dose (4 mg) of folic acid. This recommendation is connected with the results of MRC Vitamin Study (22). However, there is a strong evidence from Smithells et al. (18–20) study that a multivitamin containing 0.36 mg of folic acid can significantly reduce NTD recurrences (about 90%). In addition, two Hungarian datasets included 51 and 67 women with previous NTD, respectively, who were supplemented with a multivitamin containing 0.8 mg folic acid during the periconceptional period and the number of observed recurrence was 0 instead of the expected 3.5 (OR: 0.12 with 95% CI: 0.06–0.23). It is not possible to compare directly the data of non-randomized and randomized studies (Table 1), nevertheless, the efficacy of recurrence risk reduction was more impressive after multivitamin use. Most importantly, physicians should be aware that daily 4 mg of folic acid may mask the hematologic manifestations of vitamin B₁₂ deficiency without preventing its neurologic consequences (98). Thus, the US National Academy of Sciences (95) set a tolerable upper intake level

*However, a recent Chinese study as part of a public health campaign conducted from 1993 to 1995 indicated that periconceptual intake of 0.4 mg folic acid daily can reduce the risk of NTDs in areas with high rates of NTD (about 79%) and in areas with low rates (about 41%) (94).
of 1 mg for folic acid in healthy people for preventive purposes. Finally, after a high
dose of supplemental folic acid, more of the unmetabolised vitamin (it may amount to
25% of the dose administrated) appears in the urine (50). The embryo could be exposed
to the excess unmetabolised folic acid, a form of the vitamin that an embryo would not
encounter normally. In conclusion, it would seem to be better to use a multivitamin sup-
plement including a physiologic dose (> 1.0 mg) of folic acid for the reduction of re-
currence of NTDs.

The Hungarian trial and other recent studies indicated a preventive effect of pericon-
ceptional folic acid-containing multivitamin supplementation not only for NTD, but for
some other CA-groups, and it seems to be a great breakthrough in the prevention of major
CAs both quantitatively (an extra 30%) and qualitatively (primary prevention instead of
secondary prevention). From a practical aspect, an important public health task would be
to give a reimbursement for multivitamins for women planning their pregnancies. The
cost of treatment in cases with NTD, cardiovascular, and other CAs is extremely high,
thus their prevention would result in a significant reduction in the budget of health care.

9. FOOD FORTIFICATION

Food fortification seems to be the most practical means of supplementation with folic
acid and other vitamins for women with unplanned pregnancies. This public health ini-
tiative is comparable to the prevention of goiter by the addition of iodine to salt.

In Ireland, vitamin B12 (in 1981) and folic acid (in 1987) were added to breakfast ce-
reals, which constitute a significant part of the diet. This fortification has probably con-
tributed to the significant fall in the birth prevalence of NTDs in the 1980s (from 4.7 to
1.3/1000), because only a small part of this decline could be attributed to termination
of affected pregnancies (48).

In February 1996, the US Department of Health and Human Services (99) ordered
food fortification with folic acid of all cereal grain products at a level of 0.14 mg/100 g
from January 1998. This adds only about 0.1 mg of folic acid to the average daily diet
of women of reproductive age, nevertheless, the mean plasma folate concentrations in-
creased from 4.6 to 10.0 μg/mL and the prevalence of low folate level (<3 μg/mL) de-
creased from 20.0 to 1.7%. In addition, the mean total homocysteine concentration
decreased from 10.1 to 9.4 μmol/L and the prevalence of high homocysteine concen-
trations (>13 μmol/L) decreased from 18.7 to 9.8% (100).

In Hungary three vitamins: folic acid (160 mcg), vitamin B12 (0.8 mcg) and B6 (864
mcg) are added to 100 g flour from the third quarter of 1998 (101). The daily intake of
folic acid, vitamin B12, and B6 is about 200, 1, and 1080 mcg, respectively, by 200 g of
bread. Daily intake of 200 mcg folic acid resulted in a 41% estimated reduction in risk
of NTD (102). The use of vitamin B12 has two goals. First, it is an independent factor in
the origin of NTD. Second, about 1% of oral dose of vitamin B12 can protect the mask-
ing effect of folic acid in patient with pernicious anemia. Another goal of this food for-
tification is the prevention of coronary artery, cerebrovascular, and peripheral vascular
diseases as a result of hyperhomocysteinemia. The consumption of fortified bread with
folic acid, vitamin B12, and B6 is especially important for a large proportion of women
with low education and income who, in general, have difficulties buying food rich in fo-
late and other vitamins and who have unplanned pregnancies. However, it would be nec-
essary to change the too-conservative regulations concerning food fortification (e.g.,
0.35 mg/100 g level of flour fortification would result in a much better benefit-cost ratio).
without any possible hazard if higher dose of vitamin B<sub>12</sub> (e.g., 10 or 25 mg) is used.

As in the part of the third phase of folate-folic acid story, high- and low-risk population for NTD (and other CAs) will be differentiated in the near future because obviously different preventive strategies can be used in these two groups of women. A small segment of high-risk population can be identified on the basis of previous NTD and CAs, in addition, the low red-cell folate level (103) and MTHFR gene polymorphism (37–40) seems to be an appropriate marker of NTD risk and for the identification of high-risk women. Periconceptional care provides a unique occasion for differentiation of high- and low-risk couples as a result of preconceptional screening.

10. RECOMMENDATIONS FOR NUTRITIONAL INTERVENTIONS TO REDUCE BIRTH DEFECT RISKS

The history of NTD shows that we can modify our destiny by the help of science. Before the 1960s, nearly all NTD births were fatal. In the 1960s, physicians introduced very early complex medical intervention and the life of victims was saved in the majority of spina bifida cases. In the 1970s, the selective criteria of surgical intervention was accepted to reduce the production of multiple handicapped children. In the 1980s, prenatal screening was introduced, resulting in a significant drop in the birth of NTD fetuses, however, it increased the number of pregnancy terminations. Finally, in the 1990s, we have a chance to reduce the maldevelopment of neural-tube (and other organs) because of the intentional modification (supplementation) of the diet at least in the certain (periconceptional) period of the life. Our hope is that the extension of healthy diet for the fetal life will help us to prevent adult diseases and to promote our health significantly (104).

Recommendations defined here are based on findings and hypotheses presented in this chapter.

1. Proper preparation for conception is the earliest and probably the most important method of health promotion in general, and particularly for the prevention of birth defects. The establishment of periconception care is feasible and, on the basis of available experiences, is reasonable. This network provides an appropriate forum for nutritional interventions as well.

2. The preconceptional period is an appropriate time to change the dietary habit and to improve the lifestyle of prospective parents because their desire to have a healthy baby can ensure good compliance. It is an important task to advise all women to have a folate and other vitamin-rich diet from the preconceptional time, however, it is not enough in the exceptional case of NTD prevention.

3. Good evidence is available to advise all women capable of becoming pregnant to have a preconceptional (i.e., at least 1 mo before and until 3 mo after conception) multivitamin (including 0.4–0.8 mg of folic acid and vitamin B<sub>12</sub>) supplementation to reduce the occurrence of major CAs particularly NTDs. Multivitamins including folic acid and vitamins B<sub>12</sub>, in addition vitamin B<sub>6</sub> and C, appear to be the most effective practical way to reduce the occurrence and recurrence of NTD. Thus, we have to do our best to increase the users of appropriate multivitamin on the appropriate time period. In Hungary, a new facultative subject was introduced in the school curriculum of children aged about 15 yr entitled, “Preparation for Family Life,” including information on periconceptional multivitamin supplementation. Furthermore, a booklet was published for couples at weddings which are supplied for them when they declare their marriage (3 mo before the wedding). Finally, it is a good idea to publish a leaflet for oral-contraceptive users advising them to take a folic acid-containing multivitamin as soon as they stop using contraception. Of course, the presently
recommended 0.4 mg folic acid supplementation is an acceptable and cheaper option.

4. Fortification of food (flour, special products of bakery, juice) with folic acid, vitamin B₁₂, and possibly vitamin B₆ would be appropriate to reach the large proportion of women with unplanned pregnancies.

5. There is evidence that appropriate nutritional status of pregnant women can promote the postnatal, including adult, health of offspring. The origin of many common complex diseases (hypertension, coronary artery disease, diabetes mellitus, obesity) may be related to the quality of fetal and infant life (104).

6. Appropriately prepared parents for pregnancy can educate their children from the earliest time (after their birth) of their life for a healthy diet and lifestyle. There is a good chance that these habits will be fixed for their later life.

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1. INTRODUCTION

Deficiency of vitamins B₁₂, folic acid, B₆, niacin, C, or E, or iron, or zinc, appears to mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both (1). Oxidation is a major contributor to both cancer and aging (1–11). The percentage of the United States population that has a low intake (<50% of the RDA) for each of these eight micronutrients ranges from 2–20%; half of the population may be deficient in at least one (1). We have shown that folate deficiency breaks chromosomes due to massive incorporation of uracil in human DNA (4 million/cell) with subsequent single-strand breaks in DNA formed during base excision repair: two nearby single-strand breaks on opposite strands cause the chromosome to fall apart. Folate (10% of United States), vitamin B₁₂ (14% elderly), and B₆ (10% of United States) deficiencies all cause high uracil in human DNA. Micronutrient deficiency may explain, in good part, why the quarter of the population that eats the fewest fruits and vegetables (5 portions a day is advised) has about double the cancer rate for most types of cancer when compared to the quarter with the highest intake.

Although the public is beginning to realize that prevention of degenerative damage to somatic tissues may depend on dietary and lifestyle factors, less attention has been given to the effects that these factors may have on gametes and the possible consequences for offspring. It is generally recognized that a balanced diet that contains adequate levels of vitamins and micro- and macronutrients is desirable during gestation, and that maternal smoking and ethanol consumption should be avoided during pregnancy to reduce the risk of fetal injury. However, little attention has been given to the role that paternal lifestyle plays in contributing to the risk of damage to the infant. Evidence from biochemical and epidemiological sources suggests that the prospective male parent also requires both an adequate intake of certain nutrients and avoidance of deleterious habits, such as smoking, to limit genetic damage to sperm and possibly to reduce the risk of birth defects and childhood cancers (12).
2. OXIDANT DAMAGE TO GAMETES: SPERMATOZOA TRANSMIT HERITABLE MUTATIONS TO OFFSPRING MORE FREQUENTLY THAN OOCYTES

Endogenous oxidative damage to germline DNA may lead to heritable mutations and an increased incidence of birth defects, genetic diseases, and cancer in offspring. Ionizing radiation, an oxidative mutagen, damages gamete DNA, which results in mutations that are transmitted to the progeny in experimental animals (13). Most DNA damage is expected to be repaired. However, remaining damaged residues may be converted to mutations during the DNA replication accompanying spermatogenesis, oogenesis, or embryogenesis. Studies of genetic abnormalities and cancers that are believed to arise from germline mutations show these to have a higher frequency of paternal origin than maternal origin. One example is the increased frequency of paternally inherited mutations in the retinoblastoma gene (14). This is not surprising considering the contrasting physiology of gametogenesis between the sexes. Production of female gametes (oogenesis) occurs when the mother herself is _in utero_. The preoocytes are stored in a mitotically inactive state throughout life and only undergo further (meiotic) cell division prior to ovulation. This is in marked contrast to the production of male gametes (spermatogenesis), which are produced continuously after puberty. Mature spermatozoa are thus formed from stem cells after a much greater number of mitotic cell divisions than are oocytes, which results in an increase in the risk for mutation in sperm. Crow (15) has reviewed this area and quotes J. B. S. Haldane from 1947:

_The much greater number of cell divisions between zygote and sperm than between zygote and egg, the increased age of fathers of children with new dominant mutations, and the greater evolution rate of pseudogenes on the Y chromosome than on autosomes all point to a much higher [germline] mutation rate in males than in females._

It is estimated that the number of cell divisions between ovum and zygote is 24, whereas for sperm, this number is highly dependent on age. Therefore, for a 13-yr-old male, an estimated 36 cell divisions are completed between stem cell and spermatozoon. At age 20, 197 cell divisions; at age 30, 427 cell divisions; and at age 45, 772 cell divisions have occurred. Spermatogenesis may occur while the male is exposed to environmental fluxes in oxidant production (e.g., by smoking) and while ingesting suboptimal intakes of those nutrients (e.g., ascorbate and tocopherol) that protect the tissue against oxidative stress or are required for DNA integrity (e.g., folate). In addition, once spermatogenic cell division is completed, maturation of the spermatid into the spermatozoon involves condensation of the DNA accompanied by the apparent loss of DNA repair activity (16). Thus, any damaged residues remaining in the sperm DNA will be delivered to the oocyte upon fertilization, which may increase the possibility of mutations when the zygote divides. Based on these facts, we argue that germline damage-associated birth defects or childhood cancers are more likely due to genetic damage in the spermatozoon than in the oocyte.

3. PATERNAL SMOKING AND RISK OF MUTATION TO THE GERMLINE

Maternal smoking during pregnancy can cause many problems, such as intrauterine growth retardation leading to increased incidence of spontaneous abortion, premature birth, and low infant birthweight. However, it is pertinent to note that numerous epi-
demiologica studies over several decades and in various countries have consistently failed to find a link between maternal cigarette smoking and genetic damage to the fetus. In contrast, the few epidemiological studies on the effects of paternal smoking and risk of birth defects and childhood cancer suggest, but do not prove, an increased risk of both in the offspring of smokers as compared to nonsmokers. Schmidt (17) has made an argument comparable to ours that concerns smoking and genetic damage:

_Tobacco smoke contains numerous mutagenic substances. They reach the male gonads via the blood. They show their mutagenic action here openly much more strongly than on egg-cells because the spermatogenesis continues over the whole male reproductive period, whereas the formation of eggs is already completed in the fetal phase._

Biochemical and genetic evidence indicates that smoking indeed damages the DNA of human sperm. Smoking is a severe oxidative stress and, because of the high level of nitrogen oxides in cigarette smoke, depletes bodily antioxidant levels (18,19). Following up on previous work that showed low intake of ascorbate being associated with oxidative damage to sperm (20), Fraga et al. (18) found increased 8-oxodeoxyguanosine (oxo8dG) levels in sperm DNA of smokers vs nonsmokers. Similarly, Shen et al. (21) found that the sperm DNA of smoking men contains a significantly higher amount of oxo8dG than that of nonsmokers. The level of oxo8dG was closely correlated to seminal cotinine concentrations. Among these healthy men, other indices of semen quality were unaffected by smoking, indicating that smoking may not affect fertility in these men. Potts et al. (22) reported that sperm from smokers were significantly more sensitive to acid-induced denaturation than nonsmokers and possessed higher levels of DNA strand breaks. Smokers also have a higher percentage of sperm with fragmented DNA (4.7 ± 1.2%) as compared to nonsmokers (1.1 ± 0.2%) (23). Rubes et al. (24) found that 18 yr-old smokers had elevated frequencies of sperm aneuploidy vs 18-yr-old nonsmokers. At higher frequency in smokers were chromosome Y disomies and aggregates of chromosomes X, Y, and 8 disomies. The semen quality indices of smokers were not as high as the nonsmokers, but this may have been a result of the shorter abstinence interval among smokers. Robbins et al. (25) showed that both alcohol use and smoking were associated with sperm aneuploidy. Smoking was weakly associated with X chromosome sperm aneuploidy (p = 0.07). Alcohol use (defined as greater than six drinks/wk) was significantly associated with increased frequencies of sperm aneuploidy of the X chromosome, diploidy of chromosome 18, and the duplication phenotype XX18–18. Such defects in sperm DNA (damage and aneuploidy) may affect male fertility and may increase future chances of fathering offspring with genetic defects, such as aneuploidy syndromes, birth defects, or cancer.

3.1. Paternal Smoking and the Risk of Fetal Mortality or Defect

Comstock and Lundin (26) found that the neonatal death rate (adjusted for sex of child and education of father) among infants of smoking fathers was 17.2/1000 live births compared to 11.9 among infants of nonsmoking fathers and 26.5 among infants born to parents who both smoked. Koo et al. (27) found that wives of smoking spouses have more miscarriages and spontaneous abortions than wives of nonsmoking spouses (p = 0.06). Heary et al. (28) observed a significant association (p < 0.05) between paternal smoking and increased neural-tube defects (NTDs) in offspring (4/8 cases vs 1/17 controls).
Mau and Netter (29) studied the smoking habits of fathers of 5200 newborns. The rate of major malformations among nonsmoking fathers was 0.8%, whereas among smoking fathers it was 2.1%. Maternal smoking had no effect on the rate of birth defects. The most striking increase in birth defects concerned major facial clefts. Facial clefts occurred in 0.1% of infants from nonsmoking fathers compared to 0.5% from fathers who smoked 1–10 cigarettes/d and 0.7% from fathers who smoked more than 10 cigarettes/d. There was also an increase in perinatal mortality (unrelated to major birth defects) if the father smoked more than 10 cigarettes/d, even if the mother did not smoke (4.3% compared to 2.8%).

Savitz et al. (30) analyzed single live births among 14,685 volunteers who participated in child health studies. Paternal cigarette smoking was more common among children with cleft lip (with or without cleft palate) (odds ratio [OR] = 1.7, 95% confidence interval [CI 95%]: 0.5–6.0), hydrocephalus (OR = 2.4, CI 95%: 0.6–6.4), ventricular septal defect (OR = 2.0, CI 95%: 0.9–4.3), and urethral stenosis (OR = 2.0, CI 95%: 0.6–6.4). The authors specifically mention genetically altered sperm as a possible cause of birth defects in infants of smoking fathers. They note that several of the anomalies associated with the infants of older fathers were also increased among fathers who smoked. The concordance in defects with both advanced paternal age and paternal smoking was notable especially for ventricular septal defects and hydrocephalus.

Schmid (31), in discussing the Mau and Netter study, attributed the effect of paternal smoking on birth defects to passive smoke. However, this is not a probable explanation because the amount of smoke contents that reaches the embryo is insignificant compared with the amount that reaches the embryo when the mother herself smokes, and maternal smoking does not appear to significantly increase the risk of genetic birth defects.

Seidman (32) provides a table on the incidence of major and minor congenital malformations by paternal and maternal smoking levels. A nonsignificant increase in the incidence of major and minor malformations was seen in the offspring of smoking fathers who mate with nonsmoking mothers. Seidman does not break down the malformations by specific defect.

### 3.2. Paternal Smoking and Childhood Tumors

Gold et al. (33) examined maternal smoking, but not paternal smoking. Maternal smoking did not seem to influence the risk of brain tumors in children. A similar study by Neutel and Buck (34) took into consideration only the effects of maternal smoking on childhood cancer. For cancers of all sites, the children of mothers who smoked during pregnancy had a relative risk of 1.3 (CI 95%: 0.8–2.2), which was not significant.

Grufferman et al. (35) found that paternal smoking, but not maternal smoking, increased the risk of childhood rhabdomyosarcoma (RMS) (relative risk [RR] of 3.9, \( p = 0.003 \)). Rhabdomyosarcoma is the sixth most-common childhood cancer, with an annual incidence of about 4 cases/million children and a peak incidence at ages 3–4. The authors suggest that a direct carcinogenic effect of paternal cigarette smoke may be introduced in a prezygotic manner.

Grufferman et al. (36) interpreted their findings as follows:

*In our recent study of childhood (RMS), we found an increase in the risk of RMS among children whose fathers smoked cigarettes. However, there was no association between RMS and mothers' smoking. We hypothesize that differential germ cell dam-
age from cigarette smoking underlies our observations and that this risk of germ cell damage from cigarette smoking and from other environmental exposures is greater for men than for women. The increased susceptibility for male germ cells may be due to the number and timing of meiotic and mitotic cell divisions. In males, germ cells undergo large numbers of meiotic and mitotic cell divisions throughout the reproductive years. In contrast, in females, generally only one oocyte matures and completes meiosis each month of the reproductive years. Thus, there are very large male-female differences in the number of rapidly dividing germ cells during the reproductive years, and it is rapidly proliferating cells which are most susceptible to genetic damage.

John et al. (37) found associations between paternal smoking during the 12 mo prior to conception (in the absence of maternal smoking during the first trimester) with all childhood cancers (OR = 1.2, CI 95%: 0.8–2.1), acute lymphocytic leukemia (OR = 1.4, CI 95%: 0.6–3.1), lymphomas (OR = 1.6, CI 95%: 0.5–5.4), and brain cancer (OR = 1.6, CI 95%: 0.7–3.5). However, these correlations appear weak and the data are similar to those for maternal smoking alone. After adjustment for paternal education, and in the absence of paternal smoking, maternal smoking during the first trimester of pregnancy was associated with an increased risk for all cancers combined (OR = 1.3), acute lymphocytic leukemia (OR = 1.9), and lymphomas (OR = 2.3).

Johnston et al. (38), in their study of children with germ cell tumors, found that the smoking pattern of fathers was similar for both cases and controls. Magnani et al. (39) found that both maternal and paternal smoking up to the child’s birth were associated with non-Hodgkin’s lymphoma in childhood. After adjusting for socioeconomic status, the odds ratio for paternal smoking was 6.7 and for maternal smoking was 1.7. The author states that the odds ratio for paternal smoking was not correlated with number of cigarettes consumed. The study showed no correlation between acute lymphocytic leukemia and paternal smoking.

Preston-Martin et al. (40) found that there was a significant increase in risk of childhood brain tumors (OR = 1.5, p = 0.03) if during pregnancy the mother lived with a smoker; they state that their “finding that maternal smoking itself was not related to disease but that living with a smoker (usually the child’s father) may indicate that paternal exposures are important.”

Sandler et al. (41) analyzed cancers of all sites, except basal cell carcinoma, among people 15–59 yr old. Cancer risk was increased 50% among people whose fathers had smoked. In this study, paternal smoking was defined as the father having smoked before the child reached 10 yr of age. Increased risk associated with paternal smoking was not explained by demographic factors, social class, or individual smoking habits. There was only a slight increase in overall cancer risk associated with maternal smoking (RR = 1.2, CI 95%: 0.7–1.6). However, both maternal and paternal smoking were associated with increased risk for hematopoietic cancers, and a dose–response relationship was seen. The relative risk for hematopoietic cancers increased from 1.7 when one parent smoked to 4.6 when both parents smoked (p < 0.001). Obviously, the study included a wide range of ages and, furthermore, did not give isolated information on the teenage cases. Therefore, the study does not specifically assess the effect of paternal smoking on childhood cancer risk.

Sorahan et al. (42) examined parental use of tobacco and alcohol and the associated cancer risk among offspring. The authors found that for daily paternal consumption of cigarettes, but not pipes or cigars, there was a significant monotonic positive trend (p <
0.001) in the incidence of childhood cancer among their offspring. There was a dose–response relationship between the amount of cigarettes smoked by the father per day and the relative risk of childhood cancer. The three highest categories of paternal smoking analyzed were 20–29 cigarettes/d, with the relative risk of childhood cancer among offspring at 1.26 (95% CI: 1.05–1.5), 30–39 cigarettes/d, RR = 1.35 (95% CI: 1.03–1.78), and more than 40 cigarettes/d, RR = 1.47 (95% CI: 1.07–2.01). In this study, maternal consumption of cigarettes was not associated with an increased risk of cancer in children.

Sorahan et al. (43,44) authored two additional reports describing studies where parental smoking and childhood cancer deaths were examined for the time periods of 1953–1955 and 1971–1976. Both studies found a statistically significant positive trend between paternal daily consumption of cigarettes and the risk of childhood cancer. Again, there was no association between maternal cigarette use and cancer among offspring. For the time period of 1953–1955, about 15% of all childhood cancers in the study group were attributable to paternal smoking, as were about 14% for the 1971–1976 time period. Additional confounding factors eliminated as relative were social class, parental age at the birth of the survey child, sibship position of the child, and obstetric radiography. The authors of these reports combined the data of the two time periods to obtain further information on risks for different types of cancer (40). They found that paternal smoking was significantly associated with leukemia (p < 0.05), lymphoma (p < 0.001), CNS cancers (p < 0.05), neuroblastoma (p < 0.001), and all cancer diagnoses (p < 0.001). In the same type of analysis, maternal smoking was not positively associated with increased risk of any type of childhood cancer.

Ji et al. (45) examined paternal smoking during the preconception period and the risk of childhood cancer among offspring of nonsmoking mothers. These authors reported that paternal preconception smoking significantly elevated the risk of childhood cancers (when diagnosed in children under 5 yr of age), particularly acute leukemia and lymphoma. The risk rose with increasing pack–yr of paternal preconception smoking for acute lymphocytic leukemia (p = 0.01), lymphoma (p = 0.07), and total cancers (p = 0.006). Furthermore, children whose fathers smoked more than 5 pack–yr prior to their conception had adjusted odd ratios of 3.8 (95% CI: 1.3–12.3) for acute lymphocytic leukemia; 4.5 (95% CI: 1.2–16.8) for lymphoma; 2.7 (95% CI: 0.8–9.9) for brain tumors; and 1.7 (95% CI: 1.2–2.5) for all cancers combined.

