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## International harmonization of approaches for developing nutrient-based dietary standards

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*Executive summary*

# Harmonization of nutrient intake values

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**Key words:** Nutrient recommendations, nutrient requirements

The United Nations University's Food and Nutrition Programme, in collaboration with the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and UNICEF, convened a group of international experts to review the harmonization of approaches for developing nutrient-based dietary standards. The group met at the Innocenti Center in Florence, Italy, and was charged to:

» Identify the concepts that must be harmonized to provide a foundation for generating nutrient-based dietary standards and to define the components and terms supporting these concepts;
» Harmonize guidelines for the elaboration of methods and approaches for developing nutrient-based dietary standards; and
» Consider specific aspects of the process for developing nutrient-based dietary standards that require adjustments for unique food patterns and lifestyles of specific populations throughout the world.
» The group reviewed the need for harmonization, agreed on the definitions of key terms, developed a framework for estimating average nutrient requirements (ANRs) and upper nutrient levels (UNLs), identified criteria for establishing ANRs and UNLs, evaluated key issues related to the derivation of such values (e.g., nutrient bioavailability, extrapolation of values among diverse life-stage groups, application of standard height and weights, categorization of life-stage groups, and effects of genetic variation), and considered their uses and applications, especially their roles in the development of dietary guidelines. The group's deliberations were based on papers developed for this review and published by the *Food and Nutrition Bulletin* [1–10]. The outcome of these deliberations is summarized below.

## Why harmonize?

The group identified four basic reasons why it is important to harmonize approaches and methods for the development of nutrient intake values (NIVs), the term adopted to encompass all nutrient-based dietary standards derived from primary data. First, harmonization of the process will improve the objectivity and transparency of values that are derived by diverse national, regional, and international groups. Second, a harmonized process will provide a common basis or background for groups of experts to consider throughout processes that lead to NIV. Third, a harmonized process will permit developing countries, which often have limited access to scientific and economic resources, to convene groups of experts to identify how to modify existing NIVs to meet their populations' specific requirements, objectives, and national policies. Finally, a harmonized process will supply a common basis for the use of NIVs across countries, regions, and the globe for establishing public and clinical health objectives and food and nutrition policies, such as fortification programs, and for addressing regulatory and trade issues.

## Harmonization of key terms

The group agreed to use the term NIV to encompass the set of recommendations based on primary data that are analogous to those developed by various regional groups, e.g., dietary reference values (DRVs) by the United Kingdom, nutrient reference values (NRVs) by Australia and New Zealand, reference values for nutrient supply by Germany/Austria/Switzerland, and

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dietary reference intakes (DRIs) by the United States and Canada. The term was judged to be sufficiently neutral and descriptive of these values' broad uses.

The group agreed to recommend only two NIVs, the average nutrient requirement (ANR) and the upper nutrient level (UNL). It recognized that groups charged with the development of such recommendations have derived other values, but that these other values usually are derived from estimates of nutrient-specific ANRs or UNLs.

The exclusion of lower recommended intakes, reference nutrient intakes, safe or adequate intakes, and population-level recommendations from tables summarizing NIVs, is put forward for the following reasons:

*Lower reference nutrient intake (LRNI) or lower threshold intake (LTI).* This value is derived from the ANR, i.e., it is equivalent to the ANR minus 2 SD of the requirement. Typically it is sufficient to meet the needs of only 2% of individuals, but countries may wish to use some other analogous value (e.g., values that meet the needs of 5% or 10% of a specified population) to evaluate the likelihoods of nutrient intake sufficiency and deficiency.

The principal rationale for the exclusion of such values rests on their limited usefulness for assessing the prevalence of undernutrition in populations, and concern that such values set too low an expectation for the adequacy of nutrient intake of individuals. Their use for planning purposes is similarly too limited.

*Reference nutrient intake (RNI), recommended nutrient intake (also RNI), recommended dietary allowance (RDA), and recommended dietary intake (RDI).* This number also may be derived from the ANR as the mean plus 2 SD of the mean requirement. The process for setting it or other values intended to guide individual intakes is described in subsequent sections of this summary. Typically, this value covers the index nutrient needs of 98% of individuals. Such values are also not recommended for inclusion in tables summarizing NIVs. The group has adopted the term "individual nutrient level" ($INL_x$) for these values. The x denotes the probability of nutrient adequacy for any single individual. This term is discussed below in greater detail.

The group concluded that it would be preferable to use a more flexible approach that enables expert groups to develop values analogous to the present RNI (or its equivalent) at points in the distribution of requirement deemed to be appropriate in specific countries and regions. Thus, some may wish to use 75%, 80%, 90%, etc., rather than the 98% used currently that reflects a risk of inadequate intake of approximately 2% for an individual.

*Safe intake* (same as the adequate intake (AI) or the lower end of the range of safe intakes). Because this value often is used when data are insufficient to set an ANR, the process for setting it is greatly subjective. Ideally, such a term will be used only to describe nutrient targets for infants (based on the nutrient content of breastmilk) or other exceptional situations. Exclusion of these values from NIV tables is recommended because of the great subjectivity inherent to their derivation.

The report also recommends that the NIVs *not include population-level recommendations,* such as the upper and lower limits of the population mean intake. These standards vary with the population's intake characteristics and require several assumptions. This topic is covered in more detail in the section below on uses and applications and in Vorster et al. [1] and Murphy et al. [4] in this volume.

## The framework for estimating average nutrient requirements (ANRs)

The basic framework for estimating ANRs is based on distributions of nutrient intakes required to achieve a specific outcome in a specified healthy population [6]. If those intakes are distributed normally, the population's mean requirement is its ANR. When such intakes are not distributed normally, data should be transformed, thus enabling the resulting median intake to serve as the ANR. In many cases the distribution of requirements is unknown. Because this is not uncommon, substantial research is needed to define the distributions of nutrient requirements and to identify biological and environmental factors that influence them.

Groups charged with developing NIVs should determine which nutrients and food components to consider. The group agreed that NIVs should be established whenever possible for all nutrients and food components that are *essential OR have public health relevance.* Fiber is an example of a food component that has public health relevance but is not an essential dietary component. The group concluded that good food-composition data for a nutrient or food component are necessary to ascertain public health relevance, since such data are key to estimate exposures (or intake).

Acceptable macronutrient distribution ranges for carbohydrate, fat, and protein have been established by some groups. These ranges are derived primarily for promoting long-term health and preventing chronic (or noncommunicable) disease and will be described further in that context. Establishing an ANR for the total amount of carbohydrate and fat in the diet is not necessary. However, it is appropriate to establish ANRs for protein to achieve zero or appropriately positive nitrogen balance and for the essential fatty acids that have specific biological functions.

Population intake levels were established for some of the trace elements in the 1996 FAO/WHO report

[11]. These are levels of *intake* of a specific nutrient that can be used to plan diets or assess intakes of homogeneous populations, e.g., all girls of a similar age in a boarding school. Population intake levels for planning and assessment purposes should be derived from the ANR, assessments of the variation in nutrient requirements, and the targeted population's variation in nutrient intakes. Thus, such calculations reflect an application of the ANR. Also, assessing population intakes requires several assumptions that are not met easily. This application is considered in more detail in subsequent sections of this summary.

The group recognized that nutrient–nutrient interactions may alter nutrient requirements. Examples of such interactions are protein–energy, vitamin E–polyunsaturated fats, and calcium–protein–sodium. The potential impact of such interactions on average requirements should be considered and described whenever such interactions are likely. Ideally, such nutrient interactions should be characterized quantitatively, e.g., estimates of reductions in protein requirements with increasing energy intakes.

Finally, the group addressed the need to consider subpopulations with special needs, e.g., children with chronic diarrhea or smokers. The NIVs address the requirements of "apparently healthy" individuals. Individuals with special needs should be considered separately, and if enough data are available, NIVs may be established for them.

## Framework for estimating UNLs

The second recommended NIV is the upper nutrient level (UNL) [7]. This value was defined as the highest level of habitual nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals in the general population. As intake increases above the UNL, the potential for risk of adverse effects increases. Habitual intake was defined as chronic daily use and is usually based on the total intake of a nutrient from food (including fortificants), water, supplements, and, in some cases, medications.

