EVOLUTION OF EVIDENCE FOR SELECTED NUTRIENT AND DISEASE RELATIONSHIPS

INSTITUTE OF MEDICINE

Evolution of Evidence for Selected Nutrient and Disease Relationships

Committee on Examination of the Evolving Science for Dietary Supplements

Food and Nutrition Board

INSTITUTE OF MEDICINE

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Willing is not enough; we must do."
—Goethe

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC) Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of

the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Malden C. Nesheim, Ph.D., Cornell University, appointed by the Institute of Medicine, and Gilbert S. Omenn, M.D., Ph.D., University of Michigan, appointed by the NRC's Report Review Committee. The coordinator and monitor were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

As chair of the panel, I want to thank my fellow committee members for their commitment to the work of the committee under a rather demanding time schedule. Their quick and constructive responses to the many drafts of the report made meeting the deadline possible.

Norman I. Krinsky
Committee Chair

Contents

Executive Summary

Diet is vital to health promotion and disease prevention. Several decades of impressive evidence have documented that in addition to preventing nutrient deficiency diseases like scurvy and rickets, dietary practices can also help to prevent other diseases, including cardiovascular disease, diabetes, osteoporosis, dental caries, birth defects, and potentially some types of cancer. Although consumers, scientists, entrepreneurs, and policymakers want evidence on potential new relationships between nutrients and chronic diseases as soon as possible, conclusive evidence is typically elusive. Gathering sufficient knowledge to draw conclusions about causal relationships, especially between a given nutrient and a disease, remains a challenge.

Can the scientific method be accelerated by identifying patterns of evolving evidence to yield confirmed findings for public policy? Can patterns of research evidence be observed in identified nutrient–disease relationships that can be applied to potential nutrient–disease relationships earlier in the research process and benefit the public by enabling earlier application of such knowledge? Can unpredictable elements be identified to provide caution to consumers? The Committee on Examination of the Evolving Science for Dietary Supplements of the Institute of Medicine's Food and Nutrition Board was convened to address this topic.

THE COMMITTEE'S APPROACH

The committee was directed to review, retrospectively, selected case studies of diet and health relationships that were relevant to dietary supplements and identified as important in the National Research Council report, *Diet and Health: Implications for Chronic Disease Risk* (D&H) (NRC, 1989). It was then to determine the extent to which subsequent scientific evidence from the peer-reviewed literature used in published reports from the Dietary Reference Intakes (DRI) series (IOM, 1997, 1998, 2000a, 2001) either agreed with the preliminary evidence used to support the relationship identified originally in the 1989 review or significantly modified the original hypotheses and preliminary conclusions. The committee's analysis was to include characteristics of research with apparent high probability of predicting future confirmation by new science in support of a diet and health relationship. It also was to consider characteristics of information useful to consumers that would allow them to make scientifically informed judgments about the role that a specific food component or nutrient plays in health.

The committee based its analysis only on evidence cited in the D&H report and in nutrient-specific DRI reports published by March 2001. The DRI reports were: *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (IOM, 2001). The committee was aware of, but did not include, evidence published since release of the relevant DRI reports because that was not part of its charge.

Because the D&H and DRI reports were prepared for different purposes, precise comparison of evidence was sometimes difficult. The D&H report was an "in-depth analysis of the overall relationship between diet and the full spectrum of major chronic diseases" (D&H, p. 4). It was intended to move beyond assessment of dietary risk factors for single chronic diseases and address the complex task of determining how these risk factors influence a number of chronic diseases. The intended outcome of the assessment was recommendations for dietary patterns that would reduce risk for chronic disease. The DRI reports present "reference values that are quantitative estimates of nutrient intake to be used for planning and assessing diets for apparently healthy people" (IOM, 2000a, p. 2). In spite of differences in purpose, the D&H and DRI reports, separated by a decade or more, are deliberative reviews of evidence about many nutrient–disease relationships, and the committee determined they could be used in a qualitative description of selected case studies and an assessment of possible patterns in relationships.

BOX ES-1 Case Studies (Dyads)

- β-carotene and lung cancer
- Calcium and bone status
- Chromium and diabetes
- Folate and cervical dysplasia
- Folate and colorectal cancer*
- Folate and neural tube defects*
- Fluoride and dental caries
- Phosphorus and bone status
- Vitamin C and colds
- Vitamin C and gastric cancer
- Vitamin D and bone status
- Vitamin E and cancer (except prostate)
- Vitamin E and coronary heart disease
- Vitamin E and prostate cancer*

*Only in DRI report.

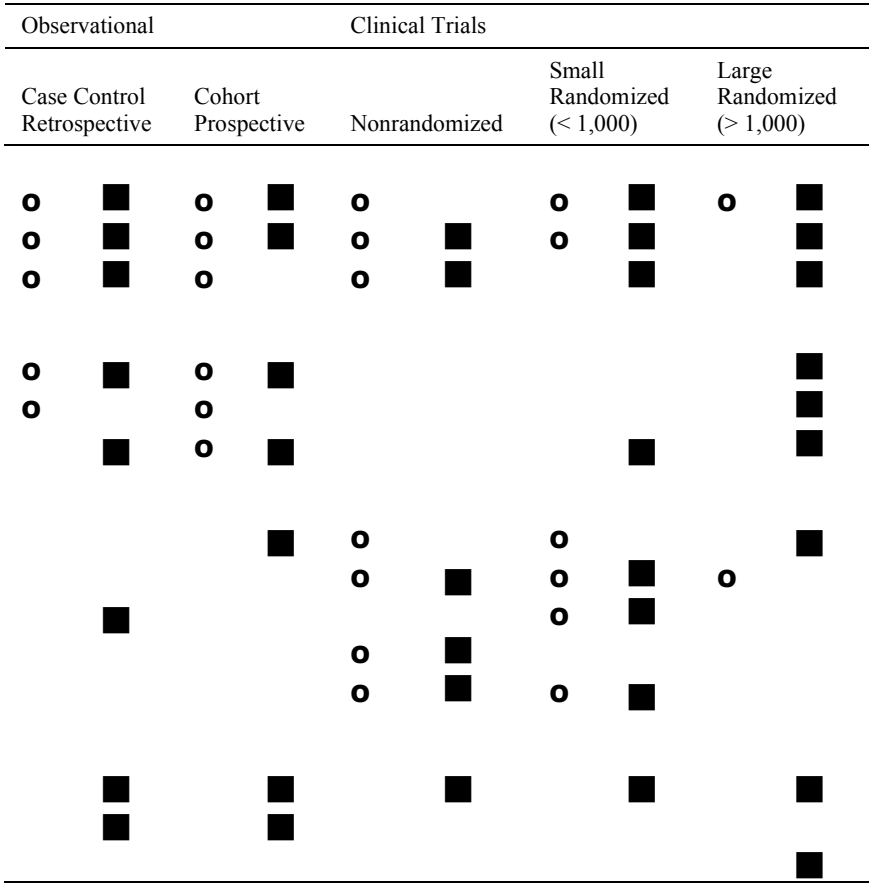
To accomplish its task, the committee developed and applied a qualitative approach to select nutrient–disease relationships (dyads), to determine the level of confidence in a positive relationship (accepted, promising, uncertain, or no relationship), and to examine differences in levels of confidence between the two reports (increased, decreased, unchanged, or not in D&H). Fourteen dyads were selected and classified, including three that were discussed only in a DRI report (see Box ES-1). The committee used the classification as a way to summarize the evidence as described in each report. It is not intended as a recommendation for or against increased consumption of a nutrient.

The evolution of evidence is described in Chapter 2 of this report. The committee prepared a tabular summary of types of evidence in the D&H and DRI reports to assess whether there were patterns of evidence that predicted an increased, decreased, or unchanged level of confidence in a positive relationship. The committee's findings and conclusions were based in large part on its review of the evidence as summarized in Table ES-1.

TABLE ES-1 Change in Confidence in a Positive Relationship by Type of Evidence for Nutrient–Disease Dyads

		Types of Evidence in D&H and DRI Reports[a]			
		Animal		Mechanistic	
Change in Confidence[b]	Dyad				
Increased					
A → A+[c]	Fluoride and dental caries	O	■	O	■
U → A	Calcium and bone status	O		O	
U → A	Vitamin D and bone status	O		O	
Decreased					
P → N[d]	β-carotene and lung cancer	O		O	■
P → U	Vitamin C and gastric cancer	O		O	■
U → N	Vitamin E and cancer[e]	O			■
Unchanged					
U → P → U	Vitamin E and CHD[f]	O	■		■
U → U	Vitamin C and colds				■
U → U	Folate and cervical dysplasia				■
U → U	Phosphorus and bone status[g]	O		O	■
U → U	Chromium and diabetes	O			
Not in D&H					
→ A	Folate and neural tube defects		■		■
→ P	Folate and colorectal cancer				■
→ P	Vitamin E and prostate cancer				

[a] O = *Diet and Health: Implications for Reducing Chronic Disease Risk* (D&H) report (NRC, 1989), ■ = Dietary Reference Intakes (DRI) reports (IOM, 1997, 1998, 2000a, 2001). See text for description of types of studies.
[b] A = accepted, P = promising, U = uncertain, N = no relationship. Some animal and mechanistic studies may have been cited in review articles in the DRI reports.
[c] Indicates extension to include adults, not just children.
[d] Confidence changes from promising to uncertain for diet and from uncertain to no relationship for dietary supplements.



[e] Except prostate cancer.
[f] CHD = coronary heart disease.
[g] For phosphorus, the D&H and DRI reports did not include any studies that directly assessed the effect of dietary intake of phosphorus on bone, but only on phosphorus absorption or serum phosphorus. In the DRI report, for young children only, data on measures of whole body bone mineral content were used to estimate accretion of phosphorus in the body during growth

FINDINGS

Confidence in nutrient–disease relationships can change, often in unexpected directions.

An important finding is that preliminary evidence in support of a nutrient–disease relationship was often not confirmed. Neither promising relationship from the D&H report (β-carotene and lung cancer, vitamin C and gastric cancer) was subsequently accepted in a DRI report. Of eight uncertain dyads from the D&H report, two were subsequently found to be accepted (calcium and bone status, vitamin D and bone status), one was found not to be a relationship (vitamin E and cancer [excluding prostate cancer]), and five remained uncertain (vitamin E and coronary heart disease [CHD], vitamin C and colds, folate and cervical dysplasia, phosphorus and bone status, chromium and diabetes). High-dose β-carotene and lung cancer in smokers is illustrative. An impressive body of evidence, including numerous observational studies, suggested that increased intake of foods rich in β-carotene might reduce the risk of developing lung cancer. This appealing hypothesis was evaluated by testing high-dose β-carotene administration in three large-scale, long-term trials, two of which focused on populations at high risk for lung cancer. In contrast to expectations, supplementation with β-carotene significantly increased the incidence of lung cancer in the two studies that enrolled persons from high-risk populations. In the third trial, involving male physicians, β-carotene supplementation had no significant effect. Hence, not only was confidence in the putative benefit of β-carotene reduced, but also the direction of the relationship changed because the available evidence suggested that β-carotene supplementation may increase the risk of lung cancer in high-risk populations.

No pattern of evidence clearly predicts change in the confidence of relationships, particularly those initially deemed uncertain or promising.

The evidence cited for each nutrient–disease dyad was heterogeneous. The committee could not identify any pattern of evidence that consistently predicted subsequent change in the level of confidence. The committee observed three instances in which confidence in a relationship decreased from the D&H report to the DRI report (β-carotene and lung cancer, vitamin C and gastric cancer, vitamin E and cancer [except prostate cancer]). In each instance, a common characteristic was an absence of trial citations (even a small trial of less than 1,000 participants) in the D&H report and the presence of trial citations in a DRI report. The case studies suggest that there is a tendency for large trials to be developed when smaller trials are promising, but the outcome of larger trials remains unpredictable. Even the citation of small clinical trials in the D&H report did not predict the nature of the relationship in a DRI report.

Large randomized trials have the greatest impact in changing the level of confidence in a nutrient–disease relationship.

Not surprisingly, large clinical trials were cited for only two dyads in the D&H report (fluoride and dental caries, vitamin C and colds) and nine dyads in the DRI reports (fluoride and dental caries, calcium and bone status, vitamin D and bone status, β-carotene and lung cancer, vitamin C and gastric cancer, vitamin E and cancer [except prostate], vitamin E and CHD, folate and neural tube defects [NTDs], vitamin E and prostate cancer). The latter two were not mentioned in the D&H report. For a dyad considered accepted in either the D&H or DRI reports, a large clinical trial was cited. In those instances with an increase or a decrease in the level of confidence in a positive relationship, a large clinical trial typically was cited in the DRI report. For vitamin E and CHD, considerable interest developed as a result of prospective observational studies published after the D&H report that suggested the relationship to be promising. However, large clinical trials published prior to issuance of the corresponding DRI report failed to demonstrate a beneficial effect of vitamin E on CHD. On the other hand, large clinical trials confirmed a role for folate in reducing the risk for NTDs.

The examples of vitamin E and CHD and of vitamin C and gastric cancer highlight the difficulty in conducting large-scale trials to investigate potential beneficial effects of single nutrients in reducing the risk for a chronic disease like CHD or cancer, especially when compared with a condition that develops over a relatively short time period, like NTDs. Chronic diseases develop over a long period, typically over decades, and may be affected by various factors at different times in the disease process. Nutrient trials have been more successful in establishing causality for conditions that develop over a much shorter time, such as was the case for trials aimed at preventing NTDs and caries.

Using a case study approach, the committee looked for patterns in types of studies that could streamline the scientific process and bring useful recommendations and information to consumers more rapidly. It did not find a "pattern express train." The committee's review of differences in evidence available at the time of the D&H report (1989) and at the time of the DRI reports (1997–2001) suggests a skeptical approach to statements about beneficial effects of single nutrients based on animal, mechanistic, or observational studies alone, and argues against premature claims of benefit. For consumers, policymakers, and regulators, the committee's assessment is as follows:

- Large, randomized controlled studies play an important role in establishing the relationship between nutrient intake and the risk of disease. Ideally, consumers should base decisions to change intake of specific nutrients on evidence from trials. Likewise, regulators and policymakers should rely heavily upon such evidence to guide nutrient recommendations.

- Caution should be exercised in using preliminary evidence from non-controlled studies as the basis for recommendations for increased intakes of a nutrient.
- Claims about nutrient–disease relationships are more easily made than scientifically supported. Because the implications for public health are so important, caution is urged prior to accepting such claims without supportive evidence from appropriately designed, typically large, clinical trials.

1

Background

Historically, the role of diet in promoting good health and preventing disease has been of great interest to many segments of society. Scientists are faced with the challenge of identifying dietary factors that influence specific diseases and defining their pathophysiological mechanisms. Government agencies, public health policymakers, the food industry, consumer groups, and others seek to interpret and translate that information as they develop regulations, policies, and products and provide advice to the public. The science relative to potential benefits or harm of specific nutrients or dietary supplements for health maintenance and disease risk reduction undergoes an evolutionary process, which frequently makes it difficult for the public to comprehend the current state of the science. The evolutionary process can at times include contradictory evidence from apparently carefully controlled studies. This may be due in part to differences in the dose of the nutrient, size or duration of the study, or characteristics of the population studied. In addition, scientists do not always agree on what constitutes sufficient scientific evidence to warrant changing recommendations to the public. It is not uncommon for a debate to ensue about how much and what kind of evidence justifies giving dietary advice to the public. The increased availability and potentially harmful use of dietary supplements has focused particular attention on nutrient–disease relationships and the role of increased nutrient intake in health promotion and disease prevention.

As in many scientific fields, early results in nutrition often receive wide public circulation and are applied or adopted without a proper evaluation of the scientific merit of the evidence, thus potentially leading to confusion and recommendations that may not be beneficial and could even be harmful. Advances

in multimedia technology and new communication channels such as the Internet have facilitated access to such information and the potential for benefit and harm (SciPICH, 1999). The resulting confusion can dilute important and substantiated public health messages and lead to a lack of confidence in many dietary recommendations. Given the exposure to and interest in an early application of evolving information about diet and health relationships, it is important to determine if it is possible to streamline the scientific method by identifying patterns in types of evidence so that one can potentially predict whether a preliminary relationship will be substantiated by further scientific study. The Committee on Examination of the Evolving Science for Dietary Supplements undertook this task with regard to nutrients and disease at the request of the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services.