In summary, the epidemiological evidence presented suggests that paternal smoking contributes to the risk of cancer in children. Maternal smoking, although deleterious to the growth of the fetus and associated with an increased risk of neonatal death, does not appear to contribute significantly to the risk of childhood cancer in the offspring. Interestingly, among these studies conducted in several different countries, there is consistency in the types of cancer that are associated with paternal cigarette use. These cancers include acute lymphocytic leukemia, lymphoma, and cancers of the CNS. Most pertinent to this discussion is the possible increased risk of cancer in the offspring of fathers who smoked at the time that the child was conceived in the absence of maternal smoking. No correlations exist between social class or other demographic factors and cancer risk in offspring that might explain such differences.

None of these studies on paternal smoking and effects on the offspring controlled for diet. If the damage to sperm DNA from smoking is mainly oxidative, then diets low in vitamin C would exacerbate the risk of damage to sperm DNA while those on diets rich in vitamin C would incur less risk. Cigarette smoke contains substantial quantities of
mutagens that may reach the testes via the blood stream to promote genetic lesions. It also contains high levels of nitrogen oxides that deplete serum antioxidants (46). The depletion of the blood plasma antioxidant pool may lead to a depletion of antioxidants in other tissues and body fluids with which it is in equilibrium, including the seminal plasma, testicular, and epididymal antioxidant pools (20), increasing the risk of oxidative damage during spermatogenesis and subsequent sperm storage. The evidence suggests that further epidemiological and intervention studies are needed and that education of prospective fathers as to their responsibility in the prevention of injury to their children, perhaps by cessation of smoking, appears warranted.

4. PATERNAL NUTRIENT REQUIREMENTS FOR THE REDUCTION OF GENETIC DAMAGE TO SPERM

Spermatogenesis in mammals requires a considerable devotion of resources that could otherwise be used to maintain somatic tissue viability. Indeed, one of the major arguments for aging is that the somatic tissues undergo degeneration because biological efforts are channeled into maintaining reproductive capacity at the expense of extended life-span (47). Some mammals have periods of seasonal reproductive activity when the female comes into estrus for a short period of time. Thus, the males of these species, such as the Silka deer, have evolved to channel their biological resources into reproductive activity, including spermatogenesis, to coincide with these periods to best conserve biological resources (48). Human females have evolved reproductive activity that is continuous throughout the year, yet fertility is limited to short periods within each month. Thus, human male spermatogenic capacity must be maintained continuously to optimize success in reproduction. This requires considerable nutrient resources to support the high rate of germline cell division with high replicative accuracy. Mutations to the male germline can occur by spontaneous error, but this is normally minimized. Alternatively, oxidative damage and/or limited resources of certain nutrients essential for accurate DNA replication may compromise the protection of sperm DNA.

4.1. Prevention of Germline Mutations Arising Via Oxidative Damage: Antioxidant Nutrients

Oxidant stress to tissues results in loss of tissue function and reduced accuracy in DNA replication, which can lead to mutations during cell division. The critical importance of preventing oxidative damage to tissues is reflected in the evolution of a multifaceted, small-molecule- and protein-based defense system to limit oxidative damage in cells (49). Furthermore, a considerable array of enzymes exists that has evolved to repair oxidative lesions to DNA, suggesting that oxidative damage to DNA has a deleterious effect on successful DNA replication and reproductive success. The evolution of specific antioxidant proteins allows organisms to limit oxidative damage in a number of ways:

1. Prevention of free-radical formation, e.g., chelation of transition metals in such proteins as ceruloplasmin and transferrin.
2. Destruction of oxidants catalytically, e.g., superoxide dismutases, catalase, and glutathione reductase.
3. Sacrificial scavenging of oxidants, e.g., albumin.
4. Enzymatic removal of oxidant-damaged biomolecules, e.g., DNA glycosylases and phospholipase A₂.
The antioxidant protein defense is reinforced by endogenous and exogenous small-molecule antioxidants that exist in the aqueous and lipid phases of cells and extracellular fluids. These small molecules include ascorbate, glutathione, urate, tocopherols, carotenoids, ubiquinols, and bilirubin (50–56). These antioxidants are a vital component of the overall antioxidant defense that acts by scavenging oxidants in a sacrificial manner or by chelating transition metal ions. Numerous studies have demonstrated that a low nutritional intake of ascorbate, tocopherols, and carotenoids is associated with increased risks for cancer, cardiovascular disease, cataract, macular degeneration, and arthritis. Therefore, there exists a nutritional requirement to maintain an adequate antioxidant defense against oxidant injury to tissues. Maintenance of adequate reproductive tissue antioxidants that are derived wholly from the diet appear to be involved in preventing oxidative damage to sperm cell DNA (18,20).

4.1.1. ASCORBIC ACID

The dietary antioxidant ascorbic acid (AA) may play a critical role in protecting spermatozoa against oxidative damage. A high concentration of AA is present in seminal fluid compared to blood plasma (400 vs 60 μM), presumably reflecting an important physiological role for this compound. Depletion of seminal plasma AA in male volunteers on controlled diets significantly increased sperm DNA oxo8dG levels, a marker of oxidative damage to DNA (20). The relationship between seminal plasma ascorbate and oxo8dG levels in sperm DNA in free-living individuals also suggests that low levels of seminal plasma AA are associated with an increased risk of oxidative damage to sperm DNA (20). Ascorbic acid is a powerful antioxidant in blood plasma and would act in the seminal plasma to limit oxidative damage in a similar manner (57). Circumstances that lower the level of seminal plasma AA, including low dietary intake, would, thus, most likely increase the risk of adduct formation in sperm DNA also. The current United States Recommended Daily Allowance (RDA) for AA is 60 mg/d. This value may be too low and is currently under evaluation to be increased. The suggested intake is meant as a prophylactic against the development of deficiency syndromes (e.g., scurvy) and may not reflect the intake required for maintenance of an effective antioxidant defense.

Smoking depletes serum antioxidants, including AA, tocopherols, and carotenoids (18,58). It is estimated that a smoker needs to consume two to three times as much AA as a nonsmoker to maintain a comparable blood plasma level (19). Smokers have lower seminal plasma AA titers and higher oxo8dG levels in sperm DNA than nonsmokers (18). Whether smoking depletes the seminal plasma AA pool directly, or it is lowered as a consequence of blood plasma AA depletion, is unknown. The latter case seems more likely, as there is some doubt that biologically significant levels of reactive oxygen species from cigarette smoke reach the testes. The total body AA pool is redistributed rapidly (59) and a depletion of blood plasma AA could account for the lowered seminal plasma AA observed in male smokers. The combined effects of low fruit and vegetable consumption and smoking may together suppress the reproductive tissue AA pool to such an extent that the level of oxidative damage in male germ cell DNA is increased. Low AA intake also reduces the fertility of the male. Males with low seminal plasma AA levels have decreased sperm viability, motility, and cell number, and increased percentage of sperm cell agglutination and abnormal morphology compared to volunteers maintained on high AA intakes (60–63). These data suggest that AA plays a vital role in the maintenance of spermatogenic activity, as well as in limiting oxidative lesion formation in sperm cell DNA and thus preventing mutations.
4.1.2. α-Tocopherol

α-Tocopherol is the major lipid-soluble antioxidant in human blood plasma that, together with AA, plays a vital role in limiting oxidative damage to membranes and serum lipoproteins (53). Unlike AA, α-tocopherol is found at much lower concentrations (0.38 μM) in the seminal plasma than in blood plasma (typically 15–40 μM) (18). Still, smokers have significantly lowered seminal plasma α-tocopherol levels compared with nonsmokers. Whether α-tocopherol has a definite role in preventing oxidative damage to sperm or is depressed in the reproductive tissues without deleterious effect on sperm genome integrity requires further study. The spermatozoon cell membrane contains an unusually high proportion of unsaturated fatty acids that are highly susceptible to peroxidation. Because membrane integrity is vital for sperm–egg fusion, and in maintaining sperm-cell viability and motility, it is possible that α-tocopherol is important for the protection of membrane lipid in the sperm cell. Limitation of spontaneous lipid peroxidation in sperm cells and seminal plasma is important in preventing the production of toxic aldehydes that may have mutagenic properties. Thus, α-tocopherol may complement AA in protecting spermatozoa from oxidative damage to the DNA. Further work to establish the role of α-tocopherol, if any, in the protection of sperm against oxidative damage is currently underway.

4.1.3. Other Antioxidants

Carotenoids, ubiquinol-10, uric acid, and bilirubin have all been proposed to be antioxidants in blood plasma. No significant levels of β-carotene or ubiquinol-10 have been reported in seminal plasma (<0.01 μM) (18,64). However, it is possible that these compounds are important components in the mechanism of protection against oxidative damage to sperm cells in male reproductive tissues.

4.2. Prevention of Mutations to Sperm DNA: Role of Nonantioxidant Nutrients

Oxidative damage is thought to be a major contributor to DNA mutations. However, mutations can also arise during DNA replication or repair if inadequate levels of certain nutrients are present. This is seen in the pathophysiological consequences of folate and vitamin B₁₂ deficiency on hematopoiesis, in which increased levels of uracil are erroneously incorporated into DNA (65,66). It is a concern that deficiencies of similar factors could be important in increasing the number of mutations during spermatogenesis or during zygote division. We discuss those nutrients that appear to be important in maintaining sperm cell DNA fidelity.

4.2.1. Folate

Folate (in the form of tetrahydrofolate) is required as a cofactor during the methylation of deoxyuridylate (dUMP) to deoxythymidylate (dTMP). dTMP is required for the synthesis of deoxythymidine residues, which are essential for DNA replication and repair. Folate (and vitamin B₁₂)-deficient patients have high levels of uracil misincorporation into the DNA of hematopoietic stem cells required for erythrocyte production (65–67). Folate deficiency is a common vitamin deficiency worldwide because of insufficient fruit and vegetable intake. Chronic alcoholics may be at particular risk. In addition to their generally poor diet and consequently low intake of both antioxidant nutrients and other vitamins, alcoholics have decreased absorption of folate across the gastric epithelium (68).
Chronic ethanol intake both lowers the activity of gastric brush-border folate hydrolase and results in necrosis of the gastric epithelium, each essential for adequate absorption of folate (69). Hence, one concern is that male alcoholics or those on poor diets may be at an increased risk of uracil misincorporation in sperm DNA and, thus, an increased risk of producing offspring with genetic defects. Repair of uracil misincorporation by uracil DNA glycosylase activity can lead to chromosomal strand breaks if nicks are formed on strands opposite to other repairable lesions such as oxo8dG (66,67,70).

Our recent evidence suggests that dietary folate is required for the proper maintenance of spermatogenesis in rats and in humans (71). Men who routinely consume folate-deficient diets may have increased rates of uracil misincorporation into sperm cell DNA. We have found uracil in sperm DNA from men (smokers and nonsmokers) who habitually eat a diet low in fruits and vegetables (unpublished data). This type of DNA damage could be delivered to the zygote upon fertilization where repair of sperm DNA damage takes place (16). Thus, if lesions exist in the sperm DNA on opposing strands, simultaneous repair of these lesions could result in DNA double strand breaks within the zygote (66). These lesions could include misincorporated uracil due to folate deficiency, as well as oxidized purines (e.g., oxo8dG), which are increased by low AA levels in tissues. Thus, paternal folate and vitamin C deficiencies may interact to increase the risk of DNA damage in the embryo.

4.2.2. ZINC

Zinc is known to be an essential trace element for testicular development and spermatogenesis (72). Zinc concentrations in seminal plasma (about 200 μg/L) are much greater than in blood plasma (about 6 μg/L), which suggests a specific function for this trace element in spermatogenesis and/or stability of spermatozoa (73). Zinc concentrations are correlated positively with sperm-cell density, and lower zinc concentrations are found in infertile men compared with fertile men (74). Zinc deficiency leads to increased oxidative damage to testicular cell DNA (as measured by oxo8dG) and increased protein carbonyl content (75). The mechanistic role of zinc in spermatogenesis is unclear, but it has been suggested that Zn^{2+} enhances the stability of sperm chromatin (76) or is required to maintain sperm function prior to zygote formation (77). Zinc, although a member of the transition metal d-block of the periodic table, does not possess any redox activity in the Zn^{2+} state. This means it is unable to participate in oxidant production like other transition metal ions, such as those of copper and iron. One possible explanation for such a high Zn^{2+} seminal plasma content would be to occupy sensitive binding sites on protein and DNA, reducing the possibility of redox-active transition metal ions binding to these sites which could promote oxidant production. Further research into the mechanisms and function of zinc is required to establish the role that this trace element plays in spermatogenesis and protection of sperm cells against oxidative damage, but there is evidence that an adequate intake of zinc may be necessary to reduce the risk of sperm cell genetic damage.

5. RECOMMENDATIONS FOR INTAKES OF NUTRIENTS TO LIMIT SPERM DAMAGE

RDA values, which are periodically reevaluated, are suggested intakes designed to prevent the development of deficiency disease. However, all RDAs may not be sufficient to maintain optimal levels of particular nutrients. Other factors, such as whether
the individual smokes or consumes large amounts of ethanol, will also affect the amount of vitamins, such as ascorbate, tocopherols, and folate, necessary to maintain adequate levels in the body. Most of the nutrients mentioned in this chapter are obtained in sufficient quantities from a well-balanced, omnivorous diet high in fresh fruits and vegetables. The current suggested intake of five servings of fruits and vegetables per day, as recommended by the National Cancer Institute, is not achieved in the United States by 80% of children and 68% of adults, and by a greater percent of the populace in some European countries (e.g., Scotland) \(^\text{(78,79)}\). The quarter of the United States population that eats the fewest fruits and vegetables has approximately double the cancer rate for most types of cancer when compared to the quarter with the highest intake \(^\text{(80,81)}\). The cost of fruits and vegetables is one factor that can discourage consumption. People with lower incomes generally have to spend a higher percentage of their income on food, eat less fruits and vegetables, and are more likely to smoke than are those with higher incomes \(^\text{(82)}\). They may experience an increased risk of genetic damage in their offspring. It would be prudent in couples attempting to conceive for the male partner to cease any smoking activity, consume limited amounts of ethanol, and follow a diet high in fresh fruits and vegetables. This practice may prevent genetic damage to sperm, and thus reduce the risk of birth defects among children.

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1. DEFINITION AND IMPORTANCE OF PRETERM DELIVERY

The United States has an infant mortality rate that ranks 20th worldwide. A major cause of this poor rank is an excess of preterm deliveries to United States women compared with lower ranking countries, such as Norway (1). Preterm delivery (< 37 completed-weeks’ gestation) contributes substantially to low birthweight (LBW, < 2500 g), but is not synonymous with it. Of all infants who weigh less than 2500 g, 60%–70% are born preterm, whereas the remainder are term infants who were growth-restricted in utero (small-for-gestational-age [SGA]) (2). Preterm delivery is held to be the strongest underlying risk factor for infant mortality, accounting for 85% of the early neonatal deaths not caused by lethal congenital defects (3). In the United States, it is estimated that nearly three-quarters of all neonatal deaths occur among infants who are delivered preterm (4).

Although there has been a long-term decline in LBW in the United States, that decline is attributable primarily to a reduction in the rate of SGA births (i.e., term LBW) (5). In contrast, the rate of preterm birth based upon gestation duration has exhibited a secular increase in the United States (9.2% in 1982 to 10.7% in 1992), rising by 14% in a decade (2,6). Between 1992 and 1995 the rate again rose (to 11.0%), but has not increased in the latest reporting interval (1996) (2,6). There has been virtually no change in the incidence of very low birthweight (VLBW, < 1500 g) (7), which reflects very preterm delivery (< 33 completed-weeks’ gestation), in particular.

In addition to increased mortality, infants delivered preterm are at increased short-term risk of complications, including respiratory distress syndrome (RDS), bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis, among others. In the longer term (1–3 yr), such children are at increased risk of seizure disorders, disabilities of the special senses (blindness and deafness), cerebral palsy, and mental retardation. Learning and behavioral deficits persist (8,9) and are associated with increased risks of hyperactivity, placement in special education programs, and grade retention among school children who entered life preterm (10,11).
2. NONNUTRITIONAL FACTORS ASSOCIATED WITH PRETERM DELIVERY

One problem hampering the identification of associated risk factors is the heterogeneous nature of preterm delivery. The most common proximate cause is idiopathic preterm labor culminating in a preterm delivery. Another etiology involves preterm premature rupture of the fetal membranes (PROM) before labor and delivery. In addition, there are numerous complications (e.g., pregnancy-induced hypertension [PIH]) which can, in turn, lead to a medical induction before term and thus 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 and very preterm delivery have been identified. These include, most importantly, a history of preterm delivery during a prior pregnancy, as well as young (< 17 yr) and older (> 35 yr) maternal age, low socioeconomic status, cigarette smoking, substance abuse, black ethnicity, bacteriuria, faulty placentation or vasculopathy, and possibly poor maternal nutritional status (low pregravid weight, anemia early in pregnancy, poor diet, and inadequate weight gain during pregnancy) (13).

3. FETAL GROWTH AND PRETERM DELIVERY

Whatever the underlying cause, preterm delivery seems to be characterized by suboptimal fetal growth. In a recent study (14), we found for a sample of young, minority mothers (about age 17 yr) that their infants who were delivered preterm (< 37 completed-weeks’ gestation) were already significantly smaller in all fetal growth dimensions by 32-wk gestation, compared to fetuses at 32-wk gestation, who later delivered at term. The diminished fetal growth was attributable to slower rates of growth from about 16–32-wk gestation. When stratified by the proximate cause of preterm delivery, infants delivered preterm for medical or obstetric indications (placental abruption, severe preeclampsia, nonreassuring heart rate patterns, chorioamnionitis, oligohydramnios) were found to have asymmetric growth patterns, which suggest a growth failure late in pregnancy. On the other hand, infants delivered preterm after PROM or after failed or no tocolytic therapy to halt their spontaneous preterm labor were proportionately smaller in all dimensions, implying an overall slowing of growth that may have originated early in pregnancy and possibly demonstrates a more chronic stress. These findings of slowed fetal growth with preterm delivery are also consistent with and expand upon the results from previous studies of fetal growth and preterm delivery in cohorts where the mothers’ ages are older (about age 25 yr).

In a prior study, Tamura et al. (15) evaluated prospectively 148 fetuses of pregnancies at risk for preterm delivery, the majority of which (63%) were measured within 7 d of delivery. They found that an increased percentage (more than three times expected) had abdominal circumferences (AC) and biparietal diameters (BPD) below the 10th percentile of standards. Two studies looked specifically at altered growth with preterm labor. Westgren et al. (16) examined the femur length (FL)-AC ratio, which is presumed to be gestational age independent, in 82 fetuses of gravidas hospitalized for preterm labor; 39 delivered preterm after failed tocolysis, whereas 43 went on to deliver at term. They found that the FL–AC ratio was significantly increased among those with failed tocolysis, implying that the growth of the fetuses delivering preterm was already suboptimal at the time when preterm labor was diagnosed. Similarly, MacGregor et al. (17)
performed ultrasounds during admission for preterm labor in 78 pregnancies at 31-wk gestation. Compared to the 30 patients who delivered after 36 completed weeks, the patients delivering before 36-wk gestation demonstrated growth measurements (BPD, head circumference [HC], AC, and FL) that were suboptimal (below the 25th percentile) or often consistent with growth restriction (below the 10th percentile).

Weiner et al. (18) used extant charts to estimate fetal weights from ultrasound and compared the estimated fetal weight (EFW) at various gestational ages to published birthweight-for-gestational-age norms. They found that the birth weights of preterm infants were significantly smaller than the EFW of same-aged fetuses who later delivered at term. Secher et al. (19) and Ott (20) drew the same conclusion based on similarly designed studies, comparing EFW derived from ultrasound to birthweights or sonographically derived standards.

Slowed fetal growth in association with preterm delivery is consistent with findings among both adolescent and adult gravidas that the risk of preterm delivery is increased with low rates of gestational weight gain in the last half of pregnancy (21–23). The low rates of gestational gain may reflect a slowed fetal growth late in pregnancy and thus be a marker for impending preterm birth.

4. MATERNAL NUTRITIONAL STATUS AND PRETERM DELIVERY

4.1. Maternal Pregravid Weight, Height, and Body Mass Index (BMI)

Maternal size (weight, height, and BMI) is often taken to reflect pregravid nutritional status, although these indicators are not very specific. In an attempt to disassociate the effects of weight (fatness) from height, the Institute of Medicine (IOM) (24) has recommended that BMI, defined as weight (kg)-for-height (m)², be used to index maternal pregravid status. At a BMI of less than 19.8, a woman is considered to be underweight; at 19.8–26.0, normal weight; above 26.0–29.0, overweight; and above 29.0, obese. Although there is abundant evidence that maternal pregravid BMI has an independent and positive effect on birthweight, the association between maternal pregravid status and preterm delivery is somewhat less clear.

Only a few studies have shown a relationship specifically between maternal height and preterm delivery. Low maternal height among women from developing countries may indicate stunting consequent to long-term nutritional stress. In their study of the risk factors for preterm LBW in India, Mavalankar et al. (25) did find that short women (< 150 cm) had a better than twofold increased risk of preterm LBW compared with their taller counterparts (155 cm), but this effect could not be disassociated from that of low maternal weight.

However, in their study of more than 13,000 women from Montreal, Kramer et al. (26) found a weak (odds ratio [OR] = 1.17, 95% confidence interval [CI] 1.05–1.30), but statistically significant, increased risk of preterm delivery (< 37 completed weeks) with short stature (≤ 157.5 cm) independent of pregravid weight. The risk of preterm delivery, however, was confined to those spontaneous deliveries before 37 wk, but not before 34 or 32 wk. Kramer et al. (26) speculated that for these women short stature may be a marker for small pelvic size, and thus an increased risk for early onset of labor as a result of pelvic filling.

In several studies, underweight has been associated with an increased risk of preterm delivery, especially when gestation duration is estimated using fundal height or ultrasound criteria (27–29), although the extent to which this represents a size bias in the estimation of gestation or the extent to which gestational weight gain and diet during
pregnancy can overcome the nutritional deficit are not well known.

4.2. Gestational Weight Gain

Most research on gestational weight gain has focused on the relationship between total weight gain and birthweight, and the body of evidence on this topic has been extensively reviewed by the IOM (24). Studies have been virtually unanimous (in developing countries and among different ethnic groups) in showing a positive relationship between weight gain and birthweight (24,30). Maternal pregravid weight or BMI (kg/m²) and weight gain appear to have independent and additive effects on birthweight outcome. Correlations between weight gain and birthweight range between 0.20–0.30. The average magnitude of the effect on birthweight (in women with a normal weight-for-height) is, assuming a base birthweight of about 3000 g, approx 20 g of birthweight for every 1 kg of total gain, and pregravid weight-for-height is a strong effect modifier on birthweight (31). The relationship between gestational weight gain and preterm delivery appears more complex and is still more controversial.

Although cumulative or total weight gain is an important predictor of birthweight, the pattern of weight gain and rates also appear to play significant roles in predicting preterm delivery (22,32). Taking a cue from several earlier studies implicating overall weight gain as a risk factor for preterm delivery (21,33,34), we found that both early and later weight gain during adolescent pregnancy have independent effects on pregnancy outcome. In a multiracial sample of 1790 teenagers from Camden County, NJ, early inadequate weight gain (< 4.3 kg by 24-wk gestation) was associated with an increased risk of SGA infants (below the 10th percentile for standards) (22,35). Preterm delivery (at < 37 completed-weeks’ gestation), on the other hand, appeared unaffected by early inadequate weight gain, but was increased for teenagers with inadequate weight gain rates (< 400 g/wk) late in pregnancy. This occurred even when the total pregnancy weight gain never fell below the targets set in clinical standards (adjusted odds ratio [AOR] = 1.69, 95% CI 1.12–2.55) (36).

Other studies of primarily adult gravidas have also indicated that low rates of weight gain, primarily in the latter half of pregnancy, are associated with preterm delivery (23,29,37,38). Wen et al. (29) in their study of over 17,000 low-income black and white gravidas from Alabama found that rates of weight gain of less than 0.24 kg/wk after 20 wk gestation increased the risk for preterm delivery (OR = 1.52, p < 0.05) compared with rates between 0.58–0.74 kg/wk. Interestingly, Wen et al. (29) also noted that women with high or excessive rates of gain (≥ 0.75 kg/wk) also had an increased risk of preterm delivery (OR = 1.71, p < 0.05), although they speculated that this association might be a function of late edema caused by PIH. Confirming the association between low weight gain late in pregnancy and preterm delivery, Siega-Riz et al. (37) found for a predominately Hispanic sample (80%) of nearly 5000 women from the West Los Angeles area that inadequate weight gain during the third trimester was predictive of preterm delivery (better than twofold increased risk).

Hickey et al. (38) studied 1015 nonobese (BMI < 26.0) black and white women from Alabama. In this study, first trimester weight gain averaged 2.48 ± 3.36 kg, second trimester rates of gain averaged 0.49 ± 0.21 kg/wk, and third trimester 0.45 ± 0.28 kg/wk. Whereas low gain or low rates of gain in the first two trimesters were not associated with preterm delivery, a low rate in the third trimester (< 0.38 kg/wk with a pregravid BMI < 19.8; < 0.37 kg/wk with a pregravid BMI 19.8–26.0) was associated
with an increased risk of preterm delivery \((OR = 2.46, 95\% CI 1.53–3.92)\).