As implied by the definition, the recommended process for deriving UNLs for all groups is the determination of a "no observed adverse effect level"* (NOAEL) or the "lowest observed adverse effect level"** (LOAEL). The group agreed that UNLs should be determined by applying an uncertainty factor to NOAELs or LOAELs and that the magnitude of uncertainty factors should be determined on a case-by-case basis. These considerations should include a careful review of the differences between values equivalent to the ANR plus 2 SD, and corresponding NOAELs or LOAELs and outcomes of a risk assessment's hazard identification and characterization. The group endorsed the use of a modification of the sequence of possible effects due to excess intakes proposed by Renwick et al. (2004) [13] to help estimate the magnitude of uncertainty factors:

1. Biochemical changes within the homeostatic range and without indication of adverse sequelae;
2. Biochemical changes outside the homeostatic range without knowing the sequelae;
3. Biochemical changes outside the homeostatic range that represent a biomarker of potential adverse effects due to excess;
4. Clinical signs and/or symptoms indicative of a minor but reversible adverse effect;
5. Clinical signs and/or symptoms of significant but reversible adverse effects;
6. Clinical signs and/or symptoms indicative of significant reversible organ damage;
7. Clinical signs and/or symptoms indicative of irreversible organ damage.

The group concluded that the magnitude of uncertainty factors is likely to increase as observations progress from items 1 to 7 in the above sequence, and with the severity of sequelae to excess intakes. It acknowledged that the earliest potentially significant adverse effects would correspond to items 2 or 3 in the above sequence.

The group's recommendation of this sequence implicitly recognizes the need for biomarkers that anticipate adverse effects, rather than focusing solely on biomarkers that reflect an adverse effect's occurrence. The availability of such biomarkers was viewed as most supportive of the protection of the public's health and most likely to minimize the role of uncertainty factors in the estimation of UNLs.

In making these recommendations, the group recognized the paucity of dose–response data available for determining UNLs and describing interindividual variation and distributions. Estimates of index exposures, particularly exposures among the most vulnerable, e.g., pregnant and lactating women, children, and the elderly, also are inadequate. The seriousness of this data gap is evident in both industrialized and less wealthy countries. Furthermore, data needed to estimate values at the upper tails of intake distributions are almost always scanty for vulnerable groups in all settings.

* "Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure" [12].

** "Lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development, or life span of a target organism distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure" [12].

## Criteria for selecting outcomes for establishing NIVs

NIVs should be based on specific outcomes related to functional capacities or the avoidance of disease or other adverse outcomes [2]. Ideally, outcomes for establishing NIVs should have the following characteristics:

» A demonstrated dose–response function;
» Responsive to inadequacy or excess of a single nutrient;
» Resistant to rapid (daily) changes in response to inadequate, adequate, or excessive intakes;
» Easily measurable or assessable with noninvasive methods;
» Not responsive to environmental changes other than nutrient intake from all sources.

Selecting outcomes that meet all of these characteristics is presently difficult; thus, research is needed that is designed to identify outcomes with these characteristics and to develop appropriate technologies for this purpose.

It is recommended strongly that a single outcome be selected for establishing NIVs for each nutrient in a specific age-physiological group. The basis for this recommendation is the likelihood that values based on more than one outcome will create confusion and unnecessary complexity. Multiple outcomes also present the risk of discriminatory application, e.g., to diverse socioeconomic or differentially privileged groups.

It also is important that experts explicitly recognize that diverse outcomes for setting requirement levels differentially affect resulting ANRs and very likely also affect their variances. For example, selecting "dark adaptation" or "saturation of liver deposits" as an outcome for setting the ANR for vitamin A will result in different ANRs and probably different variances and coefficients of variation. It also is likely that diverse diet-, host-, and environment-related factors will affect ANRs differentially. Thus, careful reviews of such influences are key to the estimation of ANRs. In practice, reliable estimates of population-specific variability are seldom available, and thus research on the determinants of variances should be a high priority.

The group stressed the importance of using all available published physiological data based on agreed-upon characteristics to determine outcomes on which to base NIVs. It did not recommend the independent development of such data by each group that is charged with estimating NIVs. The same data may be used by diverse groups as a basis for developing NIVs that are context-specific in terms of diverse population characteristics and environmental factors that may alter estimates of specific NIVs.

Acceptable distribution ranges for fat, carbohydrate, and protein intakes have been established by some groups. These ranges are derived primarily for promoting long-term health and reducing the long-term risk of noncommunicable disease. It is not necessary to establish an ANR for total dietary carbohydrate or fat. However, it is appropriate to establish ANRs for protein to achieve appropriate nitrogen balance at various life stages, and for the specific biological functions of essential fatty acids.

There is a need to be as specific as possible regarding "targeted" diseases when nutrient-based standards are recommended for disease prevention or control. Thus, for example, when targeting cancer, the site, tissue involvement, physiological stage at onset, etc. should be stated explicitly. This level of specificity is likely to support the development of biomarkers linked directly to outcomes of interest and exploitation of growing information regarding specific nutrient–gene interactions that modify the risks of diet-related long-term diseases.

Evidence linking diet to risks of long-term diseases is more often related to specific dietary patterns than to levels of intake of specific nutrients. Thus, the group stressed the need to link committees convened to develop diet-based strategies for the promotion of long-term well-being and reduction of risk of diet-related long-term diseases, with those convened to develop NIVs.

Issues related to study design and experimental errors also should be considered explicitly by groups setting NIVs. Sample size is among the more important design characteristics in this regard. For this purpose, it is necessary to consider the width of resultant confidence intervals and to minimize the likelihood of alpha or beta errors. For example, the probability of accepting a false negative conclusion with a sample size of 100 is 0.71 if an alpha value of 0.05 is used to determine statistical significance and a clinically significant difference between values of interest is set at 50%. Many nutrition studies, however, involve samples of 15 to 25 subjects rather than 100 and have a much higher risk of underpowering comparisons of interest. Such risks need to be addressed when selecting a database for estimating nutrient requirements.

## Bioavailability

Bioavailability is an important factor to consider when estimating NIVs for selected nutrients (e.g., iron, zinc, carotenoids, vitamin A, folate, protein, calcium, and magnesium). The definition of bioavailability accepted by the group was proposed by Hurrell in 2002 [14] and modified by Gibson [5], the "proportion of the ingested nutrient absorbed and utilized through normal metabolic pathways. Bioavailability is influenced by dietary factors and host related factors." Bioefficacy is the efficiency with which ingested nutrients are absorbed

and converted to an active form [15]. Both of these terms reflect the broader concept of bioequivalence of nutrients or their precursors in defining nutritional status and function. These concepts also encompass various steps in metabolic and utilization pathways of nutrients (i.e., absorption, metabolic conversion, utilization, retention, secretion, and excretion). There are multiple factors that influence the bioequivalence of nutrients and their precursors: competition for absorptive systems; role of enhancers or inhibitors of absorption; metabolic conversion efficiency in the intestine, liver, kidney, or other tissues; and interactions between or among nutrients, chemical form, and others. Also, it is important to remember that food processing, treatment, and/or preparation practices at the household level influence nutrient bioavailability.

The roles of infection (bacterial and parasitic) and the nutritional and physiological status of the host also are of key importance in defining bioequivalence of nutrients and should be considered when the impact of infections can be described quantitatively for specific populations of interest.

The importance of considering bioequivalence is especially relevant for iron and zinc, where specific approaches have been developed based on dietary components that enhance and/or inhibit absorption. Algorithms predicting the bioavailability of iron and zinc have been developed based on the amounts of enhancers and inhibitors in the diet, the nutrient's chemical form, e.g., iron, and the nutrient status of the individual. However, the validity of these models needs to be evaluated in practice and considered in setting reference values only if quantitatively significant. Retinol, tocopherol, and folate equivalents are examples in which specific conversion values depend on the relative content of precursors, the chemical form of the nutrient, the food matrix that serves as a "delivery system," and the host's physiological and health condition. Digestibility of protein sources is the key factor affecting absorbed amino nitrogen, and amino acid composition determines protein retention and urea excretion.