THE COMMITTEE AND ITS CHARGE

In accordance with the Institute of Medicine committee process, a seven-member expert committee was appointed upon recommendation of the Food and Nutrition Board. The committee's task was to retrospectively review selected case studies of diet and health relationships identified as important in the 1989 report, *Diet and Health: Implications for Reducing Chronic Disease Risk* (D&H) (NRC, 1989), with relevance to dietary supplements. The committee was to determine the extent to which subsequent scientific evidence from the peer-reviewed literature used in the published Dietary Reference Intakes (DRI) reports agreed with the preliminary evidence used to support the relationship identified originally in the 1989 review, or modified significantly the original hypothesis and preliminary conclusions. Based on the review of these studies, the committee was to provide an analysis that included characteristics of research that appear to have a high probability of predicting future confirmation by new science in support of a diet and health relationship over time. The analysis was also to include characteristics of information useful to consumers regarding the health effects of such food components or nutrients that allow them to make scientifically informed judgments regarding the role that a specific food component or nutrient plays in health.

The committee had expertise in nutritional epidemiology, evidence-based medicine, research design methodology, clinical medicine, dietetics, and nutritional sciences, including micronutrients, B vitamins, antioxidants, and calcium and related nutrients. Five members had participated in the DRI project, and one on the D&H study. Biographical sketches are included in Appendix B.

The committee met three times to consider its scope of work, review the relevant scientific evidence, and develop its findings. Once the committee completed its initial draft report, a set of reviewers familiar with the issues under discussion and approved by the National Research Council Report Review Committee individually reviewed and commented on the draft report. These

reviewers remained anonymous until the report was finalized. The review process is intended to ensure that the report addresses the committee's charge, that the conclusions and recommendations are based on scientific evidence, and that the report is presented in an effective and impartial manner.

THE REPORT

This report looks at the evolution of evidence for selected nutrient–disease relationships by comparing earlier reports prepared using similar mechanisms at different times. For selected case studies, it reviews the types of evidence cited in the D&H report and compares them with types of evidence used to draw conclusions in the DRI series (IOM, 1997, 1998, 2000a, 2001).

The report is divided into three parts. The remainder of this chapter describes how the committee approached its task, identified the case studies, and assessed the evidence. Chapter 2 describes the evidence in the D&H and DRI reports and the evolution of the evidence for each case study. Chapter 3 presents the committee's findings about patterns of evidence.

THE COMMITTEE'S APPROACH

As instructed in its charge, the committee confined its analysis to the nutrient–disease relationships and related scientific evidence specifically referenced in D&H or in one of the four reports from the DRI series published by the time it began its task. The DRI reports examined were *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (IOM, 2001). A report on macronutrients was under development and was not included in the committee's review.

The committee recognized that although the D&H and DRI reports both included assessments of scientific evidence, different purposes of each made precise comparison of types of evidence difficult. The D&H report was an "in-depth analysis of the overall relationship between diet and the full spectrum of major chronic diseases" (p. 4). It was intended to move beyond assessment of dietary risk factors for single chronic diseases and address the complex task of determining how these risk factors influence a number of chronic diseases. The intended outcome of the assessment was *recommendations for dietary patterns that would reduce risk for chronic disease*. The D&H report examined data on the association among diet, health, and chronic disease. Whenever possible, primary sources of data in the literature were used. Works of other evaluative

bodies were secondary sources. Strengths and weaknesses of each type of study were assessed and the total evidence was evaluated against six criteria: strength of association, dose–response relationship, temporally correct association, consistency of association, specificity of association, and biological plausibility. The strength, consistency, and preponderance of the data and the degree of concordance in epidemiological, clinical, and laboratory experiments determined the strength of a conclusion in the report (pp. 38, 653).

The DRI reports present "*reference values that are quantitative estimates of nutrient intake to be used for planning and assessing diets for apparently healthy people*" (IOM, 2000a, p. 2). The recommended intakes in the DRI reports were based on reviews of scientific data from observational and experimental studies published in peer-reviewed journals. When possible, the relationships between nutrient intake and prevention of disease were reviewed primarily using randomized trials, but observational studies were reviewed as well. Many of the questions raised about requirements for and recommended intakes of nutrients could not be answered fully because of inadequacies in the database. Thus, with few exceptions, following review of evidence that included examination of the extent of congruent findings, scientific judgment determined the basis for establishing recommendations for intakes of nutrients (IOM, 2000a).

In spite of the difference in purpose, the D&H and DRI reports were deliberative reviews of the scientific literature spanning a decade or more. Thus, it was felt that these reports were valuable sources for an analysis of the evaluation of scientific discovery related to the role of specific nutrients in disease prevention, and could be used in a qualitative description of selected case studies and an assessment of possible patterns in relationships.

Given its charge to look at patterns in evidence for potential relationships, not to conduct a new evidence-based review, the committee accepted as given the conclusions in the D&H and DRI reports about specific nutrient–disease relationships. The committee was aware of, but did not include, evidence published since release of the relevant DRI report because that was not part of its charge. The committee acknowledges that publication bias (i.e., the greater chance of publishing positive rather than negative findings) exists and that evaluation of nutrient–disease associations could be biased in favor of a positive association. Both reports appear to have evaluated the quality of the evidence in generally similar ways and based conclusions on the totality of the evidence available to them. The D&H report, for example, indicates that "Overall, the strength, consistency, and preponderance of data and the degree of concordance in epidemiologic, clinical, and laboratory evidence determined the strength of the conclusions in the report" (p. 39). The committee relied on the summary statements of the D&H and DRI reports; it did not reevaluate the analyses in those reports. Given that both reports were prepared with similar analyses of the quality of evidence, one would expect that any publication bias would be similar in both reports and would not be expected to affect this committee's findings.

SELECTION OF CASE STUDIES

The case studies selected were to be identified as important in the D&H report and of relevance to diet–disease relationships. For this study, the committee assumed that nutrient–disease dyads listed in the D&H report for which there was some indication of a possible relationship in a summary statement met these two conditions and were potential case studies. The committee sought to be systematic and consistent in its identification of a manageable number of case studies by using the approach described here, and recognizes that judgment was also involved in applying its classification scheme. The general guidelines for selection were: (1) either a summary statement about a relationship in the D&H report or an inconclusive statement in the D&H report and a possible or positive summary statement in the DRI report, in order to discuss evolution, (2) a dyad expressing a potential beneficial effect, and (3) high likelihood that it was an active research area, to increase the opportunity to observe any patterns that existed.

Using a staff-generated list of 85 potential nutrient–disease combinations in the D&H report, the committee members examined statements about each dyad to determine whether a conclusion was drawn about a nutrient–disease relationship. The initial list was limited to those nutrients included in the published DRI reports. If there was a summary statement in the D&H report about a possible positive, beneficial relationship, the dyad was selected for review as a case study. If the relationship was inconclusive, it was kept as a candidate for a case study unless the DRI report also indicated an inconclusive relationship. Most dyads were found to be inconclusive in both the D&H and DRI reports and were not included in the case studies (e.g., selenium and cancer). Also not included were dyads related to adverse effects (e.g., vitamin B_6 and neuropathy).

There were several exceptions. During its review the committee identified several promising or accepted dyads in the relevant DRI report that were not mentioned in the D&H report. These dyads are folate and neural tube defects (NTDs), folate and colorectal cancer, and vitamin E and prostate cancer. Because these represented an evolution of evidence since the publication of the D&H report, they were included in the committee's analysis, using the evidence in the DRI report to classify the types of evidence. Dyads discussed only in a DRI report that were inconclusive in that report (e.g., folate and homocysteine) were not selected. The dyads selected for review (case studies) are shown in Box 1-1. Once the dyads were selected, committee members reviewed the papers cited in the reports to classify types of evidence, drafted and discussed the case studies, and looked for patterns in evidence.

BOX 1-1 Case Studies (Dyads)

- β-carotene and lung cancer
- Calcium and bone status
- Chromium and diabetes
- Folate and cervical dysplasia
- Folate and colorectal cancer*
- Folate and neural tube defects*
- Fluoride and dental caries
- Phosphorus and bone status
- Vitamin C and colds
- Vitamin C and gastric cancer
- Vitamin D and bone status
- Vitamin E and cancer (except prostate)
- Vitamin E and coronary heart disease
- Vitamin E and prostate cancer*

*Only in DRI report.

EXAMINATION OF EVIDENCE

Classification of Types of Evidence

The committee used a categorization of evidence that was adapted from other classifications (IOM, 2000b; NIH, 1998; USPSTF, 1996), as shown in Box 1-2. The committee added the two categories of "animal studies" and "mechanistic studies." Thus, the studies reviewed from the examined reports were classified as either animal studies, mechanistic studies, observational studies (case control or cohort), or clinical trials (nonrandomized, small randomized [< 1,000 subjects], and large randomized [> 1,000 subjects]) (see Box 1-2).

Animal studies are experiments that test the effects of nutrient deficiency or augmentation (through either supplements or foods) on physiological variables and disease outcomes. These studies may use randomized or nonrandomized designs.

Mechanistic studies are studies that assess the effects of nutrient intake on intermediate physiological variables (e.g., the effects of vitamin E on platelet adhesion) or biochemical actions (e.g., the effects of vitamin E on protein kinase C) rather than well-accepted preclinical or clinical outcomes. *Observational case-control studies* are retrospective, observational studies that compare nutrient status, as assessed by dietary intake or biomarkers, in persons who have a

BOX 1-2 Categories of Evidence Used in Three Authoritative Documents and in this Report

U.S. Preventive Services Task Force (1996)
- Expert opinion, descriptive studies/case reports, reports of expert groups
- Uncontrolled studies
- Multiple time series (comparison between time and places)
- Well-designed cohort or case-control studies
- Well-designed, nonrandomized controlled trials
- Randomized controlled trials

NIH (1998)
- Panel consensus judgment
- Nonrandomized trials, observational studies
- Randomized controlled trials (limited body of data)
- Randomized controlled trials (rich body of data)

Institute of Medicine (2000b)
- Consensus reports and guidelines
- Observational studies
- Systematic reviews
- Some evidence from randomized controlled trials (small size or data inconsistent)
- Extensive evidence from randomized controlled trials

Evolving Science Committee
- Animal studies
- Mechanistic studies
- Observational studies
- Nonrandomized clinical trials
- Small randomized clinical trials
- Large randomized clinical trials

disease (cases) and persons without the disease (controls). *Observational cohort studies* are observational epidemiological studies that typically assess in a prospective fashion the risk of developing a disease according to baseline nutrient status in persons who are free of apparent disease at enrollment.

Nonrandomized clinical trials are a heterogeneous group of studies that test the effects of nutrient intake in the setting of a nonrandomized experimental design, for example, depletion–repletion studies. The hallmark of *randomized trials* is random allocation of individuals to different groups, typically a control group or a condition. The committee further subdivided randomized trials by the

number of participants as less than 1,000 (small) or greater than 1,000 (large). Although the cutpoint of 1,000 is somewhat arbitrary, it served to consistently distinguish between smaller and larger studies.

The committee acknowledges limitations of each type of study and the imperfections of such classifications, specifically the recognition that the strongest evidence might come from studies classified lower on this scale. For instance, a well-done study applying an appropriate animal model might be more persuasive than observational studies or small clinical trials in humans. Also, if one applies a "best evidence" approach, one large, well-done clinical trial is more persuasive than a corresponding meta-analysis of multiple trials of small size or variable quality, which may also have the inherent drawback of excluding unpublished negative studies. Limitations of studies of diets or nutrients and disease outcomes are described in the D&H and DRI reports.

Classification of Relationships by Level of Confidence

For each case study, the committee categorized the dyad by the level of confidence for a positive relationship as expressed in the D&H and then in a DRI report. The level of confidence that a relationship was real was described as accepted, promising, uncertain, or no relationship. For this analysis, a relationship was considered *accepted (A)* if the evidence was sufficiently strong that the report specifically recommended increased or decreased nutrient consumption as a means to prevent disease and possibly develop public policy. That is, the report considered the totality of the evidence strong enough to initiate or consider a public policy recommendation. A relationship was considered *promising (P)* if the evidence was sufficiently strong to initiate a large clinical trial or to advocate additional research but not strong enough for public policy recommendations. A relationship was considered *uncertain (U)* if the evidence was sparse or inconsistent and the directives for future research not prominent. A fourth category, *no relationship (N)*, indicates that the report indicated either that no relationship existed or made no concluding comment about a relationship. The committee used the four categories to classify the evidence for selected dyads reported in the D&H and DRI reports. It did not reevaluate the previous conclusions. The system should not be interpreted as a recommendation for or against increased consumption of a given nutrient.

Patterns in Evidence

The committee used a qualitative approach to examine differences in level of confidence for a positive relationship in the two reports and to search for patterns. For this analysis, an *increased* level of confidence meant that there was some progression in the level of evidence (e.g., U to A), or that the indications for augmented intake applied to a broader population (e.g., the role of fluoride as

a means to prevent caries expanded from just children to adults). A *decreased* level of confidence meant that the evidence on balance was less compelling in the DRI report than previous evidence in the D&H report. An *unchanged* level of confidence meant that there was no alteration in the apparent strength of the relationship, potentially as a result of new but inconsistent data or a lack of substantive new data. Relationships not mentioned in the D&H report that subsequently became accepted (folate and NTDs) or promising (folate and colorectal cancer, vitamin E and prostate cancer) were also considered changed and placed in a separate category, *not in D&H*. This category indicates that the D&H report did not state a potential relationship between the nutrient and disease, either because there was no hypothesized effect (e.g., vitamin E and prostate cancer) or because the D&H report focused on the impact of nutrients on chronic diseases rather than adverse outcomes during pregnancy (e.g., neural tube defects).

The committee then prepared a tabular summary of types of evidence in the D&H and DRI reports for each dyad, grouping the dyads as increased, decreased, unchanged, or not in D&H. The committee's findings about patterns were based in large part on its tabular summary. Qualitative evidence was used in lieu of factorial or regression analysis due to the small number of case studies available for review. The committee's findings are discussed in Chapter 3.

2

Case Studies

This chapter describes how the evidence base underpinning the selected nutrient–disease relationships has changed over the past decade, as expressed in the report, *Diet and Health: Implications for Reducing Chronic Disease Risk* (D&H) (NRC, 1989) and the Dietary Reference Intake (DRI) reports published by March 2001 (IOM, 1997, 1998, 2000a, 2001). Following the categorization scheme developed by the committee, the case studies are organized according to whether over time the confidence level of the nutrient–disease relationship was increased, decreased, unchanged, or only evaluated in a DRI report (i.e., not mentioned in the D&H report). For each case study, the types of evidence cited in the relevant report and the reports conclusions are summarized. Each case study concludes with a discussion of the evolution of evidence. Box 2-1 shows how the case studies are ordered.

INCREASED CONFIDENCE IN RELATIONSHIP

Fluoride and Dental Caries

Diet and Health Report

The D&H report summarizes the results of epidemiological studies that began in the 1930s and demonstrated an inverse relationship between the prevalence of dental caries and the fluoride content of water. One paper cited (Dean et al., 1942) reported the incidence of dental caries in children aged 12 to 14 years.

BOX 2-1 Change in Confidence in Relationship

Increased
- Fluoride and dental caries
- Calcium and bone status
- Vitamin D and bone status

Decreased
- -carotene and lung cancer
- Vitamin C and gastric cancer
- Vitamin E and cancer (except prostate)

Unchanged
- Vitamin E and coronary heart disease
- Vitamin C and colds
- Folate and cervical dysplasia
- Phosphorus and bone status
- Chromium and diabetes

Accepted or Promising in DRI; not in D&H
- Folate and neural tube defects
- Folate and colorectal cancer
- Vitamin E and prostate cancer

The 7,257 children included were lifetime residents of 21 cities with the fluoride content of the public water supply ranging from a not detectable level to 2.6 ppm. The incidence of caries declined markedly as fluoride content increased, up to 0.5 ppm, and declined more slowly above that level.