Most recently, Carmichael and Abrams (39) reviewed and summarized the extant literature (13 published studies) on gestational weight gain and preterm delivery. They found that 11 of these studies reported a significant association between maternal weight gain and preterm delivery, principally, that risk increased when gestational weight gain was inadequate. In their summary, gestational weight gain in later pregnancy was consistently associated with increased preterm delivery, whereas inadequate gain in early pregnancy was not.

That there is increasing evidence for an association between low rates of maternal weight gain late in pregnancy and preterm delivery does not mean that these low rates are causal. They may not reflect maternal nutrition or may reflect it indirectly. We have found in previous observational studies of both young and older gravidas that low intakes of iron and zinc are related to an increased risk of preterm delivery (40,41). In the case of zinc, the risk of preterm delivery with low dietary-zinc intake was particularly strong (threefold increased risk) for those whose rupture of membranes preceded labor (41). If, for example, maternal vaginal infection increased nutrient requirements, then the unmet maternal need, giving rise to a preterm delivery, might also be reflected by inadequate gestational gain (39). Or, inadequate weight gain may be a marker for maternal infection that is unrelated to maternal nutrition. On the other hand, the low rates of gestational gain may reflect the slowed fetal growth associated with preterm delivery and be a marker for impending preterm birth.

### 4.3. Diet and Gestational Weight Gain

One inference arising from the observation that maternal weight gain during pregnancy and infant birthweight and/or gestation duration are positively correlated is that although the maternal diet may influence pregnancy outcome, the influence probably is, at least in part, indirect. That is, during pregnancy, maternal diet affects gestational gain, which, in turn, increases the risk of preterm delivery and/or infant LBW when it (the weight gain) is poor. A poor or otherwise inadequate gestational gain thus may reflect an inadequate dietary intake. Although this association appears to be a reasonable one, a relationship between caloric intake and weight gain during pregnancy has not often been described.

The first report of a positive relationship between diet and weight gain was made by Thomson (42), who found a correlation of 0.30 between caloric intake and weight gain in Scottish primigravidas eating “to appetite.” Among Camden gravidas, a significantly lower caloric intake (by about 300 kcal/d) was associated with an inadequate gestational gain (43). After controlling for calories, women with an inadequate gestational weight gain showed little difference in macronutrients (protein, calories) or total grams of food ingested, suggesting that the differences observed were explained for the most part by lower energy intake. This relationship was confirmed subsequently with three 24-h dietary recalls taken during the course of pregnancy (41). Because intakes were obtained throughout gestation, overall caloric intake was higher (+160 kcal/d) than in the prior study. After control for confounding variables, women with inadequate gestational weight gain consumed fewer kcal/d (−173 kcal/d) than did women whose pregnancy weight gain was adequate for gestation.

### 4.4. Diet and Preterm Delivery

The relationship between poor diet and inadequate gestational weight gain, along with the observation that slowed fetal growth and low rates of weight gain late in preg-
nancy were each associated with preterm delivery, suggests that a poor maternal diet may increase the risk of preterm delivery. For example, during the Dutch Famine of 1944–1945, third-trimester exposure to intense famine shortened gestation by about 4 d, whereas exposure during the first trimester was associated with an excess of preterm birth (44). The famine is better known for its effect on fetal growth and maternal weight. The identification of preterm births probably was hampered by maternal amenorrhea during the famine. Amenorrhea would have made gestational dates insecure for most women experiencing the famine. Thus, low energy intake during the famine was probably a marker for the inadequate consumption of other essential nutrients.

4.4.1. IRON

Iron is an 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 (24). Supplementation with iron is generally recommended during pregnancy to meet the energy needs of both mother and the 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 seems to be one of the most obvious symptoms.

When detected early in pregnancy, IDA is associated with a lower caloric 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 (40,45). Maternal anemia, when diagnosed before midpregnancy, is also associated with an increased risk of preterm birth (46,47,49). During the third trimester, anemia may be a good prognostic sign reflecting expansion of the maternal plasma volume and thus usually is associated with a diminution, as opposed to an increased risk of preterm birth (48).

Most recently, Zhou (49) described the relationship of maternal hemoglobin concentrations during the first trimester in 829 Shanghai women. In Shanghai, other risk factors associated with poor pregnancy outcome (e.g., smoking, drinking) were uncommon and did not confound the relationship between anemia and preterm delivery. Preterm delivery had a U-shaped relationship with early pregnancy hemoglobin. The risk of preterm delivery was increased 1.6 times for women with hemoglobin concentrations between 10.0 and 10.9 g/dL, more than twofold for hemoglobin concentrations ranging from 9.0 to 9.9 g/dL, and greater than threefold for hemoglobins between 6.0 and 8.9 g/dL.

However, the increased risk of preterm delivery does appear to be specific to IDA, and not anemia as a result of causes other than iron deficiency. Scholl (40) reported data on 755 pregnant women receiving initial antenatal care at 16.7 +/- 5.4 wk gestation in Camden, NJ. Serum ferritin (< 12.0 μg/L) was used to characterize IDA (24). Although anemia, based upon low hemoglobin, was high (27.9%) at the initial antenatal visit, the prevalence of iron deficiency anemia (anemia with serum ferritin concentrations < 12.0 μg/L) was low, amounting to 3.5%. After controlling for confounding variables, for women with IDA early in gestation, the risk for preterm delivery increased more than twofold (AOR = 2.66), whereas anemia from other causes were not associated with any increased risk for preterm delivery. The overall attributable risk for preterm delivery to IDA was only 5.5%, meaning that although there may be direct effects of iron deficiency on preterm delivery, anemia as a result of other causes may best be viewed as a
marker for other pathologic conditions (e.g., bleeding, infection, poor nutritional status) that also increase risk for preterm delivery. On the other hand, high levels of hemoglobin or hematocrit later in pregnancy are associated with an increased risk of preterm delivery. The increased risk with high hemoglobin levels may indicate a failure of plasma-volume expansion and is correlated with an increased risk of PIH.

A number of studies (47–51) have documented this “U-shaped” relationship between low and high maternal hemoglobin and hematocrit levels and preterm delivery (see Table 1). Garn et al. (50) noted the relationship in their retrospective analysis of data from over 50,000 white and black women followed as part of the National Collaborative Perinatal Project. Using the lowest recorded pregnancy values of hemoglobin and hematocrit, they demonstrated an increased risk of preterm delivery with hemoglobin levels < 100 g/L and with levels > 120 g/L. Murphy et al. (51), in a study of nearly 55,000 women from the Cardiff Births Survey, found the same “U-shaped” relationship of preterm delivery to hemoglobin levels ascertained at entry to prenatal care (booking). Risk of preterm birth was increased with low hemoglobin levels (<104 g/L) for women entering care before 13 wk of gestation and after 20 wk, whereas for late entrants to care (wk 20–24) risk was also increased when hemoglobin levels were high. Steer et al. (48) in a multiethnic study of over 150,000 women from the North West Thames region of London showed that there was an increased risk of preterm delivery with both low (<85 g/L) and high (> 115 g/L) hemoglobin levels among all ethnic groups. Although African, Afro-Caribbean, and Indo-Pakistani women have higher rates of preterm delivery, their higher rates of anemia did not account for the increased risk of preterm delivery (48).

A high concentration of the iron-storage protein, ferritin, during the third trimester of pregnancy is also associated with an increased risk for preterm and very preterm delivery. From their studies of Alabama women, both Tamura (52) and Goldenberg (53) found high levels of third-trimester serum ferritin (> 40 ng/L) during the third trimester of pregnancy to be a marker for an increased risk for preterm and very preterm delivery. Prospective data from Camden (54) indicated that high ferritin levels (> 41.5 ng/L) during the third trimester, stemming from the failure of ferritin to decline from entry, increased risk of very preterm delivery more than eightfold. High ferritin was associated with indicators of maternal infection including clinical chorioamnionitis in Camden gravidas (54) and infant sepsis among women with pregnancies complicated by PROM (55). Maternal anemia and IDA earlier in pregnancy and inadequate expansion of maternal plasma volume appear to underlie high third trimester ferritin (54,56).

4.4.2. Micronutrients

During pregnancy, low intakes of two micronutrients, zinc (41) and folate (57) are associated with lower caloric intake, IDA at entry to care, an inadequate gestational gain during pregnancy, as well as an increased risk of preterm delivery. Zinc is an element involved either directly as a metalloenzyme in the production of enzymes, which include DNA and RNA polymerase, or as a catalyst in the synthesis of other enzymes (58). Folic acid functions as a coenzyme in the transfer of single carbon atoms from donors such as serine and histidine to intermediates in the synthesis of amino acids, purines, and thymidic acid (59). Although 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
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<td>Iron/anemia</td>
<td>Garn et al. (50)</td>
<td>Observational study of over 50,000 women followed in the National Collaborative Perinatal Project (NCPP), lowest pregnancy values of hemoglobin (Hgb) and hematocrit (Hct).</td>
<td>—</td>
<td>Mixed</td>
<td>Whites and blacks</td>
<td>Maternal Hgb and Hct levels had “U-shaped” relationship to preterm delivery (≤37 wk), with the risk being higher at Hgb &lt;100 g/L and &gt;120 g/L. The increased risk with Hgb indicated a failure of plasma-volume expansion.</td>
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<td>Murphy et al. (51)</td>
<td>Observational study of nearly 55,000 women in the Cardiff Births Survey, Hgb levels ascertained at entry to prenatal care (booking).</td>
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<td>Mixed</td>
<td>Welsh</td>
<td>Preterm delivery (&lt;37 completed weeks) showed a “U-shaped” relationship to Hgb levels. At &lt;13 wk, risk was increased about 50% with Hgb &lt;104 g/L; at wk 13–19, risk was not increased; at wk 20–24, risk was increased for low (&lt;104 g/L) and high (&gt;145 g/L) Hgb by over 50%.</td>
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<td>Klebanoff et al. (47)</td>
<td>Case-control study of 1706 (725 preterm, 981 term) deliveries from Kaiser Permanente Births Defects Study, Hct levels measured throughout gestation.</td>
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<td>14% Asian, 28% Black, 21% Mexican, 37% White</td>
<td>Hct values fell during early second trimester and began increase again at 31–33 wk. Moderate relationship between second trimester anemia (Hct &lt;10th percentile for ethnicity and gestation) and preterm delivery.</td>
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<td>Steer et al. (48)</td>
<td>Retrospective analysis of over 150,000 women from the North West Thames region of London,</td>
<td>—</td>
<td>Mixed</td>
<td>73% White, 14% Indo, Pakistani</td>
<td>Increased risk of preterm delivery (&lt;37 wk completed) with Hgb levels ≤ 85</td>
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<td>Study</td>
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<td>Hemoglobin</td>
<td>Hemoglobin (g/L)</td>
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<td>Zhou et al. (49)</td>
<td>Observational study of 829 women from Shanghai. Population homogeneous for race, parity, prenatal care, smoking. Hemoglobin sampled at 4–8 wk, 16–20 and 28–32 wk gestation.</td>
<td>27.7 ± 4.7 yr</td>
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<td>5% Black g/L (AOR = 1.62, CI 95%: 1.35–2.18) and ≥115 g/L. Inverse U-shaped relationship between maternal hemoglobin and low birth weight. Odds of preterm delivery increased 2.5-fold for women with hemoglobin &gt;130 g/L or between 90–99 g/L. Odds increased 3.5-fold for entry hemoglobin between 60–89 g/L. Risk of fetal growth retardation unrelated to entry hemoglobin.</td>
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<td>Scholl et al. (40,45)</td>
<td>Observational study of iron-deficiency anemia (IDA, anemia with serum ferritin &lt;12 µg/L) among 779 Camden, NJ women.</td>
<td>18.4 ± 3.7</td>
<td>78% Maoi (anemia)</td>
<td>77% Black (anemia)</td>
<td>56% Black (no anemia) IDA (found in 12.5% of those with anemia) was found to be associated with lower energy and iron intakes early in pregnancy. The risk of preterm delivery was better than two times higher with IDA, but was not increased with anemia from other causes.</td>
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<td>Tamura et al. (52)</td>
<td>Nested case-control study at 24-wk gestation. Samples from 94 multiparas, where cases delivered spontaneously at ≤ 32 wk, control 1 delivered spontaneously at 33–36 wk, control 2 delivered</td>
<td>16.4–36.2 yr</td>
<td>100% Low Ferritin</td>
<td>74% Black (anemia)</td>
<td>26% White (no anemia)</td>
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Table 1 (continued)

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<tr>
<th>Nutrient</th>
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<tr>
<td>Zinc</td>
<td>Cherry et al. (63)</td>
<td>Randomized, controlled trial of zinc supplementation among pregnant New Orleans teenagers, 17.6 (13.5–19.6)</td>
<td>Low 95% Black</td>
<td>With stratification by maternal weight at delivery, zinc supplementation was related</td>
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<td>Goldenberg et al. (55)</td>
<td>Observational study of 223 gravidas with PROM before 32 wk. Data from randomized antibiotic trials at five clinical centers.</td>
<td>24.8 ± 5.9 yr</td>
<td>American</td>
<td>Plasma ferritin increased between admission for PROM and delivery. Ferritin levels significantly higher (+36 μg/L) in women whose infants developed sepsis.</td>
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<td>Scholl (54,56)</td>
<td>Observational study of serum ferritin (15 and 28 wk) in 1162 gravidas from the Camden Study.</td>
<td>12–29 yr 100% Low Hispanic 33.3% Black from 90th percentile</td>
<td>At wk 28, high concentrations of ferritin (&gt;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 low birthweight (p &lt; 0.01 for each).</td>
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<td>Goldenberg et al. (53)</td>
<td>Observational study of serum ferritin (at 19, 26, and 36 wk) and preterm delivery in 580 gravidas who participated in a randomized study of zinc supplementation.</td>
<td>— 100% Low 100% Black</td>
<td>Ferritin in highest quartile at wk 28 associated with two-fold increase in odds of preterm delivery (&lt;37 wk) and infant low birthweight, threefold increase in odds of very preterm delivery (≤ 32 wk) and a fourfold increase in birthweight ≤ 1500 g.</td>
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396
assigned to placebo \((N = 556)\) or 30 mg zinc supplement \((N = 581)\). To a lower risk of preterm delivery \((38 \text{ wk})\) among normal weight women \((18.3\% \text{ vs } 28\%, p < 0.05)\). Zinc supplemented low weight multiparas had increased gestation duration \((+1.2 \text{ wk}, p < 0.008)\). There was a tendency \((p < 0.15)\) for weight gain rates to be greater among zinc supplemented women \((0.52 \text{ kg/wk})\) compared with placebo \((0.42 \text{ kg/wk})\).

Goldenberg et al. (64) 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 \((N = 286)\). Birthweight increased with zinc supplementation by 126 g \((p < 0.05)\) and gestation duration by 0.5 wk \((p = 0.06)\). Women with pregravid BMI < 26 had a birthweight increase of 248 g \((p < 0.005)\) and an increase in gestation of 0.7 wk \((p = 0.08)\).

Hunt et al. (67) Double-blind randomized, controlled trial of multivitamin mineral tablet with 20 mg zinc \((N = 106)\) or placebo \((N = 102)\). No effect of zinc supplementation on preterm delivery \((<37 \text{ wk})\) \((5.7\% \text{ zinc vs } 4.4\% \text{ controls})\) or low birthweight \((4.7\% \text{ zinc vs } 4.4\% \text{ controls})\).

Neggers et al. (60) Observational study of 476 Alabama women. Low plasma zinc at entry to prenatal care \((7.0–12.2 \mu \text{mol/L})\) increased risk of infant LBW eight times.
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<tr>
<th>Nutrient</th>
<th>Ref.</th>
<th>Study design, size, and setting</th>
<th>Age, yr</th>
<th>SES</th>
<th>Race/ethnicity</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scholl et al. (41)</td>
<td>Observational study of dietary zinc intake in 818 Camden, NJ pregnant women.</td>
<td>19.2 L. 60% Black</td>
<td>19.2 41 mg zinc/d</td>
<td>18.7 &gt;15 mg zinc/d</td>
<td>Low dietary zinc intake compared with women in the highest quartile (15.9–25.4 μmol/L, p &lt; 0.05). Plasma zinc was linearly related to gestation duration (0.17 wk/μmol/L zinc, p &lt; 0.05). Low dietary zinc intake associated with twice greater preterm delivery (&lt;37 wk) and infant LBW, 3–4 times greater very early preterm delivery (&lt;33 wk) (p &lt; 0.05). Risk or preterm delivery with PROM increased 3.5 times with low zinc (p &lt; 0.05).</td>
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</tr>
<tr>
<td>Sikorski et al. (62)</td>
<td>Observational study of zinc status in 70 women with term deliveries.</td>
<td>26 ± 4.4 Polish</td>
<td>60% Black</td>
<td>60% Black</td>
<td>Zinc index compiled from zinc assayed in blood, scalp, and pubic hair and colostrum. Zinc index in patients with PROM was lower (4.33 ± 1.18) compared with patients without PROM (5.97 ± 1.39), p &lt; 0.05. Zinc index was inversely correlated with parity (r = -0.61).</td>
<td></td>
</tr>
<tr>
<td>Folic acid/μg/L</td>
<td>Tchernia</td>
<td>Observational study of serum</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Serum folate lower by 1.1 g/L compared with women in the highest quartile (15.9–25.4 μmol/L, p &lt; 0.05). Plasma zinc was linearly related to gestation duration (0.17 wk/μmol/L zinc, p &lt; 0.05). Low dietary zinc intake associated with twice greater preterm delivery (&lt;37 wk) and infant LBW, 3–4 times greater very early preterm delivery (&lt;33 wk) (p &lt; 0.05). Risk or preterm delivery with PROM increased 3.5 times with low zinc (p &lt; 0.05). Risk or preterm delivery with PROM increased 3.5 times with low zinc (p &lt; 0.05). Risk or preterm delivery with PROM increased 3.5 times with low zinc (p &lt; 0.05).</td>
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</table>
folate in 100 high-risk women.

Supplementation trial (open) of iron vs iron + folate (350 μg/d) in 108 women.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Country</th>
<th>Mean ± SD</th>
<th>% Low</th>
<th>Race</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blot et al. (75)</td>
<td>Nonrandomized double-blind study of iron and ascorbic acid vs iron, ascorbic acid, and folate (350 μg/d) at 6-mo gestation in 200 women.</td>
<td>French</td>
<td>27.5 ± 4.5</td>
<td>34% Low</td>
<td></td>
<td>Gestation duration increased by 0.8 wk among iron and folate supplemented women (p &lt; 0.001).</td>
</tr>
<tr>
<td>Scholl et al. (57)</td>
<td>Observational study of folate from diet and supplements and serum Folate in Camden, NJ (N = 832).</td>
<td>Medicaid</td>
<td>18.8</td>
<td>67% Black</td>
<td>follic acid intake</td>
<td>Women with low folate intake (≤240 μg/d) had three-times greater preterm delivery and infant LBW (p &lt; 0.05). Risk of preterm delivery without PROM increased three times (p &lt; 0.05). Odds of preterm delivery increased 1.5% per unit decrease in serum folate (p &lt; 0.05).</td>
</tr>
</tbody>
</table>

<p>| Folic acid | Giles et al. (74) | Randomized study of folic acid (5 mg/d) or iron supplementation in 692 gravidas. Patients stratified by gestation at entry to care: &lt;10 wk, 10–20 wk, 20–30 wk, &gt; 30 wk. | Australian (Melbourne) | 24.9 ± 5.2 yr (iron) | Folic acid group contained more primigravida than placebo group. No difference between groups in gestation duration or birthweight. |
| Folic acid | Fletcher et al. (73) | Double-blind randomized study of folic acid plus iron (5 mg/d) or iron | English (London) | 27.2 ± 5.7 yr (folic acid + iron) | Gestation duration (39.7 wk iron vs 39.7 folic acid + iron) and proportion less than 38 |</p>
<table>
<thead>
<tr>
<th>Nutrient</th>
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<th>Study design, size, and setting</th>
<th>Age, yr</th>
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<th>Race/ethnicity</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fleming</td>
<td>Randomized controlled trial of folic acid (0.5 mg or 5 mg/d) with and without iron plus a placebo group in 146 gravidas.</td>
<td>—</td>
<td>—</td>
<td>Western Australia</td>
<td>Small sample sizes in each of the five trial arms (N approx 20). No difference in gestation or birth weight was detected among groups.</td>
</tr>
<tr>
<td>Folic acid/ homocysteine</td>
<td>Malinow</td>
<td>Observational study of circulating serum homocysteine and serum folate in 35 healthy nulliparous gravidas at delivery.</td>
<td>24.2 ± 5.9 yr</td>
<td>—</td>
<td>American</td>
<td>In univariable analyses, maternal homocysteine correlated (p &lt; 0.05) with gestation duration (r = -0.42) serum folate correlated (p &lt; 0.05) with gestation (r = 0.23). Maternal folate and homocysteine correlated (r = 0.54) with each other (p &lt; 0.05). Maternal serum B12 was not related to gestation duration (r = 0.08, p = 0.67).</td>
</tr>
<tr>
<td>Rajkovic</td>
<td>(79)</td>
<td>Observational study of plasma homocysteine in 20 nulliparous gravidas with preeclampsia and 20 controls.</td>
<td>20 ± 4 yr</td>
<td>—</td>
<td>45% Black 55% White</td>
<td>Preeclamptic gravidas had higher homocysteine (8.66 vs 4.99 g mol/L), and delivered significantly earlier than controls (35 ± 4 wk vs</td>
</tr>
<tr>
<td>Calcium Supplementation Study</td>
<td>Study Details</td>
<td>Baseline Mean Gestation Duration (wk)</td>
<td>Population Characteristics</td>
<td>Lower Percentage of Preterm Delivery (%)</td>
<td>Results</td>
<td></td>
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<tr>
<td>Lopez-Jaramillo et al. (85,86)</td>
<td>Randomized, controlled trial of calcium supplementation among 56 nulliparas at risk for pregnancy-induced hypertension in Quito, Ecuador.</td>
<td>19.4 ± 1.8</td>
<td>Low-income Ecuadorian clinic patients with overall low calcium intake.</td>
<td></td>
<td>Gestation duration longer for 22 supplemented women (2 g calcium/d) vs placebo group (N = 34). Gestation 39.2 ± 1.2 wk for calcium supplemented, 37.4 ± 2.3 wk for placebo (p &lt; 0.05 for each).</td>
<td></td>
</tr>
<tr>
<td>Villar and Repke (87)</td>
<td>Randomized, controlled trial of calcium supplementation, 190 teenagers in Baltimore, MD.</td>
<td>≤17</td>
<td>Low-income clinic patients 93.7% Black</td>
<td></td>
<td>Gestation duration longer for 94 supplemented teenagers (2 g calcium/d) vs placebo group (N = 95). Gestation 39.2 ± 2.7 wk for calcium supplements, 37.9 ± 3.9 wk for placebo (p &lt; 0.01). Lower percentage of preterm delivery (&lt;37 wk) among supplemented (7.4%) vs placebo (21.1%, p &lt; 0.01).</td>
<td></td>
</tr>
<tr>
<td>Belizan et al. (84)</td>
<td>Randomized, controlled trial of calcium supplementation among 194 gravidas in Rosario, Argentina.</td>
<td>23.7 ± 5.5 (calcium) 23.7 ± 5.7 (placebo)</td>
<td>Mixed Argentinian</td>
<td></td>
<td>Percent with preterm delivery (&lt;37 wk) among 579 calcium-supplemented women 6.3 vs 6.8% for 588 women receiving placebo.</td>
<td></td>
</tr>
<tr>
<td>Bucher et al. (88)</td>
<td>Medline and Embase searches for randomized trials of calcium and preeclampsia during pregnancy (2459)</td>
<td>—</td>
<td>Mixed —</td>
<td></td>
<td>The summary odds ratio for preterm delivery was 0.69 (95% CI 0.48–1.01) and not statistically significant.</td>
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continued
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Ref.</th>
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<th>Age, yr</th>
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<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Levine et al.</td>
<td>Randomized trial of calcium supplementation (500 mg/d)</td>
<td>21 ± 4</td>
<td>Mixed</td>
<td>35% White</td>
<td>Although all the trials that included preterm delivery showed a reduced risk with calcium supplementation during pregnancy, only one was statistically significant. Calcium supplemented gravidas delivered at 38.9 ± 2.5 wk vs (89) or placebo in 4589 healthy nulliparas at 13–21 wk gestation. Eligible women underwent test of compliance before enrollment in trial.</td>
</tr>
<tr>
<td>Fish oils (N-3 fatty acids)</td>
<td>Olsen et al.</td>
<td>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.</td>
<td>29.4 ± 4.4 (fish oil) 29.7 ± 4.3 (olive oil)</td>
<td>—</td>
<td>Danish</td>
<td>Mean gestation duration longer (p &lt; 0.006) for the 266 women receiving fish oil (283.3 ± 11.1 d) compared with olive oil (279.4 ± 13.1 d) or no oil groups (281.7 ± 11.6 d).</td>
</tr>
<tr>
<td>Authors</td>
<td>Study Type</td>
<td>Observations</td>
<td>Population</td>
<td>Results</td>
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</tr>
<tr>
<td>Scholl et al.</td>
<td>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.</td>
<td>12–29 yr Low 58.9% Black</td>
<td>Observations as described.</td>
<td>Prenatal supplement use associated with two to four-fold reduction in odds of preterm delivery and very preterm delivery ($p &lt; 0.001$). Significant reductions in odds of very low birth weight (six- to sevenfold) and infant low birthweight (twofold) ($p &lt; 0.001$). Prenatal supplement use corroborated by assay of circulating micronutrients at 15 and 28 wk ($p &lt; 0.05$).</td>
<td></td>
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</tr>
<tr>
<td>Fawzi et al.</td>
<td>Randomized study of 1075 HIV-1 infected gravidas from Tanzania randomly assigned to placebo, vitamin A, or multivitamins in a 2×2 factorial design.</td>
<td>Low Tanzanian</td>
<td>Women receiving multivitamins had significantly decreased ($p &lt; 0.01$) infant low birthweight by (44%), very preterm birth (39%) and fetal growth restriction (43%) CD4, CD8 and CD3 counts were significantly increased ($p &lt; 0.05$). Vitamin A had no effect on these outcomes.</td>
<td></td>
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<tr>
<td>Wu et al.</td>
<td>Livebirths from 1988 NMIH Survey (N approx 9000 analyzed to examine effect of regular of multivitamin/mineral supplements and smoking. Gravidas regularly using supplements (3+ times/wk) compared to those who used them less often.</td>
<td>25.5 ± 5.9 yr Mixed American</td>
<td>No difference between smokers who regularly used multivitamins and those using them less in odds of preterm delivery, low birthweight, or fetal growth retardation.</td>
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during times of rapid tissue growth, such as pregnancy (24).