Data on the efficiency of the biological conversion of carotenoids and various tocopherols into their bioactive forms have significant variability; however, the practical implications of this variability have not been elucidated completely. In many cases, food-composition data are scant, limiting the assessment of bioequivalence. Recent progress in FAO's data system to assess food availability (FAOSTAT II) represents an advance in this matter. The capacity to define the nutritional adequacy of local diets will remain very limited, unless efforts to improve information systems on food-composition data are strengthened. Efforts should be encouraged to advance progress in developing the International Food Data Systems Project (INFOODS) as a tool to improve the derivation of NIVs and related values.

## Derivation of life-stage groups, standard heights and weights, and NIV estimation by extrapolation

### Derivation of life-stage groups

NIVs are developed for specific life-stage groups [3]. There is no consensus, however, as to how to establish those groups. Three different options exist: chronologic age, use of functional characteristics (e.g., growth and puberty), or potential purposes for which NIVs might be used (e.g., complementary feeding programs). As an illustration of the last alternative, one might want to establish life-stage groups for infants and young children so that all children requiring complementary feeding are included in one group. It is likely that a combination of options most often will be used to establish life-stage groups. Growth and type of feeding may be used for infants and children, whereas chronologic age might be used for young, mature, and elderly adults. The same life-stage groups, however, should be used for all nutrients included in the NIV; it would be inappropriate and confusing to use one life-stage group for calcium and another for riboflavin, for example.

Pregnancy and lactation do not need to be divided into various stages such as trimesters of pregnancy or early and late lactation, because physiological adjustments in nutrient utilization generally compensate for shifts in nutrient requirements that occur at different stages of gestation or lactation. Furthermore, having more than one NIV for pregnancy and lactation is essentially impossible to implement; advising women to eat one diet during early pregnancy and another in late pregnancy is impractical.

## Standard heights and weights

Standard weights and heights should be established for each selected life-stage group to define the general characteristics of the population and to permit extrapolations of ANRs to other life-stage groups based on body size. For infants and children between 0 and 5 years of age, the new WHO growth standards are recommended as the basis for normalizing NIVs when adjustments based on weight are appropriate. For all other age groups, data from the National Center for Health Statistics/World Health Organization (NCHS/WHO) can be used to derive a standard weight and height [16]. The group recommended, however, that the average weight of men and women at 18 years of age be used throughout the adult years rather than reflecting the typical secular increase in body weight with age. It is uncertain whether this secular increase is consistent with good health. It is important to downwardly adjust energy NIVs when expressed per kilogram of body weight or per day for overweight

or obese individuals with body-mass indexes greater than 25. For all other nutrients, standard body weight uncorrected for overweight status is appropriate for estimating NIVs.

## Extrapolation

It is preferable to use original research for estimating nutrient requirements of various life-stage groups [3]. However, due to the paucity of data for some subgroups, it is often necessary to extrapolate information from other groups. Extrapolation should always be a second choice, and scientists are encouraged to develop innovative, noninvasive methods or to use existing methods (e.g., stable isotopes) to determine nutrient requirements of understudied groups, e.g., pregnant and lactating women, infants, children, and the elderly.

Until data are available for all life-stage groups, extrapolation from one group to another is necessary. Frequently, this involves extrapolation from adults to children and adolescents and from younger adults to older adults. The rationale or scientific basis for the method chosen should be completely transparent and thoroughly described for each nutrient and life-stage group. It is likely that different approaches will be used for different nutrients, or different extrapolations for diverse life-stage groups for a single nutrient. There is no one "correct" method for extrapolation, and thus scientific judgment is required. Examples of extrapolation methods that are used include body size (weight or metabolic weight), energy intakes for age, or factorial estimates of requirements for growth, pregnancy, and lactation. When the factorial approach is used, it is important to be completely transparent in describing the databases used to estimate components of the estimate, such as milk volume and composition during lactation, or composition of weight gain during pregnancy.

## Effects of genetic variation on nutrient intake values

The primary nucleotide sequence of the human genome varies by approximately 0.2% to 0.4% among humans [8]. Variations in a DNA sequence that are enriched in populations are referred to as polymorphisms, which constitute a primary molecular basis for human phenotypic variation. Human mutations expand in populations as a result of natural selection or through random drift. Historically, the nature and abundance of the food supply are among several environmental selective pressures that enabled the expansion of polymorphisms within human populations. Genetic variants that enable survival in challenging nutrient environments become enriched in populations through the process of natural selection. This process may confer differences in food tolerances or intolerances, could develop into metabolic disease alleles in different environmental contexts, and has the potential to alter NIVs. Because many human populations have existed for many generations in unique, isolated, and challenging nutrient environments, relatively rare gene variants that influence NIVs may be highly prevalent in isolated populations. Gene variants associated with human lactose intolerance and alcohol intolerance display genomic signatures of positive selection in specific geographic regions. These signatures indicate that these variants offered survival advantages related to an index food component itself and/or more broadly to the metabolic network key to a food component's broader role. Computational approaches are identifying numerous gene variants associated with nutrient transport and metabolism that display signatures of positive selection. To date, no gene variant has been demonstrated to affect nutritional requirements sufficiently to warrant genotype-specific recommendations, although the effect of the MTHFR A222V variants on folate requirements has been considered. Because polymorphisms can confer both health benefits and risks, depending on the outcome of interest, and these outcomes may respond differentially to nutrient intake levels, it may be important to consider the effects of genetic-specific recommendations on all known health outcomes. For example, the MTHFR A22V polymorphism confers increased risk for developmental anomalies but protection from colon cancer; the impact of individualized ANRs on both health outcomes should be considered for this genetic minority.

The impact of a gene variant on nutrient requirements will be dependent on its prevalence and penetrance. Penetrance is the probability that a gene variant will express a phenotype from a given genotype at a given time. In most cases, penetrance varies inversely with prevalence. Few gene variants are anticipated to be sufficiently penetrant to affect variation of ANRs to a greater degree than environmental factors. However, the identification of highly penetrant gene variants may require the derivation of more than one ANR or UNL for genetic subgroups. It is unlikely that gene–gene interactions will be a major consideration in the determination of NIVs because of the low prevalence associated with highly penetrant gene–gene interactions. Furthermore, because chronic diseases are polygenic complex traits, individual SNPs are unlikely to impact NIVs that target the reduction of diet-related risk of long-term disease.

Thus, the group concluded that other than that for folate, no other specific polymorphisms have been identified that should be considered in the derivation of NIVs beyond those subsumed in estimates of interindividual variation. This field is, however, progressing

very rapidly and our understanding of human genetic variation is expected to improve steadily in the near and mid-term future. Linking specific gene variants to known nutrient-sensitive ethnic or geographic populations, such as salt sensitivity in African Americans, may enable population-specific recommendations for genetic subgroups. Therefore, advances in understanding the impact of genetic variation on NIVs merit the close attention of all groups charged with their derivation.

## Methodological approaches and applications of NIVs

The term "uses" frequently has been used to refer to all of the various applications of a set of NIVs. The group felt, however, that it is important to distinguish between the terms "uses" and "applications." Common uses of NIVs are for planning diets (of groups and individuals) and assessing intakes (of groups and individuals). The group decided to refer to this set of uses as "methodological approaches [4]." "Applications," then, refers to specific ways in which methods can be applied to various tasks (e.g., setting fortification levels, developing food-based dietary guidelines) [1, 9, 10].

Theoretical approaches to using the NIV for assessment of dietary intakes for individuals requires calculating the probability of an inadequate intake using the ANR and its distribution. At any intake on the x-axis one can calculate the probability of inadequacy for an individual. For example, if the intake equals the ANR, then the probability of inadequacy for an individual is 50%. For the assessment of groups, the prevalence of inadequacy can be estimated as the percentage of the population below the ANR if certain criteria are met.*

For planning diets for individuals, one must first establish a "recommended intake" or individual nutrient level ($INL_x$, where x indicates the likelihood of meeting an individual's nutrient requirement, historically 98%). The group suggests that the $INL_x$ should be based on the ANR adjusted for the level of acceptable risk for deficiency. For example, if 2 SD of the requirement are added to the ANR, then the likelihood of meeting an individual's needs is 98%, or conversely the individual's risk of inadequacy is 2%.

