The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes was charged with developing a research agenda to provide a basis for public policy decisions related to recommended intakes of the B vitamins and choline and ways to achieve them, along with information needed to establish tolerable upper intake levels (ULs). This chapter describes the approach used to develop the research agenda, briefly summarizes gaps in knowledge, and presents a prioritized research agenda. A section at the end of each nutrient chapter (Chapters 4 through 12) presents a prioritized list of research topics for the nutrient.

APPROACH

The following approach was used to develop the research agenda:

• Gaps in knowledge were identified in
  – nutrient requirements,
  – methodological problems related to the assessment of intake of these nutrients and to the assessment of adequacy of intake,
  – relationships of nutrient intake to public health, and
  – adverse effects of nutrients.
• Data were examined to identify any major discrepancies between intake and the Estimated Average Requirements (EARs), and possible reasons for such discrepancies were considered.
• Listings of studies currently funded by the National Institutes of
Health were obtained and examined along with other listings by expert groups and comments submitted by experts.

- The need to protect individuals with extreme or distinct vulnerabilities resulting from genetic predisposition or disease conditions was considered.
- Expert opinion was used to weigh alternatives and set priorities.

**IMPORTANT FEATURES OF STUDIES TO ESTIMATE REQUIREMENTS**

Derivation of an Estimated Average Requirement (EAR) involves identification of the criterion for a particular status indicator or combination of indicators that is consistent with impaired status as defined by some clinical consequence. For many of the B vitamins, there is a dearth of information on the biochemical values that reflect abnormal function. One priority should be determination of the relationship of existing status indicators to clinical endpoints to allow their use for setting EARs. For some nutrients new clinical endpoints of impaired function need to be identified and related to status indicators.

The depletion-repletion research paradigms that are often used in studies of requirements, although not ideal, are still probably the best approach to determining vitamin requirements. However, these studies should be designed to meet three important criteria:

- An indicator of vitamin status is needed for which a cutoff point has been identified, below (or above) which vitamin status is documented to be impaired. (In the case of folate, an erythrocyte level of 300 nmol/L [140 ng/mL] fits this criterion because lower levels are associated with megaloblastic changes in blood cells. In the case of vitamin B₆ and several other B vitamins, however, there is little information relating levels of status indicators to functional sufficiency or insufficiency. Instead, the levels of indicators normally used to assess requirements are those exhibited by subjects on a baseline adequate diet—even though there is no information regarding whether this level of intake is greatly in excess of adequate, barely adequate, or deficient.) The amount needed for restoration of biochemical status indicators to baseline values is not necessarily equivalent to the requirement for the nutrient.

- The depletion and repletion periods should be sufficiently long to allow a new steady state to be reached. This can be very problematic because turnover rates of total body content for B vitamins range from less than 1 to about 3 percent per day, which suggests
that long periods are needed for equilibrium. In the case of erythrocyte folate, theoretically the erythrocytes have to turn over completely (approximately 90 days). Study design should allow for examination of the effects of initial status on response to maintenance or depletion and repletion

- Intakes used in repletion regimens should bracket the expected EAR intake to assess the EAR more accurately and to allow for a measure of variance. In addition, an accurate assessment of variance requires a sufficient number of subjects.

A relatively new and increasingly popular approach to determining requirements is kinetic modeling of body pools using steady-state compartmental analyses. This approach is unlikely to supplant depletion-repletion studies because it has a number of drawbacks; for example, assumptions that cannot be tested experimentally are often needed and the numbers obtained for body pool sizes are inherently imprecise. Even if accurate assessments of body pools were possible and were obtained, such information would be useful in setting a requirement only if the size of the body pool at which functional deficiency occurs could be established.

**MAJOR KNOWLEDGE GAPS**

**Requirements**

For all the B vitamins and choline, there is a serious lack of data useful for setting Estimated Average Requirements (EARs) for children, adolescents, pregnant and lactating women, and the elderly. Studies should use graded levels of nutrient intake and a combination of response indices and should consider other points raised in the preceding section. For some of the B vitamins (e.g., folate), studies should examine whether the requirement varies substantially by trimester of pregnancy. The nutrients and life stage and gender groups for which studies of requirements appear to be priorities from a public health perspective are vitamin B₁₂ requirements of the elderly and folate requirements by trimester of pregnancy. In addition, priority should be given to the identification of indicators on which to base vitamin B₆ requirements.

This short list does not imply that studies of requirements of other nutrients or age groups are not important, merely that it seems less likely that such studies will produce results that will have significant benefit on the health of the U.S. or Canadian populations. Research
topics for each of the B vitamins and choline appear at the end of Chapters 4 through 12.