4.4.2.1. Zinc. Neggers et al. (60) used plasma zinc as an indicator of zinc status and demonstrated a positive association between the maternal serum zinc at 16 wk gestation and birthweight in a large population of low income Alabama women. Serum zinc in the lowest quartile was associated with eightfold increased risk of LBW, suggesting that a threshold effect for circulating levels of zinc during pregnancy (Table 1). Kirksey et al. (61) examined the relationship of maternal zinc nutriture to birthweight and found that plasma zinc concentrations in the second trimester, along with pregnancy weight at 3-mo gestation, formed the best predictor model of birthweight for these pregnancies, accounting for 39% of the variance. Scholl and colleagues examined the relationship of dietary zinc intake in 818 Camden women (41) and reported increased risks of complications and adverse pregnancy outcomes in association with low intakes of dietary zinc (6 mg/d or less). These complications include increased inadequate gestational weight gain and a greater risk of IDA at entry to care. Poor outcomes associated with low zinc intake included risks of LBW, preterm delivery, and very preterm delivery that were two to three times greater than controls. In the presence of IDA, the risk of very preterm delivery was greater than fivefold. Sikorski et al. (62) also reported a lower zinc index at delivery (composite of zinc in hair, colostrum, and plasma) in women with premature rupture of the membranes at term (Table 1).

Although observational studies have been suggestive, clinical trials of zinc supplementation have focused on entire groups of low-income women where the mean zinc intake is below the RDA for pregnancy. These trials have yielded equivocal results, perhaps because of an approach that selects a population, as opposed to individuals, at risk.

Two trials have shown effects that were conditional on maternal weight (63,64) that is, a lower rate of preterm delivery in zinc-supplemented women who were not overweight (Table 1). Cherry et al. (63) reported a response to supplementation for pregnant adolescents less than 25-wk gestation. Low serum zinc concentrations were more common in underweight and multiparous women. The frequency of preterm delivery was less in the zinc-treated normal-weight women compared to the placebo group. Zinc treatment of underweight multiparous women was associated with a gestational age increase of nearly 3 wk.

The trial conducted by Goldenberg and colleagues (64) took a more targeted approach and recruited women with plasma zinc levels below the median and randomly assigned them to zinc or placebo. The analysis was stratified after the fact by BMI ($\geq 26$ and BMI $< 26$). Zinc supplementation was associated with increased gestation duration of approximately half a week ($p = 0.06$) and an increase in birthweight about half of which was explained by the longer duration of gestation. Women with a BMI $< 26$ benefited most from zinc treatment with a 248 g increase in infant birthweight and a 0.7-cm-larger infant head circumference. Consistent with prior results, effects were increased for women with lower pregravid BMI (Table 1).

Kynast and Saling (65) found that zinc treatment between 20–34-wk gestation was associated with a lower incidence of preterm labor, and fetal growth retardation when zinc supplemented gravidas were compared to control subjects. In contrast, other randomized controlled trials (66,67) found no differences in maternal or fetal outcomes with zinc supplementation.

In addition to its effect on protein synthesis, zinc also has antiseptic action (58). In theory, a low zinc intake may be associated with an increased risk of infection during pregnancy leading to fragile fetal membranes (and PROM); conversely, a low plasma
zinc level may be an acute phase response to a stressor such as maternal infection.

4.4.2.2. Folate and Homocysteine. During gestation, marginal maternal folate nutriture has the potential to impair cellular growth and replication in the fetus and/or the placenta, which, in turn, could increase the risk of preterm delivery and infant LBW (24). Such adverse outcomes are more prevalent among poor women than those who are better off financially. Pregnant women who live under circumstances where preterm delivery is prevalent have been reported to consume diets with a lower density of vitamins and minerals, including folate (68,69) and to limit consumption of folate-containing dietary supplements (70,71).

The influence of dietary and circulating folate on preterm delivery and infant LBW were studied in 832 women from Camden, one of the poorest cities in the continental United States (57). Low intakes of folate from diet and supplements were associated with maternal characteristics reflecting poorer maternal nutritional status including lower caloric intake, low rate of gestational weight gain, and a higher frequency of IDA at entry to prenatal care. After control for gestation at entry and the time in pregnancy when the samples was drawn, there was a significant relationship between dietary folate intake and serum folate at wk 28 (r = 0.17). Low folate intake (< 240 ng/d) was associated with a greater-than-threefold increase in risk of infant LBW and of preterm delivery, after control for maternal age, parity, ethnicity, smoking rate of gestational weight gain, and intake of energy, and other nutrients (zinc, fiber, and vitamin B12). Circulating folate at wk 28 also was associated with risk; the adjusted odds for LBW increased by 1.5% and preterm delivery increased by 1.6%/U (nmol/L) decrease in serum folate at wk 28 after controlling for potentially confounding variables (Table 1). Thus, lower concentrations of folate at wk 28 were associated with a greater risk of preterm delivery and LBW.

Randomized studies of routine folate supplementation in combination with iron suggested that folate was associated with increased maternal hemoglobin, greater gestational weight gain, as well as an increase in mean gestation duration (Table 1). Fleming (72) enrolled 146 Australian women before midpregnancy (wk 20) into a randomized trial, which included folic acid at a high and a low dose (folic acid 5 mg/d, folic acid 0.5 mg/d) as two of the study arms. A total of 89 women completed the study with sample sizes of 15–20 per group. There were no significant differences in placental weight, birthweight, or gestation duration among the groups. Small sample size and poor statistical power would limit the ability to detect an effect of folate, if any.

Fletcher (73) conducted a randomized controlled study comparing iron (200 mg ferrous sulphate) to iron with folate (5 mg). Supplements were administered to 643 London women at entry to care. No significant differences in birth weight, gestation, or the incidence of congenital defects were found.

Giles (74) performed a double-blind randomized controlled trial of 692 Melbourne (Australia) women assigned to folate (5 mg/d) or placebo. Four groups were stratified by gestation at entry. There were small differences in birth weight and gestation among the groups. None, however, were statistically significant.

Blot and colleagues (75) assigned 200 French women to iron (105 mg elemental iron) alone or in combination with folic acid (350 mg/d). Of these, 109 were reevaluated at delivery. Women treated with folate had higher serum and red cell folate at delivery. The main effect of folic acid was to extend gestation by nearly 1 wk; gestation duration was increased among the folate supplemented women (40.7 ± 1.2 vs 39.9 ± 1.2 wk) as was birthweight (3461 ± 430 g vs 3303 ± 375 g) and placental weight (660 ± 130 g vs 604 ± 115 g). Tscherina and colleagues (76) also found that shorter gestation correlated
with lower serum folate and red-cell folate.

A metaanalysis of existing supplementation studies (77) recommended further study of the effect of folate during pregnancy on preterm delivery and LBW as an “urgent priority.” Many of the trials demonstrated a beneficial effect when a folate supplement was used in combination with iron during pregnancy, although these trials were said to be methodologically flawed.

Homocysteine and dietary folate intake are related. High homocysteine concentrations may stem from a relative folate shortage involving an underlying genetic defect in folate metabolism, from diet alone or from both sources. Homocysteine may do harm to the conceptus by increasing risk of serious maternal complications and thus increasing risk of preterm birth (including indicated preterm delivery) and intrauterine growth restriction.

In their case series, de Vries and colleagues (78) reported increased frequency of hyperhomocysteinemia (24% vs 2–3% expected) among women with serious complications of pregnancy (placental abruption, fetal demise, IUGR). Growth-restricted infants averaged 1327 ± 498 g at birth, and were delivered preterm at 33 ± 3.6 wk. Likewise with placental abruption, mean birthweight was low (1518 ± 981 g) and gestation was shortened (30.8 ± 5.7 wk).

Further, preeclamptic gravidas studied by Rajkovic (79) had significantly higher plasma homocysteine and delivered at an average of 35 ± 4 wk gestation, compared to 40 ± 1 wk among controls.

Malinow (80) assessed the influence of maternal homocysteine and serum folate at term (37–42 wk) on infant birthweight and gestation duration in 35 healthy nulliparas. They reported that higher maternal homocysteine was associated with significantly lower infant birthweight and shorter gestation while higher maternal folate correlated with increased birth weight (univariate analyses). A concentration gradient was detected; homocysteine declined from maternal vein to umbilical vein to umbilical artery by approx 1 μmol/L for each phase suggesting uptake of maternal homocysteine by the fetus.

### 4.4.3. OTHER NUTRIENTS

#### 4.4.3.1. Calcium

Another element that has received attention for its possible association with preterm delivery is a macromineral, calcium. During pregnancy, there is an increased physiologic demand for calcium. A full-term infant accretes about 30 g of calcium, primarily in the third trimester when the fetal skeleton is actively ossifying. To meet these needs, there is enhanced absorption of calcium from the gut (81,82). Diets low in calcium, both in general and especially during pregnancy, have been associated with an increased blood pressure levels because smooth muscle reactivity is heightened. During pregnancy, this results in an increased risk of PIH, hypothetically leading to preterm delivery. Calcium supplementation trials during pregnancy have been shown to lower blood pressure levels (83,84).

Two calcium-supplementation trials among high-risk women (women with very low intakes in Quito, Ecuador, and teenagers in Baltimore, MD) showed promising results in decreasing the incidence of preterm delivery (Table 1). In Ecuador (85,86), length of gestation was increased from 37.4 ± 2.3 wk for the placebo group (N = 34) to 39.2 ± 1.2 weeks (p < 0.01) for the calcium-supplemented group (N = 22). Among teenagers (aged 16 yr) in Baltimore, (87) with similar overall dietary calcium-intakes, the calcium-supplemented group had a lower incidence of preterm delivery (7.4%) compared with
the placebo group (21.1%, \( p < 0.007 \)). Further, life-table analysis demonstrated an overall shift to a higher gestational age in the calcium supplemented group (Table 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 PIH, but no effect on preterm delivery (84).

A metaanalysis (88) of 14 randomized controlled trials of calcium supplementation involving several thousand gravidas showed significant reductions in systolic and diastolic blood pressure with the administration of calcium salts. Risk of preeclampsia was reduced more than twofold among calcium supplemented women in these trials. An examination of the studies indicates that only two of nine examined yielded statistically significant findings. However, Bucher’s analysis yielded no effect of calcium intake on preterm delivery or fetal growth restriction (Table 1).

A large, randomized, double-blind placebo controlled trial of more than 5000 low-risk nulliparous women enrolled patients at medical centers throughout the United States, after prescreening them for compliance with supplement use (89). Women were supplemented with elemental calcium or placebo before midpregnancy and this was continued until they delivered. Calcium did not reduce risk of preeclampsia, or PIH or blood pressure. Likewise, calcium had no effect on obstetrical outcomes including preterm delivery or infant birthweight. However, it should be borne in mind that the women recruited into this trial had calcium intakes that were atypically high, averaging 1100 mg/d before supplementation. Thus, the ability of supplemental calcium to decrease the risk of preterm delivery may be confined to high-risk populations where there is either a dietary restriction of calcium intake or where, as in the case of adolescents, there is an increased demand for calcium both to meet the needs of the growing fetus and the mother herself.

4.4.3.2. N-3 Fatty Acids. Consumption of marine foods rich in n-3 fatty acids is also associated with a longer gestation duration. Ecologic data from the Faroe Islands, where 50% of the diet is derived from marine sources, show that island women bear infants with substantially higher birthweights (about 200 g) and longer gestations than the Danes (90). This difference in gestation was hypothesized to arise from a diminution in prostaglandin F2 and E2 production, or an increase in prostacyclin production with high consumption of n-3 fatty acids.

The clinical trial conducted by the People’s League of Health (91) on 5022 London women between 1938–1939 suggested that supplementing the usual British diet that, on the average, was not markedly deficient, with minerals, vitamins, and halibut oil extended gestation. Fewer infants were born before the 40th wk of gestation to the supplemented women (20% among supplemented born before wk 40 vs 24% among the unsupplemented) although there was no difference in mean birthweight.

A recent clinical trial (92) of 533 Danish women who received fish oil, olive oil, or no supplement by wk 30 gestation showed that women taking the fish-oil supplement had longer average gestations (4 d) and bore infants with higher average weights (+107 g), which was mostly attributable to the change in gestation. The effect of fish oil was strongest for women with low fish consumption at entry and amounted to an increase of 7.4 d in this group. There was little effect of the supplement on women with high consumption at entry (−1.6 d) (Table 1). Thus, the hypothesized effect of n-3 fatty acids on gestation appears to have a threshold, beyond which there is no effect. Furthermore, in this trial, the effect appeared to be specific to the mechanism underlying the initiation
of idiopathic preterm labor rather than PROM.

Finally, ecological studies have indicated that the consumption of large quantities of fish during pregnancy is associated with lower blood pressure and thus, in theory, with a lower incidence of PIH (93). Consistent with this is the reduction in the incidence of preeclampsia in the People’s League of Health study (91). However, because of the multiple supplementation regimen, it could not be attributed to the use of the fish oil per se. Thus, use of fish oils during pregnancy may, in some populations, potentially be associated with lower risk of indicated preterm delivery through a reduction in the incidence of PIH.

4.4.3.3. Multivitamin-Mineral Supplements. In the United States survey, data suggest limited supplement use by reproductive-age women; overall about one-quarter of women (26% white, 15.5% black) report regularly taking vitamin or mineral supplements (94). In one study (71), only 16% of low income Massachusetts gravidae took vitamins before pregnancy. This varied by ethnicity: with white gravidae (23%) reporting use about twice as frequently as blacks (11%) or Hispanics (10%). During pregnancy, 9% of whites, 20% of blacks, and 13% of Hispanics used prenatal vitamins erratically (1–3 times/wk) or not at all. Reasons for noncompliance included: maternal confidence that diet was good, an unstable home life, or side effects attributed by the women to the supplement.

In Camden, NJ, 17% of low-income minority gravidae reported using supplements before they were pregnant (95). Periconceptional use was more likely to occur among women with a history of an adverse pregnancy outcome, principally spontaneous abortion in past pregnancies, and was associated with increased spotting and bleeding during the current pregnancy. Thus, low-income women appeared to use supplements when they previously had or currently anticipated a problem with their pregnancies. Based upon data from the Maternal Infant Health Survey (96), other predictors of the failure to use vitamin/mineral supplements before or during include: being black, unmarried, under the age of 20 with less than a high school education. In Camden, multiparity and late entry to prenatal care were additional factors associated with supplement nonuse (95).

Information on the effects of prenatal multivitamin/mineral supplements on pregnancy outcome is limited. Camden women who use such supplements during pregnancy have 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). Prenatal supplement use was corroborated by assays of circulating micronutrients. At entry to care, there were no differences among women who went on to use supplements or not, whereas at wk 28, circulating levels of ferritin and folate were higher for supplement users (95).

Fawzi and colleagues (97) examined the effect of supplementing more than 1000 HIV-positive women from Tanzania with multivitamins, vitamin A, and placebo in a double-blind placebo-controlled trial. The trial also used a factorial design which allowed the quantification of the effect of multivitamins with and without vitamin A.

Multivitamin supplementation decreased the risk of very preterm delivery (approximate twofold reduction) along with decreasing risk of infant LBW and fetal growth restriction. The multivitamin supplement had the bonus of increasing CD4, CD8, and CD3 counts in the women. Vitamin A supplementation had no effect on pregnancy outcome or on T-cell counts.

A third study (98) examined multivitamin use from survey data (the 1988 National Maternal and Infant Health Survey). They reported no effect of multivitamin use on
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pregnancy outcomes including preterm delivery; multivitamin use also did not ameliorate the effect of maternal smoking on the fetus. However, these conclusions are limited by the fact that survey questionnaire was geared toward regular multivitamin use (3 d/wk or more) and did not differentiate women who used vitamins from those who did not. Thus, there was no control group—women not using multivitamins during pregnancy.

5. RECOMMENDATIONS

Although the relationship between nutrition and growth, both prenatal and postnatal, is well established, maternal nutrition has newly emerged as a risk factor for preterm delivery. As of yet, only a few observational and experimental studies have examined the influence of maternal diet and nutritional status on gestation duration and preterm birth; thus, for greater security, the relationship will require much further study. In many cases, the influence of maternal diet probably is indirect (e.g., poor diet gives rise to an inadequate gestational weight gain). In other instances, it is more complex. An indicator of poor maternal nutrition (e.g., inadequate third-trimester weight gain) may be a surrogate measure (for poor fetal growth), with an underlying factor (possibly maternal nutrition) giving rise to both the observed risk factor and the poor outcome (increased risk of preterm birth).

Further studies that group outcome measures according to the proximate causes of preterm delivery and target individuals (vs populations) at risk are required to determine whether poor maternal nutrition is a marker for or a cause of preterm birth. These studies should not focus primarily on macronutrients because evidence suggests that populations at high risk of preterm birth appear to have a poor-quality diet. For example, among minority groups from the inner cities of the United States, a high intake of fat (> 30% caloric intake from fat) is common, whereas the consumption of fresh fruits and vegetables and whole grains is virtually nonexistent (99). Likewise, lower-social-class women from London also eat diets that fail to meet “... basic maternal needs for a range of nutrients characteristic of whole grain, vegetable and fruit and dairy produce” (68). Thus, it may be that the poor of the industrialized world rely more on high-fat convenience-type foods that do not spoil readily, than on perishables with higher nutrient density. An alternative nutrient source, vitamin and mineral supplements, are used more frequently by the middle classes than the poor (71). Thus, as a corollary for clinical practice, it may be prudent to encourage women to eat a healthful diet and complement it with folate-containing multivitamin/mineral supplements if pregnancy is contemplated. And, because a poor diet rarely occurs in isolation, it is also important to seek prenatal care, and to maintain a lifestyle free of cigarettes, alcohol, and drugs.

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Dietary Polyunsaturated Fatty Acids for Optimal Neurodevelopment

Recommendations for Perinatal Nutrition

Ricardo Uauy, Patricia Mena, and Patricio Peirano

1. INTRODUCTION

Multiple studies over the past four decades have addressed the evaluation of effects of early human malnutrition on central nervous system (CNS) development in experimental animals and man. From the results of these studies, one can conclude that a reduction in energy and/or essential nutrient supply during the first stages of life have profound effects on somatic growth and organ structural and functional development, especially for the brain. Malnutrition impairs brain development, reducing cell replication cycles and dendritic connections. Different regions of the brain are impacted in specific ways; cell number as measured by DNA content is especially affected by intrauterine malnutrition and early postnatal malnutrition; synaptic connectivity is particularly affected if malnutrition occurs after birth, but before the third year of life (1). Beyond the “brain’s growth spurt,” alterations in dietary precursors may determine in part neurotransmitter levels (serotonin, norepinephrine, dopamine, acetylcholine) in specific brain regions, essential and nonessential lipid supply may affect the structural composition of the brain and of myelin sheaths (2).

The linkage between retarded somatic growth and altered brain development is strong if the nutritional deprivation model is one of early protein energy malnutrition (PEM). This principle has guided many experimental studies and epidemiological evaluations of the nutrition mental development relationships. Yet, there are multiple instances where somatic growth may proceed unabated, although brain structure and functions are significantly altered. The effect of early anemia on brain function does not affect somatic growth; the impact of taurine deficiency on retinal and brain development on nonhuman primates and on human infants is not dependent on structural proteins because this sulfur amino acid is not incorporated in protein synthesis; the role of n-3 fatty acids as structural components of brain is not associated with effects on growth, but rather on modifying membrane function and electric responses. Examples in the opposite direction, namely of normal mental development and poor growth are
more difficult to find, suggesting that somatic growth is a necessary, but insufficient, condition to attain normal mental development. Early malnutrition from pyloric stenosis or cystic fibrosis illustrate the capacity of the brain to recover from malnutrition, but are not by themselves sufficient to negate the effect of sustained nutritional deprivation on the developing CNS. An important contribution to the approach of the relationship between nutritional deficiencies and brain development has been the concept that direct effects of PEM on child development seems no longer evident, because PEM coexists with other nutritional deficiencies that can also disrupt child development (3).

The study of the influence of specific nutrients on brain development in preterm neonates is in its infancy. Fetal growth retardation is not an isolated nutritional deprivation, but rather a combination of many restrictions: oxygen, blood supply, overall nutrients, and various growth factors and hormones. It is likely that better nutritional practices in early life are contributing significantly not only to improved survival, but also to better growth and development of very immature infants. Yet this rather obvious conclusion is not well substantiated by objective data. One of the few controlled observations in premature infants revealed that head size at 12 mo was related to the time after birth when very low birthweight (LBW) infants had reached full caloric intake. Because the relationship between head size and later development in preterm infants has been established, this is indirect evidence for an effect of better nutrition on developmental indices in later life. Additional supportive evidence can be derived from a study of 526 full-term born in Groningen, Holland between 1975 and 1979 and followed for 9 yr. There was a significantly lower occurrence of neurological dysfunction in the 135 breast-fed infants (fed for more than 3 wk) after adjusting for obstetric, perinatal, neonatal neurological, and social differences (4). Randomized studies by A. Lucas in preterm infants fed differently, have confirmed the long-term effects in IQ of even a 1-mo nutritional intervention. Male infants are biologically more vulnerable, they have a lower IQ at age 7–8 yr of age, if fed a routine formula as compared to those receiving a nutrient-enriched preparation. In addition, the risk of cerebral palsy and a verbal IQ < 85 was lower in the group fed the enriched formula. It is possible that suboptimal nutrition does not permit full CNS recovery after an early insult (5–7). As an example of the complexity of this problem, the increase in protein intake in preterm infants required to optimize somatic growth as studied in the late 1940s was associated to lower IQ scores in later life (8). The classic Harlem Columbia study revealed that higher protein intake fed to pregnant women who where at risk of delivering LBW was in fact associated with increased prevalence of prematurity and to lower developmental indices at 12 mo (9). The research on the effects of energy and caloric intake is reviewed in classic publications (1–3,10). This chapter primarily addresses the role of essential PUFAs on neural development in the fetus, preterm, and term infants.

2. BACKGROUND ON THE ESSENTIAL PUFAS AND THEIR METABOLISM

2.1. Essentiality of Polyunsaturated Fatty Acids

The concept that specific components of fat may be necessary for the proper growth and development of animals and possibly humans was introduced in 1929 by George and Mildred Burr (11). The essential fatty acids (EFA) were considered of marginal nu-
tritional importance for the human until the 1960s when clinical signs of EFA deficiency became apparent in infants fed skim milk-based formula and in those given lipid-free parenteral nutrition. Hansen firmly established that linoleic acid (LA) is essential for normal infant nutrition in a clinical and biochemical study of 428 infants fed cow’s milk-based formulations with different types of fat (12). Daily LA intake of study infants ranged from 10 mg/kg while fed a fully skimmed milk preparation to 800 mg/kg when a corn/coconut oil-based preparation was fed. He observed dryness, desquamation, and thickening of the skin and growth faltering as frequent manifestations of LA deficiency in young infants. More subtle clinical symptoms appear in n-3 EFA deficiency. They include skin changes unresponsive to LA supplementation, abnormal visual function, and peripheral neuropathy (13,14). The nervous system manifestations of n-3 deficit are likely caused by an insufficiency of the specific metabolic derivative of LNA, namely DHA. Indeed, the high concentrations of DHA in cerebral cortex and retina would suggest its participation in neural and visual function (15,16).

2.2. LCPUFA Metabolic Pathway

The structural role of long-chain polyunsaturated fatty acids (LCPUFA) derived from EFA and the functional correlates of specific fatty acids are being increasingly recognized. The LCPUFAs arachidonic acid, (AA [20:4 n-6]), eicosapentaenoic acid (EPA [20:5 n-3]), and docosahexaenoic acid (DHA [22:6 n-3]) are important membrane components and precursors of potent bioactive oxygenated products. Eicosanoids, such as prostaglandins, leukotrienes, and epoxides derived from AA and EPA modulate or are required in numerous physiologic processes, a myriad of clinical correlates associated with deficient or excessive EFA intake have been observed (17-21).