When planning diets for groups, one should aim for a distribution of intakes that results in an acceptably low prevalence of inadequacy (estimated as the proportion below the ANR) and also a low prevalence of nutrient excess (estimated as the proportion above the UNL). To reduce the prevalence of inadequacy, one could either shift the entire intake distribution to a higher level, or change the shape of the intake distribution by improving the intakes of those at the lower end. Either way, the goal is to identify an intake distribution that represents an acceptable level of inadequacy, such as only 2% to 3% of the population being below the ANR. This may be achieved through education in relevant nutrition practices or by a targeted food supply (e.g., fortification of staple foods) to ensure that the intake distribution curve has only a small proportion of the population below the ANR or above the UNL. For most groups, it is not appropriate to use the $INL_x$ as the target for the group's mean intake. Due to significant interindividual differences in high variance individuals in a group, targeting mean group intakes at an $INL_x$ usually results in a high prevalence of inadequacy (as much as 25% to 30%, for some nutrients, even when $INL_{98}$ is targeted) because of commonly high levels of interindividual differences in nutrient intakes. For this reason, intake distributions should be examined, not just group mean intakes.

In summary, NIVs should form the basis of planning and assessment of diets, and this requires at a minimum an ANR and a UNL. The $INL_x$ is derived from the ANR by adding a factor to cover a specified percentage of the population (x). The specific application of the INL will drive the x factor that is applied [6]. Graphs and charts illustrate the relationship among the ANR, UNL, and $INL_x$ and the appropriate use of these NIVs for nutrient assessment and dietary planning purposes**.** Groups charged with developing NIVs may choose to include values for $INL_x$ in basic tables, but this latter value is derived basically from the ANR and its distribution.

## Trade and regulatory issues

The group agreed that issues related to international and domestic trade, and the important roles played by the Codex Alimentarius Commission and the World Trade Organization (WTO), should be considered when developing harmonized processes and approaches for deriving NIVs [9]. Also, it is important that scientific advice regarding nutrient requirements and their applications be made available to specific groups of the Codex Alimentarius Commission, such as the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) and the Codex Committee on Food Labeling (CCFL). Information on developing dietary guidelines for health and consumer protection also should be provided to these specific groups. It is crucial to understand the important role

*Among these criteria is that requirements must have a reasonably normal distribution; thus, the estimated average requirement (EAR) cutpoint method that is the basis for estimating the prevalence of nutrient adequacy or inadequacy in a targeted population cannot be used for assessing iron intakes of menstruating women, because the distribution of iron requirements for this group is highly skewed to the left.

that the Codex Alimentarius Commission plays in setting food standards and guidelines for protecting consumer health and ensuring fair practices in domestic and international trade.

Food labeling is an important component of trade and regulatory nutrition issues. Harmonizing label procedures also will improve trade opportunities within regions and worldwide. The process of developing food labels can be harmonized among regional, national, and international groups. To establish food labels, some have used the $INL_x$ weighted by the distribution of the various life-stage groups in populations as a basis for food labels. Others have used the highest nutrient level recommended for individuals in a population.

Food fortification is another application of NIVs relevant to trade and regulatory issues. Food fortification may be mandatory or voluntary. Fortification programs should be designed so that the prevalence of intakes of target nutrients that are below the ANR or above the UNL is low [17]. This will ensure that very few individuals have either inadequate or excessive intakes of targeted nutrients.

## Application of NIVs to dietary guidelines

Explicit food-based dietary guidelines (FBDGs), or similar recommendations, have been developed by many countries [10]. In some countries, such as the United States and Canada, FBDGs are the basis for national nutrition education activities and food assistance programs. FBDGs generally provide a comprehensive set of guidelines that are intended to reduce long-term disease risk and improve general health. In addition to specific guidelines regarding the intake of fruits, vegetables, whole grains, and dairy foods, statements often are included regarding physical activity, food safety, and the types of carbohydrate and fat for reducing long-term disease risk. Thus, FBDGs serve as the basis for healthy lifestyles. In most countries, resources for disseminating information embodied in FBDGs are inadequate and their use and implementation by the general public are limited. Thus, not surprisingly, direct evidence that dietary guidelines are an effective means to improve the overall health of a population is lacking in practically all countries.

It is not possible to harmonize food-based dietary guidelines across countries, cultures, and regions, because these guidelines stem from social influences on food patterns, culturally sensitive issues regarding food within a country or subpopulation, and nutrition and health problems of a specific population. However, *methods* for developing dietary guidelines can be harmonized around the world. As is the case for food labeling and fortification programs, NIVs form the basis for FBDGs. A harmonized method for developing FBDGs based on ANR, $INL_x$, and UNL values is described by Vorster et al. [1].

## Summary

The conceptual framework for the various NIVs is depicted in **figure 1** along with the methodological approaches and applications. The NIVs consist of two values derived from a statistical evaluation of data on nutrient requirements, the average nutrient requirement (ANR), or nutrient toxicities, the upper nutrient level (UNL). The individual nutrient $level_x$ ($INL_x$) is derived from the distribution of average nutrient requirements. The percentile chosen is often 98%, which is equivalent to 2 SD above the mean requirement. Concepts underlying the NIVs include criteria for establishing a nutrient requirement, e.g., ferritin stores, nitrogen balance, or serum vitamin C. Once the requirement for the absorbed nutrient is determined, it may be necessary to adjust the value for food sources, i.e., bioavailability, or host factors, such as the effect of infection on nutrient utilization. Other concepts that committees may want to consider when establishing NIVs include the effects of genetic variation on nutrient requirements and the role of the nutrient in preventing long-term disease.

Two fundamental uses of NIVs are for assessing the adequacy of nutrient intakes and for planning diets for individuals and populations. Establishing the NIV using the statistical framework proposed in this report improves the efficacy of the values for identifying risks of nutrient deficiency or excess among individuals and populations. NIVs also are applied to a number of aspects of food and nutrition policy. Some examples include regulatory issues and trade, labeling, planning programs for alleviating public health nutrition problems, food fortification, and dietary guidance.

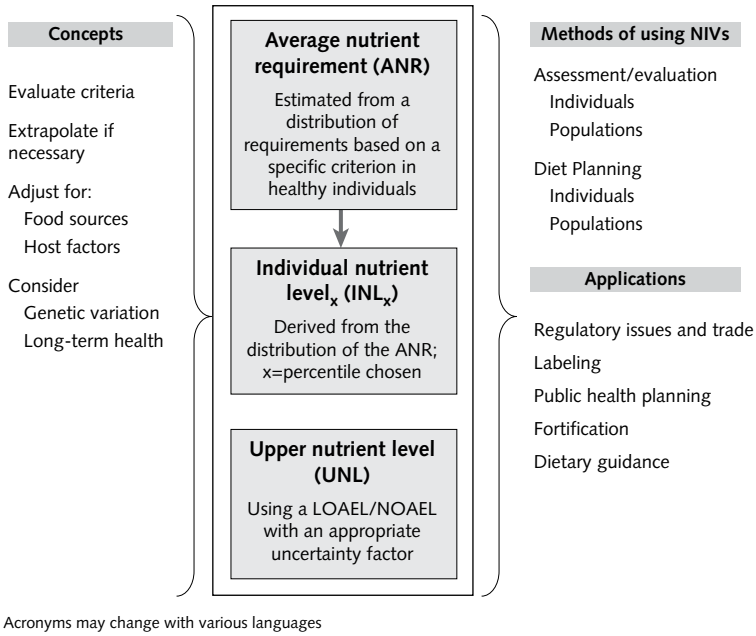


FIG. 1. The two nutrient intake values (NIVs) are the average nutrient requirement (ANR) and the upper nutrient level (UNL). Other NIVs may be derived from these two values, i.e., the individual nutrient level$_x$ (INL$_x$), which is the ANR plus some percentile of the mean used for guiding individual intakes. The ANR and UNL are derived from estimates of amounts needed for a specific physiological criterion, e.g., tissue stores, metabolic balance, or a biochemical function. The NIVs are modified for population differences in the food supply, host factors such as infection, genetic variations, and needs for sustaining long-term health. The methods of using NIVs to assess/evaluate intakes of individuals and populations differ from that used for planning diets for individuals and populations. NIVs are the basis for a number of policy applications. Examples include food labeling and fortification, food-based dietary guidance, planning public health nutrition programs, and establishing food regulatory policies.