Two papers cited reported the results of additional fluoride on the incidence of decayed, missing, and filled teeth. One report (Driscoll et al., 1981) was a 9.5-year clinical trial. Fluoride tablets were given to first and second grade children in nine schools once or twice a day for 6 years. A control group received a placebo. The tablets were discontinued when two of the communities fluoridated their water, but children were evaluated after 9.5 years. The incidence of caries decreased during the 6 years of fluoride supplements, and the protective effect continued after the fluoride was discontinued. Another study (Lemke et al., 1970) evaluated the effect of fluoridation of the water supply in one city for over 11 years, and then again 4 years after discontinuation of fluoridation. Children were examined before and after the discontinuation of fluoride. The incidence of dental caries was 50 to 60 percent lower after water was fluoridated than before, but increased to prefluoridation levels when fluoridation was discontinued.

Other studies (Anonymous, 1987; Stamm and Banting, 1980) evaluated the effects of differing levels of fluoride in the water supply on root surface caries in adults. Two of the studies compared the incidence of root surface caries in lifetime residents of two communities that had different levels of fluoride in the water (Anonymous, 1987). Both found a lower incidence of caries with the higher level of fluoride. Increased fluoride concentration in the cementum was also observed with increased fluoride in the water. A large trial in which water was fluoridated in three communities (Grand Rapids, Michigan; Newburgh, New York; and Brantford, Ontario) found a 50 to 60 percent reduction in caries prevalence, and no major adverse effects were noted in residents of any age (McClure, 1970).

The results of these studies provide conclusive evidence that fluoridation of the water supply or supplemental fluoride reduces dental caries, and the D&H report stated that "of all dietary components exhibiting a protective effect against caries, the most effective is fluoride" (p. 640).

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997), used the evidence discussed in the D&H report on the cariostatic effect of fluoride as an indicator for an Adequate Intake (AI) for fluoride. An AI, one of the DRI reference values, is a recommended intake value based on observed or experimentally determined approximation or estimate of nutrient intake by a group (or groups) of healthy people. An AI is used when a Recommended Dietary Allowance cannot be determined (IOM, 2001).

The relationship between dental caries, fluorosis, and fluoride concentration in drinking water, based on earlier work by Dean (Dean, 1942; Dean et al., 1942), was examined. In addition, the DRI report cited more recent evidence from observational and clinical interventions indicating that pre- and posteruptive exposures to fluoride have cariostatic effects (Hargreaves, 1992), and that the best results are achieved when fluoride is consumed beginning at birth (Groeneveld et al., 1990). Additional information was also obtained after the D&H report was published on physiological activity by which fluoride produces its cariostatic effect (Marquis, 1995; Whitford, 1996). The DRI report concluded, based on several retrospective clinical studies, that "the earlier children are exposed to fluoridated water or dietary fluoride supplements, the greater the reduction in dental caries in both the primary and permanent teeth" (p. 299), and that "fluoridated drinking water increases resistance to dental caries at all ages" (p. 299). The conclusions of the DRI report agreed with the conclusions of the D&H report and provided additional supporting evidence, demonstrating that exposure to fluoride at all ages prevents dental caries and that both pre- and posteruptive exposure to fluoride has cariostatic effects.

However, studies of the effect of prenatal fluoride supplements produced conflicting results. A prospective study did not support the hypothesis that prenatal fluoride exposure reduces dental caries (Leverett et al., 1997). These data led to the conclusion that "scientific evidence is insufficient to support a recommendation for prenatal fluoride supplementation" (p. 304).

Evolution—Accepted to Accepted +

Large retrospective and intervention studies conducted before publication of the D&H report provided conclusive evidence that fluoridated water and dietary fluoride supplements reduce dental caries. The science in this area had already evolved to the point that the level of fluoride required to reduce caries, but not result in fluorosis, was established. Exploration of the mechanisms of the cariostatic action of fluoride began before the D&H report was prepared and has advanced since it was published. The research results presented in the D&H report were used to establish an AI for fluoride in the DRI report. Studies conducted after the D&H report was published led to the conclusion that there is no evidence to support the need for additional fluoride during pregnancy. Additional studies conducted after the D&H report and considered in the DRI report provided evidence that fluoridated water, dietary supplements, and topical application of fluoride reduce dental caries at all ages.

Calcium and Bone Status

This section discusses dietary calcium in relation to bone status and osteoporosis and in relation to fracture risk.

Diet and Health Report

Osteoporosis. In the D&H report three major lines of evidence were reviewed with respect to the association between calcium intake, bone mass accretion or maintenance, and osteoporosis: (1) the pathophysiological relationships among dietary calcium, intestinal absorption, and bone mass, (2) epidemiological studies, and (3) clinical studies of calcium supplementation. In the first case, achievement of optimal peak bone mass and minimizing bone loss in later life were identified as important factors that could modify risk of osteoporosis. However, experimental evidence for the amount of calcium intake and retention needed to support optimal bone gain and minimal bone loss was unknown. It was concluded that the efficiency of calcium absorption declines in the elderly, but the quantitative contribution of this decline to aging-related bone loss and increase in incidence of fractures in the elderly remained undetermined.

The D&H report reviewed evidence from epidemiological studies that centered on the relationship between dietary calcium intake (either lifetime by diet

history, recent intake by food frequency, or measured intakes) and measures of bone mineral density (BMD) or osteoporosis by one of a variety of techniques. The techniques included single and dual photon absorptiometry, radiology, and computed tomography. The findings did not consistently support a relationship between calcium intake and bone mass or rate of bone loss if measured in women after menopause. In observational studies reporting that people with diagnosed osteoporosis consumed less calcium than age-matched controls, the intakes were usually less than 800 mg of calcium/day. Interpretation of the findings was complicated by a lack of information about activity of the subjects and with respect to consumption of dietary compounds known to interfere with calcium at the absorptive or excretion levels.

Evidence from clinical trials, including both nonrandomized and randomized designs, was summarized in the report as, "the long-term effects of calcium supplementation on bone mass are not yet established" (p. 352). Nearly all studies were conducted in postmenopausal women for whom the primary outcome was bone loss as a function of intervention with calcium (in some studies with estrogen replacement therapy compared to estrogen alone, or in addition to vitamin D or fluoride). Most of the studies reviewed demonstrated that the higher calcium intakes reduced the amount of bone loss, although some found no effect. The studies were limited in that they were relatively short in duration, the methods of measurement of bone mass and site of measurement were highly variable, and the populations in the different studies varied in age and osteoporosis status. None of the studies was designed to demonstrate an association between lifetime intake of calcium and risk of developing osteoporosis.

The report identified postmenopausal women and elderly persons as target groups for interventions with calcium (and vitamin D) since it was well established that production of the active form of vitamin D [1-25-dihydroxyvitamin D] declines with age, and efficiency of calcium absorption is compromised in later life. However, the report concluded that "There is no direct evidence that the impaired intestinal calcium absorption observed during menopause and aging can be overcome by increased calcium intake. Moreover, the evidence that calcium supplementation prevents the trabecular bone loss associated with the menopause is, at best, weak" (p. 360). And further, "calcium supplementation should therefore not be used as a substitute for sex hormone replacement, which prevents postmenopausal bone loss in most patients" (p. 617).

Bone Fracture. Information about an association between calcium intake and fracture risk was limited at the time of the D&H report. Only one study (Riggs et al., 1982) was cited in the D&H report, and it was not a randomized controlled trial (RCT). All participants were postmenopausal women who had generalized osteopenia and one or more nontraumatic vertebral fractures. The calcium intervention of 1,500 to 2,000 mg/day was combined with high, intermittent amounts of vitamin D of 1,250 μg (50,000 IU) once or twice a week.

Fracture risk was reduced by 50 percent with higher calcium and vitamin intakes. Thus, preliminary evidence was provided for reduction of risk of bone fracture in older women with established osteoporosis on a combination of these therapies, but it was impossible to identify a separate role for calcium alone.

Dietary Reference Intake Report

Osteoporosis. Calcium was discussed in the report, *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (IOM, 1997). The basis for derivation of the DRI for calcium varied somewhat with age group. It reflected a combination of data from classic metabolic studies of calcium balance (chosen after rigorous review to meet specific criteria) and clinical trials. In the clinical trials, response to an intervention of two or more levels of calcium given for at least 2 years was assessed by bone mineral content (BMC) or BMD at one or more bone sites. Such measures of bone mass are known to be strong predictors of fracture risk (Cummings et al., 1993).

An example follows that uses a combination of balance studies and trials measuring bone mass to derive an AI for calcium for the age group of 51 to 70 years. Results of balance studies showed that calcium intakes between approximately 1,100 and 1,500 mg were associated with higher calcium retention. However, lack of appropriate balance data in women precluded calculation of a plateau in calcium retention. Data were also reviewed from randomized trials of calcium intervention that measured bone mass as an outcome (Table 4-1 of the DRI report, reprinted in Appendix A) and assessed according to bone site measured and time after menopause. This analysis indicated that the effectiveness of calcium intervention varies by bone site measured, menopausal stage, and usual intakes of the participants. Studies in which calcium intake was increased above 750 mg/day (Reid et al., 1995), 800 mg/day (Prince et al., 1995), or 1,000 mg/day (Riis et al., 1987) showed a reduction in loss of bone mineral from cortical-rich skeletal sites in postmenopausal women. Taking the two approaches together, an AI of 1,200 mg was chosen.

For persons over 70 years of age, there were insufficient data to use balance studies as a predictor of calcium needs. Thus, the report relied on data from four randomized longitudinal studies published during the 1990s (Chapuy et al., 1992; Chevalley et al., 1994; Dawson-Hughes et al., 1997; Recker et al., 1996) in which BMC and BMD were outcome measures. In each of the four studies, supplemental calcium (with or without added vitamin D) resulted in less loss of bone than the lower calcium intake. Bone loss was measured by BMD at one or more skeletal sites including the proximal femur, femoral shaft, spine, forearm, and the total body.

The information from randomized trials was more useful than cross-sectional studies because in the latter, "calcium intake is not accurately measured, calcium intake at one point in time may not reflect lifetime calcium intake,

and bone mass at a single point in time is the result of the lifelong influence of many confounding variables that are not measured" (p. 86). Epidemiological data were also reviewed to assess possible associations between life-long dietary intakes of calcium and osteoporosis, but no direct evidence was available.

The DRI report reviewed the literature on other dietary components (protein, sodium, and caffeine) or lifestyle habits (physical activity) that affect the utilization of calcium, thus ultimately influencing its availability for deposition in bone. In all cases, any nutrient interaction identified was unlikely to have a significant impact on calcium homeostasis and require a calcium intake above what had been set based on the balance studies and bone mineral measures.

Bone Fracture. With respect to bone fracture as the major outcome, several calcium intervention studies conducted during the 1990s in both institutionalized and noninstitutionalized persons revealed a linkage between calcium intake, reduced bone resumption, and a reduction in bone fractures. Of the four studies reviewed, two studies (Chapuy et al., 1992; Dawson-Hughes et al., 1997) were conducted in men and women with participants randomized to placebo or calcium supplement groups. Calcium supplements (500 to 1,200 mg/day) provided a total calcium intake of 1,200 mg/day or more for up to 3 years. In both studies, supplementation reduced bone loss moderately (for whole body bone this was significant up to 3 years), and a reduction of nonvertebral fracture rates occurred. In two other studies (Chevalley et al., 1994; Recker et al., 1996), calcium supplementation of 800 to 1,200 mg/day resulted in a reduction in vertebral fracture rate, although in one study in women with low habitual calcium intakes (Recker et al., 1996), the reduction in vertebral fractures was noted only in those with a previous fracture but not first vertebral fractures. In conclusion, the DRI report cautioned "additional studies are needed to estimate the magnitude of the impact of calcium intake on fracture rates. Available data do not allow use of fracture outcomes to identify the AI for calcium" (p. 116).

Evolution—Uncertain to Accepted

The evidence compiled for the D&H report relied primarily on descriptive studies. Outcome measures consisted primarily of circulating bone-regulating hormones, such as vitamin D metabolites and parathyroid hormone, mineral balance, urinary excretion of calcium or phosphorus, or early generation bone densitometry. Studies reviewed in the DRI report consisted mostly of randomized trials conducted over the past decade.

The use of measures of bone mass by dual energy x-ray absorptiometry (DXA), only available since the early 1990s, allowed for a more quantifiable and bone-site specific outcome measure. This technique used a stable photon source of an x-ray tube rather than a radioactive source (such as iodine-125 or gadolinium), providing greater precision and better ability to detect real changes

or differences in BMC. The availability of DXA thus provided new knowledge about a functional measure of calcium intake as indicated by an accretion of bone mass during growth or prevention of bone loss in the elderly population, the age of achievement of peak bone mass (middle to late teen years as opposed to ages 25 to 30 as previously thought), and the peak bone mass velocity from which estimates of deposition of calcium during peak growth could be calculated. Biochemical markers of bone turnover have also been proven to be sensitive measures of change in bone status. Many recent studies have assessed change in bone status in response to diet by using plasma osteocalcin to reflect bone formation and urinary pyridinoline, deoxypyridinoline, or plasma C-terminal telopeptide of type 1 collagen to reflect bone resorption.

Statements on directions for research from the D&H report such as, "Long-term studies should be conducted to determine the effect of calcium supplementation on the rate at which bone mass is lost in this age group" (in reference to people 65 years of age and older in whom intestinal absorption of calcium is decreased) (p. 622), appear to have set the stage for research in the past decade. Although the DRI report is cautious in its interpretation, data from clinical trials designed to study the effect of varying calcium intakes on bone mass were used along with data on calcium retention to establish the AI of calcium for adult populations.

It is unlikely that evidence from epidemiological, ecological, or clinical trials will ever define a direct causal link between dietary calcium intake in early life and osteoporosis risk later in life. There are too many confounding variables, both dietary and lifestyle-related, that would obscure a direct link, and controlled trials of lifetime duration are not feasible and are extremely costly. Research over the past decade has provided fairly convincing evidence that bone mass influences fracture risk and calcium influences bone mass at any age. Particularly revealing in this regard is that for persons over approximately 66 to 70 years of age, calcium intake not only reduces bone loss but also reduces risk of fracture. Application of this knowledge may have an enormous impact on health-care costs in this population.

From the perspective of fracture prevention, information has evolved in line with the research agenda set out by the D&H report, specifically with reference to the elderly population. In the four well-designed studies reported over the past decade summarized in the DRI report, the interventions were calcium supplements of 500 to 1,200 mg/day (in addition to vitamin D supplements of 8.8 to 20 μg/day [352 to 800 IU/day]) (p. 116), or calcium supplements alone given over 2 to 3 years. For the studies with men and women, the intervention of calcium and vitamin D led to a reduction in fracture rates. In two studies where the intervention was calcium alone and the population was only women, fracture rates were reduced in persons with prior or first-time vertebral fractures. Thus, although the DRI report indicated that the evidence was not sufficiently robust to use in setting an Estimated Average Requirement (EAR), the studies of the past

decade have gone a long way to fulfilling the recommendation of the D&H report for additional research to better understand calcium and bone status. (An EAR, one of the DRI reference values, is a nutrient intake value that is estimated to meet the requirements of half the healthy individuals in a life stage and gender group [IOM, 2001]).

Vitamin D and Bone Status

Diet and Health Report

Most of the evidence linking vitamin D and osteoporosis and bone fractures was indirect in the D&H report, emerging from observational studies on changes in vitamin D status and calcium absorption with aging. In older persons, circulating plasma 25-hydroxyvitamin D (and 1,25-dihydroxyvitamin D) were lower than in younger persons. Possible reasons suggested for this difference are less exposure to sunlight, a reduced capacity for de novo synthesis of the parent compound in the skin, and also perhaps a decline in the activation of the 1-α-hydroxylase enzyme that increases synthesis of the active hormone in response to hypocalcemia via parathyroid hormone (PTH). These studies established a relationship between declining vitamin D status in the elderly and lower bone mass or greater incidence of hip fracture. In four studies (Caniggia et al., 1986; Crilly et al., 1981; Gallagher et al., 1982; Riggs et al., 1976) there was a positive relationship between vitamin D status (or intake of vitamin D or the active metabolite) and an increase in calcium absorption or bone mass or reduction in bone loss. In summary, the D&H report stated, "Numerous studies during the past two decades suggest that elderly people in the United States, Israel, Great Britain, and Europe are at increased risk for developing vitamin D deficiency" (p. 618). It was speculated that vitamin D deficiency in this population may be a key factor in reduced calcium absorption leading to reduced bone mass.