Animal tissues, especially the liver, are capable of further elongating and desaturating the parent EFAs generating a family of compounds for the respective families as shown in Fig. 1. As depicted in the figure, AA can be formed from LA, it becomes essential only if the capacity for elongation and desaturation of linoleic acid is limited. This in fact occurs in the cat and other felines. Further details on EFA metabolism can be found in referenced reviews (20–23). The competitive desaturation of the n-3, n-6, and n-9 series by δ-6 desaturase is of major significance because this is the controlling step of the pathway. If n-3 FAs are absent or deficient in the diet the elongation/desaturation of the n-6 compounds generates a significant elevation of docosapentenoic acid [DPA (22:5n-6)]; if both EFAs are lacking eicosatrienoic acid (ETA) [20:3 n-9] accumulates (22,23).

The conversion of parent EFAs to LCPUFA is under active regulation, therefore, providing the equivalent amount of LA or LNA (24,25) cannot reproduce the effects of providing AA, EPA, or DHA. The uniqueness of the biological effects of feeding human milk on EFA metabolism is based on the direct supply of LCPUFAs bypassing the regulatory step of the δ-6 desaturase. Excess dietary LA associated with some vegetable oils, particularly safflower, sunflower, and corn oils, may decrease the formation of DHA from LNA because the δ-6 desaturase is inhibited by excess substrate. In addition, AA formation is lower when excess LA is provided (22–25). The inhibitory effect of EPA on δ-5 desaturase activity has been considered responsible for the lower AA observed when marine oil is consumed. Excess LA, as seen in enterally or parenterally fed infants receiving corn oil or safflower oil as the predominant FA supply, will inhibit the elongation/desaturation of the parent EFAs and thus lower the LCPUFA supply neces-
3. STUDIES OF ESSENTIAL FATTY ACID METABOLISM USING STABLE ISOTOPE PRECURSORS

Using stable isotope-labeled precursors, several groups have evaluated the formation of LCPUFA in term and preterm neonates. We administered a single dose of 50 mg/kg of deuterated precursors by oral route, labeled precursors and products were measured in plasma using negative ion mass spectrometry of pentafluorobenzyl derivatives. Peak concentrations of labeled precursor EFA were reached during the first day after dosing, deuterated products increase concentrations with time reaching peak by 48 h in the term infants and closer to 96 h in the preterm infants. The ratio of concentration of labeled products relative to precursors, and indexes of metabolic conversion, is in the order of 1–100 for the n-6 series and 1–40 for the n-3 series. There is also evidence for metabolic retroconversion in these studies. The results of all these studies suggest greater biosynthesis of LCPUFA at younger gestational and postnatal ages. Alternatively, the data indicate a reduced turnover rate for LCPUFA with advancing age, this could be related to the decrease in growth rate and overall metabolic rate during the first year of life. These results are limited by the lack of evaluation of tissue pools, especially neural tissue accretion. High activity of δ-6 desaturase in neural tissues suggests a high LCPUFA synthesis in those tissues. However, present information on both chemical composition and functional assessment indicates that endogenous synthesis from precursors is insufficient to meet the needs imposed by rapid growth, specially in premature infants. The study of infants who died suddenly of an unexplained cause has served to document a strong
correlation between the composition of the brain cortex and RBC total lipids and that brain cortex composition is clearly affected by early dietary LCPUFA supply (33, 34).

### 3.1. Effects of PUFAs on Neural Membrane Function

The dry weight of the human brain is predominantly lipid; 22% of the cerebral cortex and 24% of white matter consist of phospholipids. Studies of several animal species and recent evidence from humans have established that brain phospholipid AA and DHA decrease, although n-9 and n-7 mono- and polyunsaturated fatty acids increase when LA and LNA or only n-3 fatty acids are deficient in the diet (25, 33–35). Typically, n-3 fatty acid deficient cells have decreased DHA and increased levels of the end product of n-6 metabolism, DPA. Within the subcellular organelles, synaptosomes and mitochondria seem to be more sensitive to a low dietary n-3 supply as evidenced by the relative abundance of DHA and the changes in composition of these organelles in response to dietary deprivation.

The functional implications of diet-induced changes in structural lipids have been the subject of much research (36–38). Fatty acid composition of structural membrane lipids can affect membrane function by modifying membrane fluidity, affecting membrane thickness, changing lipid phase properties, or by specifically interacting with membrane proteins (36–40). The changes in neural membranes of greatest potential significance during development are those related to changes in physical properties and to changes that affect membrane excitability. Diet-induced changes in structural lipids affect the functional characteristics of excitable membranes in several animal species and in human neural cell lines (41–42).

A deficiency of n-3 fatty acids can change membrane physical properties, including membrane-bound enzymes, receptor activity, antigenic recognition, and signal transduction (43, 44). Also, n-3 fatty acid deficiency affects rotational mobility, as measured by fluorescent diphenylhexatriene probes, less than lateral mobility, as measured by pyrene dimer formation within the lipid bilayer (45). We found that excited dimer (eximer) pyrene formation in human retinal cells, which occurs when two monomers collide, is increased by DHA supplementation of the culture media (46). Membrane composition changes in human retinoblastoma and neuroblastoma cell lines also affect membrane transport mechanisms. A higher DHA content increases the affinity and transport rates for choline and taurine (47). Furthermore, preliminary studies suggest that the dietary n-3 fatty acid supply may affect nucleotide cyclase activity and rybosylation of guanine nucleotide-binding proteins (48).

The role of membrane lipid composition in determining the electrical properties of cultured neuronal cells exposed to exogenous fatty acids has been investigated (49, 50). Both n-3 and n-6 fatty acids induced slower rates of rise, and to a lesser extent, lower amplitude of Na+ action potentials. The reverse effects were observed when saturated or trans-monoenoic fatty acids were added. It seems likely that these effects were mediated by a change in the number of active Na+ channels, a change in membrane composition or altered fatty acid availability to the cells may explain these findings (49). These functions are very important for brain development. LCPUFA have a role in growth-related events in the neurons and also affect the development of synaptic processes for neural cell interaction. DHA is a major constituent of synaptic end sites, whereas AA is present in growth cones and synaptosomes (51). AA is preferentially released from membrane phospholipids by the action of endogenous phospholipase A2, and participates in signal transduction events that regulate growth cone activity and ul-
ultimately conversion of growth cones to mature synaptic endings (52). DHA plays a role as a structural component of the membranes, particularly at the synapse site, where membrane microenvironment influences neurotransmitter uptake and release (42). AA and DHA also affect the process of interaction between nerve cells during development, by activating the transcription of genes responsible for brain lipid-binding protein. This protein is crucial in a signaling pathway of the response of glial cells to neurons, DHA binding regulates its activity (53). AA is also released, specifically by glutamate and N-methyl-D-aspartate by stimulating calcium depending phospholipase A2. These receptors have been implicated in long-term potentiation, which is considered a likely mechanism for memory and learning (54). In addition, fatty acids, specifically, DHA affect the expression of genes that regulate cell differentiation and growth of retinal neuronal differentiation (55).

3.2. Sources of Dietary Essential PUFAs

The principal source of PUFA for infants is human milk (HM). The amount of PUFA in HM depends, principally, on mother’s diet during pregnancy. It lactation and varies according to postpartum age, preterm or term delivery, and maternal diseases affecting lipid metabolism, such as diabetes, cystic fibrosis, and abetalipoproteinemia. AA is the main n-6 PUFA and DHA is the most important of the n-3 series. The ratio of total n-6 to n-3 is 5–10:1, ranging up to 18:1 if high linoleic acid oils are consumed. The ratio of AA to DHA is most commonly 1.5–2:1. The variability in LCPUFA in HM is high and determined mainly by diet. EPA is found in minimal amounts except in populations consuming high fish intake (56). DHA levels range from close to 0.1% reported from Germany to 1.4% in Inuits of North America; however, typical values range from 0.3% to 0.4% (23,56). Makrides reported a longitudinal reduction in breast milk DHA from Australian women on Western diets from 0.32% in 1981 to 0.21% in 1995 (57). A higher fish consumption in the maternal diet or DHA supplementation can increase DHA content within a short time frame (58,59).

Vegetable oils derived from corn, safflower, and sunflower contain predominantly LA; others, such as soy and linseed oils, contain LNA. This latter fatty acid has higher concentrations in chloroplast membranes of green leafy vegetables rather than in oily seeds. Thus products from animals fed in the wild have different fatty acid composition than grain-fed animals. This is of interest in terms of the higher DHA content of eggs from range-fed chicken. The introduction of egg yolk as a weaning food in some areas of the world may represent a useful dietary practice to assure LCPUFAs supply in early infancy. In addition, the use of evening primrose oil or black current oil provides 18:3 n-6 γ linolenic acid (GLA) thus bypassing the controlling enzyme, δ-6 desaturase, necessary for AA formation. This latter approach has been used by some formula manufacturers to improve AA status in young infants.

n-3 polyunsaturated fatty acids of marine origin are formed in the chloroplast of the phytoplankton or microalgae consumed by fish. The main source for the de novo synthesis of n-3 fatty acids are marine autotrophic bacteria, microalgae, and protozoa, which constitute the zooplankton and phytoplankton. Fish, higher in the food chain, incorporate the n-3 PUFA and further elongate them to 20 and 22 carbon atom fatty acids containing 4, 5 and up to 6 double bonds by the action of specific desaturases. Thus, fish will concentrate EPA and DHA as triglycerides, mainly in the adipose tissue and in the fat of muscle and visceral organs. The higher fat content of fish, the higher its con-
tent of n-3 fatty acids. Some marine mammals, such as seals and polar bears, which feed predominantly on fish, also accumulate relatively high quantities of n-3 fatty acids in their adipose tissue (60,61). Another important source of LCPUFA is egg yolk phospholipids. The concentrations of PUFA is different depending on the feed given, the ample use of fish meal in chicken feed has increased egg yolk DHA (63). LCPUFA products for blend in infants formulas can be successfully produced if chicken feed is carefully monitored and refined lipid extraction procedures are used. This is presently an important LCPUFA source used in European infant formulas.

Bacterial strains and microalgae isolated from the intestinal content of some fish show a remarkably high content of EPA and DHA, therefore efforts have been made to grow these microorganism in natural or artificial sea water to obtain EPA and DHA for nutritional or pharmacological use. In addition, selected fungal strains produce concentrated arachidonic acid which is suitable for human consumption (63–65). The industrial production of AA, EPA, and DHA from strains of these single-cell organisms has been successful, however their expanded use will depend on price and demand for them relative to the concentrates obtained from marine oils. Single cell oils offer a promising new source of LCPUFAs provided mass production becomes commercially profitable (65). Table 1 summarizes the main sources of LCPUFAs for use in supplementation.

The definition of what to feed is not simply answered by deciding how much do breast-fed babies receive on the upper range, the lower range, or the midpoint of LCPUFA content of human milk. Rather, it must be based on functional response to a given level of DHA. If the effort is primarily focused on demonstrating efficacy, selecting a value in the upper range is preferable. On the other hand, if serious safety concerns were an issue, selecting a value in the lower range would be more appropriate. Supplementation of formula fed preterm with graded levels of AA (0–1.1%) and DHA (0–0.76% of total fat) compared to human milk-fed babies demonstrated that plasma and RBC LCPUFA levels can be mimicked by supplementing formula at levels of 0.54% AA and 0.3% DHA (58,66).

4. PUFA IN HUMAN GROWTH AND DEVELOPMENT

4.1. Significance of PUFAs During Fetal Development

The fetus and the placenta are fully dependent on maternal EFA supply for their growth and development. The major fat deposition in the human fetus occurs during the third trimester, but key phospholipids in placental vessels and uterine vasculature are dependent on EFA supplied by the mother for eicosanoid formation from the moment of conception (67,68).

Maternal dietary LA and LNA supply serve as precursors for n-3 and n-6 LCPUFA synthesis by the maternal liver. The placental transfer of fatty acids is regulated in part by the transplacental fatty acid gradient. Serum albumin concentration and (α) fetoprotein have a high binding affinity for free fatty acids, thus may be important for placental fatty acid transfer (69,70). Mammalian fetuin is a placental protein with a 50-fold greater efficiency in binding fatty acids relative to albumin. Lipoprotein lipase on the maternal surface of the sincipitotrophoblast hydrolyzes maternal triacylglycerol releasing free fatty acids. Fetal erythrocytes also appear to perform a significant role in the placental DHA transfer (70). There is a progressive enrichment in the concentration of AA and DHA in circulating lipids in the fetus during the third trimester, at a time when
fetal demands for vascular and especially neural growth are greatest (71). Significant increases in the AA and DHA content of fetal brain tissue during the last trimester of gestation and initial postnatal months have been observed (72). A total of 600 g of EFA are transferred from mother to fetus during a full-term gestation; net uptake approx 2.2 g/d. AA and DHA are supplied to the fetus from the maternal diet and by endogenous fetal biosynthesis (liver desaturation/elongation).

Studies conducted in different populations using similar methodology have shown that the pattern of change in fatty acid composition during gestation and pregnancy is similar across different ethnic/diet groups (73). Populations with higher maternal plasma concentrations of n-6 fatty acids have lower n-3 content and vice versa. DHA concentrations decrease during late gestation, unless mother diet is very high in DHA. N-6 cord levels in term infants are less dependent on maternal levels than cord DHA. Most n-3 fatty acids, which come into the fetal circulation, will be accrued by the fetus despite low maternal n-3 concentrations. A need for adequate dietary LCPUFA during pregnancy is suggested by these results. This may be particularly important for populations with low n-3 EFA intake, for multiparous women, or during multiple pregnancies (68,74). The changes in maternal fatty acid plasma and red-cell content are paralleled by changes in fetal levels measured by cordocentesis. The latter are similar to values obtained at birth except for a significantly higher cord LA level measured during centesis (71). The results confirm that fetal DHA relative blood content and absolute concentration increase with gestational age. In the case of AA, levels increase or decrease with gestation depending on study, although there is a better positive correlation with birthweight than that observed with DHA (75,76). Differences in fatty acids profiles at 34-wk gestation in women delivering preterm and those delivering term babies have been noted. Red blood cell and plasma AA are higher in mothers delivering preterm babies. The comparison suggests that maternal AA mobilization and availability may be altered in women delivering preterm. Preterm maternal n-3/n-6 ratios suggests that perinatal n-3 EFA metabolism plays an important role in preterm birth (77). The fatty acid profile of preeclamptic pregnant women shows a lower unsaturation index, and is particularly low in n-6 LCPUFAs relative to controls (78). The PUFA status in pregnancy is of special significance since prostaglandins play a key role in the onset of labor, they also induce abortion and labor. AA in amniotic fluid increases at the initial process of human parturition. The use of marine oil supplementation has been proposed in the prevention of preeclampsia because maternal blood pressure responses are

Table 1
Sources of PUFAs for Use in Infant Formula

<table>
<thead>
<tr>
<th>Source</th>
<th>n-6</th>
<th>n-3</th>
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<tbody>
<tr>
<td>Black current seed oil</td>
<td>GLA</td>
<td>GLA</td>
</tr>
<tr>
<td>Borage oil</td>
<td>GLA</td>
<td>GLA</td>
</tr>
<tr>
<td>Egg phospholipids</td>
<td>AA</td>
<td>DHA</td>
</tr>
<tr>
<td>Evening primrose oil</td>
<td>GLA</td>
<td>EPA/DHA</td>
</tr>
<tr>
<td>Fish oil</td>
<td>DHA</td>
<td>DHA</td>
</tr>
<tr>
<td>Marine oil fractions</td>
<td>AA</td>
<td>DHA</td>
</tr>
<tr>
<td>Animal phospholipids</td>
<td>AA</td>
<td>DHA</td>
</tr>
<tr>
<td>Single-cell oil</td>
<td>AA</td>
<td>DHA</td>
</tr>
</tbody>
</table>
dependent on AA/EPA balance in the vessel wall. EPA derived prostanoids oppose the action of AA derivatives, namely EPA has vasodilatory effects and decreases rather than enhances platelet aggregation. Epidemiologic studies and a randomized controlled trial of fish oil supplementation in normal pregnancy have demonstrated prolonged gestation with higher birthweight in the n-3 supplemented. Despite the lack of efficacy of fish oil supplements given in the second trimester of gestation in reducing the risk of preeclampsia, IUGR, and preterm labor, the potential benefit of supplementation given periconceptionally remains open.

4.2. Significance of PUFAs for Preterm Infants

The brain growth and LCPUFA deposit during the last trimester of pregnancy suggest that preterm infants may be especially vulnerable to insufficient dietary essential fatty acid. Preterm infants show a progressive decrease in LCPUFA of plasma and red blood cell lipids, especially if the early diet lacks LCPUFA. Human milk-fed infants receiving DHA and AA in formula have higher levels in plasma, red blood cells, and buccal mucosal cells.

4.2.1. Controlled Studies in Preterm Infants

We have studied preterm infants receiving HM or randomly assigned to formulas with different EFA content from 30-wk gestation to early infancy. The functional impact of n-3 fatty supplementation included enhanced maturation of the rod photoreceptor responses in LCPUFA supplemented infants, mimicking matched human milk-fed preterm infants of similar postconceptional age (36 wk). By 57 wk, a time when retinal development is nearly complete, the difference in photoreceptor function was not apparent, except for changes in oscillatory potentials, which reflect inner retinal signal processing. Visual acuity test throughout the 6-mo study were also less mature in infants receiving formula devoid of n-3 LCPUFA despite ample provision of LNA. The LCPUFA supplemented group had significantly better visual acuity as measured by VEP and FPL than the control group. Highly significant correlations were found for both VEP and FLP visual acuity when compared to the level of DHA in multiple lipid fractions from study infants. In our more recent studies conducted in Chile, no LCPUFA effect on auditory brain-stem evoked responses were demonstrated, this is in agreement with Faldella. There was an increased maturation of the sleep–wake cycle in terms of less indeterminate sleep and more quiet (non-REM) sleep, in the human milk-fed compared with the randomized formula-fed groups. Bayley scale at 18 mo demonstrated better developmental indices in the human milk-fed, but no differences within formula-fed groups. The findings in developmental indices suggest that environmental influences may have a greater effect than the early diet.

Carlson’s randomized clinical study in preterm infants supplemented with LCPUFA, demonstrated better visual acuity using behavioral measures in infants up to 4 mo of age. After this time, control infants “caught-up” in visual function measures. These investigators also report evidence of more rapid visual processing as measured by the Fagan test of visual recognition at 6–12 mo of age in LCPUFA supplemented infants. The reduction in AA, when fish oil was provided as a source of n-3 fatty acids, was associated with reduced weight and length growth. In a second preterm infant study using low EPA marine oil for up to 2-mo corrected age, Carlson demonstrated improved visual development at the 2 mo follow-up and a 10-point IQ difference favoring the DHA supplemented group at 12 mo. No significant drop in AA or deleteri-
ous effects on growth was observed when low EPA marine oil was used. The DHA supplemented group had shorter look times in the novelty preference test at 9 mo, suggesting better visual processing.

4.3. Significance of PUFAs for Term Infants

Similar questions to those addressed in the previous section on LBW infants have been posed more recently in healthy full-term infants. The finding of lower plasma DHA concentrations in infants fed formula compared to that of breast-fed infants suggests that present formulas provide insufficient LNA or that chain elongation-desaturation enzymes are not sufficiently active during early life to support tissue accretion of DHA. Furthermore, necropsy studies in infants born at term who died from sudden infant death syndrome, revealed brain composition is affected by type of feeding. HM feeding resulted in higher DHA content in the brain cortex as compared to cow’s milk-based formula feeding (33,34,97).

The first controlled randomized study compared infants fed a formula supplemented with 0.36% DHA, a formula providing ample LNA, but no DHA and a breast-fed reference group. This study revealed delayed visual acuity at 4 and 6 mo of age in the group on formula lacking DHA, relative to those receiving formula supplemented with LNA + DHA, these latter infants were similar to the breast-fed control group (98).

Studies by Innis (99–101) concluded that the need for DHA supplementation should not be based on the alleged improved visual acuity of breast-fed infants because, despite differences in DHA content in blood, no differences in preferential looking acuity between BF and unsupplemented formula-fed infants were found. The formula used in this study contained 2.1% of total fat as LNA, but no LCPUFAs. On the other hand, a multicenter trial using formula supplemented with 0.2% DHA and 0.4% AA did not show an effect of this level of supplementation on visual acuity, neither on growth (102). A study with different levels of LNA, 0.4–3.2% of total lipids in term infants did not find effect over transient visual evoked responses at 120 and 240 d of age (103). In a small trial with LCPUFA-supplemented formula and breast milk infants, the visual acuity measured by swept steady-state visual evoked potential was different. The LCPUFA supplemented group was better than standard formula and worse than breast milk infants (104).

Recently, we have shown a persistent effect in visual acuity development during the first year of life in HM and in DHA-supplemented formula-fed infants, compared with infants fed with formula without LCPUFA during the first 17 wk. The formula was supplemented with 0.35% DHA with or without 0.72% of AA from single-cell oil sources. The dietary effects on visual acuity development were evident during the first year of life using the more accurate sweep VEP acuity, but were not demonstrable using the behavioral testing of acuity. The difference in visual acuity were significantly correlated to the DHA content of red blood cell phospholipids (105). A beneficial effect of DHA, AA, and GLA supplementation on psychomotor development at 4 and 12 mo assessed by the Brunet-Lezine method has been reported (106,107), but not at 24 mo of age. These authors report a strong association between the erythrocyte phosphatidylcholine arachidonic /linoleic acid ratio and developmental quotient at 24 mo, but there is no relation with the dietary intervention in the first 4 mo of age (108). A randomized trial in term infants fed formula containing no LCPUFA, DHA, or AA + DHA found no differences on 12 mo Bayley Scale scores, but found a lower language comprehension and production scores measured on the MacArthur Communicative Development Inventory
in the DHA group, compared to the AA + DHA supplemented group (109). These children have now been followed to age 3 yr and do not show differences on psychomotor development and vocabulary (110).

Willats studied 44 term infants fed with a DHA and AA supplemented formula or a non-LCPUFA formula during the first 4 mo. Infant cognitive behavior was assessed at 10 mo of age by a means-end problem-solving test. The LCPUFA-supplemented group had significantly more intentional solutions and scores than infants with non-LCPUFA formula. Higher problem-solving scores in infancy are related to higher childhood IQ scores (111). In an interesting model to study effects of DHA present in HM, Gibson supplemented mothers to produce breast milk with DHA concentration ranged from 0.1 to 1.7% of total fatty acids. Infant plasma and erythrocytes phospholipids was related with breast milk DHA in a saturable manner. Specifically, there was no further increase with breast milk DHA above 0.8% of total fatty acids. At 12 mo, the developmental quotient had a low, but significant, correlation with erythrocytes DHA. This correlation was not evident at 24 mo (58). In a 3-yr follow-up of visual acuity maturation study, we found formula-fed full-term infants had lower operant preferential looking (OPL) than HM fed infants (112). The cohorts were breast fed from birth to at least 4 mo or fed for 12 mo formula containing ample LA and 0.5% of the total fat as LNA. The breast-fed group was weaned to an oleic acid (18:1)-predominant formula and received egg yolk through 12 mo of age. The breast-fed group maintained higher plasma and RBC membrane phospholipid DHA concentrations throughout the first year of life. At 3 yr of age, stereo acuity, as measured by OPL techniques, was more mature in the breast-fed infants relative to the formula-fed group; 92% of the breast-fed group had mature OPL stereo acuity whereas 35% of the infants in the formula-fed group met the maturity criteria. Visual recognition in the breast-fed group was also better, whereas only 61% of the formula-fed infants had a perfect score, 93% of the breast-fed group had a perfect score (112). Human milk provides a unique nutrient mix, hormones, and special growth-promoting factors; these, as well as LCPUFAs, may play role in CNS development. Other observational studies with breast-fed and formula-fed infants in relation to cognitive development have been published since 1929 (113–123). A long-term effect of early feeding on brain development is suggested by results from the controlled trial of preterm infants indicating that feeding HM which contains LCPUFAs by nasogastric tube for 28 d was associated with a + 8.3-point IQ difference at 8 yr of age relative to a formula-fed group (devoid of LCPUFAs) after controlling for socioeconomic and other maternal variables (5). These controlled observations in preterm infants indicate that HM may, in fact, offer unique advantages for brain development. Except this study, done with HM by tube feeding, all the others has been done with not-randomized breast-fed infants.

Mothers who succeed at breast feeding are different from those who feed formula: they have higher socioeconomic status, better educational level and educational achievement, higher intelligence, less symptoms indicative of maternal depression, and greater preoccupation with infant development. The act of feeding provides a mode of mother–infant interaction, contribute to bonding and may enhance cognitive development. The most recent study using extensive covaried adjustment showed that the benefit of breast feeding for cognitive development at 11 yr of age was no longer significant after maternal IQ and home environment were incorporated into the analysis (123).
In addition, the follow-up of phenylketonuric infants, treated with early protein restriction, may also serve to evaluate the effect of LCPUFA deficit. The impact of DHA supplementation on functional development is presently in progress (124). In peroxisomal diseases, there is an alteration of the n-3 partial β-oxidation necessary to produce DHA. The relevance of DHA supplementation in this condition may be especially relevant if less generalized disease is present (125). The benefit of DHA supplementation in patients with peroxisomal diseases suggests that DHA is also involved in myelogenesis, because there is an improvement of myelination in MRI after long-term DHA supplementation (126). This could be of potential importance in white matter disease of micropremies.