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# Introduction

Janet C. King and Cutberto Garza

The important roles of wholesome food supplies lead national governments or their designated agents to name expert groups periodically to derive and promulgate nutrient-based dietary standards, e.g., estimated average requirements, recommended intakes for individuals, and upper tolerable intake levels. Discrepancies often arise among diverse national efforts, in part because there is no global consensus regarding concepts and approaches for their derivation. These discrepancies create problems for health, trade, and other national authorities responsible for those sectors.

The lack of a global consensus on the most appropriate concepts and approaches for the determination of national standards makes it difficult to resolve differences that arise in setting national and international nutrition standards and public and clinical health objectives, designing national and international food policies, and enhancing the transparency of national standards to trade and other regulatory and normative activities with economic, health, and safety implications. Resolution of these differences is most problematic for developing countries that often have to sift through disparate recommendations without the needed infrastructures to make decisions.

## Project objective

To address these discrepancies in dietary standards worldwide that lead to international discrepancies in health, food policies, and trade, a working group was convened *to harmonize concepts and approaches* (as opposed to deriving specific recommendations) for developing nutrient-based dietary standards. A major outcome of this effort is an improvement in the transparency of methods used to derive nutrient-based dietary standards and how to apply them to various functions.

## Approach

The United Nations University (UNU) Food and Nutrition Programme in collaboration with the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) Nutrition Department for Health and Development commissioned 10 papers from leaders in establishing and applying nutrient intake recommendations. Each paper focused on distinct aspects of the process for developing harmonized nutrient-based dietary standards. A brief description of the papers follows.

King et al. [1] review the terminology used by various countries and regions for defining dietary standards. A general framework for establishing nutrient intake values is proposed and the rationale for the proposed framework is discussed. Aggett [2] reviews the approaches for identifying upper nutrient levels and proposes a framework for defining upper nutrient levels.

Yates [3] reviews the possible approaches for identifying physiological criteria for establishing dietary standards (i.e., determining what physiological functions requirements will satisfy). Important components of this paper are how to estimate the numbers of subjects needed to estimate function-specific nutrient requirements and interindividual variation, how to identify the basis for that variation, and the assessment of approaches for identifying the physiological states or ages for which data are required.

Murphy and Vorster [4] review the specific methologic approaches to plan and assess intakes for individuals and populations. The advantages of basing dietary

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assessments and plans on the NIV are discussed.

Atkinson and Koletzko [5] review the bases for extrapolation and interpolation among and between age groups, environments, and physiological states for which insufficient data are available.

Gibson [6] reviews the biological factors that influence recommended intakes of specific nutrients (e.g., composition of usual diets, bioavailability, biological value, interindividual variability, nutrient–nutrient interactions, etc.).

Stover [7] reviews the implications of expanding understanding of the human genome and the technological capabilities that have made that understanding possible. Special attention is focused on the role of population-wide versus individual recommendations and on the likely magnitude of inter- and intrapopulation genetically based differences that relate to nutrient requirements.

Vorster et al. [8] review the diverse applications of nutrient intake values (NIVs) for dietary assessment and planning. Examples of how to use NIV for food labeling, food fortification, and food-based dietary guidelines are provided.

Ramaswamy and Viswanathan [9] review regulatory and trade issues of importance to the harmonization of approaches for setting nutrient-based dietary standards and, ultimately, quantitative estimates of standards.

Smitasiri and Uauy [10] review principles and approaches for the translation of nutrient-based dietary standards to food-based guidelines, with special care being taken to address the multiple uses that food-based guidelines have served (e.g., consumer education and feeding programs).

Following an initial review and modification of the papers, the authors and staff from the UNU, FAO, WHO, and UNICEF met at the UNICEF Innocenti Center in Florence, Italy, in December 2005, to discuss the papers and develop the final report on harmonizing dietary standards. Following the December meeting, the authors revised their reports based on discussion and decisions regarding the framework, criteria, uses, and applications of dietary standards. The papers included in this supplement to the *Food and Nutrition Bulletin* are the final product of this process. An Executive Summary [11] is also included in the report that outlines the discussion and decisions made by the group.

## Members of the Working Group on International Harmonization of Approaches for Developing Nutrient-Based Dietary Standards

The members of the working group are Professor Peter J. Aggett, Head of School, Lancashire Postgraduate School of Medicine and Health, University of Central Lancashire, UK; Lindsay Allen, Director, Western Human Nutrition Research Center, University of California, Davis, USA; Stephanie A. Atkinson, Professor, Department of Pediatrics, McMaster University, Ontario, Canada; Cutberto Garza, Boston College, Boston, Massachusetts, USA; Rosalind S. Gibson, Department of Human Nutrition, University of Otago, New Zealand; Janet C. King, Children's Hospital Oakland Research Institute and the University of California at Berkeley and Davis, California, USA; Berthold Koletzko, Division of Metabolic Diseases and Nutritional Medicine, Ludwig-Maximilians-University of Munich, Germany; Suzanne P. Murphy, University of Hawaii, Cancer Research Center of Hawaii, Honolulu, USA; Professor Pirjo Pietinen, National Public Health Institute, Nutrition Unit, Helsinki; Suttilak Smitasiri, Head, Division of Communication and Behavioral Science, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand; Sunder Ramaswamy, Frederick C. Dirks Professor of Economics, Middlebury College, Middlebury, Vermont, USA; Prakash Shetty, Kasturba Medical College, Karnataka, India; Patrick Stover, Cornell University, Ithaca, New York, USA; Professor Daniel Tome, Institut National Agronomique Paris-Grignon, Paris; Ricardo Uauy, INTA University of Chile, Santiago, Chile; Brinda Viswanathan, Institute for Social and Economic Change (ISEC), Nagarbhavi, Bangalore, India; Hester H. Vorster, Faculty of Health Sciences, North-West University Potchefstroom Campus, Potchefstroom, South Africa; and Allison A. Yates, Director, Beltsville Human Nutrition Center, Beltsville, Maryland, USA.

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# Nutrient intake values (NIVs): A recommended terminology and framework for the derivation of values

Janet C. King, Hester H. Vorster, and Daniel G. Tome

## Abstract

*Although most countries and regions around the world set recommended nutrient intake values for their populations, there is no standardized terminology or framework for establishing these standards. Different terms used for various components of a set of dietary standards are described in this paper and a common set of terminology is proposed. The recommended terminology suggests that the set of values be called nutrient intake values (NIVs) and that the set be composed of three different values. The average nutrient requirement (ANR) reflects the median requirement for a nutrient in a specific population. The individual nutrient level ($INL_x$) is the recommended level of nutrient intake for all healthy people in the population, which is set at a certain level x above the mean requirement. For example, a value set at 2 standard deviations above the mean requirement would cover the needs of 98% of the population and would be $INL_{98}$. The third component of the NIVs is an upper nutrient level (UNL), which is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in a specified life-stage group. The proposed framework for deriving a set of NIVs is based on a statistical approach for determining the midpoint of a distribution of requirements for a set of nutrients in a population (the ANR), the standard deviation of the requirements, and an individual nutrient level that assures health at some point above the mean, e.g., 2 standard deviations. Ideally, a second set of distributions of risk of excessive intakes is used as the basis for a UNL.*

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**Key words:** Nutrient requirements, nutrient recommendations, recommended dietary allowances, recommended nutrient intakes

## Introduction

Most countries or regions around the world recommend nutrient intake values for their populations. These recommendations serve as a basis for national or regional nutrition policies, nutritional educational programs, and food regulations and action programs. Traditionally, these standards have been set at a level that would cover the requirements of practically all healthy persons in the population. As new knowledge of human nutrient requirements becomes available, the standards need to undergo a reassessment and revision as appropriate. Most countries or regions review the status of knowledge and update their nutrient intake values about every 5 to 10 years.