Dietary Reference Intake Report

The observations of the prevalence of vitamin D deficiency in postmenopausal women and the elderly and the association with osteoporosis noted in the D&H report were further substantiated by studies conducted in the 1990s (Chapuy et al., 1992; Honkanen et al., 1990; McKenna, 1992; Pietschmann et al., 1990) and reported in *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997). These led to several randomized, double-blind clinical trials (either in women alone or in men and women) to investigate the effectiveness of vitamin D supplementation above that previously recommended to impede bone loss and reduce fracture risk. The response to a vitamin D supplement of 10 to 25 μg/day (400 to 1,000 IU/day) was maintenance of normal serum 25-hydroxyvitamin D, reduction in elevated

PTH, and reduced bone resorption as indicated by various urinary markers (Brazier et al., 1995; Chapuy et al., 1992; Fardellone et al., 1995; Kamel et al., 1996; Lips et al., 1988; Sebert et al., 1995; Sorva et al., 1991). Only in one study (Lips et al., 1996) was the supplement of vitamin D associated with a significant gain in BMD at the femoral neck (but not other bone sites) in women. However, in a classic study by Chapuy and colleagues (1992) that was replicated by Dawson-Hughes and colleagues (1997) in the United States, a vitamin D supplement of 20 μg/day (800 IU/day) given with calcium for 18 months resulted in a significant increase in BMC and a reduction in vertebral and nonvertebral bone fractures.

Evolution—Uncertain to Accepted

Based on the observational studies cited in the D&H report, it was predicted that older persons require higher intakes of vitamin D (or its metabolite, 1,25-dihydroxyvitamin D) to maintain normal vitamin D status, but no clinical trials of the effects of increased vitamin D intake on osteoporosis or fracture were available (or reviewed) for the report. The studies reviewed in the DRI report support the earlier statement about increased risk of vitamin D deficiency with aging, and led to an AI of 15 μg/day (600 IU/day) for men and women over 70 years of age. Of important functional significance, recently published clinical trials summarized in the DRI report that used DXA technology to track fracture occurrence and were of sufficient duration demonstrated that vitamin D intakes at or greater than the AI level are associated with a reduction in rate of nonvertebral fracture. A persistent limiting factor to the studies on fracture outcome in the elderly is that both supplemental calcium and vitamin D were given. Thus, the relative contribution of each nutrient to the increase in bone loss and reduced fracture rate remains to be explored. In this respect, further research is required to delineate the distinct roles for each of the nutrients in fracture prevention.

DECREASED CONFIDENCE IN RELATIONSHIP

β-Carotene and Lung Cancer

Diet and Health Report

At the time of the D&H report, the hypothesis that dietary β-carotene could prevent lung cancer was promising. The D&H report cited 12 epidemiological studies, including both case-control and cohort studies, that found inverse associations between either dietary intake of β-carotene or plasma or serum levels of β-carotene and risk of lung cancer. While it was recognized that the estimates of intake were derived from measures of fruit and vegetable consumption, there were supportive data from animal studies indicating that β-carotene as a single

agent could inhibit carcinogenesis. The animal studies reviewed were not of respiratory tract carcinogenesis, but rather prevention of skin carcinogenesis, where efficacy in skin carcinogenesis was observed in 4 of 5 animal studies cited.

While a promising association was noted, the D&H report also noted several caveats about this relationship. The report noted that "Consumption of carotenoid-rich foods does not necessarily serve as a protective factor against lung cancer for persons who smoke. The magnitude of the relative risk . . . has not yet been well characterized" (p. 322). The report also noted that "Evidence does not yet permit a conclusion that the association is with β-carotene specifically rather than some other carotenoid" (p. 322). Given this limitation, the report recommended that "consumption of the relevant foods—not the putative protective components of those foods—should be encouraged" (p. 488). The report also stated that "Clinical trials to determine the effect of dietary β-carotene supplements on lung cancer are in progress, but results are not yet available" (p. 313). Thus, the report was cautious and did not advocate supplemental β-carotene as a general approach for lung cancer prevention.

Dietary Reference Intake Report

In the years between publication of the D&H report and the DRI report *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a), considerable research evolution occurred. Three major clinical trials investigating supplemental β-carotene (as a single agent or in combination with another nutrient) and cancer prevention were completed. Two of these trials targeted the primary prevention of lung cancer (ATBC, 1994; Omenn et al., 1996), and a third targeted the primary prevention of total cancers (Hennekens et al., 1996). These trials failed to substantiate a possible preventive role for β-carotene (high-dose supplements) with regard to lung cancer, and contrary to expectations, lung cancer incidence was significantly increased rather than reduced in two of the three trials. A possible modifying role for tobacco and/or asbestos exposure was observed. β-carotene supplementation increased the risk of lung cancer only in persons who were currently smoking or who had significant prior exposure to asbestos in the two trials where an increased risk was noted. The DRI report stated, "These trials indicate a lack of evidence of overall benefit on total cancer or cardiovascular disease and possible harm in certain subgroups such as current smokers or asbestos-exposed subjects" (p. 371).

Although this indicates a failure to substantiate the hypothesis that β-carotene might prevent lung cancer, several caveats were noted in the DRI report. Plasma concentrations achieved in the clinical trials where adverse effects on lung cancer were noted are outside the range achieved through dietary intake (p. 370). Also, the trials were not designed to test the hypothesis that dietary β-carotene obtained from foods (fruits and vegetables) would reduce the risk of

lung cancer. This issue remains unresolved. On the basis of the evaluation of observational data, the report suggested that "3 to 6 mg/day of β-carotene from food sources is prudent to maintain plasma β-carotene concentrations in the range associated with a lower risk of various chronic disease outcomes" (p. 353), a suggested increase in intake from current intake estimates. The report also noted that carotenoid-rich foods are complex mixtures and "it is not clear whether observed health benefits are due to carotenoids per se or to other substances found in carotenoid-rich foods" (p. 351).

Evolution—Promising to Uncertain (Diet) and Uncertain to No Relationship (Supplements)

A possible role for supplemental β-carotene in lung cancer prevention has not been substantiated. Furthermore, the randomized clinical trial data indicated that high-dose supplements of a nutrient could have the opposite effect of that intended, particularly when the doses tested were widely variant from the doses where benefit is inferred in observational research. This case study also highlights the notion that lifestyle factors influence whether a given nutrient intervention is beneficial or harmful. In this case, it appeared that cigarette smokers and asbestos-exposed individuals were harmed, but former smokers had no increase in risk of lung cancer with supplementation. These advances in understanding were the result of time and a substantial financial investment in randomized clinical trials.

A clearer understanding of the role of carotenoid-rich foods in the prevention of lung and other cancers has yet to emerge, despite some methodological advances. For example, since 1993 there has been a database of the carotenoid content of foods (Mangels et al., 1993). Prior to this development, researchers were unable to evaluate the association of carotenoids other than β-carotene with risk of lung cancer or other cancers. As noted in the DRI report (p. 343), more recent observational studies of carotenoids and lung cancer risk indicate that β-carotene is not uniquely associated with lung cancer risk; protective associations have been noted for several other carotenoids, such as α-carotene and lutein (Le Marchand et al., 1993; Ziegler et al., 1996). The role of these and other carotenoids from foods in the risk of lung and other cancers remains uncertain.

This case study also illustrates that the evidence base and evolution of understanding the role of a nutrient in a food matrix and a nutrient provided as a supplement may differ. Because of the differences found in the confidence in the relationship between lung cancer and β-carotene from supplements and from diet, the two sources of intake are considered separately in the discussion of characteristics of evidence.

Vitamin C and Gastric Cancer

Diet and Health Report

At the time of the D&H report, the hypothesis that vitamin C could prevent cancer was most promising for gastric cancer. With regard to this cancer site, the report discussed seven observational epidemiological studies that showed inverse associations between consumption of vitamin C-rich foods or vitamin C status and gastric cancer risk (pp. 331–332). The studies included case-control and cohort studies, with vitamin C exposures assessed by dietary intake estimates and by blood measures of ascorbate status. The observational epidemiological data were supplemented by nearly 20 studies of vitamin C in animal carcinogenesis models (none of gastric cancer), and a limited number of mechanistic studies demonstrating inhibition of nitrosation by vitamin C. The report also mentioned four studies on the use of vitamin C supplements in cancer patients (p. 514), but did not draw on these reports because they were therapeutic studies rather than preventive investigations and therefore not in keeping with the preventive focus of the D&H report. The summary statement in the D&H report concluded, "Epidemiologic studies suggest that vitamin C-containing foods and possibly vitamin C itself either may protect against cancer or have no association with the disease. The strongest evidence for a protective effect seems to be for stomach cancer" (p. 341). Also, recognizing that the epidemiological studies were based on vitamin C-containing foods (fruits and vegetables), the report recommended ". . . in considering appropriate preventive measures, consumption of the relevant foods—not the putative protective components of those foods [vitamin C for stomach cancer]—should be encouraged" (p. 488).

Dietary Reference Intake Report

The DRI text on vitamin C and gastric cancer was limited in that the emphasis of this report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a), was the identification of possible biomarkers and their modulation by vitamin C in order to establish a nutrient requirement for vitamin C. The observational epidemiological literature on vitamin C and gastric cancer was not discussed, but studies of vitamin C supplementation effects on biomarkers of gastric and bladder cancer were summarized in a table (Table 5-2 of the DRI report, reprinted in Appendix A). Three of four studies showed positive effects of vitamin C on biomarkers of gastric cancer. The summary noted that "Despite the epidemiological associations and the evidence that gastric juice vitamin C is protective against nitrosation and oxidant damage, the two vitamin C supplementation studies conducted to date have not shown a subsequent decrease in gastric cancer incidence. Although many of the . . . studies suggest a protective effect of vitamin C against specific cancers

by site, the data are not consistent or specific enough to estimate a vitamin C requirement based on cancer" (p. 125).

Evolution—Promising to Uncertain

A possible preventive role for vitamin C in gastric cancer has not been demonstrated, although the mechanistic basis for such a role has grown considerably. More specifically, an understanding of the role of *Helicobacter pylori* infection in the etiology of gastric cancer was not available at the time of the D&H report, whereas newer studies of the effects of vitamin C supplementation on biomarkers in patients with *H. pylori* infection are becoming available (Mannick et al., 1996). However, 5.25 years of vitamin C supplementation (120 mg/day, in combination with molybdenum) to a large population of poorly nourished men and women at high risk of gastric cancer from Linxian County, China, did not reduce the incidence of gastric cancer (Blot et al., 1993). The relative risk for the study was 1.10, with a 95 percent confidence interval of 0.92 to 1.30. Thus, there appeared to be "no demonstrable short-term benefit" (Blot et al., 1993). While an understanding of gastric cancer etiology has evolved considerably over the past several years, including identification of a possible mechanistic basis for vitamin C preventive effects, large-scale randomized trials were unable to demonstrate this benefit. The negative trial results do not conclusively show that vitamin C is unrelated to gastric cancer risk, given the limitations of trials of chronic disease prevention (e.g., short duration of intervention, only one dose tested, or results may not be generalizable to other populations with different risk factor profiles).

Vitamin E and Cancer

Diet and Health Report

At the time of the publication of the D&H report, animal studies and observational cohort studies of vitamin E and cancer were available. The epidemiological studies mentioned in the D&H report used plasma or serum vitamin E levels to assess vitamin E status. While there was no consistent observed effect of only vitamin E on cancer risk, it was noted that "low serum levels of vitamin E and selenium may be related to increased risk of some cancers, such as breast and lung cancers" (p. 322). With regard to the animal studies, the report noted that "the role of vitamin E in cancer inhibition is inconclusive at this time" (p. 320).

Dietary Reference Intake Report

In the time that elapsed between the D&H report and DRI report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a), several observational and interventional studies of relevance to vitamin E and cancer were published. The largest was a clinical trial of vitamin E for the primary prevention of lung cancer in heavy smoking men from Finland (ATBC, 1994). No reduction in lung cancer risk was noted in men randomized to receive supplemental vitamin E. The relative risk was 0.98, with a 95 percent confidence interval of 0.86 to 1.12. At least five clinical trials with vitamin E to prevent the recurrence of colorectal adenomatous polyps were reported, but none found a benefit (pp. 219–220). Thus, a possible role for vitamin E in the prevention of lung and colorectal cancers has not been substantiated, based on data from large, randomized clinical trials. However, a possible preventive role in prostate cancer was suggested in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC) and is discussed later in this chapter.

Evolution—Uncertain to No Relationship

A possible role for vitamin E in lung cancer prevention has not been substantiated. This is based largely upon a large clinical trial, the ATBC trial discussed above. However, the data from existing trials cannot rule out a possible preventive effect of vitamin E for lung or other cancers in persons with low selenium status, as none of the trials involving vitamin E targeted populations with this status. Low selenium status is believed to lead to biochemical changes that increase risk of illness associated with other stresses (IOM, 2000a).

UNCHANGED CONFIDENCE IN RELATIONSHIP

Vitamin E and Coronary Heart Disease

Diet and Health Report

The D&H report mentions a possible association between large doses of vitamin E and a reduced risk of coronary heart disease (CHD), but does not highlight this association. The initial citation is a letter published in the periodical *Nature* in 1946 that provides a conclusion without actual results; the methods are scant, and it is unclear whether the study is a case series or clinical trial (Vogalsang and Shute, 1946). Subsequently, the D&H report mentions several clinical trials of persons with angina that are nonconfirmatory (Anderson and Reid, 1974; Donegan et al., 1949; Makinson et al., 1948; Rinzler et al., 1950). The trials were typically small in size and of suboptimal design. A trial of persons with intermittent claudication was likewise inconclusive (Farrell, 1980).

The D&H report then mentions a possibility that vitamin E could raise high-density lipoprotein cholesterol, based on observations in two uncontrolled trials (Barboriak et al., 1982; Hermann et al., 1979). This finding was not confirmed in a small, randomized controlled trial (Stampfer et al., 1983). The section concludes by mentioning that vitamin E might raise triglycerides and that vitamin E had no effect on atherosclerosis in monkeys (Hayes, 1974). Of the two studies suggesting that vitamin E might raise triglycerides, one was a case-control study (Farrell and Bieri, 1975), and the other a relatively large clinical trial (Tsai et al., 1978).

The relationship of vitamin E and CHD appears to be uncertain in the D&H report. Summary statements in the D&H report do not mention a possible protective effect of vitamin E from either diet or supplements. Also, in contrast to recommendations for additional research on the effects of antioxidants (particularly β-carotene) on cancer, the D&H report makes no recommendations for additional research on the effects of vitamin E on CHD. Overall, it appears that the role of vitamin E in preventing CHD was not considered a highly promising line of investigation.

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM 2000a), differs substantially from the D&H report. The DRI report presents a large body of evidence, from a variety of study designs, on the potential impact of vitamin E on CHD. More importantly, the DRI report highlights the potential relevance of oxidized low density lipoproteins (LDL) in development of CHD and the role of vitamin E in inhibiting LDL oxidation.

The DRI report cites Steinberg's seminal paper that states the basic hypothesis on oxidative modification of LDL (Steinberg et al., 1989). Potential mechanisms are then summarized (Azzi et al., 1995; Devaraj et al., 1996; Freedman et al., 1996; Rota et al., 1998), followed by a brief overview of animal studies (Parker et al., 1995). A large section then summarizes the results of four prospective observational studies (Table 6-3 of the DRI report, reprinted in Appendix A). These studies document significant inverse relationships between vitamin E intake on subsequent CHD events (Knekt et al., 1994; Kushi et al., 1996; Rimm et al., 1993; Stampfer et al., 1993). Two of the observational studies suggest an inverse association between dietary intake and CHD (Knekt et al., 1994; Kushi et al., 1996), while the other two studies suggest an inverse relationship between total (dietary and supplemental) vitamin E intake (Rimm et al., 1993) or just supplemental vitamin E intake (Stampfer et al., 1993) and subsequent CHD. On the basis of the mechanistic studies, the animal studies, the prospective observational studies, and one clinical trial of high-dose vitamin E

supplements (Stephens et al., 1996), the relationship between vitamin E intake and CHD appeared very promising in the 1990s.