4.4. Recommendations for Formula-Fed Infants

Because we recognize not only n-6 but also n-3 as essential nutrients for premature infants, the following provisional recommendations are suggested for the formula-fed infant for the first 6 mo of life:

1. The total EFA requirement (n-6 + n-3) for premature infants should be set at 5–6% of total energy although up to 13% can be provided safely. This represents approx 0.6–0.8 g/kg/d with an upper limit of 1.5 g/kg.

2. The parent n-6 EFA (linoleic acid) supply should be 0.5–0.6 g/kg daily and because desaturase and elongating enzymatic activity may be limited in the premature infant, formulas for these infants should provide 60–100 mg/kg/d as preformed arachidonic acid.

3. The total n-3 FA supply should be 70–150 mg/kg daily. Because desaturase and elongase enzyme activity may be limited in the premature infant, formulas for these infants should provide 35–70 mg n-3 long-chain per kg body weight/d, as DHA.

4. The total linoleic acid supply should not exceed 12% of total energy since excess linoleic acid may adversely affect the formation of LCPUFAs.

5. The ratio of total n-6 to n-3 FA, present in the early diet should be maintained within a range of 5:1 to a maximum of 15:1. The ratio of DHA to AA should be from 1:1 to 1:2 because excess DHA may lower conversion of linoleic acid to AA.

5. CONCLUSIONS/RECOMMENDATIONS

Studies presented in this chapter provide clear evidence that dietary n-3 fatty acid deficiency affects eye and brain function of preterm infants as measured by ERG, cortical visual evoked potentials and behavioral testing of visual acuity. Preterm infants require DHA in their diet because they are unable to form these in sufficient quantity from LNA provided by soy oil-based formula products. Dietary n-3 and n-6 fatty PUFA supply results in discernible differences in the fatty acid composition of plasma and RBC membrane lipids. Changes in membrane chemical structure are likely responsible for the observed functional effects. The evidence from term infants is controversial. Some, but not all, studies find that DHA-supplemented group obtains a better, transient, visual acuity, and eventually a better cognitive development. In any case supplementation with both DHA and AA has not been demonstrated to induce any adverse effects. Long-chain PUFAs have demonstrable benefits during development. The effects on neural development are of particular interest. Human milk is the best and only time-proven source of fat and EFAs in the infant diet. Technological procedures based on chemical and physical separation of the unsaturated fatty acids have permitted the elaboration of concentrated EPA, DHA, and AA for clinical use. The development of single-cell oil sources has
allowed the provision of novel forms of LCPUFA delivery. Before the 1990s, low LNA was found in most infant formulas, by now virtually all infant formula in developed countries is supplemented with LNA and several manufacturers in Europe, Australia, Asia, Latin America, and Japan have added DHA, DHA plus AA, or also included GLA in preterm and term formula. The public health and cost benefit implications of these changes need to be fully evaluated in order to support this practice on a global scale.

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97. Ghebremsikel K, Leightfelds M, Leaf A, Costelone K, Crawford M. Fatty acid composition of
plasma and red cell phospholipids of preterm babies fed on breast milk and formulae. Eur J Pediatr
98. Ghebremsikel K, Leightfelds M, Leaf A, Costelone K, Crawford M. Fatty acid composition of
plasma and red cell phospholipids of preterm babies fed on breast milk and formulae. Eur J Pediatr


GLOBAL PREVENTIVE NUTRITION STRATEGIES
1. INTRODUCTION

Infectious diseases and malnutrition were the major medical problems in most Asian countries before World War II and are still so today in some parts of Asia. The structure of diseases has been dramatically changed in many Asian countries after World War II, in parallel with a rapid development of the economy followed by Westernization of lifestyles. The GNP has been increased by 20 times in Japan, 43 times in Korea, and 17 times in Thailand in the past 25 yr and lifestyles in housing, clothing, and eating have been westernized in these countries. Westernization of diets is seen in an increase of protein and fat consumption at meals and the prevalence of American-style fast foods, such as hamburgers and fried chicken. In Japan, Korea, and Thailand, the three leading causes of death today are neoplasms, heart disease, and cerebrovascular disease, as they are in Europe and North America. The cost for cardiovascular disease in Japan in 1996 was $48 billion, accounting for 7% of the national budget. Chronic diseases resulting from atherosclerosis are not only a medical, but also a growing economic problem in Asian, as well as Western countries. Thus, more attention is required, aimed at prevention of risk factors for these chronic diseases by nutritional intervention. In this chapter, the relationship between Westernization of diets and chronic diseases based on atherosclerosis or their risk factors in Asian countries is reviewed. Nutritional recommendations are described in reference to prevention of risk factors for chronic diseases based on atherosclerosis.

2. EPIDEMIOLOGY OF CHRONIC DISEASES AND THEIR RISK FACTORS

2.1. Chronic Diseases

Heart disease, which develops from atherosclerosis and/or hypertension, has been linearly increasing year by year and has recently become one of the major causes of death in Japan, Korea, and Thailand (1). Although the death rate from ischemic heart disease has increased severalfold in Japan and Korea in the last 20–50 yr, it is still sub-
stantially lower than in Europe and North America; 9.9/100,000 in 1950 to 46.7/100,000 in 1994 in Japan (2), and 2.2/100,000 in 1983 to 12.5/100,000 in 1992 in Korea (3) (Fig. 1). In Thailand, the death rate from heart disease increased from 40.3/100,000 in 1987 to 56.0/100,000 in 1992, a period of only 5 yr. The prevalence of ischemic heart disease in one Thai community serially studied increased from 7/1000 in 1976 to 17/1000 in 1983 (4). Cerebrovascular diseases are also emerging as a leading cause of death in Japan and other Asian countries (1) (Fig. 1). Although the death rate from total cerebrovascular diseases in Japan has gradually decreased during the past 25 yr, the death rate from cerebral infarction and its contribution to total cerebrovascular diseases have greatly increased over 40 yr; from 4.0/100,000 in 1950 to 54.4/100,000 in 1994 (2) (Fig. 1). In Korea, the death rate from cerebrovascular diseases increased from 65.4/100,000 in 1983 to 80.4/100,000 in 1992 (3). In Thailand, the death rate from hypertension and cerebrovascular diseases increased from 12.8/100,000 in 1987 to 16.9/100,000 in 1992 and the prevalence of hemiplegia as a result of cerebrovascular diseases in one town increased from 1.5/1000 in 1976 to 6.6/1000 in 1983 (4).

2.2. Risk Factors

Both hypercholesterolemia and hypertension are now proven to be major risk factors for ischemic heart disease and cerebrovascular diseases among Japanese, similar to Caucasian populations (5,6). Nationwide surveys demonstrate that serum total cholesterol levels have increased by 20–30 mg/dL between 1960 and 1990 in both adults and children in Japan (7–11) (Table 1). The average total cholesterol levels of Japanese adults today are still lower than those of American adults, but those of Japanese children are now similar or even slightly higher than those of American children. All school children 9–12 yr old (approx 10,000 school children) have been screened for serum cholesterol, blood pressure, and obesity every year in the town of Matsuyama since 1989. Their serum total cholesterol levels have increased by 5 mg/dL for these 7 yr. The prevalence of hypercholesterolemia (≥ 200 mg/dL) is 9.6% (441/4590) in school children of 9–10 yr and 8.2% (374/4579) in those of 12–13 yr in Matsuyama; their average total cholesterol levels are 168.3; 24.5 at the age of 9–10 yr (n = 4972) and 166.3; 25.3 at the age of 13 yr (n = 4579) (Kida K, Matsuyama Study 1995). The prevalence of hypertensive diseases (hypertension and diseases from hypertension) in Japan has increased from 8.0/1000 in 1965 to 15.6/1000 in 1975, and 30.7/1000 in 1985 (12). The prevalence of hypertension among adults 30 yr old or more in 1990 was 29.7% in men and 26.2% in women (10). That among school children is 11/1000 in boys and girls of 9–10 yr and 15/1000 in boys and 16/1000 in girls of 12–13 yr (Kida K, Matsuyama Study 1998). It is well established that obesity is associated with hyperlipidemia and hypertension among Japanese (13,14). The average BMI (weight/height$^2$) among Japanese adults has been increasing during the past 40 yr and estimated prevalence of adulthood obesity (BMI ≥ 30) in Japan today is 2.1% in men and 3.3% in women, which is still lower than those in Western countries (15). Nevertheless, the prevalence of obesity (160 or more of Rohrer Index, weight/height$^3$) among Japanese school children has remarkably increased by 3–5 times during the past 30 yr (Kida K, Matsuyama Study 1995) (Fig. 2). School children with hypercholesterolemia or obesity in Matsuyama are provided
with health education, including diet and exercise recommendations, by school dietitians and school nurses. Improvement of serum total cholesterol or obesity is achieved in more than 50% of children after this instruction (Kida K, Matsuyama Study) (Fig. 3, Table 2).

In Korea, the average total cholesterol levels of children are comparable with those of Japanese or American children and the prevalence of hypercholesterolemia (≥ 200 mg/dL) among school children is reported to be 5–23% (16). The prevalence of obesity has increased by 70% in boys and by 35% in girls over only 4 yr, from 1984 to 1988; these rates are now 12.4% among boys and 11.6% among girls in Seoul and Cheju (17,18). In Thailand, a survey of coronary risk factors and nutritional conditions among 3495 workers was done in 1985 and reported that the prevalence of hypercholesterolemia (≥ 200 mg/dL), hypertension (≥ 141/91) and obesity (BMI ≥ 25) was, respectively, 71.3, 9.6, and 25.5% in men and 65.4, 4.3, and 21.4% in women. In 1991, the later survey was done among 519 hospital staff people, who were expected to be more motivated for health. This study demonstrated that the prevalence of hypercholesterolemia, hypertension, and obesity was 33.4, 4.6, and 18.2% in men and 40.2, 1.8, and 27.0% in women, lower than those in the former survey, but still high (4,19). This suggests that motivation and education play an important role in reducing these risk fac-

Fig. 1. Yearly change in death rates from ischemic heart disease and cerebral infarction in Japan and Korea.
<table>
<thead>
<tr>
<th>Yearly Change in Serum Total Cholesterol Levels in Japan</th>
<th>1960 (7)</th>
<th>1970 (8)</th>
<th>1980 (9)</th>
<th>1990 (10)</th>
<th>1997 (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–39 yr</td>
<td>167 ( n = 676 )</td>
<td>188 ± 33 ( n = 239 )</td>
<td>192 ± 38 ( n = 897 )</td>
<td>196 ± 35 ( n = 620 )</td>
<td>197 ± 33 ( n = 328 )</td>
</tr>
<tr>
<td>40–49 yr</td>
<td>175 ( n = 1043 )</td>
<td>194 ± 45 ( n = 1043 )</td>
<td>197 ± 38 ( n = 1533 )</td>
<td>204 ± 37 ( n = 788 )</td>
<td>207 ± 35 ( n = 460 )</td>
</tr>
<tr>
<td>50–59 yr</td>
<td>175 ( n = 878 )</td>
<td>181 ± 45 ( n = 623 )</td>
<td>199 ± 39 ( n = 1081 )</td>
<td>200 ± 37 ( n = 758 )</td>
<td>204 ± 33 ( n = 503 )</td>
</tr>
<tr>
<td>60–69 yr</td>
<td>179 ( n = 428 )</td>
<td>169 ± 43 ( n = 334 )</td>
<td>193 ± 40 ( n = 594 )</td>
<td>197 ± 38 ( n = 674 )</td>
<td>199 ± 35 ( n = 552 )</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–39 yr</td>
<td>187 ( n = 396 )</td>
<td>184 ± 42 ( n = 184 )</td>
<td>178 ± 34 ( n = 591 )</td>
<td>186 ± 32 ( n = 992 )</td>
<td>188 ± 31 ( n = 571 )</td>
</tr>
<tr>
<td>40–49 yr</td>
<td>192 ( n = 325 )</td>
<td>179 ± 36 ( n = 377 )</td>
<td>191 ± 39 ( n = 705 )</td>
<td>200 ± 36 ( n = 1124 )</td>
<td>201 ± 34 ( n = 724 )</td>
</tr>
<tr>
<td>50–59 yr</td>
<td>207 ( n = 216 )</td>
<td>194 ± 44 ( n = 142 )</td>
<td>211 ± 41 ( n = 735 )</td>
<td>218 ± 37 ( n = 995 )</td>
<td>219 ± 35 ( n = 791 )</td>
</tr>
<tr>
<td>60–69 yr</td>
<td>194 ( n = 104 )</td>
<td>189 ± 44 ( n = 33 )</td>
<td>213 ± 39 ( n = 597 )</td>
<td>223 ± 38 ( n = 870 )</td>
<td>218 ± 36 ( n = 688 )</td>
</tr>
</tbody>
</table>
Fig. 2. Yearly changes in obesity among school children (Rohrer Index ≥ 160) in Matsuyama (Japan).

Fig. 3. Effect of intervention on LDL-cholesterol levels and ratio of ApoB/ApoA1. Children with 140 mg/dL or more serum LDL-cholesterol and those with 0.18 or more of ApoB/ApoA1 ratio were given nutritional education by school dietitians and followed up for 2 yr.
In Japan, the total energy intake per capita per day has not significantly changed for the past 40 yr; 2098 kcal in 1950, 2184 kcal in 1965, 2119 kcal in 1980, and 2007 Kcal in 1997 (11) (Fig. 4). On the other hand, the percent energy intake from fat has increased 3.3 times during these 40 yr; 7.7% in 1950, 14.8% in 1965, 23.6% in 1980, and 26.6% in 1997. Intake of fat of animal, plant, and fish origin has been increased, respectively, by 4.2, 1.9, and only 1.7 times during these 35 yr, and the ratio of fat from animal, plant, and fish is now 4:5:1 in Japan (11) (Fig. 4). The percent energy intake from saturated fatty acids (S), monounsaturated fatty acids (M), and polyunsaturated fatty acids (P) in Japan was 2, 3, and 4%, respectively, in 1955, but it became 8, 9, and 9%, respectively, in 1985, which indicates that fat intake, particularly fat of animal origin, greatly increased in these 30 yr (20). The ratio of n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids increased from 2.8 in 1955 to 3.8 in 1985 (20) and 4.1 in 1990 (21). The increase in the ratio of n-6/n-3 polyunsaturated fatty acids might be attributed to a relative decrease in intake of fat of fish origin and an increase in intake of plant oils containing a large amount of linoleic acid. In fact, the energy intake from linoleic acid was increased 2.5 times, from 2.6% in 1955 to 6.4% in 1985 in Japan (20,22). The protein intake per capita per day has not changed in the past 30 yr in Japan, but protein of animal origin increased 1.7 times and protein of plant origin decreased by 34% in this period (11). The change in the quantity and quality of nutrients might reflect Westernization of diets of Japanese people. Furthermore, occasions to eat meals outside at restaurants or even at American-style fast-foods bars, where fat-rich foods, such as hamburgers and fried chicken, are served, have remarkably increased in Japan during the past 30 yr; 11.3% in 1965, 16.9% in 1980, and 17.8% in 1997 (11). The eating habits of Japanese have, thus, been Westernized in quantity, quality, and manner. A similar pattern is seen in Korea (23) (Table 3). Eating habits have been greatly Westernized during the past 20 yr. The total energy intake per capita per day has slightly decreased, whereas the percent energy from fat has been increased by three times; 5.7% in 1971, 9.6% in 1981, and 16.6% in 1992 (23). The ratio of fat from animal, plant, and fish was 19:69:12 in 1971 and was 25:62:13 in 1990. The ratio of S:M:P is reported to be 2:2:8:1 and that of n-6/n-3 to be 4.9 in 1990 (23). The protein intake per capita per day was increased only by 1.4 times, whereas protein of animal origin increased 4.4 times from 1981 to 1990.
The expense for fast foods and instant foods increased from 7.7% in 1973 to 12.9% in 1983 and occasions to eat meals outside the home also increased, from 2.8% in 1977 to 8.9% in 1986 (23). In Thailand as well, Westernization of diets can be seen in increased fat intake by people in Bangkok. Fat currently accounts for 30% of total energy intake (19).

4. ATHEROSCLEROSIS AND NUTRITION

It is well established that the origin of dietary fat and its composition of fatty acids are related to the risk of cardiovascular diseases resulting from atherosclerosis. The epidemiological study with Eskimos in Greenland and many other studies demonstrate that fish oils or n-3 polyunsaturated fatty acids could play a role in preventing cardiovascular diseases (see Chapter 9) (24–28). Epidemiological studies with Japanese have shown similar results. The death rates from ischemic heart disease and cerebral infarction were lower by 2.6 and 9.2 times, respectively, in the town of Higashi-Izu, where people eat more fish than in Tokyo (29). More recently, a large-scale epidemiological investigation was done to look at the effect of eating fish on the risk of chronic diseases among adults in Japan. The 55,523 deaths out of 265,118 cohort from 1966–1982 were analyzed in reference to their intake of fish. The study revealed that the total death rate and the death rate from cerebrovascular disease, heart disease, hypertension, and cancer were significantly lower in people who ate fish every day compared to those who did so sometimes, rarely, or never (30) (Table 4). Looking at the degree of development of
atherosclerosis in living subjects by measuring pulsewave velocity of the aorta, it was 7.0, 1.1 m/s in a fishermen’s village where 90% of people eat fish every day, and death from ischemic heart disease is low. The pulsewave velocity was significantly slower (less sclerotic) than the value of 7.7, 1.3 m/s in a farmer’s village where only 11% of people eat fish every day, and death from ischemic heart disease is high (31).

Furthermore, renarrowing of coronary arteries after percutaneous transluminal coronary angioplasty (PTCA) for ischemic heart disease was significantly decreased, from 37% in controls \((n = 11005)\) to 20% in subjects who were orally given 1.6 g/d of eicosapentenoic acid (EPA) for 3–4 mo \((n = 30)\) (32), as confirmed by a large-scale double-blind investigation (33). A multicenter study in Japan demonstrated that EPA was effective in arteriosclerosis obliterans (ASO) as assessed by the diameter of ulcers on the skin; 7.4, 1.87 mm in controls \((n = 25)\) vs 6.0, 1.59 mm in subjects treated with 1.8 g/d of EPA for 6 wk \((n = 18)\) (34). These data indicate that oils of fish origin or n-3 polyunsaturated fatty acids could be beneficial in preventing atherosclerosis and related chronic diseases.

Another characteristic of the Japanese diet is a high intake of salt (NaCl), which likely plays an essential role in hypertension as an important risk factor for atherosclerosis. The salt intake per capita per day in Japan today is 12.8 g, i.e., 6.4 g/1000 kcal, which has not changed in the past 30 yr (11). About 50% of total salt is from soy sauce, soy bean paste, and salted vegetables (11). People in the eastern part of Japan consume 1.3 times more salt than those in the Kinki district, including Osaka and Kyoto (11). Public education in a community for 8 wk successfully reduced salt intake of the residents by 3.8 g/d and lowered the systolic blood pressure by 4.7 mmHg and diastolic blood pressure by 3.9 mm Hg (35).

5. NUTRITIONAL RECOMMENDATIONS

Taking the rapid increase in chronic diseases based on atherosclerosis and Westernization of diets into consideration, much attention is paid to reduction of chronic diseases by establishing a nutritionally appropriate lifestyle in Japan. The most

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Eating of fish</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Every day</td>
<td>Sometimes</td>
<td>Rarely</td>
<td>Never</td>
<td>(\chi^2)</td>
</tr>
<tr>
<td>Total death</td>
<td>1.0</td>
<td>1.07</td>
<td>1.12</td>
<td>1.32</td>
<td>9.13</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>1.0</td>
<td>1.08</td>
<td>1.10</td>
<td>1.10</td>
<td>4.54</td>
</tr>
<tr>
<td>Heart diseases</td>
<td>1.0</td>
<td>1.09</td>
<td>1.13</td>
<td>1.24</td>
<td>3.92</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.0</td>
<td>1.55</td>
<td>1.89</td>
<td>1.79</td>
<td>4.14</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>1.0</td>
<td>1.21</td>
<td>1.30</td>
<td>1.74</td>
<td>3.77</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>1.0</td>
<td>1.04</td>
<td>1.04</td>
<td>1.44</td>
<td>2.14</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>1.0</td>
<td>1.03</td>
<td>1.16</td>
<td>2.62</td>
<td>2.11</td>
</tr>
<tr>
<td>Uterus cervix cancer</td>
<td>1.0</td>
<td>1.28</td>
<td>1.71</td>
<td>2.37</td>
<td>4.14</td>
</tr>
</tbody>
</table>

| Observed person • year | 1,412,740 | 2,186,368 | 203,945 | 28,943 |
recent recommendations appear in the sixth edition of Recommended Dietary Allowance for Japanese issued by the Ministry of Health and Welfare of Japan in 1999 (37) (Table 5). The recommended energy intake was calculated by the formula:

\[
E = (1 + A) \times B \times 10/9
\]

where \( E \) is the recommended energy intake (kcal/d), \( B \) is the basal metabolic rate (kcal/d) estimated from body surface, and \( A \) is the coefficient for intensity of daily activities. The energy intake from fat is recommended to be 25–30% of total energy intake for young people of 1–17 yr and 20–25% of total energy intake for adults of 18 yr or more. The ratio of S:M:P among fatty acids is recommended to be 3:4:3 and the ratio of n-6/n-3 of polyunsaturated fatty acids to be 4.0 according to the results of analyses of the present nutritional conditions of Japanese people. The recommendation of S:M:P is in contrast to the previous recommendations, which was 1:1.5:1 (37). The intake of salt (NaCl) is recommended now at 10 g/d or less and should be reduced to 7–8 g/d if possible. There are a few arguments relating to these recommendations. There are no apparent reasons to elevate the upper limit of fat intake to 30% of the total energy for young people from 25% for adults. In fact, hypercholesterolemia is found in 8.2–9.6% of school children (Kida K, Matsuyama Study, 1998). Furthermore, pathological studies in Japan, as well as in the United States have revealed that atherosclerosis takes place with high frequency even in young people and is related to their blood cholesterol levels (38-40). Our study group of Matsuyama, therefore, recommends that fat intake should not exceed 25% of the total energy in school children and adolescents of 6–17 yr. Even infants, except babies and young infants, should not be exposed to fat-rich diets because the period of infancy is critical toward setting eating habits in later life (Table 5). Although no definite consensus on the ideal composition of fat and fatty acids is established, it is obvious from animal experiments and epidemiological studies that fat of animal origin should be reduced and the ratio of n-6 to n-3 of polyunsaturated fatty acids should be lowered as much as possible. The authors do not think it is rational to set the recommended values for the composition of fat and fatty acids from the results of analyses of the average diets in Japan today that are unfavorably Westernized in terms of fat. It might be feasible for Japanese to take more fat of fish origin and less of animal origin and at the same time to reduce linoleic acid (n-6

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Nutritional Recommendations in Japan, Korea, and Thailand</th>
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<tbody>
<tr>
<td></td>
<td>Japan (36)</td>
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<tr>
<td>Fat Intake (%) total energy</td>
<td>20–25 (18 yr)</td>
</tr>
<tr>
<td>Fat intake from animal:plant:fish</td>
<td>4:5:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish meat (g), animal meat (g)</td>
<td></td>
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<tr>
<td>S:M:P</td>
<td>3:4:3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>n-6/n-3</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> Reasonable at present.
<table>
<thead>
<tr>
<th></th>
<th>Japan</th>
<th>Korea</th>
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<tr>
<td></td>
<td>Breakfast</td>
<td>Lunch</td>
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<tr>
<td>Steamed rice</td>
<td>Steamed rice</td>
<td>Steamed rice</td>
</tr>
<tr>
<td>Soybean soup</td>
<td>Beef and vegetable in sukiyaki style</td>
<td>Grilled mackerel pike</td>
</tr>
<tr>
<td>Grilled salmon</td>
<td>Salad</td>
<td>Stewed radish</td>
</tr>
<tr>
<td>Pickled radish</td>
<td>Fruit</td>
<td>Grilled salmon</td>
</tr>
<tr>
<td>Stewed vegetable</td>
<td>Low-fat milk</td>
<td>Grated yam</td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td>Spinach with egg in soup</td>
</tr>
<tr>
<td>Total energy</td>
<td>1967 kcal</td>
<td>% energy</td>
</tr>
<tr>
<td>(carbohydrate/fat/protein)</td>
<td>1:1.2:1.1</td>
<td></td>
</tr>
<tr>
<td>S:M:P</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>7.7 g</td>
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polyunsaturated fatty acid) intake by avoiding oil or food fortified with linoleic acid, so that the ratio of n-6/n-3 of polyunsaturated fatty acids may be lowered to the level of 2.8, which was the ratio in 1955. Our study group of Matsuyama, accordingly, recommends the ratios of S:M:P among fatty acids and of n-6/n-3 of polyunsaturated fatty acids be 1:1.5:1.5 and 3.0:(2.0), respectively (Table 5). Regarding salt intake, the recommended intake of salt, 10.0 g/d, is still high compared with those in many other parts of the world. Our study group of Matsuyama also recommends intake of salt to be 8.0 g/d. The recommended salt intake of 8.0 g/d is not far below the 10.8 g/d level found in the Kinki district, one of the most traditional, and at the same time, most modernized places in Japan.