The amounts of nutrient intakes recommended vary considerably from country to country. Also, the terms used to describe the intake values differ. For example, some countries recommend a single value that serves as a recommended intake for all members of a population subgroup, whereas other countries recommend four different values: a lower reference intake, an average requirement, a recommended intake for nearly all members of a population group, and an upper tolerable level or limit. There is no standardized, commonly agreed-upon terminology for these terms. Furthermore, there is no standard method or approach for deriving these different nutrient intake values.

In this paper, we will review the different terms and definitions used to describe a set of dietary recommendations from different countries and regions around the world. We will then propose a set of terms and definitions for harmonizing nutrient intake values worldwide. Finally, we will suggest a theoretical framework for deriving specific nutrient intake values.

Throughout this paper, a set of dietary intake recommendations derived from primary research data is

referred to as nutrient intake values (NIVs). The term *nutrient* was chosen instead of *dietary* to denote that these values are for the intakes of specific nutrients, not food components, such as cereals, fruits, vegetables, etc. Also, the term *value* was selected instead of *reference* to reflect the wide range of uses and applications of these standards. In addition to being a set of standards for assessing the adequacy of intakes of a population, they also serve as important values for setting nutrition policies that influence agricultural, economic, and legislative decisions of a country or region. Thus, they serve as values for assessing nutrient status as well as making policy decisions.

## Terminology: expressions and definitions of nutrient intake values

In recent years, the number of terms used to describe a set of nutrient intake values has increased from a single recommended intake to multiple recommendations spanning a range of nutrient requirements from inadequate to excessive. This expansion is due, in part, to the increased uses and applications of nutrient intake values [1, 2]. Definitions of the various terms used to describe the nutrient intake values defined by representative countries and regions follow; they are also summarized in **table 1**.

### US and Canadian terminology

#### *DRI (dietary reference intake)*

This term was developed by a joint US-Canadian Committee charged with establishing reference values for planning and assessing diets of healthy populations as well as serving as a basis for nutrition policies. The DRIs refer to the complete set of reference intakes, including the RDA (recommended dietary allowance), AI (adequate intake), UL (tolerable upper intake level), and EAR (estimated average requirement) [3–7]. DRIs are expressed as intakes per day but are meant to represent average intakes of individuals over time. It is thought that the nutrient intake can vary substantially from day to day without ill effects [2, 3]. Each DRI expression (RDA, AI, UL, and EAR) has specific uses for planning and assessing diets or for applications to nutrition policy and education.

#### *RDA (recommended dietary allowance)*

The RDA is the original term introduced by the US Food and Nutrition Board of the National Research Council in the 1940s [8]. It was defined as the level of

TABLE 1. Comparison of the suggested "harmonized" terminology with terms in use at present around the world

| Recommendation | Harmonized terms | USA/Canada | UK | European Communities | Mexico | WHO/FAO |
|---|---|---|---|---|---|---|
| Umbrella term for the set of recommendations | NIV | DRI | DRV | | VNR | |
| Average requirement | ANR | EAR | EAR | AR | RN | |
| Recommended intake level | $INL_X$ | RDA | RNI | PRI | IDR | RNI |
| Lower reference intake | | | LRNI | LTI | | |
| Safe intake | | AI | Lower end of safe intake range | Lower end of safe intake range | IDS | |
| Upper level of safe intake | UNL | UL | Upper end of safe intake range | Upper end of safe intake range | LSC | UL |
| Appropriate macronutrient distribution range | | | AMDR | Minimum and maximum population ranges | | Population mean intake goals |

AI, adequate intake; AMDR, adequate macronutrient distribution range; ANR, average nutrient requirement; AR, average requirement; DRI, dietary reference intake; DRV, dietary reference value; EAR, estimated average requirement; IDR, ingestión diaria recomendada; IDS, ingestión diaria sugerida; $INL_X$, individual nutrient level, x = percentile chosen; LRNI, lower reference nutrient intake; LSC, límite superior de consumo; LTI, lowest threshold intake; NIV, nutrient intake value; PRI, population reference intake; RDA, recommended dietary allowance; RN, promedio de los requerimientos nutrimentales; RNI, reference nutrient intake; UL, upper tolerable nutrient intake level; UNL, upper nutrient level; VNR, valores nutrimentales de referencia

intake of an essential nutrient that, on the basis of scientific knowledge, is judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy people. The RDA continues to be used as one of the nutrient intake values included in the US/Canadian DRIs. The RDA is based on a statistical distribution of individual requirements for meeting a chosen criterion of adequacy for the target nutrient, such as calcium, vitamin A, or protein. Based on the statistical distribution of requirements, the RDA is set at a level of intake that meets the needs of 97% to 98% of healthy individuals in a particular age- and sex-specific group.

#### *EAR (estimated average requirement)*

Using the same statistical distributions for nutrient requirements, the EAR is the average or mean daily nutrient intake that meets the requirement of half the healthy individuals in a particular age- and sex-specific group.

#### *AI (adequate intake)*

The AI is defined as the observed or experimentally derived intake by a defined population group that appears to sustain health [3–7]. An AI is used when there are insufficient primary data to establish a statistical distribution of individual requirements and, therefore, an EAR and RDA. The AI is estimated in a number of different ways. For some nutrients, it is based on the observed mean intakes of groups with an apparent low prevalence of nutrient inadequacy. For example, an AI is used to represent the nutrient intake values for infants because they were derived from the nutrients supplied by human milk. The AI has also been derived from the results of experimental studies when data were thought to be inadequate to describe a statistical distribution of requirements for a specific function or criterion. Examples include calcium, vitamin D, fluoride, and sodium.

#### *AMDR (adequate macronutrient distribution range)*

The AMDR specifies the upper and lower boundaries for percentage of energy from macronutrients (i.e., carbohydrate, fat, *n*-6 and *n*-3 polyunsaturated fatty acids, and protein) [6]. These boundaries are generally based on intakes associated with reducing the risk of chronic disease.

#### *UL (tolerable upper intake level)*

The UL is defined as the highest level of daily nutrient intake likely to pose no risk of adverse health effects for nearly all individuals in the group (general population) [9]. The level is also estimated from a statistical analysis of the risk assessment associated with a range of high nutrient intakes. The term *tolerable intake* was chosen to avoid implying a possible beneficial effect [9].

### British terminology

#### *DRV (dietary reference value)*

Dietary reference values (DRVs) are nutrient-based dietary standards recommended by the United Kingdom in 1991 [10, 11]. The DRVs apply to groups of healthy people and are not appropriate for those with disease or metabolic abnormalities. As for US/Canadian DRIs, the DRVs for a nutrient assume that requirements for energy and all other nutrients are met when deriving a specific reference value. The British DRVs provide three values for most nutrients: the lower reference nutrient intake (LRNI), the estimated average requirement (EAR), and the reference nutrient intake (RNI). For some nutrients, a "safe intake" is given, and for carbohydrate and fat, individual minimum, maximum, and population averages are specified [10].

#### *LRNI (lower reference nutrient intake)*

Using a statistical distribution of nutrient requirements, the LRNI is set at 2 standard deviations (SD) below the mean or average intake (EAR).

#### *EAR (estimated average requirement)*

The British EAR is the intake that meets the estimated nutrient needs of half of the individuals in a group, assuming a normal, statistical distribution of requirements.

#### *RNI (reference nutrient intake)*

This term is set at 2 SD of the requirement above the EAR and will meet the needs of 97% to 98% of the population; it is similar to the US/Canadian RDA.

#### *Safe intake*

A safe intake is specified for a nutrient for which there is insufficient data to determine a statistical distribution of requirements. The safe intake is judged to be *a level* or *range of intakes* at which there is no risk of deficiency and below a level where there is a risk of undesirable effects. The safe intake corresponds conceptually to the US/Canadian AI.

#### *Individual minimum, maximum, and population averages*

These terms are used by the British to specify recommended intakes of carbohydrate and fat [9, 10].

### European Communities terminology

#### *PRI (population reference intake)*

This term was introduced by the Commission of the European Communities in 1993 [12] to refer to an intake acceptable for a defined age- and sex-specific group. Like the US/Canadian RDA and the British RNI, it is based on a statistical distribution of requirements for a nutrient and is set at 2 SD above the mean requirement.

