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## A Research Agenda

The Panel on Antioxidants and Related Compounds and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (DRI Committee) and its subcommittees were charged with developing a research agenda to provide a basis for public policy decisions related to recommended intakes of vitamin C, vitamin E, selenium, and  $\beta$ -carotene and other carotenoids, and ways to achieve them. 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 5 through 8) presents a prioritized list of research topics for each nutrient.

### APPROACH

The following approach resulted in the research agenda identified in this chapter:

1. Identify gaps in knowledge;
2. Examine data to identify any major discrepancies between intake and the Estimated Average Requirements (EARs); consider possible reasons for such discrepancies;
3. Consider the need to protect individuals with extreme or distinct vulnerabilities due to genetic predisposition or disease conditions; and
4. Weigh alternatives and set priorities based on expert judgment.

As a result of this approach, the following four areas were identified:

- 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.

## MAJOR KNOWLEDGE GAPS

### *Requirements*

To derive an Estimated Average Requirement (EAR), the criterion must be known for a particular status indicator or combination of indicators that is consistent with impaired status as defined by some clinical consequence. For the nutrients considered in this report, there is a dearth of information on the biochemical values that reflect abnormal function. A priority should be the determination of the relationship of existing status indicators to clinical endpoints in the same subjects to determine if a correlation exists. For some nutrients, either new clinical endpoints or intermediate endpoints of impaired function have 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 nutrient requirements. However, these studies should be designed to meet three important criteria:

1. An indicator of nutrient status is needed for which a cutoff point has been identified, below which nutrient status is documented to be impaired. (In the case of vitamin E, values are based on induced vitamin E deficiency and the correlation with hydrogen peroxide-induced hemolysis and plasma  $\alpha$ -tocopherol concentrations, because there is little information relating levels of status indicators to functional sufficiency or insufficiency. Also with vitamin C, there is little information relating levels of status indicators to functional sufficiency or insufficiency, because dose-dependent absorption and renal regulation of ascorbate allow body conservation during low intakes and limitation of plasma levels at high intakes.)

2. The depletion and repletion periods should be sufficiently long to allow a new steady state to be reached. This can be very problematic for vitamin C because biological half-life ranges from

8 to 40 days and is inversely related to ascorbate body pool. For  $\beta$ -carotene and other carotenoids, no long-term depletion-repletion studies with validated intermediate endpoints exist. Study design should allow examination of the effects of initial status on response to maintenance or depletion-repletion.

3. Repletion regimen intakes 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. Although this approach is unlikely to supplant depletion-repletion studies, it may be the only technique available to obtain this type of information, despite a number of drawbacks. A number of assumptions that cannot be tested experimentally are often needed, and the estimates 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 one could establish the body pool size at which functional deficiency occurs. The amount needed for restoration of biochemical status indicators to baseline values is not necessarily equivalent to the requirement for the nutrient.

For vitamin C, vitamin E, selenium, and  $\beta$ -carotene and other carotenoids, useful data are seriously lacking for setting requirements for infants, 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 above. For some of these nutrients, studies should examine whether or not the requirement varies substantially by trimester of pregnancy. In addition, more information is needed for groups at increased risk for oxidative stress, especially those who smoke or who are subjected to second-hand smoke, athletes, and individuals living at high altitudes.

Data are lacking about gender issues with respect to metabolism and requirements of these nutrients. For example, women and children with low intakes of selenium are at higher risk of Keshan disease than are men with similar intakes. Women are at higher risk of macular degeneration even at similar plasma concentrations of carotenoids.

The understanding of the health effects of carotenoids is rudimentary compared with that of the other nutrients in this report.

Little information is available on bioavailability, toxicity, and effects of these compounds, apart from  $\beta$ -carotene. Although the only known validated function for carotenoids in humans is to act as a source of vitamin A in the diet, little is known about the relative contribution of dietary provitamin A carotenoids to vitamin A status.

Research to date has indicated little cause for concern about the adequacy of vitamin E intake for apparently healthy people; deficiency states can be produced only as a result of genetic abnormalities in  $\alpha$ -tocopherol transfer protein, as a result of various fat malabsorption syndromes, or as a result of protein-energy malnutrition. However, the prevalence of these genetic abnormalities is unknown.

A growing number of studies suggest that there are complex interrelationships among nutrients, particularly those involved in protecting against oxidation (e.g., vitamin C, vitamin E, and selenium), 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 intake level for one or more of the nutrients.

### *Methodology*

For some nutrients, serious limitations exist 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. Although the analytical methodology for serum carotenoid status is becoming more routine, methods for the analysis of the major dietary carotenoids remain as a limiting methodological factor. A related gap, which is not strictly methodological, is the bioavailability of the various isomers of vitamin E. Major needs include: (1) a comparison of the biological potencies of the various forms of food vitamin E from mixed diets; (2) validation of dietary intake instruments to assess intake of vitamin E and of the major carotenoids in food; and (3) an examination of the mechanisms by which bioavailability is altered by food matrices.

### *Relationships of Intake to Public Health*

Although interest is high and numerous studies have been conducted, serious gaps still exist in knowledge of the relationship of intakes of vitamin C, vitamin E, selenium, and  $\beta$ -carotene and other carotenoids to the risk of coronary heart disease, cancer, and other

chronic degenerative diseases. An imbalance in oxidant stress and defenses can lead to the formation and excretion of oxidized products of nucleic acids, lipids, and proteins, which may play a role in chronic disease.