In Korea, the recommended fat intake is 20% of total energy intake and recommended ratios of S:M:P and n-6/n-3 are 1.0:1.0–1.5:1.0 and 4–10, respectively (41). In Thailand, it is recommended that the fat intake should not exceed 30% of total energy intake with equal distribution of S, M, and P among fatty acids (42).

Table 6 presents a typical sample of menus of Japanese and Korean meals for a breakfast, lunch, and dinner. The Japanese menu fits the recommendation of our study group of Matsuyama. The menus of meals are neither too Westernized nor too traditional or “old fashioned” so that all generations of people may accept it as part of daily life today. Efforts are needed to educate the community, governmental organizations, and nongovernmental organizations, including food industries and mass medias, to understand the role of nutrition and usefulness of Asian traditional diets in prevention of chronic diseases based on atherosclerosis and their risk factors.

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Potential Benefits of Preventive Nutrition Strategies
Lessons for the United States

Walter C. Willett

1. INTRODUCTION

For years, the Recommended Dietary Allowances (RDAs) served as nutritional guidelines for individuals and institutions (1). However, these have developed from minimalist criteria primarily aimed at prevention of clinical deficiencies. These guidelines were later supplemented with recommendations to reduce dietary fat and cholesterol. In recent years, attention has focused on specific types of fat and suboptimal intake of dietary factors, even in the absence of recognized clinical deficiency. Dietary factors, including substances not generally considered to be nutrients, now appear involved in the cause or prevention of conditions as diverse as coronary heart disease (CHD), stroke, many cancers, cataracts, and birth defects. The National Research Council (NRC) and the Surgeon General’s office (2,3) have examined the relation of diet to health more broadly and issued new recommendations. This brief overview will build on the 1989 NRC report on Diet and Health (2) emphasizing subsequent findings.

2. 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. These analyses highlighted the large differences in disease rates worldwide and provided many hypotheses; however, such studies are limited as many other factors besides diet vary across cultures and the data are inherently aggregated. The next generation of studies were primarily case-control investigations, which mainly examined dietary factors retrospectively in relation to risk of cancer and other diseases. Now, large prospective studies of many thousands of persons are beginning to provide data based on both biochemical indicators of diet and dietary questionnaires that have been rigorously validated (4). 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 infeasible 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 endpoints, 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, metabolic, animal, and mechanistic studies.

3. SPECIFIC DIETARY COMPONENTS

3.1. Dietary Fat

Major reviews on diet and health have consistently recommended reducing total fat intake, usually to 30% of energy or less (2,3), to decrease 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 (5) and Hegsted (6), 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 (7–10).

Substitution of carbohydrate for saturated fat (the basis of the American Heart Association diets) tends to reduce HDL as well as total and low-density lipoprotein (LDL) cholesterol; thus, the ratio does not change appreciably (8–10). In contrast, substituting monounsaturated fat for saturated fat reduces LDL without affecting HDL, thus providing an improved ratio (10). In addition, monounsaturated fats, compared to carbohydrate, reduce blood sugar and triglycerides in adult onset diabetics (11). 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 (12). Although this is difficult to address directly, other factors that influence HDL levels, including alcohol, estrogens, obesity, smoking, exercise, and medications, affect CHD risk in the predicted direction (13,14). 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 (10,15).

The optimal amount of polyunsaturated fat intake in the diet remains uncertain. The earlier metabolic studies predicting total serum cholesterol (5,6) suggested that intakes should be maximized, and the American Heart Association recommended intakes of 10% of energy (compared to United States averages of about 3% in the 1950s and 6% at present). However, in more recent metabolic studies, the benefits of polyunsaturated fat have been less clear (10) and concerns have arisen from animal studies in which ω-6 polyunsaturated fat (typically as corn oil) has promoted tumor growth (16), and the possibility that high intakes of ω-6 relative to ω-3 fatty acids might promote coronary thrombosis (17,18).
Evidence based on clinical endpoints (as opposed to blood lipid levels) is remarkably sparse. In Keys’ pioneering study of diets and CHD in seven countries (19,20) 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 prospective studies of individuals (21–23). Some studies, however, tend to support a modest association between dietary cholesterol and CHD risk (24), and inverse associations have been seen with polyunsaturated fat (21,23). 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 reduced incidence of CHD (25–28). In the one trial that clearly reduced CHD mortality (29), many components of the diet as well as weight and smoking were changed simultaneously.

High intake of ω-3 fatty acids, found primarily in marine fish, but also in some vegetable oils and plants, reduces platelet aggregability and prolongs bleeding time (18), slightly reduces blood pressure (30), decreases serum triglycerides, but increases LDL-cholesterol (31). Fish consumption was associated with a greatly reduced risk of myocardial infarction (MI) in one prospective study (32) and in a randomized trial among postinfarction patients (33). Subsequent data have been less supportive of a major effect of fish consumption on overall risk of CHD (34–36), but the benefits of ω-3 fatty acids may be primarily in prevention of fatal arrhythmias that can complicate CHD, rather than in prevention of infarction (37,38).

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. Intake of 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 (39). Trans-fatty acids increase LDL and decrease HDL (39–44), as well as raise Lp(a), another lipid fraction implicated in CHD etiology (43,45). Positive associations between intake of trans-fatty acids and CHD have been seen among regions in the Seven Countries Study (46), a case-control study of men and women (47), and a cross-sectional angiographic study (48). In a multicenter European case-control study, adipose levels of trans-fatty acids were by far lowest in Spain, also the country with the lowest CHD rates (49). In the same study, the risk of CHD within countries was 40–50% higher among individuals with the highest trans-fatty acid levels, but this did not quite obtain statistical significance. In the most detailed prospective study, trans-fatty acid intake was strongly associated with risk of CHD (23) and, as predicted by metabolic studies, this association was stronger than for saturated fat.

Understanding of the interrelationships between dietary fats, blood lipids, and CHD risk has been further complicated by the recognition that antioxidants are likely to play a critical role in preventing atherosclerosis. Experimental evidence suggests that lipid soluble antioxidants such as vitamin E can block the oxidative modification of LDL, an important step in atherogenesis (50). Within Europe, countries with higher blood antioxidant levels have lower rates of CHD (51), and, as described below, vitamin E supplements have been inversely associated with CHD risk. As liquid vegetable oils, particularly those that are minimally processed, are the primary source of vitamin E in our diets, reduction of these fats could have adverse effects on CHD risk. Soybean oil
may be an exception because it is highly polyunsaturated, yet its primary form of vitamin E, \( \gamma \)-tocopherol, is rapidly excreted and poorly incorporated into tissues and lipoproteins. Thus, diets high in soybean oil, the major fat in the United States diet, might result in LDL particularly susceptible to oxidation. In contrast, LDL particles formed on a diet high in monounsaturated fat in the form of olive oil appear to be relatively resistant to oxidation (52,53).

3.2. Dietary Fat and Cancer

Another major justification for decreasing dietary fat has been anticipated reductions in the risk of cancers of the breast, colon and rectum, and prostate (2,54). The primary evidence has been that countries with low fat intake, also the less affluent areas, have had low rates of these cancers (54,55). 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 animal models (56,57), although no association was seen in a large study that did not use an inducing agent (58). 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 (16,56,58). In most case-control studies, no association between fat intake and breast cancer was observed, although a weak positive association (relative risk [RR] = approx 1.07 for 40 vs 30% of energy from fat) was seen in the pooled data from 12 such studies (59). However, these studies are now of diminishing relevance as data from six large prospective studies, including 3400 cases among 280,000 women, have recently been published (60–65). In none of these studies was the risk of breast cancer significantly elevated among those with the highest fat intake, and the summary relative risk for the highest vs lowest category of dietary fat composition is 1.03 (66). In the largest study (67), no reduction in risk was seen even below 20% of energy from fat. Thus, over the range of fat intake consumed by middle-aged women in these studies, which included the present dietary recommendations, dietary fat did not appear to increase breast cancer risk. Effects of much greater fat reductions or influences at early times in life could not be excluded.

The large differences, approximately fivefold, in breast cancer rates among countries are probably the result of many factors. High consumption of soy products, which contain antiestrogenic isoflavonoids (68) has been hypothesized to account for the low rates of breast cancer in Japan. Although deserving further research, similarly low rates of breast cancer are uniformly seen in nonindustrialized countries, most of which do not consume soy products. Also, no relation between intake of soy products and breast cancer risk was seen in two recent case-control studies in China (69). Indirect evidence is growing that the powerful effect of energy and growth restriction on mammary cancer incidence in animals also applies to humans. Adult height, in part a marker of early energy balance, is associated with breast cancer rates internationally and in many case-control and cohort studies (70–72). Some of this effect is mediated through delayed ovulation (73); childhood weight gain is the primary determinant of age at menstruation (74), which still averages about 18 yr in China (75).

Associations between animal fat consumption and colon cancer incidence have been seen more consistently (76–78), although not in all studies (79), whereas little relation has been seen with vegetable fat. Positive associations with animal fat have also been observed for adenomatous polyps, a precursor lesion (80). However, the associations
between red meat consumption and colon cancer have been even stronger than the effect of fat in some analyses (77,78), suggesting that relationships with red meat may be because of other components of cooked flesh, such as heat-induced carcinogens (81) or the high content of readily available iron (82).

Like breast and colon cancer, prostate cancer rates are much higher in affluent compared to poor and Eastern countries (55). More detailed epidemiologic studies are few; in subsets of several case-control studies, men with prostate cancer reported higher fat intake than did controls (83,84). A positive association has been seen between intake of α-linolenic acid, primarily attributable to consumption of fat from red meat (85).

### 3.3. Dietary Fat and Body Fatness

Overweight is an important cause of morbidity and mortality (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 (86). 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 (75). 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 to 20–25% of energy had at most a 1.5-kg effect on overall long-term body weight (87–89). In a trial among obese women, a reduction in fat intake to ~18% of energy reduced weight by only 3.4 kg, and no effect on waist/hip ratio or percent body fat was observed (90). 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 (91), but long-term studies are needed. However, available evidence suggests that reductions in dietary fat composition over the ranges currently recommended are not likely to have sustained and substantial effects on body fatness.

What can we now say about dietary fat and health? As noted in the executive summary of the NRC report (87), but generally ignored, there is little evidence that dietary fat per se is associated with risk of CHD. Metabolic and epidemiological data are presently consistent in suggesting that intake of partially hydrogenated vegetable fats should be minimized. Metabolic data and epidemiologic data support a reduction in saturated fats, particularly from dairy sources, to as low as feasible, but these data suggest that the benefits will be small unless unsaturated fats replace the saturated fat. Definitive data are not available on the optimal intake of polyunsaturated and monounsaturated fats, but the 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. Available evidence also suggests that fat reduction would have little effect on breast cancer risk, although reducing red meat intake may well decrease the incidence of colon cancer. Browner et al. have estimated that decreasing saturated fat intake so that total fat was reduced from 37 to 30% of energy would reduce mortality in the United States only by 2% (92). This is likely to be an overly optimistic estimate, however, as the calculations did not account for reductions in HDL and assumed major causal association with breast and colon cancer.
3.4. Vegetables and Fruits

Recommendations to eat a generous amount of vegetables and fruits (2) are supported by a wealth of epidemiologic data, primarily relating to cancer incidence. In over 200 case-control or cohort studies, persons consuming higher amounts of these foods or having higher levels of carotenoids in their blood experienced reduced risk of various malignancies (93,94). An inverse relation with lung cancer is most strongly suggested (95). This has led to the suggestion that β-carotene might be the protective factor (96), but this has not been supported by randomized trials (97–99). Intake of fruits and vegetables has also been related to lower risk of stomach cancer in many case-control studies (3,94,100); both the epidemiologic evidence and mechanistic studies (101) suggest a possible protective role for vitamin C. Vegetable and fruit consumption has also been inversely related to risk of colon cancer (102) that has been attributed to beneficial effects of dietary fiber, but recent evidence suggests that folic acid might also account for the reduced risk (78,103). Although the numbers of studies are smaller, apparent protective effects have also been seen for cancers of the oral cavity, larynx, pancreas, bladder, and cervix (93,94,100). In a large prospective study, breast cancer incidence was about 25% higher among women with low vegetable intake (104).

Plants contain numerous components, in addition to the micronutrients noted above, that have potential anticancer activity (94); such chemicals could reduce the formation of carcinogens, induce detoxifying enzymes, and block the effects of endogenous estrogens. Although the epidemiologic data on cancer provide solid support for recommendations to consume an abundance of vegetables and fruits, further details about the types and amounts of these foods could permit more precise recommendations.

In the more limited literature on coronary heart disease, fiber intake has been related to decreased risk (22,105,106), but the possibility that this association could have been because of other factors in plants has generally not been explored. Evidence that elevated blood homocysteine is an independent risk factor for coronary heart and cerebrovascular disease (107–109), and that levels can be reduced by supplements of folic acid and vitamin B6 (110,111) suggest another mechanism. Vegetarians generally have reduced blood pressures and higher intake of fruits and vegetables has been associated with lower blood pressure even in nonvegetarians (112); the active factor remains unclear, but potassium is a likely contributing factor (113).

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 (114,115) and may account for more than half of these cases. The effect of low folate intake may be particularly adverse among the approx 10% of the population who are genetically less efficient in utilizing the ingested form of this vitamin (116).

In both case-control (117) and prospective studies (118), intake of dietary antioxidants including carotenoids and vitamin C has been inversely related to risk of cataracts. As cataract formation, which is increased by sunlight and cigarette smoking (119), 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 greatly decreased risk of macular degeneration (120). 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 (121).
3.5. 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 (10). These adverse responses may be aggravated in the context of insulin resistance (122,123), which is highly prevalent to some degree in western populations.

Although direct evidence for benefit in reducing disease risk is limited, several reasons exist to emphasize whole grain and other less refined complex carbohydrates as opposed to the highly refined products and sugar generally consumed in the United States. 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 (124). The more 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 (124) to a greater degree than with less refined foods. Diets with a high-glycemic index, particularly when associated with low-fiber intake, appear to increase the risk of noninsulin-dependent diabetes (125,126), and possibly risk of CHD (127). Anticipated reductions in colon cancer risk by diets high in grain fiber diets have been difficult to document epidemiologically (128,129). However, reduced constipation and risk of colonic diverticular disease (119) 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 (130). 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 (131).

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 (132); this may be nutritionally critical for persons with otherwise marginal intakes. Thiamin, riboflavin, and niacin are presently replaced by fortification, but other nutrients remain substantially reduced.

3.6. Protein

Average protein consumption in the United States substantially exceeds requirements (2) 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 (133), contribute to homocysteinemia (134), and has been hypothesized to increase risk of various cancers (135); however, evidence for the latter effect is limited.

3.7. Calcium and Dairy Products

Recommendations to “maintain adequate calcium intake” (2) and to consume dairy products on a daily basis (136) derive primarily from the importance of calcium in maintaining bone strength. Although calcium supplements (in conjunction with vitamin
D) have reduced fracture incidence in older adults (137,138), uncertainty remains regarding the optimal intake. Intakes as high as 1500 mg/d have been recommended for postmenopausal women at risk of fractures (139), which are difficult to achieve without supplements. However, some populations have low-fracture rates despite minimal dairy product consumption and low overall calcium intake by adults (140). 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 (133); few studies have directly addressed the relation of dairy product consumption and fracture incidence; with the exception of one small study (141); higher consumption of calcium or dairy products as an adult has been associated with a higher or no difference in fracture incidence (142–144).

Inverse associations have been observed between calcium intake and blood pressure in some studies (145), but in a review of trials of supplementation little overall effect was seen (146). Low-calcium intake has been associated with risk of colon cancer, but evidence has not been consistent and deserves further examination (147).

Although recommended calcium intakes can be achieved by a high consumption of greens and certain other vegetables, greatly increased intakes would be required for most women to achieve currently recommended levels by diet without regular use of milk and other dairy products. However, calcium supplements have been shown to increase bone density and are an inexpensive form of calcium. Thus, dairy product consumption can be considered an optional rather than an necessary dietary component.

3.8. Salt and Processed Meats

Reduction of salt (sodium chloride) intake from an average of approx 8–10 g/d to less than 6 g/d will, on average, decrease blood pressure to a small degree. In a recent review, Law et al. (148) concluded that a 3-g/d reduction 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 the salt consumption, particularly in processed foods and by institutions.

In a number of case-control studies, the consumption of salty and pickled foods has been associated with stomach cancer (149). However, as this cancer is relatively rare in the United States, further benefit from reducing salt intake would be small.

4. BODY WEIGHT

Although the NRC made the recommendation to “balance food intake and physical activity to maintain appropriate body weight” (2) without any specific weight recommendations, the document was cited as the justification for the revised 1990 United States guidelines for weight. These guidelines included substantial increases after age 35 to much heavier weights than the earlier and widely used Metropolitan Life recommendations (150). Unfortunately, the 1990 guidelines appear to have been based on unpublished data 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 (151,152). More detailed analyses indicate that middle-aged persons of even average weight have a high prevalence of abnormalities of
blood glucose and lipids as well as blood pressure (153), and experience substantial increases in MI (154,155), diabetes (156), hypertension (157), gallstones (158), and total mortality rates (159,160) compared to their leaner counterparts. Thus, the 1995 guidelines based on a BMI range of 19–25 kg/m², are probably closer to optimal, and the best health experience is achieved by avoiding increases in weight during adulthood (151).

As noted earlier, dietary fat composition over a wide range appears to have little relationship with weight maintenance; in contrast, regular exercise and avoidance of extreme inactivity such as excessive television watching is crucial (161).

5. 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. Overwhelming epidemiologic data indicate that moderate consumption reduces risk of MI (162,163), two drinks a day decrease risk by approx 30–50%. Although it has been suggested that this effect may be a result of antioxidants in red wine (164), similar protective effects for equivalent amounts of alcohol have been seen for all types of alcoholic beverages (165). On the other hand, modest positive associations with risk of breast cancer incidence have been observed in approx 30 studies (166,167) for similar levels of alcohol intake, possibly because alcohol appears to increase endogenous estrogen levels (168,169). The overall effect of alcohol, as represented by total mortality, appears beneficial up to about two drinks per day in men (170). 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 their age or lack of coronary risk factors (171). Furthermore, the risk of transition from moderate alcohol consumption to addiction and uncontrolled drinking has not been well quantified.

6. VITAMIN SUPPLEMENTS

As populations can be identified with very low rates of almost every major disease, vitamin supplements may not further enhance health among populations consuming ideal diets. Only recently are data emerging that address the effects of vitamin supplements against the background of actual diets in the United States, which appear far from ideal (100).

The most firmly established benefit, 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 approx 70% (114,172). This is probably only a sentinel indicator of suboptimal folate intakes, as associations have also been seen with colonic neoplasias, and low-folate intake, along with suboptimal vitamin B₆, is likely to contribute to elevated blood homocysteine levels and risk of cardiovascular disease (108,109).

As noted above, vitamin E supplements well beyond the dietary range have been associated with decreased risk of CHD. In prospective studies men and women who consumed the highest amounts of vitamin E (mostly from supplements) had an approx 40% lower risk of MI compared to those having low vitamin E intakes (173,174). The maximum reduction in risk appeared to be at levels of 100 or more international units (IUS) per day, well above intakes achievable by diet alone. In one case-control study use of
vitamin E supplements was associated with a 50% reduced risk of oral cancer (175). Vitamin C supplementation was associated with lower risk of CHD in one national study (176), but the data available did not distinguish vitamin C from other supplements. No protective effect of vitamin C was observed in another prospective study (174). Apart from a possible reduction in risk of cataracts (119), little evidence at present that high doses of vitamin C have substantial benefits. Although no overall benefit of supplemental vitamin A was seen in a large prospective study of breast cancer, an inverse relation with risk was seen among women with below-average vitamin A intakes (104).

The effects of vitamin supplementation on risk of chronic diseases have been examined in only a few randomized trials. In a randomized trial conducted in a region of China with low consumption of fruits and vegetables, a supplement containing β-carotene, vitamin E, and selenium reduced incidence of stomach cancer (177). Benefits from additional micronutrients may not be limited to those in extreme poverty; in a Canadian trial a multivitamin/mineral supplement reduced risks of infections by 50% among older persons (178), possibly owing to an enhancement of immune status (see Chapter 18).

Current evidence, although far from complete, suggests that supplements of folate and possibly other vitamins, at the RDA level, contained in most nonprescription multivitamin preparations, may have substantial benefits for at least an important, but unidentified, subgroup of the United States population, perhaps characterized by increased requirements as well as by suboptimal diets. As intakes of folate as well as other micronutrients appear marginal for many Americans (93,100), the risks of using multivitamins appear nonexistent, and the cost of supplements is low (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.

Seven RECOMMENDATIONS

Any set of dietary recommendations must be made with the clear qualification that information is currently incomplete and conclusions are subject to change with new data. Most of the major causes of morbidity and mortality in the United States develop over many decades and large-scale nutritional epidemiologic studies have only begun in the last 15–20 yr; 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 (see Fig. 1).
1. Stay lean and active throughout life. For most individuals, body weight should not increase by more than 5–10 pounds 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 calories.

2. Vegetables and fruits should be consumed in abundance (five servings/d is minimal) and include green leafy and orange vegetables daily.

3. Grains should be consumed primarily in a minimally refined, whole grain form and intake of simple sugars should be low.

4. 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.

5. Animal fats and trans-fatty acids from partially hydrogenated vegetable oils should be avoided as much as possible. On the other hand, olive oil and possibly other primarily monounsaturated fats appear to be healthy alternatives. Highly polyunsaturated fats such as corn and soybean oil may also be healthy alternatives, but theoretical concerns exist regarding high consumption of these oils. Unless explicitly stated otherwise, it is safest to assume that deep-fried fast foods and most commercially prepared foods contain trans-fatty acids.

6. The optimal consumption of dairy products and calcium intake is not known, but high consumption of milk is not likely to be necessary or beneficial for middle-aged and older adults. 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 provides a sensible nutritional safety net. Definitive evidence exists that use of a folic acid-containing multivitamin supplement during the early weeks of pregnancy can prevent a large fraction of

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**Fig. 1.** USDA food pyramid, annotated. Optimal food intakes for children and pregnant or lactating women may need further consideration. (Modified figure reproduced with permission of Nasco Nutrition Teaching Aids.)
neural tube defects and the Public Health Service recommends that women who are potentially child-bearing consume 400 μg of synthetic folic acid daily, the level found in most multivitamin supplements (181). For most people, this level of intake is difficult and unreliable to achieve by diet alone. As noted above, benefits of folic acid supplementation may extend to prevention of myocardial infarction, stroke, and colon cancer, although this is not proven. Because menstrual losses of iron may not be adequately replaced by iron intake on the low-energy diets of women in a sedentary society, it may make sense for most premenopausal women to use a multiple vitamin that also contains iron. Pending further data, the use of a vitamin E supplement at 400–800 IU/d is reasonable for most middle-aged and older persons as substantial evidence suggests that this may reduce risk of MI. Although not proven, evidence also suggests that use of vitamin C supplements at the level of 300–500 mg/d may reduce risk of cataract, which may be sufficient reason for some individuals to consider using this. Although there is no documented harm from these supplements at the levels of intake suggested, long-term effects need to be examined in further studies. Also, 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 adventurous in eating! Unfortunately, most of us in the United States are heirs to the rather monotonous Northern European dietary tradition centered on the consumption of meat and dairy products. Contemporary food processing has added to the deleterious effects of this diet by the removal of dietary fiber and micronutrients through overrefining 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 both healthy as well as interesting and enjoyable.

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Chapter 20 / Preventive Nutrition Strategies


1. INTRODUCTION

Developing countries increasingly face the dilemma of dealing simultaneously with problems of persistent endemic malnutrition affecting primarily children and women of reproductive age and increasing prevalence of obesity and of diet-related chronic diseases among adults. Unless this dilemma is squarely faced and rationally addressed, there is a real possibility that scarce preventive health and nutrition resources will be fragmented, resulting in the deterioration rather than the progression toward health for all. The health transition (and by implication, the nutrition transition) has widely different dynamics in different countries, but it seems that countries all over this world are bringing the need to seriously reassess goals and objectives for disease control and for preventive nutrition into focus.

2. THE HEALTH TRANSITION

Figure 1 shows the general relationships among the processes termed the “health transition,” and which include both the “demographic transition” marked by declines in mortality and fertility, and the “epidemiologic transition” in which as the population ages there is an emergence of adult chronic disease and a change in the major causes of mortality.