***LTI (lowest threshold intake)***

This European Communities term is similar to the British LRNI and describes the mean intake minus 2 SD. Intakes below this level are assumed to be inadequate for nearly all of the individuals in a group.

***AR (average requirement)***

This term reflects the average requirement, i.e., the estimated nutrient need of half the individuals in a group; it is equivalent to the US/Canadian and British EAR.

***Acceptable range***

A range of safe intake values is given where insufficient information is available. The acceptable range is similar to the British safe intake, and the lower end of the range is similar to the US/Canadian AI. The upper end of the range is conceptually similar to the UL for the US/Canada.

### Dietary reference intakes for Koreans (KDRIs)

Korea released a set of DRIs in 2005 [13]. Four values were proposed that are very similar to those used by the US/Canada: an estimated average requirement (EAR), recommended intake (RI), adequate intake (AI), and tolerable upper intake level (UL). They also established an AMDR (acceptable macronutrient distribution range) for the distribution of energy among the macronutrients.

### Southeast Asia RDAs

The region of Southeast Asia, including Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam, released a set of recommended dietary allowances (RDAs) for their region in 2005 [14]. One nutrient intake value, an RDA, was recommended for each nutrient. In view of the limited studies done in most Southeast Asian countries, the Recommended Dietary Allowance Committee drew heavily from FAO/WHO/UNU Expert Panel reports on nutrient requirements and the US/Canadian reports on dietary reference intakes.

### German language societies

The "reference values for nutrient intakes" (Referenzwerte für die Nährstoffzufuhr) have been established jointly by the Nutrition Societies of Germany, Austria, and Switzerland and adopted also for Slovenia [15]. Provided are **average requirements** (*Durchschnittlicher Bedarf*) for energy intake; **reference intakes** (*Referenzwerte*) for most essential nutrients (protein, *n*-6 fatty acids, and most vitamins, minerals, and trace elements), based on mean intakes plus assumed 2 SD of population variation, or in the absence of adequate information on standard deviation, based on mean intakes plus 20% to 30%; **estimated values** (*Schätzwerte*) for nutrients where human requirements cannot be calculated with the desired accuracy, such as *n*-3 fatty acids, vitamin E, vitamin K, β-carotene, biotin, pantothenic acid, and some trace elements; and **orientation values** (*Richtwerte*) for nutrients where for health reasons a certain range of intakes is desirable for some substances, even though strict borderlines cannot be drawn. Lower orientation values are provided for water, fluoride, and dietary fiber and upper orientation values for total fat, cholesterol, alcohol, and salt.

### Australia and New Zealand

Australia and New Zealand [16] recently defined a series of nutrient reference values (NRVs) broadly following the approach outlined in the US/Canadian publications on dietary reference intakes and applications in dietary assessment [3–7, 17]. thus, the terms estimated average requirement (EAR), recommended dietary intake (RDI, which is equivalent to the US/Canadian RDA), adequate intake (AI), and upper level of intake (UL) are used in the same way as defined in the US/Canadian report. To address the issue of chronic disease prevention, two additional sets of reference values were developed for selected nutrients for which sufficient evidence existed. The acceptable macronutrient distribution range (AMDR) was made as an estimate of the range of intakes for each macronutrient for individuals (expressed as percentage contribution to energy), which would allow for an adequate intake of all the other nutrients while maximizing general health outcome. The suggested dietary target (SDT) is the daily average intake from food and beverages for certain nutrients that may help in the prevention of chronic disease. These two additional sets of reference values apply only to adults and adolescents over 14 years of age.

### Mexico

Mexico released a new edition of values for reference nutrients (*valores nutrimentales de referencia* [VNR]) in 2005 [18]. Four standards were recommended. The RN50 (*promedio de los requerimientos nutrimentales*) is the mean nutritional requirements of a population; this is equivalent to the estimated average requirement used by the US/Canadian report. The daily recommended intake (*ingestión diaria recomendada* [IDR]) is the value obtained by adding 2 SD to the mean of the requirements in order to cover the needs of 97.5% of the individuals in the population. If the standard deviation is unknown, the RN50 is multiplied by 1.2, assuming a coefficient of variation (standard deviation divided by the mean and multiplied by 100 to give a percentage) of 10%. The daily suggested intake (*ingestión diaria sugerida* [IDS]) is used in place of the IDR

when information about requirements is insufficient to determine an RN; and the upper limit of consumption (*límite superior de consumo* [LSC]) establishes the daily intake that one should not surpass and that has, therefore, a precautionary sense.

### WHO and FAO terminology

The World Health Organization/Food and Agriculture Organization (WHO/FAO), sometimes together with the United Nations University (UNU), has published a number of recommendations (reference values) for different groups of nutrients over time [19–24]. The most recent report on vitamin and mineral standards, published in 2004 [23], uses the terms described below.

#### *RNI (recommended nutrient intake)*

The RNI is the daily intake that meets the nutrient requirements of almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group. The most recent RNIs (for 6 minerals and 11 vitamins) are based on nutrient-specific criteria. A statistical distribution of requirements is derived from primary data, and the RNI equals the mean requirement plus 2 SD. It is equivalent, therefore, to the US/Canadian RDA, the British RNI, and the European PRI. Insufficient data were available to establish an RNI for vitamins E and A. An acceptable intake that supports the known function of vitamin E was determined and used as the best estimate of requirements. A recommended safe intake level was specified for vitamin A as the level of intake that prevents clinical signs of deficiency and allows normal growth, but it does not protect vitamin A status during prolonged periods of infection or other stresses.

#### *UL (upper tolerable nutrient intake level)*

ULs were defined for some nutrients. The definition is similar to that used for the US/Canadian UL: the maximum intake from food that is unlikely to pose a risk of adverse health effects in almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group.

#### *Basal vs. normative requirements*

Two earlier publications from the UN agencies distinguished between basal and normative nutrient requirements [19, 24]. The basal requirement was defined as the mean requirement to prevent clinically detectable signs of impaired function attributable to inadequacy of the nutrient; the normative requirement was the mean requirement to maintain a level of tissue storage that is judged to be desirable.

#### *Population-level requirements*

Population-level intake recommendations were established in the WHO/FAO Trace Element Report in 1996 [24]. Population-level intake recommendations establish a safe range of intakes for a population by considering both the variability of intakes and the variability of requirements within a defined group of similar individuals, e.g., girls between 7 and 9 years of age. The upper limit of safe ranges of population mean intakes was set so that only 2% to 3% of individuals would have intakes above the average threshold of toxicity for a nutrient, whereas the lower limit of safe ranges of population mean intakes was set so that only 2% to 3% of individuals would have usual intakes below the average requirement. More recent WHO/FAO reports do not specify population intake ranges. This is because these standards require knowledge of the variance of intakes, which usually differs markedly across populations, even within a country. Furthermore, the magnitude of these variances is often not known.

WHO recently set population goals for macronutrient intakes as a percentage of energy intakes [22]. Although the values are similar to those specified by US/Canadian adequate macronutrient distribution ranges (AMDRs), they are interpreted differently [22]. The US/Canadian standards refer to *adequate ranges* for usual intakes of individuals, whereas the WHO standards refer to *mean intake goals* for populations or large groups. For example, the WHO *mean* intake goal for total fat intake is 15% to 30% of energy, whereas the US/Canadian AMDR for adults is 20% to 35% of energy intake. The AMDR recommends that *most individuals* should have fat intakes above 20% of energy; an acceptable mean intake, therefore, would be substantially above 20% of energy from fat. The WHO standard states that a healthy population could have a *mean* fat intake as low as 15% of energy intake, implying that it is acceptable for half of the population to have intakes below 15% of energy from fat.

## Summary of current terminology of dietary standards

All countries or regions establish recommended nutrient intake values using a statistical distribution of individual nutrient requirements derived from primary research data. Using those data, most establish an estimated average requirement and a recommended nutrient intake value that is usually set at 2 SD above the average requirement. Although defined summarily, these two values are given several different names (**table 1**). The average requirement is referred to as the estimated average requirement (EAR) or average reference intake (ARI). The recommended intake level is called the recommended dietary allowance (RDA), reference nutrient intake (RNI), population reference intake (PRI), or recommended nutrient intake (RNI). The terminology used for the recommended intake value is confusing, since the letter R is used to refer to

both "recommended" and "reference."