The results of clinical trials that tested the impact of vitamin E supplements on CHD events were also summarized in the DRI report. In 1994, the ATBC trial, which tested the impact of vitamin E on lung cancer in 29,133 Finnish male smokers, documented a nonsignificant impact of this supplement (50 mg/day of *all rac*-α-tocopherol acetate) on CHD events over 5 to 8 years of follow-up, yet a potential increase in hemorrhagic stroke (ATBC, 1994). In 1996, the Cambridge Heart Antioxidant Study, which enrolled 2,002 persons with angiographic evidence of CHD, reported a significant (77 percent) reduction in nonfatal myocardial infarction from vitamin E (400 or 800 IU [268 or 567 mg]/day of *RRR*-α-tocopherol) over a median follow-up period of 1.4 years, but no significant impact on fatal myocardial infarction or total mortality (Stephens et al., 1996). In 1999, the GISSI-Prevenzione Trial documented no significant impact of vitamin E (300 mg/day of *all rac*-α-tocopherol) on CHD clinical events over 3.5 years in 11,324 survivors of a myocardial infarction (GISSI-Prevenzione Investigators, 1999). In 2000, the Heart Outcomes Prevention Evaluation (HOPE) trial documented no significant effect of vitamin E (400 IU [268 mg]/day of *RRR*-α-tocopherol) on CHD events over 4.5 years of follow-up in 9,541 persons at high risk for CHD (HOPE Study Investigators, 2000). The final trial cited (DeMaio et al., 1992) documented a nonsignificant reduction in the extent of coronary stenosis from 1,200 IU (804 mg)/day of *all rac*-α-tocopherol in 100 persons who had a percutaneous transluminal coronary angioplasty.

The discordance between the nonsignificant large-scale trials and the voluminous and promising evidence from bench research and observational studies is unclear but might be related to the duration of therapy or the stage of disease. In each of the major trials, participants either had advanced CHD or were at high risk of CHD. Still, in view of the inconsistent data, particularly the results of two major trials (GISSI-Prevenzione Investigators, 1999; HOPE Study Investigators, 2000), the DRI report concludes "as of this date, there are insufficient data on which to base a recommendation of supplemental vitamin E as a heart disease preventative for the general population" (p. 217).

Evolution—Uncertain to Promising to Uncertain

The section on vitamin E and CHD in the D&H report is very brief, the evidence is at best inconclusive, and the topic is not highlighted as promising. One animal study is cited; no observational or mechanistic studies are cited. The summary statement did not mention the potential impact of vitamin E on CHD. This presentation of evidence suggests that at the time of the D&H report, the oxidative modifications hypothesis had not matured and certainly had not caught

the attention of the general public or a broad cadre of researchers. In other words, the relationship was uncertain.

In contrast, the DRI report presents a qualitative overview of available evidence from a variety of categories. While the mechanistic and animal studies strongly support the oxidative modification hypothesis, it is likely that the prospective observational studies, published in the early 1990s, fueled interest in the potential impact of vitamin E supplements on CHD and converted the relationship from uncertain to promising. In the cohort studies, the magnitude of potential risk reduction was substantial, suggesting that increased intake of vitamin E, particularly from supplements, might reduce the risk of CHD by more than 30 percent. Still, the GISSI and HOPE trials effectively ruled out a benefit of this magnitude, at least in the populations studied, namely, persons with pre-existing CHD or those at high risk for CHD. Hence, the overall pattern of evidence evolved from uncertain, as implied in the D&H report, to highly promising in the 1990s, and back to uncertain by the time the DRI report was published.

Vitamin C and Colds

Diet and Health Report

Vitamin C and the common cold was not a major emphasis of the D&H report, and the number of citations was limited. The focus was on vitamin C supplements and not dietary components. One large and three small, double-blind, placebo-controlled studies of vitamin C from supplements were specifically referred to in the report. One small, randomized clinical trial found a statistical difference between the placebo group and the vitamin C-supplemented group in the number of persons who remained free of illness throughout the study period, and a highly significant 30 percent decrease in the total days of disability (Anderson et al., 1972). A large, randomized clinical trial by this same group did not find any significant difference between the placebo groups and supplemented groups with respect to the incidence of colds, but the high-dose treatment groups (4 or 8 g on the first day of illness) demonstrated a significant reduction in the severity of the symptoms, similar to the results of their earlier study (Anderson et al., 1974).

A small, double-blind, placebo-controlled study of children in a boarding school reported no difference in the number of respiratory episodes between placebo and vitamin C-supplemented groups, but did report a significant reduction in days of morbidity and a significant increase in the number of children with no sick days reported during the 14-week period in the vitamin C group (Coulehan et al., 1974). Coulehan and colleagues also found that both boys and girls in the lower grades (6 to 10 years of age) had a significant decrease in sick

days, while only the girls in the higher grades (10 to 15 years of age) showed this effect associated with vitamin C supplementation.

Karlowski and colleagues (1975) conducted a small, double-blind study with 311 employees of the National Institutes of Health and concluded that vitamin C had "at best only a minor influence on the duration and severity of colds," and "the effects demonstrated might be explained equally well by a break in the double blind." The break in the double-blind study may have been due to the curiosity of the scientist participants. The D&H report summarizes the effect of vitamin C on colds by indicating that "Several studies . . . generally indicate that vitamin C taken even in gram quantities does not prevent colds and at best only reduces the frequency and severity of symptoms in cold sufferers" (p. 515).

Dietary Reference Intake Report

The relationship of vitamin C to colds was also not a major focus in the DRI report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a). The report cited several small trials in which supplemental vitamin C had some effect on colds. Of the ten articles cited, four were published before 1988 (Chalmers, 1975; Coulehan et al., 1976; Ludvigsson et al., 1977; Miller et al., 1977). Of the other six, five were reviews (Hemila 1996, 1997; Hemila and Herman, 1995; Herbert, 1995; Jariwalla and Harakeh, 1996), and the last was a study that dealt primarily with the antihistaminic effect of vitamin C (Johnston et al., 1992).

The studies included one by Coulehan and colleagues (1976) that followed up their 1974 study (also cited in the D&H report) with a double-blind, placebo-controlled study (868 subjects). They gave 1 g/day of vitamin C, and reported that there was no difference in the number of people becoming ill, number of episodes, or mean illness duration between the supplement and placebo groups. They also reported that children with a high plasma vitamin C level had significantly longer illness days on average than those with low levels. Coulehan's group was one of the first to suggest that vitamin C may have an antihistaminic effect.

Miller and colleagues (1977) carried out a study with 44 school-aged monozygotic twins (6 to 15 years of age) who received 0.5 to 1.0 g of vitamin C/day, depending on body size. They reported no significant overall treatment effect on cold symptoms, but the younger girls receiving treatment had significantly shorter and less severe illness episodes, as did the younger boys. Ludvigsson et al. (1977) carried out a double-blind study with 8- and 9-year-old children, using 1 g/day of vitamin C. They reported a reduction in the duration and severity of colds. The incidence was either unaltered or even increased with high doses of vitamin C. They concluded that "vitamin C in large doses thus had no definitely proved effect against colds."

The reviews presented different evaluations of the role of vitamin C and colds, and were not used for the DRI conclusion. Because of the limitations and variations in the response of colds to vitamin C, the DRI report concluded that "the data are not consistent or specific enough to estimate the vitamin C requirement based on the common cold" (p. 127).

Evolution—Uncertain to Uncertain

The selection of the literature on vitamin C and colds in the D&H report was sparse. It did not include several references available at the time it was published that were used 11 years later in the DRI report. Because of the conflicting nature of the studies, it is doubtful that the D&H report would have been significantly altered by including these studies. The conclusion from the D&H report was that supplemental vitamin C could not prevent colds; the report was inconclusive on the question of the frequency and severity of symptoms. Although the DRI report took a systematic approach, the committee concluded that the data were not consistent or specific enough to estimate the vitamin C requirement based on colds. Thus, the report did not discuss whether vitamin C may reduce the duration of colds.

Folate and Cervical Dysplasia

Diet and Health Report

The D&H report cited data from one randomized, controlled intervention study, which suggested a possible benefit of supplemental folate on the progression of cervical dysplasia. In this small-scale study (n = 47), conducted by Butterworth and colleagues (1982), the progression of cervical dysplasia in women treated with large (10 mg) daily doses of folate was compared with that of placebo-treated subjects. Cervical dysplasia, which may or may not progress to cervical cancer, improved only in the subjects consuming folate. The D&H report indicated that the data from this study "suggest that oral folate supplements may prevent the progression of cervical dysplasia or promote regression to normalcy" (p. 338).

Since cervical dysplasia may spontaneously revert to normal or progress to cervical cancer, the statement in the D&H report about data from this small, randomized trial was cautious. Although there is no concluding statement in the report about cervical dysplasia or cervical cancer, the trial that was suggestive of an effect prompted additional studies related to this possible association.

Dietary Reference Intake Report

The section in the DRI report, *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998), related to folate and cancer begins with the statement, "Experimental data indicate that changes in folate status may influence the process of neoplastic changes in certain epithelial tissue: a negative change in folate status may stimulate carcinogenesis. It is unclear if supraphysiological doses obtained from supplements afford any protection" (p. 264). The report presented a separate review of data relating folate intake and/or status with risk of cervical dysplasia and with risk of advanced cervical neoplasia. The findings of the intervention study by Butterworth and colleagues (1982) were also cited in the DRI report, which indicated that "the positive alteration in cytology may have been an attenuation of dysplasia or simply a reduction in megaloblastic cellular changes" (p. 264). The DRI report also cited a subsequent larger (n = 235) randomized, controlled intervention trial conducted by the same research group that failed to reproduce the previous findings (Butterworth et al., 1992b). The hypothesis that poor folate status itself might not be carcinogenic but may exacerbate an underlying predisposition to cancer was investigated by Butterworth and coworkers in a case-control study (n = 464) reported in 1992 and cited in the DRI report (Butterworth et al., 1992a). In this study, risk was five times greater for cervical dysplasia in patients with the human papilloma virus-16 (HPV-16) infection who also had somewhat lower (not clinically deficient) red blood cell folate concentrations. Since cervical infection with HPV-16 significantly increases the risk for cervical cancer, this study provides evidence that suboptimal body reserves of folate, as reflected in low red blood cell levels, influence a key risk factor associated with early cervical cancer.

The mechanistic animal studies also cited in the DRI report provided supporting evidence for the role of impaired folate status on carcinogenesis. The DRI report cited two publications that resulted from a case-control study by Ziegler and coworkers (1990, 1991) of invasive cervical cancer with community controls, conducted in five areas of the United States, in which no association between risk of invasive cervical cancer and folate intake was found. An Australian case-control study (Brock et al., 1988) that did not support an association between invasive cervical cancer and dietary factors including folate was also cited. This study was specifically designed to examine the hypothesis of a retinal/carotene protective effect. Folate was included only as an ancillary interest. The DRI report also cited two other case-control studies. One by Verreault and coworkers (1989) failed to find an association between folate intake and cervical cancer, and one conducted in Latin American countries by Potischman and coworkers (1991) failed to find an association between serum folate concentrations and invasive cervical cancer. Potischman and coworkers had previously reported that folate intake was not associated with risk for cervical cancer in the same

study population. The DRI report concluded, "the effect of folate status on carcinogenesis in the cervix remains uncertain" (p. 265).

Evolution—Uncertain to Uncertain

The scientific evidence has evolved to include larger, controlled intervention trials that do not support an inverse association between supplementation with folate and precancerous cervical dysplasia. Studies cited in the DRI report do support the hypothesis that less than adequate folate status may increase the risk for cervical dysplasia when coexisting risk factors are present. However, data do not support an inverse relationship between cervical cancer and folate supplementation.

Butterworth's small intervention trial (Butterworth et al., 1982) suggested that folate supplements might positively affect the progression of cervical dysplasia. These researchers subsequently conducted a larger and more comprehensive study with two major phases. The first phase was a case-control study with 464 participants (Butterworth et al., 1992a). Approximately 50 percent of the subjects in the first phase also volunteered to participate in the second phase, which was a randomized, placebo-controlled, double-blind, clinical intervention trial (Butterworth et al., 1992b). The controlled intervention trial was designed to test the hypothesis that high-dose folate supplements will modify the course of cervical dysplasia and improve its cytological and histological manifestations. The findings from this second intervention trial reject that hypothesis. Possible explanations for the difference between the later trial (Butterworth et al., 1992b) and the earlier study (Butterworth et al., 1982) include a more adequate sample size, exclusion of persons classified as having atypia less than dysplasia, and a longer period of observation. The high rate of apparent regression of dysplasia in both the placebo-treated and folate-supplemented groups of the intervention trial may also have contributed to the lack of significant differences.

Case-control study findings indicate that inadequate tissue folate concentration (reflected by red cell folate content) enhances the effects of HPV-16 infection. These data suggest that for less than optimal folate status to enhance cervical carcinogenesis, concurrent factors must be present that predispose to carcinogenesis.

The early report by Butterworth et al. (1982) that suggested folate supplementation could lead to regression of dysplastic cervical lesions also led to a series of case-control studies by other investigators to examine the relationship between folate intake and risk of invasive cervical cancer. Interpretation of these case-control studies, including several cited in the DRI report, was constrained by methodological weaknesses of the studies such as: (a) folate intake was assessed with food-frequency instruments that were not validated for folate intake, (b) there was a lack of stratification of subjects for known risk factors such as

HPV infection, and (c) the subjects had advanced stages of neoplasia that may be unresponsive to folate.

In summary, scientists have been unable to confirm the data cited in the D&H report that suggested folate supplementation may reduce the risk for cervical dysplasia. The scientific evidence has evolved to support the hypothesis that less than adequate folate status may increase the risk for cervical dysplasia when coexisting risk factors are present. In addition, research evidence suggests that once the cervical dysplasia has advanced to the neoplastic stage, the inverse association between folate status and disease risk is no longer detectable.

Phosphorus and Bone Status

Diet and Health Report

The review of phosphorus in the D&H report centered on the importance of the ratio of dietary calcium to phosphorus (Ca:P ratio) in determining the effect of excessive dietary phosphorus on calcium absorption, excretion, and bone resorption. In rats, high phosphorus intake depressed circulating calcium thereby inducing a mild hyperparathyroidism (Draper et al., 1972). At the time of the D&H report, the research on calcium and phosphorus interactions focused primarily on calcium balance measurements. Also, some biochemical evidence (hyperparathyroidism and/or increased urinary hydroxyproline) indicated that high phosphorus diets induced bone resorption in growing aged animals (Draper and Bell, 1979; Draper et al., 1972). As a result, the prevailing opinion was that the ratio of dietary calcium to phosphorus was critical to establishing the recommended intake for phosphorus. In small, nonrandomized, descriptive studies in humans who served as their own controls (Bell et al., 1977; Spencer et al., 1978), varying high intakes of dietary phosphorus were provided as phosphate additives in foods. These intakes reduced calcium absorption (especially at low calcium intakes) and induced secondary hyperparathyroidism. Compensatory reduction in urinary calcium also occurred. In contrast, in a study with a very small sample size (n = 2 to 4 subjects per diet test group) involving prison inmates and staff (Malm, 1953), addition of phosphate salts to the diet (for a total intake of 2,000 mg/day) with moderate calcium intake (500 to 600 mg/day) showed no overall detriment to calcium balances. Thus, while there was some evidence in humans of a negative interaction of high phosphorus intakes (up to 3,000 mg/d) on calcium homeostasis (Portale et al., 1984, 1986), there was no evidence of an effect of high dietary phosphorus on bone mass or as a factor in the etiology of osteoporosis. The D&H report concluded, "High-phosphorus diets may decrease calcium bioavailability, but they also reduce urinary calcium excretion and their influence on bone mass and the risk of osteoporosis is unknown" (p. 360). In the directions for research included in the chapter on "Osteoporosis," the effect of dietary phosphorus (alone or with protein and fiber) on

calcium economy was stated as a research need, especially for adolescents and the elderly who have greater calcium requirements.

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride* (IOM, 1997), summarized the clinical trials of the past decade by stating, "In balance studies in human adults, Ca:P molar ratios ranging from 0.08:1 to 2.40:1 (a 30-fold range) had no effect on either calcium balance or calcium absorption. Thus, for the reasons cited, there is little or no evidence for relating the two nutrients, one to the other, during most of human life" (p. 154). Thus, for the first time in the history of setting nutrient-based dietary recommendations in the United States, the recommendation for dietary phosphorus intake was not tied to that of calcium. Rather, the DRI values for phosphorus for children and adolescents were based on tissue accretion applied to a factorial model. For adults, the DRI values were based on the physiological response of maintenance of plasma phosphorus at the bottom end of the normal range (0.87 mmol/L [2.7 mg/dl]) as an indicator to reflect adequacy of phosphorus intake. No information on a possible relationship between phosphorus intake and bone mass was included.