The understanding of the nutrition of pantothenic acid, biotin, and choline is rudimentary compared with that of the other B vitamins. Little information is available on human requirements, intake, bioavailability, toxicity, and metabolic effects of these compounds. Although choline can be formed in the human body from endogenous precursors, little is known about the relative amounts of choline derived from the diet and from endogenous synthesis. Research to date has indicated little cause for concern about the adequacy of pantothenic acid or biotin intake for healthy people; deficiency states can be produced only by actively interfering with the absorption or bacterial production of these vitamins. On the other hand, animal studies suggest that choline intake may affect long-term health.

A growing number of studies suggests that there are complex interrelationships among nutrients (e.g., vitamin B6, folate, vitamin B12, and perhaps choline, methionine, and riboflavin), but these are not well understood in relation to the maintenance of normal nutritional status and to the prevention of chronic degenerative disease. These interactions may affect the need for one or more of the nutrients.

Methodology

For some nutrients there are serious limitations in the methods available to analyze laboratory values indicative of nutrient status, to determine the nutrient content of foods, or both. These limitations have slowed progress in conducting or interpreting studies of nutrient requirements. The most serious gaps were judged to be those relating to analytical methodology for blood folate analysis and methods for the analysis of the folate content of food. A related gap, which is not strictly methodological, concerns the bioavailability of various forms of folate. Major needs include a comparison of the bioavailability of food folate from mixed diets and of folate in the form of folic acid (from supplements or fortification) consumed with food and an examination of the mechanisms by which bioavailability is altered by food matrices.
Developmental Disorders

For the B vitamins the developmental disorder of greatest concern is neural tube defect (NTD). Major gaps in knowledge include the mechanisms by which maternal folate sufficiency reduces the occurrence of NTD in the infant (e.g., evaluation of whether increased NTD risk is due to folate deficiency or to the mode of action of folate sufficiency [does it act on mother, embryo, or both?]); the relative efficacy of food folate, folate added to food, and folate supplements in reducing NTD risk; the process, if any, by which folate influences the embryonic process of neurulation; and the genes that are responsible for the heritability and folate-responsiveness of NTD. This latter area could include (1) linkage analyses in suitable genetically homogeneous human populations to assess the etiologic relationship between NTD and a variety of genetic alterations (including the thermolabile variant of 5,10-methylenetetrahydrofolate reductase) and in the genes responsible for NTD in the curly tail mouse; (2) investigation of whether alterations in any of these genes produce NTD when induced in mouse models, yield folate-responsive NTD in mouse models, and provide suitable markers for assessing NTD risk in human populations; and (3) identification of an animal model for common human NTDs that is responsive to relevant levels of folate.

Chronic Degenerative Disease Risk

Although interest is high and numerous studies have been conducted, there are still serious gaps in knowledge of the relationship of B vitamin intake to risk of vascular disease and other chronic degenerative diseases. With the new U.S. regulations on the fortification of cereal grains with folate, it is now possible to investigate the health effects, both positive and negative, of folate fortification on folate intake and health status by life stage and gender.

Adverse Effects

For B vitamins and choline as a group, only a few studies have been conducted that were explicitly designed to address adverse effects of chronic high intake. Thus, information on which to base Tolerable Upper Intake Levels (ULs) is extremely scanty. Because it appears that vitamin B_{12} deficiency greatly increases the potential
of folate to cause adverse effects, efforts are needed to improve methods to detect and correct vitamin B$_{12}$ deficiency before adverse hematological or neurological changes occur and to determine the prevalence of vitamin B$_{12}$ deficiency.

**THE RESEARCH AGENDA**

The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes agreed to assign highest priority to research that has potential to prevent or retard human disease processes and to prevent deficiencies with functional consequences. The following five areas for research were assigned the highest priority:

- Studies to provide the basic data for constructing risk curves and benefit curves across the exposures to food folate and to folate added to foods and taken as a supplement. Such studies would provide estimates of the risk of developing neural tube defects, vascular disease, and neurological complications in susceptible individuals consuming different levels of folate.
- Studies of the magnitude of effect of folate, vitamin B$_6$, vitamin B$_{12}$, and related nutrients for the prevention of vascular disease and of possible mechanisms for the influence of genetic variation.
- Studies to overcome the methodological problems in the analysis of folate. This includes the development of sensitive and specific indicators of deficiency and the development of practical, improved methods for analyzing the folate content of foods and determining its bioavailability.
- Studies to develop economical, sensitive, and specific methods for assessing the prevalence, causes, and consequences of vitamin B$_{12}$ malabsorption and deficiency and for preventing and treating these conditions.
- Studies of how folate and related nutrients influence normal cellular differentiation and development, including embryogenesis and neoplastic transformation.

Although data are not sufficient for deriving a Tolerable Upper Intake Level (UL) for most of the B vitamins and additional research is required on the adverse effects of B vitamins and choline, it was concluded that higher priority should be given to the areas listed above because of relatively low expectation of adverse effects or toxicity.