In the original formulation by Omran (1), there were three proposed eras through which countries passed at different stages of socioeconomic development: the era of “pestilence and famine,” in which life expectancy was low and the major causes of death were those associated with malnutrition, infection, and reproduction; the era of “receding pandemics” where life expectancy rises to 30–50 yr, morbidity is still dominated by nutritional and infectious causes, and major mortality fluctuations are less common; and finally the era of “degenerative and man-made diseases,” with life expectancy over 50 yr and major causes of death including cardiovascular diseases, cancer, diabetes, and other chronic ailments. Acknowledging the heterogeneity of social and economic development among human societies, Omran suggested that there were at least three models of the transition: the traditional or Western model, an accelerated model (typified by Japan), and a delayed or contemporary model. The latter describes the incomplete transition that characterizes most developing countries today, in which pretransition and posttransition problems must be dealt with simultaneously (2).
Frenk et al. (3) further elaborated the model, based on observations from several large, middle-income countries, to take account of widening within-country gaps in health status among social classes and geographical regions which they have termed “epidemiologic polarization.” Features of this “protracted-polarized model” of health transition include recognition that the eras postulated by Omran are not necessarily sequential, but may overlap; that incomplete coverage of interventions to manage various health problems occurs; and the occurrence and persistence of major inequities in health status and even the reemergence of epidemic diseases that had been controlled or eradicated.

Table 1, from Jamison and Mosley (4), shows one widely-utilized summary by age group of the “unfinished agenda” of health problems associated with underdevelopment and those “neglected and emerging problems” that are increasingly coming to dominate the health agenda. The relative importance of the “emerging problems” on the health agenda is a function not only of socioeconomic development but also of demographic shifts, lifestyle, and health services. As populations age, adult problems become more important as causes of morbidity, mortality, disability and health care costs. The elderly are becoming more numerous and more visible almost everywhere. Throughout the developing world, it has been estimated that populations over 65 yr of age will increase by about 150% between 1985 and 2015 (4).

3. THE NUTRITION TRANSITION

Food consumption patterns, nutritional status, and diet-related morbidity and mortality likewise show characteristic shifts with socioeconomic development, urbanization, education, and industrialization; these shifts have been most thoroughly articulated by Popkin (5–9), who has postulated that the pace of dietary change seems to have recently
accelerated to varying degrees in different regions of the world. The transition seems to converge on a rapid shift, when economic resources allow, to a dietary pattern characterized by higher intakes of fat, animal products, sugar and refined foods and lower intakes of fiber. The associated changes in nutritional status, often attributed to dietary changes because of their parallel in time but also affected by changes in other risk factors such as sanitation and physical activity patterns, include declines in general undernutrition and micronutrient deficiencies in children and increased prevalence of obesity and the chronic diseases for which obesity is a risk factor (cardiovascular disease and type II diabetes) among adults. It is evident, however, that the “polarization” noted by Frenk et al. (3) for general health status also applies to nutrition, with many countries facing the emergence of diet-related chronic diseases while still dealing with problems of undernutrition in children and among the poor.

Popkin (10) has pointed out several contrasting dynamics of the dietary transition; the Western high-income model that characterized the United States and a number of other countries, in which dietary transition occurred slowly; the “Japanese and Korean accelerated model” in which similar changes have occurred in less than 30 yr; “emerging Asian models” epitomized by China and Thailand, in which urbanization and economic development have provoked very rapid dietary change among city dwellers and the higher socioeconomic classes; and the “Latin American pattern” which is similar to the US experience in occurring slowly and permeating the entire society, and in which obesity is as much or more a problem of the poor than of the well-to-do.

The pace of dietary change in many developing countries is very rapid, fueled largely by urbanization. It is estimated that 40% of the population of the developing worlds live in cities in the year 2000, and 50% will be urban dwellers by 2015 (11). In 1990, seven

### Table 1

<table>
<thead>
<tr>
<th>Age group</th>
<th>Problems on the unfinished agenda</th>
<th>Neglected and emerging problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young children</td>
<td>Diarrheal disease</td>
<td>Injury</td>
</tr>
<tr>
<td>(0–4 yr)</td>
<td>ARI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Measles, tetanus, polio</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Micronutrient deficiencies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaria</td>
<td></td>
</tr>
<tr>
<td>School-age children</td>
<td>Schistosomiasis</td>
<td>Adolescence pregnancy</td>
</tr>
<tr>
<td>(5–14 yr)</td>
<td>Geohelminth infections</td>
<td>Disability</td>
</tr>
<tr>
<td>Young adults</td>
<td>Maternal mortality</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>(15–44 yr)</td>
<td>Malaria</td>
<td>Injury</td>
</tr>
<tr>
<td></td>
<td>Excess fertility</td>
<td>AIDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STDs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mental illness</td>
</tr>
<tr>
<td>Middle-aged</td>
<td></td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>(45–64 yr)</td>
<td></td>
<td>Cancers</td>
</tr>
<tr>
<td>Elderly</td>
<td></td>
<td>Disability</td>
</tr>
<tr>
<td>(65 + yr)</td>
<td></td>
<td>Depression</td>
</tr>
</tbody>
</table>

*a Adapted from ref. 4.*
of the world’s “megacities” (population more than 10 million) were in developing countries, and six more were added in the decade 1990–2000 (4). The urban environment is generally more conducive to good nutritional status in children, but urbanization also brings disadvantages. Declines in breastfeeding rates and duration, household food insecurity linked to the cash economy for very poor urban families, and increases in obesity associated with sedentary lifestyles bring new nutritional risks which residents of the world’s large cities face in unprecedented numbers (12).

Table 2, constructed from large-sample surveys in China (1989) and Egypt (1994), shows rural/urban contrasts in proportion of adult dietary energy intakes from fat, one of the most sensitive and predictable changes in diet with urbanization. The increased average proportion of energy from fat among urban dwellers in both countries substantially decreases the proportion of the population with very low fat intakes (< 10% of energy) and increases the proportion with intakes of fat higher than optimal (> 30% of energy). The Chinese data were analyzed additionally by income, showing a direct relationship between household income and percent of dietary energy from fat in both urban and rural households (13).

Nutritional status as judged by body mass index (BMI) in adults has a complex relationship with diet and socioeconomic status (SES). In general, pretransitional societies show positive relationships between adult BMI and SES, while in posttransitional societies the relationship is reversed, particularly for women. Figure 2 shows an analysis of data on women’s BMI as a function of family income in Brazil in 1989 (14). Among poor families, women’s BMI increased linearly with household income, with the slope of the relationship steepest among the poorest families. Among the 30% of families with the highest household incomes, income showed no relationship to women’s BMI. In post-transitional societies, obesity is emerging as a problem of poverty rather than affluence, paralleling the distribution seen by socioeconomic status in the US and the UK (15).

The prevalence of obesity is increasing in developing countries, and the associated morbidities and mortality risks are emerging as public health problems (13,15–24). Most of the literature comes from specific cohorts and localized community data, but the available national surveys support the notion that the prevalence of obesity is increasing rapidly in many countries and that this phenomenon is taking place in the presence of continuing problems of undernutrition and micronutrient deficiencies in children. In the Egypt Food Consumption Monitoring Survey of 1994, the prevalence of obesity among adult women was shown to be high (> 30%) even in the presence of continued endemic short stature and underweight in preschool children (31% and 11% respectively). Risk of obesity was significantly higher for urban than for rural women (25). The China Health and Nutrition Survey showed that the percentage of the population overweight increased between 1989 and 1991, and was strongest in the highest income groups (13).

Whether early undernutrition might predispose to later adult obesity and associated conditions is a question with potentially important implications. In the United States, adult obesity shows no relationship to stature (26). However, studies in the United States and Norway have demonstrated an inverse association between height and coronary heart disease, as well as other chronic disease causes of death, in adulthood (27,28).

Possible relationships between undernutrition early in life and later risk of obesity and related chronic disease has received recent attention largely because of the body of work published by Barker and colleagues from England (29,30). Briefly, these investi-
gators have observed that in the United Kingdom (as in the United States) obesity and related morbidity and mortality risks are more prevalent among the lower socioeconomic portion of the population, as is the risk of low birth weight and poor postnatal growth. In a series of correlational studies, they have shown a relationship between low birth weight and risk in adult middle age of high blood pressure, noninsulin-dependent diabetes, and other risk factors for coronary heart disease. They hypothesize several possible mechanisms by which early undernutrition, either in utero or in early infancy, may “program” the individual for increased risk of adult chronic disease; these include modification of gene expression by the nutrient environment, modification of the endocrine milieu through permanent influences on endocrine structures or responses, reduced cell number and altered organ structure, and selection of clones of cells resulting in differences in proportions of different cell types. While intriguing, the hypotheses of Barker et al. require extensive examination in a variety of settings and designs (30). If correct, then part of what has been regarded as the genetic contribution to ischemic heart disease may in fact be the effect of the nutritional environment during early development; the implications for developing countries are potentially great.

3.1. The Variety of Nutritional Patterns in Developing Countries

The differences among developing countries with regard to the distribution and prevalence of various nutrition problems and risks are at least as great as those between industrialized and less developed countries. The major determinants of the prevalence of various forms of malnutrition as well as other health problems are extremely variable. For example, the population of children under age 5 yr is expected to increase by only 2% in Latin America between 1985 and 2015, and 5% in Asia, but 38% in the Middle East and 70% in sub-Saharan Africa (13). Some of the specific challenges fac-
Public health nutrition planners in different economic contexts are discussed below.

### 3.2. The Lowest-Income Countries: The Unfinished Agenda Dominates

It has been estimated that over the next decade perhaps 30–40 countries will remain in the lowest income bracket, in which health problems are dominated by infectious diseases of childhood, undernutrition, and high fertility (4,29,31). The major problems continue to be diarrheal disease, acute respiratory illnesses in young children, measles, tetanus, polio, malaria, micronutrient deficiencies, parasitic diseases, and reproductive mortality. In these predominantly sub-Saharan and South Asian countries, the focus of nutrition goals must continue to be on the establishment of basic primary health care infrastructure, breastfeeding, maternal nutrition, immunization against vaccine-preventable diseases, control of diarrheal disease, and family planning. A recent analysis of trends in child undernutrition in the 15-year period between 1970 and 1995 (33) shows a 15.5% reduction in rates of child underweight, attributable primarily to improvement in formal education, in national food availability, and in basic sanitation and health services.

### 3.3. Middle-Income Countries: Social Polarization of Priorities

In a larger group of somewhat higher-income countries, predominantly in East Asia, Latin America, and the Middle East, the infectious disease, malnutrition, and reproductive health burdens remain substantial at the same time as rapid urbanization, industri-
alization, and economic development are leading to the emergence of new health problems including injuries, occupational injuries, and preventable chronic diseases. Diet-related chronic disease emerges as a major priority quickly, since it affects first the higher-income, urban, educated segment of the population.

3.4. High-Income Countries: The Challenge of Equity

In high-income countries diet-related chronic diseases dominate the public health nutrition agenda. Problems of undernutrition and access to food affect mainly the lower income (and politically marginalized) segments of the population and generally have to fight for space on the political agenda and for resource allocation. Further, at least in the United States and the United Kingdom, obesity and its attendant problems also are most prevalent among low socioeconomic status segments of the population. So-called “diseases of affluence” are actually diseases of poverty in some of the richer countries. Policy makers often find it difficult to conceptualize the simultaneous occurrence of undernutrition and obesity, with the result that improvement of access to food and dietary quality often get a back seat to other priorities.

The nations which comprise Eastern Europe and the former Soviet Union represent rather special cases; these have been categorized as “industrialized, nonmarket economies” (32). Having long since passed through demographic, epidemiologic, and nutrition transitions, they have been recently undergoing an epidemic of excess mortality among relatively young adults from preventable causes (34). These include trauma, poisoning, respiratory diseases (often secondary to smoking), and complications of pregnancy and child birth (35). Dietary and nutritional problems include high alcohol consumption, very high costs of food, limited dietary variety in terms of fruits and vegetables, micronutrient deficiencies in some groups, including iodine deficiency disease, and undernutrition particularly in the elderly (36). Recognition of the substantial inequities in nutritional well-being and food security within present-day Europe characterizes a recent draft Food and Nutrition Action Plan for the region (37).

3.5. The Challenge of Protecting the Nutritionally Vulnerable in the Course of Economic Adjustment Programs

The economic adjustment or “restructuring” programs that began in the 1980s and continue today resulted from a combination of pressures including reduced foreign loans to governments of less-developed countries, inability to generate internal monies through taxation or borrowing, and declines in real per capita incomes in a number of countries. Although the specifics vary, restructuring generally involves increased domestic savings, decreased government expenditures, increased economic efficiency, and contraction of public infrastructure.

In a number of countries a major feature has been the elimination or drastic curtailment of untargeted or minimally targeted consumer subsidy programs. Without specific protection, one might expect such adjustments to have the greatest impact on the poorest consumers, with resultant increases in food insecurity and risk of undernutrition. Egypt is a major case in point (25). Prior to 1990, a complex but largely untargeted subsidy and price control program made available to consumers at very minimal cost a basic and nutritionally adequate diet. The subsidy program has been largely dismantled except for wheat flour and local bread. The prices of basic food commodities, as well as other consumer goods and utilities, increased three- to ten-fold during the period 1990–1994, while real wages only dou-
bled. In 1994, two-thirds of households in a large survey of almost 7000 households reported having changed their food intake in the previous year because of rising food prices and almost half (48%) reported spending more than three-quarters of their household income on food. The prevalence of household food insecurity (measured by reporting spending > 75% of household income on food plus giving a food response to an open-ended question about how any hypothetical additional household income would be used) ranged from 4–21% in various regions. There is some suggestion that the prevalence of underweight in preschool during the same period may have increased slightly, after several years of steady decrease.

Protecting the poorest consumers from the negative short-term impact of structural adjustment programs requires the formulation of explicit goals and strategies to redirect a portion of resources into direct targeted programs to protect and improve the situation of vulnerable groups, and to redirect economic and social policies to promote the availability and consumption of nutritionally adequate diets among low-income families and children. Examples are the integration of explicit food and nutrition objectives and programs into adjustment strategies and programs of assistance through World Bank loans in Venezuela, Mexico, El Salvador, Costa Rica, and Jamaica (38).

4. GOALS FOR PREVENTIVE NUTRITION

4.1. Readiness to Take Advantage of New Information and Technology

While nutritional improvements have generally paralleled economic development, there is far from a one-to-one or deterministic relationship between the two. Experience to date indicates that medical and public health interventions, per capita incomes, and behavior and lifestyle variables must all be taken into consideration. A few countries and regions have managed to improve nutritional status dramatically without major increases in per capita incomes, while others having experienced large increases in income have not made concomitant strides in nutritional improvements. To cite a few extreme examples (39), Sri Lanka, China, and Vietnam, with per capita incomes in the range of $330–420, have life expectancies between 66 and 71 yr, while Saudi Arabia, Libya, and Oman exhibit life expectancies of 61–64 yr against per capita incomes over $5000.

Caldwell (40) has analyzed data on health and income, identifying eleven countries (plus the state of Kerala in India) with health achievements over and above what would be expected by per capita incomes and another eleven countries with the poorest performance. The areas which did the best in achieving favorable health indicators were (in descending order) Kerala, Sri Lanka, China, Burma (now Myanmar), Jamaica, India, Zaire, Tanzania, Kenya, Costa Rica, Ghana and Thailand. They averaged a life expectancy of 61 yr and infant mortality of 64/1000 live births, and a per capita income average of $501. The countries that did worst in this analysis were (in order from the poorest performance) Oman, Saudi Arabia, Iran, Libya, Algeria, Iraq, Yemen AR, Morocco, Ivory Coast, Senegal, and Sierra Leone, with average per capita incomes of $4462, life expectancy 51 yr and infant mortality rates 124/1000 live births. The analysis showed that the strongest relationships with improved health status at low cost where variables which reflected the education of women of reproductive age, the practice of family planning, and the education of men. Medical care variables were less strongly related to health indices, and income showed even weaker relationships.

These analyses strongly support the idea that societies with an egalitarian tradition that includes relative independence for women and an emphasis on the value of educa-
tion, have been able to take advantage of advances in medical and public health knowledge and information to improve their situations. Those that have severe differentials between male and female educational opportunities, and for which other cultural and behavioral characteristics mitigate against reduced fertility, have done poorly with regard to health even with rapidly and drastically increased incomes.

4.2. The Importance of Setting Goals

The explicit articulation of goals for protection and improvement of nutritional status has the effect of continuous reinforcement of the importance of nutrition to policy makers and program administrators. It also provides the basis for monitoring of progress. The incorporation of specific and measurable nutrition and dietary goals into national health policy plans has characterized some of the most successful national experiences, including those of Norway (41), Costa Rica (42), the US (43), Cuba (44), and others.

The World Declaration and Plan of Action for Nutrition, agreed upon at the International Conference on Nutrition (ICN) in Rome in December 1992, set an ambitious agenda before the year 2000 (45). These included elimination of famine and famine-related deaths; starvation and nutritional deficiency diseases in communities affected by natural and man-made disasters; and iodine and vitamin A deficiencies. The goals also included substantial reduction by the year 2000 in starvation and widespread chronic hunger; undernutrition among children, women and the aged; other important micronutrient deficiencies including iron; diet-related communicable and noncommunicable diseases; social and other impediments to optimal breast-feeding; and inadequate sanitation and poor hygiene, including unsafe drinking water.

It has become clear that the goals of the ICN were unrealistic. At mid-decade, only 46% of participating countries and territories had finalized or drafted their national plans of action for nutrition; 29% had not yet begun (46). Some progress has occurred. There has been remarkable response to universal salt iodization, where iodized salt is reaching remote areas in several developing countries and the global prevalence of IDD has been reduced to 14% in 1997 from 30% in the early part of the decade (47). However, elimination of IDD, iron deficiency, and vitamin A deficiency will require much coordinated and committed action. Progress toward reduction in protein-energy malnutrition and other types of malnutrition is far, in every case, from the levels required if the goals are to be met.

4.3. Primary Prevention vs Secondary Interventions and the Polarization of Priorities

While the simultaneous existence of problems of deficit and excess is now generally acknowledged, the informed articulation of realistic goals for overall nutritional improvement in developing countries is far behind where it needs to be. As Popkin (8) has pointed out, a failure to acknowledge the role of nutrition in coronary heart disease, cancer, and other chronic diseases will lead to the domination of medical interventions in the allocation of resources. The experience of the industrialized countries to date argues that primary prevention strategies associated with lifestyle changes are the most effective strategies with regard to these post-transition health problems. The challenge will be to articulate goals for preventive nutrition clearly and to avoid the polarization that results from a conceptual framework that pits undernutrition and children and the poor
against emerging problems associated with diets dense in energy and fat and with sedentary lifestyles. If the hypotheses of Barker et al. (30) prove to be correct, then the best weapon against chronic diseases of adulthood will prove to be protection and improvement of the nutritional status of mothers and children.

5. RECOMMENDATIONS

Our recommendations for policy makers and planners in developing countries are several:

1. Make explicit goals for reduction of malnutrition, undernutrition and food insecurity in vulnerable population groups,
2. Allocate resources and direct policies to assure the achievement of these goals even in the face of overall economic stress,
3. Embrace primary prevention strategies first for damming the flood of diet-related chronic diseases of adulthood, and
4. Give priority to the development and maintenance of nutrition surveillance systems which will allow the tracking of the nutrition transition in specific circumstances.

On the research agenda, priority should be very high for efforts to better understand the relationships between early (in utero and early childhood) nutritional status and adult health, in a variety of contexts, and to discover ways to assure food and nutrition security (including prevention of obesity and related chronic disease) for entire populations.

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476 Part V / Global Preventive Nutrition Strategies

VI  NUTRITION-RELATED RESOURCES
Books Related to Preventive Nutrition

Websites of Interest

http://www.ifst.org/
IFST (Institute of Food Science & 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, PhD, “The Blonz Guide” focuses on the fields of nutrition, foods, food science & health supplying links and search engines to find quality sources, news, publication and entertainment sites.

http://www.hnrc.tufts.edu/
The Jean Mayer United States 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 American Dietetic Association is the largest group of food and nutrition professionals in the US, 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 (The American Dietetic Association); and lobbying for federal legislation. Also available from: Preventive Nutrition: The Comprehensive Guide for Health Professionals, 2nd ed.
Edited by: A. Bendich and R. J. Deckerbaum © Humana Press Inc., Totowa, NJ
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.faseb.org/ain/hometest2.html
The American Society for Nutritional Sciences (ASNS) located in Bethesda, MD, is a research society facilitating, for example, animal and human nutrition studies, official publication (The Journal of Nutrition, available through nutrition.org), annual meetings, education and training opportunities, and professional networking. Categories for membership include the following: regular, associate, student, and emeritus.

http://www.faseb.org
The Federation of American Societies for Experimental Biology (FASEB) is a coalition of member societies with the purpose of enhancing the profession of biomedical and life scientists, emphasizing public policy issues. FASEB offers logistical and operational support as well as sponsoring scientific conferences and publications (The FASEB Journal).

http://www.ificinfo.health.org/
The International Food Information Council (IFIC) is a non-profit organization whose purpose is to provide access to health and nutrition resources, data, and information based on science to professionals, educators, journalists, government officials and others in order to facilitate the communication of health and nutrition information to consumers.

http://www.ifis.org/home.html
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.veris-online.org/
The VERIS Research Information Service is a non-profit corporation, focusing on antioxidants, providing professionals with reliable sources on the role of nutrition in health. Data in VERIS publications, distributed without fee to those who qualify, is based on technical peer-reviewed journals. Quarterly written reports and newsletters, research summaries, annual abstract books, vitamin E fact book and educational programs are among the available VERIS publications and communications. Links to helpful web resources are also accessible.
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://www.calciuminfo.com
This is an online information source created, copyrighted, and maintained by SmithKline Beecham Consumer Healthcare Research and Development. The nutritional and physiological role of calcium is presented in formats designed for healthcare professionals, consumers, and kids. References and related links, educational games for kids, calcium tutorials, and a calcium calculator are easily accessible.

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.dsqi.org/
The Dietary Supplement Quality Initiative (DSQI) is designed to educate consumers on the health benefits, safety, standards and regulations, and labeling of dietary supplements. Industry news, interviews, editorials, and DSQI resources and services provide useful tools for consumers, practitioners, producers and distributors.
Martindale’s Health Science Guide-2000, “The Virtual Nutrition Center”, provides a large volume of information. The Nutrition Overview lists resources such as travel warnings and immunization, on-line nutrition calculators, nutrition journals, literature and patent searches, conferences, and dictionaries. Nutrition Interactive allows access to databases, courses and tutorials. All sections are accessed through a single site, use caution when printing!

The United States 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.

The National Agriculture Library (NAL), a primary resource for agriculture information, is one of four national libraries in the US 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.

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.

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.

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.

Nutritional Science Journals

Annual Review of Nutrition

American Journal of Clinical Nutrition
http://www.erlbaum.com/1065.htm
Nutrition and Cancer

http://www.faseb.org/asns/journal/journal/html
The Journal of Nutrition

http://www.crcpress.com/jour/catalog/foods.htm
Critical Reviews in Food Science and Nutrition

Proceedings of the Nutritional Society

http://www.stockton-press.co.uk/ijo/index.html
International Journal of Obesity

http://www.wiley.com/Home.html
International Journal of Eating Disorders

http://www.cup.org/Journals_JNLS_CAT/nut/nut.html
The British Journal of Nutrition

http://www.ils.org/dnutrition.html
Nutrition Reviews

http://www.peakcom.com/clinnutr.org/jabs.html
Journal of Parenteral and Enteral Nutrition

http://www hbuk.co.uk/ap/journals/ap.htm
Appetite

http://www.stockton-press.co.uk/ejcn/index.html
European Journal of Clinical Nutrition

http://www.lrpub.com/journals/j1013.htm
Journal of Pediatric Gastroenterology and Nutrition

http://www.eatright.org/journaltoc.html
Journal of the American Dietetic Association

http://www elsevier.nl:80/inca/publications/store/5/2/5/0/1/3/
Journal of Nutritional Biochemistry

http://www.karger.com/journals/anm/anm_jh.htm
Annals of Nutrition and Metabolism

http://www.hcsyr.edu/inutrition/
Nutrition: The International Journal of Applied and Basic Nutritional Sciences
http://www.elsevier.nl/inca/publications/store/5/2/5/4/8/3/
Nutrition Research

http://www.mcb.co.uk/liblink/nfs/jourhome/htm
Nutrition and Food Science

http://www.tandf.co.uk/journals/carfax/09637486.html
International Journal of Food Sciences and Nutrition

Journal of Renal Nutrition

http://www.ajnd.org/au/index.html
Australian Journal of Nutrition and Dietetics
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Preventive Nutrition
The Comprehensive Guide for Health Professionals
Second Edition

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Since the widely acclaimed first edition of Preventive Nutrition: The Comprehensive Guide for Health Professionals was published in 1997, many new studies have significantly extended our understanding of the health effects of nutrition. In this richly enhanced second edition, leading nutritionists, public health experts, and clinicians update and extend this now classic guide to improving individual health outcomes through appropriate nutrition. The new edition encompasses the broadest range of topics, from cancer and cardiovascular disease to infectious disease in children, from reproductive and prenatal nutrition to global public health nutrition strategies. Each chapter provides an overview of the field, a discussion of the author’s own research and its implications, and recommendations for various patient groups based on the totality of evidence rather than on the findings of any single study.

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■ Illuminates nutritional factors in cancer, cardiovascular disease, diabetes, cataracts, and osteoporosis
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