Evolution—Uncertain to Uncertain

The shift in perspective about the importance of the ratio of dietary calcium to phosphorus may be credited to the many balance studies in humans that did not demonstrate abnormalities in calcium homeostasis imposed by high dietary phosphorus. This led to a search for an alternate approach to establishing a nutrient-based recommendation for phosphorus as described above. With a better understanding of the endocrine regulation at the intestinal mucosa and renal levels that maintain normal plasma phosphorus, there is more confidence that the human body adapts to phosphorus intakes over a relatively wide range. To date, no attempts have been made to relate phosphorus intake to outcomes of bone status or risk of osteoporosis in humans.

The importance of the ratio of dietary calcium to phosphorus in humans may need to be reevaluated if concerns about a population-level increase in phosphorus intake through such sources as cola beverages and food phosphate additives are realized, a point noted in the DRI report (IOM 1997). The studies in animals and humans suggest that in the situation when diets of very low calcium and very high phosphorus are consumed over a long period of time or when renal function is not normal, the calcium economy of the body *might* be compromised. Target populations for such a concern are children and adolescents who substitute cola beverages (which have a high phosphoric acid content) for milk (which has a high calcium content). The issue of the influence of high

dietary intake of phosphorus (especially in combination with low dietary calcium) on bone accretion in children or age-related bone loss later in life remains to be explored.

Chromium and Diabetes

Diet and Health Report

In the D&H report, small intervention trials of 12 to 76 persons were reported. Two of the studies found a positive effect of chromium on insulin or glucose (Riales and Albrink, 1981; Simonoff, 1984) and two did not (Anderson, 1986; Rabinowitz et al., 1983). Another found a relationship in a subset of the participants and no relationship in a larger group (Anderson, 1986). The D&H report indicates that "no population data have been reported implicating chromium deficiency in humans with diabetes, and chromium supplementation does not improve blood glucose or insulin levels in those with the disease" (p. 628). The report concludes, "the possible role of chromium deficiency in the etiology of diabetes is unresolved" (p. 630).

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (IOM, 2001), cited a study conducted in China in which a positive effect of chromium supplementation was observed on some responses related to diabetes (Anderson et al., 1997), but dietary chromium intake was not reported and other limitations of the study were noted. The DRI report recommended, "investigation of possible relationships between chromium status and insulin resistance, impaired glucose tolerance, and Type II diabetes" (p. 6-15). The relationship remained inconclusive.

Evolution—Uncertain to Uncertain

Conflicting results of studies related to chromium and diabetes led the D&H report to conclude that evidence for a relationship was inconclusive. The DRI report cited a study in China suggesting that chromium supplementation in Type II diabetics improved some aspects of glucose tolerance, but evidence from only one study was considered not sufficiently strong to be conclusive.

RELATIONSHIP DISCUSSED ONLY IN A DIETARY REFERENCE INTAKE REPORT

Folate and Neural Tube Defects

Diet and Health Report

The topic of the prevention of birth defects, including neural tube defects (NTDs), was not included in the D&H report.

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998), made a definitive conclusion about an inverse association between supplemental folate intake taken during the periconceptional period and risk of NTDs. The conclusion stated in the DRI report to reduce the risk of neural tube defects was, "women capable of becoming pregnant consume 400 μg of folate daily from supplements, fortified foods, or both in addition to consuming food folate from a varied diet. At this time the evidence for a protective effect from folate supplements is much stronger than that for food folate" (p. 259). The primary studies on which this conclusion was based are summarized in the DRI report and include six observational studies and six controlled trials (Tables 8-7 and 8-8 of the DRI report, reprinted in Appendix A). The observational studies were published between 1988 and 1995 and with one exception found a significant reduction in risk for NTDs associated with periconceptional consumption of folate-containing multivitamins. The controlled trials, conducted between 1981 and 1992, provided the most definitive data on which subsequent public health policy was based.

Evolution—Accepted in DRI

The case study of folate and NTD risk reduction provides an excellent example of the evolution of scientific evidence from very preliminary suggestive observations to definitive data from randomized, controlled intervention trials.

Hibbard (1964) first suggested that folate might be involved in the etiology of NTDs. Following publication of this hypothesis, two controlled trials were conducted. In the first study by Smithells and coworkers (1981), which was not randomized, women were given a mixture of eight vitamins including 360 μg/day of folic acid. The risk of recurrence in the supplemented group was about one-seventh that of the unsupplemented group. In the second study, which was a small randomized trial using 4 mg/day (4,000 μg/day) of folic acid, a significantly lower recurrence rate was noted when the "non-compliers" were moved

to the unsupplemented group (thus changing the protocol to nonrandomized) (Smithells et al., 1983). Due to the nonrandomized design of these controlled trials, it was impossible to conclude that the difference in NTD rates between the groups receiving supplements and the unsupplemented groups was due to folate and not some other factor associated with the groups taking the supplements. Another major constraint of the Smithells studies was the fact that folate was provided as one component of a multivitamin supplement; therefore, it was not possible to definitively state that the risk reduction was due to folate alone.

To avoid bias and address the lack of acceptance of these early controlled trials, a large Medical Research Council (MRC) study was begun in 1983 that involved an international, multicenter, double-blind randomized trial (MRC Vitamin Study Research Group, 1991). The effect of supplementation was investigated by a factorial study design in which folate alone was compared with other vitamins alone, with both folate and other vitamins, and with neither (placebo). By 1991, the results were definitive enough to warrant stopping the trial—the evidence showed a 72 percent protective effect of folate alone. It was also concluded that the other vitamins showed no protective effect for NTD. A second significant trial from Hungary was a randomized controlled study that showed that the occurrence of NTDs could be significantly reduced by giving periconceptional multivitamin supplements that included 800 μg of folic acid (Czeizel and Dudas, 1992).

Data from the early controlled trials did provide scientific evidence that periconceptional folate-containing multivitamin supplements reduced the incidence of NTDs. However the data were considered inconclusive by the scientific community (Smithells et al., 1983; Vergel et al., 1990). Due to the methodological constraints including nonrandomization and provision of folate as a component of a multivitamin supplement, these data were not translated into public health policy in the 1980s. It took scientists an entire decade from these early reports to design, implement, and report conclusive data from appropriately designed intervention trials. The results of the controlled intervention trials reported in 1991 and 1992 were supported by the collective findings of observational studies published primarily between 1988 and 1995. None of the observational studies could identify folate specifically as the vitamin responsible for reducing NTDs.

The definitive data from the MRC and Hungarian controlled trials led scientists and public health policymakers to accept the positive association between supplemental periconceptional folate use and NTD risk reduction as conclusive. This evolution of scientific data culminated with the U.S. Public Health Service (USPHS) policy statement issued in 1992 that all women of childbearing age who are capable of becoming pregnant should take 400 μg/day of folic acid to reduce the risk of NTDs (CDC, 1992). The USPHS recommendation was followed several years later by a federal regulation that required all "enriched"

cereal grain products to contain specified quantities of folic acid by January 1, 1998.

The DRI report included a review of both the controlled trials and observational studies and provided additional supporting data published since 1992. The DRI report also included a recommendation that all women capable of becoming pregnant consume 400 □g/day of folic acid from fortified foods, supplements, or both in addition to consuming food folate from a varied diet to reduce the risk of NTDs. The DRI recommendation thus reinforces and refines the 1992 USPHS public health policy statement. The science related to folate and NTDs has now evolved as discussed in the DRI report to research questions related to mechanisms by which supplemental folate reduces the risk of NTDs, and questions related to genetic predisposition and response to folate supplementation.

Folate and Colorectal Cancer

Diet and Health Report

The D&H report does not discuss folate and colorectal cancer. The only related statement was that "an increased incidence of tumors in the liver, colon, and esophagus results from diets deficient in methyl groups" (p. 338). There was no mention of folate and colorectal cancer in the summary of the D&H report.

Dietary Reference Intake Report

The DRI report, *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998), stated, "experimental data indicate that changes in folate status may influence the process of neoplastic changes in certain epithelial tissue: a negative change in folate status may stimulate carcinogenesis. It is unclear if supraphysiological doses obtained from supplements afford any protection" (p. 264). The DRI report presented supporting evidence for modulation of colorectal cancer by folate status. Data from a case-control study by Lashner and coworkers (1989), although nonsignificant, indicated that chronic ulcerative colitis patients who were treated with a drug that inhibits folate absorption (sulfasalazine) were at higher risk for colonic dysplasia. When these patients were treated with folate supplements, the risk for colonic neoplasia was reduced. In a subsequent case-control study by the same research group (Lashner, 1993), red blood cell folate concentration was significantly lower in patients with colorectal neoplasia. The DRI report cited the large-scale observational study of Giovannucci and coworkers (1993), who found in two cohorts that risk of colorectal adenoma was elevated in individuals with low folate intake and high intakes of alcohol. The possible association with colorectal cancer was assessed in a subsequent large-scale observational study (Giovannucci et al., 1995) in which folate intake was

assessed and associated with new cases of colorectal cancer in U.S. male health professionals during a 6-year follow-up period. The work by Giovannucci and coworkers supports the hypothesis that increased folate intake is inversely associated with the incidence of colorectal adenomatous polyps and colorectal cancers. This study confirmed an inverse relationship between folate intake and colorectal cancer that was striking when combined with high alcohol consumption. The large size of the population, the prospective design of the two studies, and the effective control of a number of confounding dietary variables strengthen the conclusion that increased folate intake is inversely associated with the incidence of colorectal adenomatous polyps and of colorectal cancers.

The DRI report also cited data from a case-control study in male smokers that was nested within the ATBC study (Glynn et al., 1996). In that study, neither dietary folate intake nor blood folate level alone was significantly associated with colorectal cancer. In contrast, the combination of high alcohol consumption and a low folate diet was associated with a significant increase in colorectal cancer risk. The potential influence of folate-related genetic polymorphisms on colorectal cancer risk, with supporting evidence for a protective role for folate related to colorectal cancer risk, was referred to in the DRI report (Ma et al., 1997). Studies investigating the hypothesis that a folate deficiency localized within the colonic mucosa may increase the risk for colorectal cancer were not supported by clinical observational data cited in the DRI report (Meenan et al., 1997).

In summary, the DRI report stated, "data supporting the modulation of carcinogenesis by folate status are the strongest for the colorectum" (p. 265). In addition, the DRI report indicated, "more evidence for or against a causal relationship between folate status and colorectal cancer will be provided by data from prospective controlled intervention trials that are currently under way" (p. 266).

Evolution—Promising in DRI

Observational studies generally support the hypothesis that diminished folate status is associated with an increased rate of colorectal neoplasia. Data have evolved that strengthen the inverse association between folate status and colorectal cancer. Definitive conclusions related to the impact of folic acid supplementation on colorectal neoplasia await the results of ongoing controlled intervention trials.

Lashner and coworkers observed that low folate status appears to increase the risk of colorectal adenomas or cancer in a case-control study of patients with chronic ulcerative colitis. Although the data fell slightly short of statistical significance, the study established the importance of examining this issue. Later work by Lashner's group confirmed these observations by prospectively

comparing red blood cell folate levels in ulcerative colitis patients with neoplastic colorectal tissue with matched controls without neoplasia.

Observational studies generally support the hypothesis that diminished folate status is associated with an increased rate of colorectal neoplasia, although the nature of the relationship is different in each study. Some of the most convincing observational evidence to establish an association between folate status and colorectal neoplasia has come from two observational studies that have carefully examined interactions between folate and other dietary components, especially alcohol. In both studies, moderate to high alcohol intake significantly increased the neoplastic risk of a low-folate diet. In contrast to the early reports in persons with ulcerative colitis, the observational studies in the general population are particularly important since they show an association in individuals who do not have coexisting conditions that predispose them to colorectal cancer. Even though the data from all of the epidemiological studies related to folate and colorectal cancer are not uniformly supportive of an inverse relationship, a composite of the data provides a supportive argument for the existence of a relationship that is stronger than that observed for cervical cancer. More definitive evidence for a causal relationship between folate status and colorectal cancer may be provided by the ongoing prospective, controlled intervention trials.

Vitamin E and Prostate Cancer

Diet and Health Report

Vitamin E and prostate cancer was not specifically discussed in the D&H report.

Dietary Reference Intake Report

Vitamin E was discussed in the report, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000a). A possible preventive role in prostate cancer was suggested in the ATBC study (ATBC, 1994), a large clinical trial of vitamin E for the primary prevention of lung cancer in men from Finland who were heavy smokers. In that trial, a significant 34 percent lower incidence of prostate cancer was seen in the men who received supplemental vitamin E (Heinonen et al., 1998). Thus, the DRI report concluded that "At present, the data from intervention trials are most suggestive for the ability of vitamin E to prevent prostate cancer, but only a single trial has yet been reported, and prostate cancer was not the primary endpoint of that study" (p. 220).

Evolution—Promising in DRI

The D&H report made no mention of a role for vitamin E in this cancer. As a result of information now available, largely the ATBC trial results, the National Cancer Institute is funding a large, 12-year, prostate cancer primary prevention trial of supplemental vitamin E and selenium, the Selenium and Vitamin E Cancer Prevention Trial. Thus, the relationship between supplemental vitamin E and prostate cancer prevention is perhaps best characterized as promising.

3

Findings

FINDINGS

Using a qualitative case study approach, the committee assessed changes in the levels of confidence in 14 nutrient–disease relationships (dyads). Eleven were identified in both the D&H and DRI reports; three, for which evidence had evolved, were discussed only in a DRI report.

Applying its classifications of *accepted*, *promising*, *uncertain*, and *no relationship* to the selected dyads in the D&H report, the committee found that only fluoride and dental caries fell in the accepted category. Two were promising (β-carotene and lung cancer, vitamin C and gastric cancer), and eight were uncertain (calcium and bone status, vitamin D and bone status, vitamin E and cancer, vitamin E and coronary heart disease [CHD], vitamin C and colds, folate and cervical dysplasia, phosphorus and bone status, chromium and diabetes). By publication of the DRI reports between 1997 and 2001, the level of confidence had changed for six dyads, in some cases in surprising ways. Of the three dyads reviewed that were discussed only in the DRI reports, one was accepted (folate and neural tube defects [NTD]) and two were promising (folate and colorectal cancer, vitamin E and prostate cancer). The dyads are shown in Table 3-1. Table 3-2, which displays the types of evidence and changes in confidence, is the basis for the committee's findings, described in the following paragraphs.

Confidence in nutrient–disease relationships can change, often in unexpected directions.

An important finding is that preliminary evidence in support of a nutrient–disease relationship is often not confirmed. Neither promising relationship

TABLE 3-1 Description of Relationships

Change in Confidence About Existence of Positive Relationship	Level of Confidence (D&H → DRI)[a]	Dyad
Increased	A → A+	Fluoride and dental caries
	U → A	Calcium and bone status
	U → A	Vitamin D and bone status
Decreased	P → N[b]	β-carotene and lung cancer
	P → U	Vitamin C and gastric cancer
	U → N	Vitamin E and cancer (except prostate)
Unchanged	U → P → U	Vitamin E and coronary heart disease
	U → U	Vitamin C and colds
	U → U	Folate and cervical dysplasia
	U → U	Phosphorus and bone status
	U → U	Chromium and diabetes
Not in *Diet and Health*	→ A	Folate and neural tube defects
	→ P	Folate and colorectal cancer
	→ P	Vitamin E and prostate cancer

[a] D&H = *Diet and Health: Implications for Reducing Chronic Disease Risk* (NRC, 1989), DRI = Dietary Reference Intake reports (IOM, 1997, 1998, 2000a, 2001), A = accepted, U = uncertain, P = promising, N = no relationship.
[b] P to U for dietary β-carotene and U to N for supplementary β-carotene.

from the D&H report (β-carotene and lung cancer, vitamin C and gastric cancer) was subsequently accepted in a DRI report. Of the eight uncertain dyads from the D&H report, two were subsequently found to be accepted (calcium and bone status, vitamin D and bone status), one was found not to be a relationship (vitamin E and cancer [excluding prostate cancer]), and five remained uncertain (vitamin E and CHD, vitamin C and colds, folate and cervical dysplasia, phosphorus and bone status, chromium and diabetes). High-dose β-carotene and lung cancer is illustrative. An impressive body of evidence, including numerous observational studies, suggested that an increased intake of foods rich in β-carotene might reduce the risk of developing lung cancer. This appealing hypothesis was evaluated by testing high-dose β-carotene administration in three large-scale, long-term trials, two of which focused on populations at high risk for lung cancer. In contrast to expectations, supplementation with β-carotene significantly increased the risk of lung cancer in the two studies that enrolled persons from high-risk populations. In the third trial, involving male physicians, β-carotene supplementation had no significant effect. Hence, not only was confidence in the putative benefit of β-carotene reduced, but also the direction of the relationship changed because the available evidence suggested that β-carotene supplementation may increase the risk of lung cancer in high-risk populations.