Many of these studies have been conducted with supplemental intakes that are far above those that can be obtained from food, and some questions and controversy remain regarding the linkage of these antioxidant nutrients with increased risk of chronic disease. For example, some clinical intervention trials have shown that in male long-term smokers, high doses of  $\beta$ -carotene supplements did not decrease and may have increased their risk of lung cancer.

Multiple factors are probably involved in disease, but in some cases, one particular nutrient may contribute significantly. For example, vitamin E is known to protect *ex vivo* low-density lipoprotein oxidation, whereas  $\beta$ -carotene offers no protection. Questions that have to be answered include the following: (1) what are the tissue uptake and subcellular distributions of these nutrients in humans; (2) what is the mechanism by which these nutrients are taken up and regulated by the cells; (3) what is the turnover of these nutrients in the various tissues; (4) in which tissues are they degraded and how rapidly; and (5) what are the major metabolic intermediates during degradation and do they have biological function?

Additional randomized clinical trials are needed to test whether or not supplementation with vitamin C, vitamin E, selenium, and/or  $\beta$ -carotene and other carotenoids can reduce the risk of chronic disease. A number of clinical intervention trials involving more than 100,000 people are in progress. However, whether the results are positive or negative, additional studies will be necessary. For example, if the results are negative, the question will arise as to whether treatment was instituted early enough and whether even longer trials starting at an earlier age are needed to test the hypothesis properly. If the results are positive, the relative importance of vitamin C, vitamin E, selenium, and  $\beta$ -carotene will have to be sorted out, because they are being used in combination in several of the studies. Also, the issue of dose will arise. Most of these studies are using doses that may be unnecessarily high. The questions of who should be treated, at what dosage, and at what age will have to be addressed, along with the impact of treatment on various subgroups (older adults, those who smoke, those with other chronic diseases such as diabetes, etc.). Again, if the results are positive, indicating that antioxidants do indeed offer protection, it will be important to determine if combinations of antioxidants in various doses can further increase the beneficial effect.

### *Adverse Effects*

Considering these nutrients as a group, only a few studies have been conducted that were explicitly designed to address adverse effects of chronically high intake. Thus, information on which to base Tolerable Upper Intake Levels (ULs) is extremely limited. Although an unexpected result of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was a non-prespecified 50 percent increase in mortality from hemorrhagic stroke in Finnish men who smoked and were supplemented with 50 mg/day of vitamin E, additional randomized trial evidence is needed for confirmation or refutation of this result. Because data on the potential for  $\beta$ -carotene to produce increased lung cancer rates in smokers are conflicting, ongoing studies are needed to help resolve this issue.

## THE RESEARCH AGENDA

### *Reporting Data*

Because the various forms of vitamin E are not interconvertible and because plasma concentrations of vitamin E depend on the affinity of hepatic  $\alpha$ -tocopherol transfer protein for the various forms, it is recommended that the relative biological potencies of the different forms of vitamin E be reevaluated. Until this is done, the actual concentrations of each of the various vitamin E forms in food and biological samples should be reported separately whenever possible.

### *Research*

Five major types of information gaps were noted: (1) a dearth of studies designed specifically to estimate average requirements in presumably healthy humans; (2) a nearly complete lack of usable data on the nutrient needs of infants, children, adolescents, and pregnant women; (3) a lack of definitive studies to determine the role of these nutrients in reducing the risk of certain chronic diseases; (4) a lack of validated biomarkers to evaluate oxidative stress and the relationship between antioxidant intake and health and disease; and (5) a lack of studies designed to detect adverse effects of chronic high intakes of these nutrients.

Highest priority is thus given to research that has the potential to prevent or retard human disease processes and to prevent deficiencies with functional consequences. The following six areas for re-

search were assigned the highest priority (other research recommendations are found at the end of Chapters 5 through 8):

- Studies to provide the basic data for constructing risk curves and benefit curves across the exposures to dietary and supplemental intakes of vitamin C, vitamin E, selenium,  $\beta$ -carotene and other carotenoids. Studies should be designed to determine the relationship of nutrient intakes to validated biomarkers of oxidative stress. These studies should be followed by nested case-control studies to determine the relationship of the biomarkers of oxidative stress to chronic disease. Finally, full-scale intervention trials should be done to establish the preventive potential of a nutrient for chronic disease.

- Investigations of the gender specificity of the metabolism and requirements for vitamin C, vitamin E, selenium, and  $\beta$ -carotene and the other carotenoids.

- Studies to validate methods and possible models for determining Dietary Reference Intakes in the absence of data for some life stage groups, such as children, pregnant and lactating women, and older adults.

- Research to determine the interactions and possible synergisms of vitamin C, vitamin E, selenium, and  $\beta$ -carotene with each other, with other nutrients and other food components, and with endogenous antioxidants. Multifactorial studies are needed to demonstrate *in vivo* actions as well as synergisms that have been shown *in vitro*.

- Studies to develop economical, sensitive, and specific methods to assess the associations of vitamin C, vitamin E, selenium, and  $\beta$ -carotene and the other carotenoids with the causation, prevalence, prevention, and treatment of specific viral or other infections.

- Investigations of the magnitude and role of genetic polymorphisms in the mechanisms of actions of vitamin C, vitamin E, selenium, and  $\beta$ -carotene and the other carotenoids.

Because of inconsistent data, a Tolerable Upper Intake Level (UL) could not be established for  $\beta$ -carotene, and due to a lack of sufficient data, ULs could not be set for other carotenoids from food. Thus, research is needed concerning the ULs for the carotenoids. In addition, research would be helpful relative to the adverse effects of vitamin C, vitamin E, and selenium. However, it was concluded that higher priority should be given to the areas listed above because of the possibility of adverse effects at intakes consumed from food and supplements in the United States and Canada.