No pattern of evidence clearly predicts change in the confidence of relationships, particularly those initially deemed uncertain or promising.

The evidence cited for each dyad was quite heterogeneous. The committee could not identify any pattern of evidence that consistently predicted a change in the level of confidence that a positive relationship existed. The committee observed three instances in which confidence in a relationship decreased from the D&H report to the DRI report (β-carotene and lung cancer, vitamin C and gastric cancer, vitamin E and cancer [except prostate cancer]). In each instance, a common characteristic was an absence of trial citations (even a small trial of less than 1,000 participants) in the D&H report and the presence of trial citations in a DRI report. The case studies suggest that there is a tendency for large trials to be developed when smaller trials are promising, but the outcome of larger trials remains unpredictable. Even the citation of small clinical trials in the D&H report did not predict the nature of the relationship of the corresponding dyads in the DRI reports. The committee also observed that few meta-analyses and systematic reviews were referenced in the D&H and DRI reports. This may have occurred because those techniques were not commonly used prior to publication of the D&H report and because the DRI reports drew on original research studies to set nutrient requirements. For the dyads discussed in both the D&H and DRI reports, the committee also observed references to animal studies more often in the D&H report (nine dyads) than in the DRI reports (two dyads). The differential citation of animal studies likely reflects the different purposes of the reports and the reliance on human studies to set nutrient requirements.

Large randomized trials have the greatest impact in changing the level of confidence in a nutrient–disease relationship.

Not surprisingly, large clinical trials were cited for only two dyads in the D&H report (fluoride and dental caries, vitamin C and colds) and nine dyads in the DRI reports (fluoride and dental caries, calcium and bone status, vitamin D and bone status, β-carotene and lung cancer, vitamin C and gastric cancer, vitamin E and cancer [except prostate cancer], vitamin E and CHD, folate and NTDs, and vitamin E and prostate cancer). The latter two dyads were not mentioned in the D&H report (see Table 3-2). For a dyad considered accepted in either the D&H or DRI reports, a large clinical trial was cited. Also, in those instances with an increase or decrease in the level of confidence in a positive relationship, a large clinical trial typically was cited in the DRI report. For vitamin E and CHD, considerable interest developed as a result of prospective observational studies published after the D&H report that suggested the relationship to be promising. However, large clinical trials published prior to issuance of the corresponding DRI report failed to demonstrate a beneficial effect of vitamin E on CHD.

TABLE 3-2 Change in Confidence in a Positive Relationship by Type of Evidence for Nutrient–Disease Dyads

		Types of Evidence in D&H and DRI Reports[a]			
		Animal		Mechanistic	
Change in Confidence[b]	Dyad				
Increased					
A → A+[c]	Fluoride and dental caries	O	■	O	■
U → A	Calcium and bone status	O		O	
U → A	Vitamin D and bone status	O		O	
Decreased					
P → N[d]	β-carotene and lung cancer	O		O	■
P → U	Vitamin C and gastric cancer	O		O	■
U → N	Vitamin E and cancer[e]	O			■
Unchanged					
U → P → U	Vitamin E and CHD[f]	O	■		■
U → U	Vitamin C and colds				■
U → U	Folate and cervical dysplasia				■
U → U	Phosphorus and bone status[g]	O		O	■
U → U	Chromium and diabetes	O			
Not in D&H					
→ A	Folate and neural tube defects		■		■
→ P	Folate and colorectal cancer				■
→ P	Vitamin E and prostate cancer				

[a] O = *Diet and Health: Implications for Reducing Chronic Disease Risk* (D&H) report (NRC, 1989), ■ = Dietary Reference Intake (DRI) reports (IOM, 1997, 1998, 2000a, 2001). See text for description of types of studies.
[b] A = accepted, P = promising, U = uncertain, N = no relationship. Some animal and mechanistic studies may have been cited in review articles in the DRI reports.
[c] Indicates extension to include adults, not just children.
[d] Confidence changes from promising to uncertain for diet and from uncertain to no relationship for dietary supplements.

Observational				Clinical Trials					
Case Control Retrospective		Cohort Prospective		Nonrandomized		Small Randomized (< 1,000)		Large Randomized (> 1,000)	
o	■	o	■	o		o	■	o	■
o	■	o	■	o	■	o	■		■
o	■	o		o	■		■		■
o	■	o	■						■
o		o							■
	■	o	■				■		■
			■	o		o			■
				o	■	o	■	o	
	■					o	■		
				o	■				
				o	■	o	■		
	■		■		■		■		■
	■		■						
									■

[e] Except prostate cancer.
[f] CHD = coronary heart disease.
[g] For phosphorus, the D&H and DRI reports did not include any studies that directly assessed the effect of dietary intake of phosphorus on bone, but only on phosphorus absorption or serum phosphorus. In the DRI report, for young children only, data on measures of whole body bone mineral content were used to estimate accretion of phosphorus in the body during growth

In the case of folate and NTDs, large clinical trials substantiated the role of folate in NTD risk reduction. The large trials reported in 1991 and 1992 were supported by the collective findings of observational studies published primarily between 1988 and 1995. None of the observational studies were able to identify folate specifically as the responsible vitamin. The definitive data from the clinical trials led scientists and public health policymakers to accept as conclusive the positive association between supplemental periconceptional folate use and NTD risk reduction.

Large, randomized controlled trials are generally given greater weight in evaluating nutrient–disease relationships. In such trials, participants are assigned randomly to a study group that receives the treatment or to a control group. This assignment is intended to evenly distribute known and unknown confounding factors so that the randomized groups are comparable. Even under these circumstances, large-scale trials have limitations (IOM, 1997, 1998, 2000a, 2001; NRC, 1989) that may lead to failure to find an effect, either positive or negative. These include short duration of trial, level of nutrient supplement given, confounding by other nutrients administered, and incomplete compliance of the study group. Trends in nutrient intake or disease risk factors can also alter a relationship over time.

Perhaps most importantly, the case studies of vitamin C and gastric cancer and of vitamin E and CHD highlight the difficulty in conducting large-scale trials to investigate potential beneficial effects of single nutrients in reducing risk for a chronic disease like cancer or CHD, especially when compared with conditions that develop over a relatively short time, like NTDs. Chronic diseases develop over a long period of time, typically over decades, and may be affected by various factors at different times in the disease process. Nutrient trials have been more successful in establishing causality for conditions that develop over a much shorter time, such as was the case for trials aimed at preventing birth defects and caries.

CONCLUDING REMARKS

Conclusive evidence about a relationship between specific nutrients and a disease or diseases remains typically elusive for a number of reasons. First, scientific advances in understanding relationships between specific nutrients and diseases do not necessarily emerge within a short time, and progress is often erratic. Some gaps are filled while others are created. Initial findings based on preliminary evidence are sometimes confirmed and other times not supported. Not surprisingly, the process is time consuming and costly. Second, while preliminary evidence, typically from mechanistic studies, animal studies, and observational studies in humans, can generate exciting new hypotheses about nutrient–disease relationships, evidence from these studies has limitations. For instance, even in well-designed, large-scale observational studies, it is difficult

to isolate the effects of a single nutrient under investigation from the confounding effects of other nutrients and from non-nutritional factors, or a mechanistic study may not consider multiple actions of a nutrient. Third, the etiology of disease, especially chronic disease, is commonly multifactorial. Even if diet has a prominent role, it is extremely unlikely that a single nutrient is directly responsible for a chronic disease, and conversely that addition of a single nutrient will eliminate disease risk. Fourth, clinical trials, which are generally considered to provide the strongest evidence about the effects of nutrient intake on subsequent disease, are complex, expensive, and time consuming, especially for chronic diseases, which develop over decades and are influenced by a host of genetic, physiological, and environmental factors that may also affect risk.

Fifth, it is possible that a focus on specific nutrients as risk factors for diseases in relatively homogenous populations has led to a number of spurious associations that are not subsequently supported by clinical trials. Historically, the substantial success of understanding the relationships between nutrients and classic nutrient deficiency diseases in humans has been driven by clinical observations where the risk of disease was much higher in a subset of the population. Additionally, the etiology was specific to one disease, and short-term intervention trials could prevent the disease. Although the multifactorial nature of chronic diseases in general increases the complexity of nutrient–disease relationships, greater emphasis on understanding the interrelationships between genetic factors and specific nutrients might contribute to more developed hypotheses for further clinical testing, as might development of biomarkers and surrogate endpoints.

Unfortunately, society's expectation of a rapid translation of scientific discovery into application lacks an appreciation of the constraints on defining nutrient–disease relationships as described above. Advances are commonplace in other fields, and advancement in medical research is likewise expected to occur quickly. It is understandable that persons at high risk for a disease or afflicted with a disease sometimes view the scientific method as tedious and unresponsive to their needs. Likewise, entrepreneurs and businesses that have invested in research and development want their products to reach and succeed in the marketplace as quickly as possible.

In its qualitative assessment of 14 case studies of nutrient–disease relationships, the committee was unable to find a way to predict from preliminary evidence whether there is a real beneficial effect of a single nutrient in reducing risk for a given disease or condition. Indeed, while the committee recognizes the urgency of understanding the potential benefits of nutrients in disease prevention, the findings suggest a cautious approach. The dearth of confirmatory evidence on aspects of vitamin E in preventing CHD, and the discovery of possible harmful effects from trials of high-dose β-carotene supplements, illustrate the committee's concerns.

Using a case study approach, the committee looked for patterns that could streamline the scientific process and bring useful recommendations and information to consumers more rapidly. It did not find a "pattern express train." The committee's review of differences in evidence available in the D&H report (1989) and the DRI reports (1997–2001) suggests a skeptical approach to statements about beneficial effects of single nutrients based on animal, mechanistic, or observational studies alone, and argues against premature claims of benefit. For consumers, policymakers, and regulators, the committee's assessment is as follows:

- Large, randomized controlled studies play an important role in establishing the relationship between nutrient intake and the risk of disease. Ideally, consumers should base decisions to change intake of specific nutrients on evidence from trials. Likewise, regulators and policymakers should rely heavily upon such evidence to guide nutrient recommendations.
- Caution should be exercised in using preliminary evidence from non-controlled studies as the basis for recommendations for increased intakes of a nutrient.
- Claims about nutrient–disease relationships are more easily made than scientifically supported. Because the implications for public health are so important, caution is urged prior to accepting such claims without supportive evidence from appropriately designed, typically large, clinical trials.

4

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Appendix A

Tables from the Dietary Reference Intake Reports

TABLE 4-1 Randomized Controlled Calcium Intervention Trials in Postmenopausal Women

Site	Calcium Intake (mg/d)			Relative Change in BMD or BMC in Calcium Group Compared with Placebo		Statistically Significant Change in BMD or BMC in Calcium Group Compared with Placebo
	N	Diet	Supplement[a]	Year 1	Year 2 or More, Annualized	
Spine						
Early postmenopausal						
Aloia et al., 1994[b]	70	500	1,700	P[j]	S or N	no
Dawson-Hughes et al., 1990	67	< 400	500	S[h] or N[i]	S	No
Elders et al., 1991[d]	248	1,150	1,000 and 2,000	P	S or N	Yes
Riis et al., 1987	25	~ 1,000[c]	2,000	P	S or N	No
Late menopausal						
Dawson-Hughes et al., 1990	169	< 400	500 (CCM)	P	S or N	yes
			500 (CC)	P	S or N	no
		400–65	500 (CCM)	P	S or N	no
			500 (CC)	S or N	S or N	no
Prince et al., 1995	126	800	1,000	P	S or N	No
Reid et al., 1995	78	750	1,000	P	S or N	Yes
Radius (Proximal)						
Early postmenopausal						
Aloia et al., 1994[b]	70	500	1,700	S or N	P	no
Dawson-Hughes et al., 1990	67	< 400	500	P	P	no
Prince et al., 1991[e]	80	800	1,000	P	P	no
Riis et al., 1987	25	~ 1,000	2,000	P	P	yes

Late postmenopausal						
Dawson-Hughes et al., 1990	169	< 400	500 (CCM)	P	P	yes
			500 (CC)	P	S or N	yes
		400–65	500 (CCM)	P	P	no
			500 (CC)	P	P	no
Recker et al., 1996						
Prevalent vertebral fracture group	94	~ 450	1,200 (CC)	—	—	yes
Non-prevalent vertebral fracture group	99	~ 450	1,200 (CC)	—	—	no
Femoral neck						
Early postmenopausal						
Aloia et al., 1994[b]	70	500	1,700	P	P	yes
Dawson-Hughes et al., 1990	67	< 400	500	P	S	no
Late menopausal						
Chevalley et al., 1994	93	600	800	P[f]		no
Dawson-Hughes et al., 1990	169	< 400	500 (CCM)	P	P	yes
			500 (CC)	P	P	no
		400–65	500 (CCM)	P	S or N	no
			500 (CC)	P	S or N	no
Prince et al., 1995[g]	126	800	1,000	P	P	no
Reid et al., 1995	78	750	1,000	P	S or N	yes

continued

TABLE 4-1 Continued

Site	Calcium Intake (mg/d)			Relative Change in BMD or BMC in Calcium Group Compared with Placebo		Statistically Significant Change in BMD or BMC in Calcium Group Compared with Placebo
	N	Diet	Supplement[a]	Year 1	Year 2 or More, Annualized	
Total body						
Early postmenopausal						
Aloia et al., 1994[b]	70	500	1,700	P	P	yes
Late postmenopausal						
Reid et al., 1995	78	750	1,000	P	P	yes

[a] Calcium sources: Dawson-Hughes: citrate malate (CCM), carbonate (CC); Aloia, Ettinger, and Riis: CC; Prince: lactate-gluconate (1991, 1995), milk powder (1995); Elders and Reid: (lactate-gluconate + CC) or citrate; Chevalley: CC or osseino-mineral complex.
[b] All women treated with 400 IU (10 µg) vitamin D per day.
[c] Estimate based on national norm rather than on intake of study subjects.
[d] Randomized open trial.
[e] All women participated in an exercise program.
[f] An 18-month study in 82 women and 11 men.
[g] Supplement tablets and milk powder significantly reduced bone loss at the trochanter.
[h] S = Similar change in BMD or BMC when compared with placebo.
[i] N = Negative, but not necessarily significant, change in BMC or BMD when compared with placebo.
[j] P = Positive, but not necessarily significant, change in BMC or BMD when compared with placebo.
SOURCE: Adapted, with permission, from Dawson-Hughes B. ©1996. Calcium. In: Marcus R, Feldman D, Kelsey J, eds. *Osteoporosis*. San Diego: Academinc Press, Inc. Pp. 1103 and 1105.

Reprinted from: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (IOM, 1997, pp. 78–79).

TABLE 5-2 Vitamin C Intake and Biomarkers of Gastric and Bladder Cancer

Reference	Subjects	Vitamin C Dose (mg/d)	Duration	Findings
Leaf et al., 1987	7 men	2–1,000	5–12 d	↓ In vivo nitrosation (*N*-nitrosoproline)
Young et al., 1990	18 healthy men	1,500	1 wk	↓ Urinary ß-glucuronidase activity (linked to bladder cancer)
Dyke et al., 1994a	43 patients with gastritis	1,000	4 wk	↓ Gastric mucosa DNA[a] adduct formation
Dyke et al., 1994b	48 patients with gastritis	1,000	4 wk	↑ O^6-alkyltransferase DNA repair enzyme
Drake et al., 1996	82 patients with dyspepsia	None	—	Significant ($p < .001$) correlation between gastric mucosa ascorbyl radical concentration and ROS[b] activity
Mannick et al., 1996	84 patients with *Helicobacter pylori* infection	2,000	4–12 mo	↓ Nitrotyrosine in gastric mucosa (measure of RNS[c] activity)
Satarug et al., 1996	31 healthy men, 80 men with liver fluke infection	300 with 300 mg/d proline	1 d	↓ In vivo nitrosation by urinary nitrosoproline products

[a] DNA = deoxyribonucleic acid.
[b] ROS = reactive oxygen species.
[c] RNS = reactive nitrogen species.

Reprinted from: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000, pp. 106–107).

TABLE 6-3 Vitamin E Intake and Risk of Coronary Heart Disease in Men and Women

Reference	Variable	Quintile/Tertile 1	2	3	4	5	*p* Value for Trend
Rimm et al., 1993	Intake from food (IU[a]/d)	1.6–6.9	7.0–8.1	8.2–9.3	9.4–11.0	11.1	
	Relative risk of CHD[b]	1.0	1.10	1.17	0.97	0.79	
	95% CI[c]	—	0.80–1.51	0.84–1.62	0.69–1.37	0.54–1.15	NS[d]
	Intake from supplements (IU/d)	0	< 25	25–99	100–249	≥ 250	
	Relative risk of CHD	1.0	0.85	0.78	0.54	0.70	
	95% CI	—	0.69–1.05	0.59–1.08	0.33–0.88	0.55–0.89	NS
Stampfer et al., 1993	Intake from food (IU/d)	0.3–3.1	3.2–3.9	4.0–4.8	4.9–6.2	6.3–100	
	Relative risk of CHD	1	1.04	0.77	1.14	0.95	
	95% CI	—	0.8–1.35	0.66–1.14	0.89–1.47	0.72–1.23	NS
	Intake from food and supplements (IU/d)	1.2–3.5	3.6–4.9	5.0–8.0	8.1–21.5	21.6–1,000	
	Relative risk of CHD	1	1	1.15	0.74	0.66	
	95% CI	—	0.78–1.28	0.9–1.48	0.57–0.98	0.5–0.87	< 0.001

Knekt et al., 1994	Intake from food (mg/d)	≤ 5.3	5.4–7.1	> 7.1			
	Relative risk of CHD	1	0.73	0.35			
	95% CI	—	0.38–1.39	0.14–0.88			< 0.01
Kushi et al., 1996	Intake from food (IU/d)	≤ 4.91	4.92–6.24	6.25–7.62	7.63–9.63	≥ 9.64	
	Relative risk of CHD	1	0.70	0.76	0.32	0.38	
	95% CI	—	0.41–1.18	0.44–1.29	0.17–0.63	0.18–0.80	< 0.004

[a] IU = international unit.
[b] CHD = coronary heart disease.
[c] CI = confidence interval.
[d] NS = not significant.

Reprinted from: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM 2000a, pp. 214–215).

TABLE 8-7 Observational Studies of Folate and Risk of Neural Tube Defect

Study	Design	Subjects	Exposure	Results	Comments
Mulinare et al., 1988 (as reported in CDC, 1992)	Case/control in metropolitan Atlanta	NTD[a] case infants and normal control infants Pregnant women without a prior NTD-affected pregnancy	Multivitamin supplement containing 0–0.8 mg of folic acid at least 1 mo before conception through the 1st trimester	24 NTD cases in infants from women supplemented and 157 cases in infants from women unsupplemented 405 normal cases in infants from supplemented mothers and 1,075 normal cases in infants from unsupplemented women controls Odd ratio = 0.40, $p < 0.05$	60% reduction in risk
Bower and Stanley, 1989 (as reported in CDC, 1992)	Case/control in Western Australia	Spina bifida case infants and normal control infants Pregnant women without a prior NTD-affected pregnancy	Dietary folate and multivitamin supplement at least 1 mo before conception through the 1st trimester	77 NTD cases and 154 control mothers in study. The highest folate quartile was compared with the lowest. An increasing protective effect was observed from the lowest to the highest quartile. Odds ratio = 0.25, $p < 0.05$	75% reduction in risk

Mills et al., 1989 (as reported in CDC, 1992)	Case/control in California and Illinois	NTD case infants and normal control infants Pregnant women without a prior NTD-affected pregnancy	Multivitamin plus folate supplement containing up to 0.8 mg of folic acid plus diet at least 1 mo before conception through the 1st trimester	89 NTD cases in infants from supplemented women and 214 cases in infants from unsupplemented women 90 normal infants from supplemented women and 196 normal infants from unsupplemented women controls Odds ratio = 0.91, not statistically significant	No protective effect
Milunsky et al., 1989 (as reported in CDC, 1992)	Prospective cohort in New England	NTD case infants and normal control infants Pregnant women without a prior NTD-affected pregnancy	Multivitamin plus folate supplement containing 0.1–1.0 mg of folic acid plus diet at least 1 mo before conception through the 1st trimester	10 NTD pregnancies among 10,713 women who took multivitamin plus folate 39 NTD pregnancies among 11,944 women who took multivitamins without folate Relative risk = 0.28, $p < 0.05$	72% reduction in risk
Werler et al., 1993	Case/control in Boston, Philadelphia, and Toronto	NTD cases and controls with other major malformations Mothers of cases and controls	Daily use of multivitamins, mostly 0.4 mg of folic acid, from 28 d before through 28 d after last menstrual period	34 supplemented and 250 unsupplemented NTD case women 339 supplemented and 1,253 unsupplemented women controls Adjusted odds ratio = 0.6 (95% CI = 0.4-0.8)	40% reduction in risk

continued

TABLE 8-7 Continued

Study	Design	Subjects	Exposure	Results	Comments
Shaw et al., 1995	Case/control in California	NTD cases and normal control infants	Any use of folate-containing vitamins in the 3 mo before conception	88 supplemented and 207 unsupplemented NTD case women 98 supplemented and 149 unsupplemented women controls Odds ratio = 0.65 (95% confidence interval = 0.45–0.94)	35% reduction in risk

[a] NTD = neural tube defect.

Reprinted from: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998, pp. 248–251).

TABLE 8-8 Controlled Trials Relating Folate Supplementation and Risk of Neural Tube Defect in the Periconceptual Period

Study	Design	Subjects	Exposure	Results	Comments
Randomized controlled trials—previous NTD pregnancy					
Laurence et al., 1981	Randomized controlled trial in Wales	Pregnant women with prior NTD[a]-affected pregnancy; supplemented mothers took 4 mg of folic acid daily Unsupplemented mothers took a placebo	4 mg of folic acid or placebo daily at least 1 mo before conception through the 1st trimester	2 NTD pregnancies in 60 supplemented women 4 NTD pregnancies in 51 placebo-treated women Relative risk = 0.40, not statistically significant	60% reduction in risk
Wald et al., 1991	Randomized controlled multicenter trial in United Kingdom and Hungary	Pregnant women with prior NTD-affected pregnancy Supplemented mothers took 4 mg of folic acid daily Unsupplemented mothers took a placebo	4 mg of folic acid or placebo daily at least 1 mo before conception through the 1st trimester	6 NTD pregnancies in 593 supplemented women 21 NTD pregnancies in 602 unsupplemented women Relative risk = 0.28, $p <$ 0.05	72% reduction in risk

continued

TABLE 8-8 Continued

Study	Design	Subjects	Exposure	Results	Comments
Kirke et al., 1992	Randomized controlled multicenter trial in Ireland	Pregnant women with prior NTD-affected pregnancy Supplemented women took 0.36 mg of folic acid with or without multivitamins daily Unsupplemented women took multivitamins daily excluding folic acid	Supplements taken for at least 2 mo before conception and until the date of the third missed menstrual period	0 NTD in 172 infants/fetuses of supplemented women 1 NTD in 89 infants/fetuses of unsupplemented women Indeterminant protective effect, not statistically significant	Trial was prematurely terminated
Nonrandomized controlled trials—previous NTD pregnancy					
Smithells et al., 1983	Nonrandomized controlled multicenter trial in UK	Pregnant women with prior NTD-affected pregnancy Supplemented mothers took 0.36 mg of folic acid plus multivitamins daily Unsupplemented mothers took nothing	0.36 mg of folic acid plus multivitamins or no use from 1 mo before conception through the 1st trimester	3 NTD pregnancies in 454 supplemented women 24 NTD pregnancies in 519 unsupplemented women Relative risk = 0.14, $p < 0.05$	86% reduction in risk

Vergel et al., 1990	Nonrandomized controlled trial in Cuba	Pregnant women with prior NTD-affected pregnancy Supplemented mothers took 5 mg of folic acid daily Unsupplemented mothers took nothing	5 mg of folic acid or no use from 1 mo before conception through the 1st trimester	0 NTD pregnancies in 81 supplemented women 4 NTD pregnancies in 114 untreated women Indeterminant protective effect, not statistically significant	Complete protective effect
Randomized controlled trial—all women planning pregnancy					
Czeizel and Dudas, 1992	Randomized controlled trial in Hungary	Women planning a pregnancy Supplemented women took 0.8 mg of folic acid plus multivitamins daily Unsupplemented women took a trace-element supplement	Supplements taken for at least 1 mo before conception and until the date of the second missed period	0 NTD pregnancies in 2,104 supplemented women 6 NTD pregnancies in 2,052 unsupplemented women Relative risk = 0.0, p = 0.029	Complete protective effect

[a] NTD = neural tube defect.
SOURCE: Adapted from CDC (1992).

Reprinted from: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (IOM, 1998, pp. 250–253).

APPENDIX TABLE REFERENCES

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Appendix B

Committee Member Biographical Sketches

NORMAN I. KRINSKY, Ph.D. (Chair) received his Ph.D. in biochemistry from the University of Southern California. He is currently professor emeritus in the Department of Biochemistry, School of Medicine, Tufts University, Boston, and a scientist at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, also at Tufts. Dr. Krinsky is a member of the Advisory Committee of the International Antioxidant Research Centre at King's College in London. He is also the president of the New England Free Radical/Oxygen Society. Dr. Krinsky served as chair of the Food and Nutrition Board/Institute of Medicine (FNB/IOM) Panel on Dietary Reference Intakes for Dietary Antioxidants and Related Compounds. Currently, Dr. Krinsky's research is directed at examining the biological activity of cleavage products of carotenoids; the interaction between smoking, carotenoid intake, and lung cancer; and the functions of antioxidants.

LAWRENCE J. APPEL, M.D., M.P.H. is a professor of medicine, epidemiology, and international health at the Johns Hopkins University Medical Institutions. He holds an M.D. from the New York University School of Medicine and an M.P.H. from Johns Hopkins University. Dr. Appel has been the principal or coprincipal investigator in numerous studies that examined the effects of life-style modification, particularly nutrition interventions, on blood pressure. In addition, Dr. Appel is the course director of the class, "Clinical Trials Issues and Controversies," at Johns Hopkins. Dr. Appel previously served on the FNB/IOM Committee on Nutrition Services for Medicare Beneficiaries and

currently serves as chair of the FNB/IOM Panel on Dietary Reference Intakes for Electrolytes and Water.

STEPHANIE A. ATKINSON, Ph.D., R.D. is a professor of nutrition in the Department of Pediatrics and associate member of the Department of Biochemistry in the Faculty of Health Sciences, McMaster University in Hamilton, Ontario. She contributes her expertise as a consultant in clinical nutrition as a member of the special professional staff at The Children's Hospital in Hamilton. Dr. Atkinson received her Ph.D. in nutritional sciences from the University of Toronto. Her research focuses on nutrition for prematurely born infants and on developmental aspects of bone, particularly related to the impact of nutrition, childhood diseases such as leukemia, epilepsy, and inflammatory bowel disease, and steroid therapy, on skeletal development in infants and children. Dr. Atkinson currently holds an appointment as a member of the inaugural Governing Council of the new Canadian Institutes of Health Research. Previously, she served the nutrition community as councilor for the American Society of Clinical Nutrition, scientific chair of the 16th International Congress of Nutrition held in 1997 in Montreal, and as a member of the Board of Trustees of the National Institute of Nutrition in Canada. Dr. Atkinson also serves on the FNB/IOM Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and was chair of the Panel on Dietary Reference Intakes for Calcium and Related Nutrients. She is a member of the Canadian and American Societies for Nutritional Sciences, American Society for Clinical Nutrition, American Society for Bone and Mineral Research, and Dietitians of Canada, and is a fellow of the American College of Nutrition.

LYNN B. BAILEY, Ph.D. is a professor of nutrition in the University of Florida's Food Science and Human Nutrition Department. Before joining the faculty in 1977, Dr. Bailey completed her Ph.D. and postdoctoral training at Purdue University in the area of human nutrient requirements. Her research has focused on the estimation of folate requirements and the evaluation of folate status in different life stages, including adolescence, young adulthood, pregnancy, and postmenopause. She was the editor of the book *Folate in Health and Disease*. She has served on numerous expert scientific panels, including the Food and Drug Administration's Folic Acid Subcommittee, which addressed the fortification of cereal grain products with folic acid in an effort to reduce the risk of neural tube defects. Dr. Bailey was the recipient of a national U.S. Department of Agriculture Award for Superior Service for her research accomplishments related to estimating folate requirements. Dr. Bailey previously served on the FNB/IOM Panel on Dietary Reference Intakes for Folate, Other B Vitamins, and Choline.

SUSAN TAYLOR MAYNE, Ph.D. is an associate professor in chronic disease epidemiology at the Yale University School of Medicine and associate director of the Yale Comprehensive Cancer Center for which she leads the Cancer Prevention and Control Research Program. The primary focus of her

research is in the area of nutrition and cancer prevention. She directed a large cancer prevention clinical trial to determine whether supplemental β-carotene reduces the incidence of mouth and throat cancer. Additionally, she participated in the working group on carotenoids and cancer of the International Agency for Research on Cancer. She is a member of the Executive Committee of the International Carotenoid Society and the Steering Committee of the Carotenoid/Vitamin A Research Interaction Group (CARIG), and cochaired the CARIG Annual Conference at the Federation of American Societies for Experimental Biology in 1996 and 2001. Dr. Mayne has served on the FNB/IOM Panel on Dietary Reference Intakes for Dietary Antioxidants and Related Compounds. She has a Ph.D. in nutritional biochemistry with minors in biochemistry and toxicology from Cornell University, and received post-doctoral training in epidemiology at Yale University.

PAUL D. STOLLEY, M.D., M.P.H. is a professor and former chair of the Department of Epidemiology and Preventive Medicine at the University of Maryland at Baltimore. Dr. Stolley is an epidemiologist and internist, and trained at the Johns Hopkins School of Hygiene and Public Health, where he previously served on the faculty in the Department of Epidemiology. He is currently on loan to the Food and Drug Administration's Center for Drug Evaluation and Research, where he is assigned to a project evaluating drugs used in pregnancy. He founded and led the Clinical Epidemiology Unit at the University of Pennsylvania where he served as the Herbert Rorer Professor of Medicine. Dr. Stolley has had a long interest and experience in the investigation of obscure illnesses and epidemics. He is a member of IOM and is past president of the American College of Epidemiology, Society of Epidemiology Research, and American Epidemiological Society. He served as a liaison to the Committee on Diet and Health in 1989. Dr. Stolley's research interests include epidemiology, public health, stroke, and violence.

JUDITH R. TURNLUND, Ph.D., R.D. is a research nutrition scientist at the U.S. Department of Agriculture Western Human Nutrition Research Center at the University of California at Davis, and is an adjunct professor in the Department of Nutrition. She earned her B.S. in chemistry and psychology at Gustavus Adolphus College in Minnesota and holds a Ph.D. in nutritional sciences from the University of California at Berkeley. She is a registered dietitian. Her research interests include human requirements for and bioavailability of trace elements (copper, molybdenum, zinc, and iron) and nutrition and aging. Dr. Turnlund is a member of the American Society for Nutritional Sciences, American Society for Clinical Nutrition, and American Dietetic Association. She served on the FNB/IOM Panel on Dietary Reference Intakes for Micronutrients, and has served on trace element task groups for the World Health Organization. Dr. Turnlund received the American Institute of Nutrition Lederle Award for outstanding accomplishments in human nutrition.