In addition to an average and recommended (or reference) nutrient intake value, recommendations include a lower reference intake that defines a value that is below the requirements of most individuals in the population, an upper intake value that is the highest intake unlikely to pose any health risks, a safe intake value (or range) or adequate intake that is used when the statistical distribution of requirements is unknown, and a range of intakes for macronutrients (adequate macronutrient distribution range [AMDR]) associated with a reduced risk of chronic disease.

## Proposed terminology for nutrient intake values (NIVs)

To avoid confusion about the meaning of acronyms and the use of multiple acronyms for the same nutrient intake standard, we propose that a common set of terms and definitions be used by all countries and regions establishing NIVs. We recognize, however, that the absolute values will probably differ among the various countries and regions, because differences in body size, the bioavailability of food sources, and environmental conditions that affect nutrient needs (e.g., sunshine exposure or altitude) will influence the statistical distribution of nutrient requirements in a particular population. Nevertheless, we believe that the same terms and definitions can be used worldwide. The following terms are recommended.

### Average nutrient requirement (ANR)

The ANR is the average or median requirement estimated from a statistical distribution of requirements for a specific criterion and for a particular age- and sex-specific group.

### Individual nutrient level$_x$ (INL$_x$)

The INL$_x$ is the recommended nutrient level for all healthy individuals in a specific subpopulation. Often committees add 2 SD to ANR, which will cover the needs of most (i.e., 98%) of the population, assuming that the distribution is symmetrical. In that case, the INL$_x$ would be INL$_{98}$. But one might choose a lower level in the distribution of requirements for the recommended intake if the current nutrient policies of the country or region conclude that it is not practical to achieve an intake equal to the 98th percentile for the entire population. This might be the case, for example, if the additional cost of providing fish rich in omega-3 fatty acids is inconsistent with the food supply for a country. Then one might choose to make a recommendation at a lower level, such as 1.5 SD above the ANR, or at the 93rd percentile. In that case, the INL$_{93}$ would meet the needs of 93% of the population. Thus, the INL$_x$ for a specific country or region should be consistent with the overall nutrition policy.

### Upper nutrient level (UNL)

The UNL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in a specified life-stage group. Ideally, it is based on an analysis of the statistical distribution of risk for high nutrient intakes. The UNL is usually set at a level where the risk of excessive intakes is close to zero. Also, the UNL is a level of intake that should be avoided on a chronic basis [9].

## General framework for estimating nutrient intake values (NIVs)

Starting with the report by the United Kingdom recommending a multiple reference framework for quantitative nutrient standards [10] and the subsequent reports of the US/Canadian dietary reference intakes (DRIs) [3–7], a new statistical approach was used for making dietary recommendations. The general features of this model were adopted by a number of other countries or regions, such as the Germanic language nutrition societies [15], South Korea [13], Southeast Asia [14], and Australia and New Zealand [16], as well as WHO/FAO [23]. The framework for assessing intakes of population groups stems from the work of a subcommittee of the US Food and Nutrition Board [25] that related the probability of inadequacy to nutrient requirements. The framework has since been expanded to include both assessment and planning of nutrient intakes and can now be applied to both individuals and groups [17, 26].

### Determining the average nutrient requirement

The first step in estimating nutrient requirements is to determine the average nutrient requirement (ANR) for a defined population from primary research data (**fig. 1**). Once the distribution of requirements is known, the ANR can be estimated. Often the ANR for a population is estimated from experimental data derived from a subsample of individuals in the defined group, because in practice, rarely, if ever, are the specific nutrient requirements known for each person in a group of interest. However, it is usually not possible to mirror completely the diversity of the group. For most nutrients, data on individual responses to varying levels of nutrient intake are scant, resulting in the need to make many assumptions about the attributes of the population group from data obtained from a small subsample that has been studied in depth. With each assumption, there is potential error and a decrease in

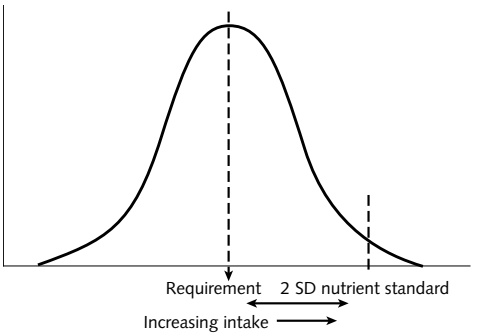


FIG. 1. Frequency distribution of individual requirements

the level of confidence or reliability in the resulting reference standard. This is perhaps one of the main reasons why previous nutrient reference values were frequently developed by determining the minimum amount of a nutrient needed by all who were evaluated or sampled, and then adding a safety factor to create an "allowance" that should meet everyone's need [27]. However, if an allowance or reference standard is derived from the addition of safety factors, its use is limited to being a goal for an individual. It cannot be used to predict the adequacy of the diet for a group or to plan what a group's intake should be. The statistical approach described here permits the application of the nutrient intake value for dietary assessment and planning [1, 2, 17, 26].

The estimated requirements will obviously vary with the criterion chosen. For example, an ANR that prevents the clinical symptoms of a nutrient deficiency will be much lower than one that sustains nutrient stores or reserves. The process of selecting a specific criterion is one of the more difficult tasks for a committee charged with establishing NIVs since the criterion chosen can lead to high standards and, therefore, a higher prevalence of inadequate intakes, or vice versa. Different methods for deriving human nutrient requirements and a description of the criterion are given by Yates [28].

There are two sources of variation that affect nutrient requirements: the level of intake within the individual and variation among the individuals studied. Within an individual, nutrient needs at the cellular level vary depending on environmental differences, immune status, energy expenditure level, and type of activity; thus, one day is not necessarily like the next in the actual demand for and use of a nutrient. This difference in day-to-day need must be considered when estimating an ANR. Therefore, the condition under which the population is studied is important. If the ANR obtained is not close to the actual median requirement, then the estimate of the percentage of individuals within a group that have inadequate diets will be less precise, and one could over- or underestimate the level of inadequacy in the population.

Often committees charged with establishing dietary standards do not have information on the average nutrient requirements for all possible different populations. This is especially true for infants, children, pregnant and lactating women, and older individuals. When experimental data are missing, nutrient requirements and their variability are generally extrapolated and interpolated among and between age- and sex-specific groups and physiological states from other population data [29].

### Estimating variation in requirements

The second step in estimating nutrient requirements using the statistical framework is to estimate the variation in requirements. A normal distribution of requirements is an underlying assumption of the theoretical model. This is not always the case, however. It is known, for example, that menstrual iron losses are not normally distributed in women. If the data are not normally distributed, they can be transformed to normality, an ANR and $INL_x$ can be calculated, and then these numbers can be transformed back into the original units [5]. If normality cannot be assumed for all components of a requirement (e.g., intestinal, urinary, and integumental losses), then a Monte Carlo simulation may be used to sum up all of the components. This approach involves simulating a large population of individuals (e.g., 100,000), each with his or her own requirement for a particular nutrient. To accomplish this, the component parts of nutrient needs (i.e., the factorial losses) are treated as coming from independent random distributions. The total requirement is then calculated for each individual, and the 50th and individual nutrient level percentiles (e.g., the 98th percentile) are calculated directly.

Although the estimated *mean* or *median* of requirements (the ANR) that is obtained from a small sample of individuals in the group may be close to the actual mean of that group, the *variation* in requirement among individuals in the sample may not be nearly as close to the true variation in the population without a significant increase in the number of observations (individuals sampled). Nutrient needs vary between individuals: individuals of the same body size performing the same types of activity for similar periods may utilize and need different amounts of a nutrient, based on their rate of metabolism, organ function, and a host of other aspects that affect requirements (**fig. 2**). This is one of the reasons that in studies in which nutrient requirements are evaluated at various levels of intake, the subjects usually serve as their own controls, dampening some of the effects of individual responses.

The disparity between the true variation in requirements in a population and what is measured or estimated among individuals in a sample can be due to lack of randomness in selecting the sample (i.e., a

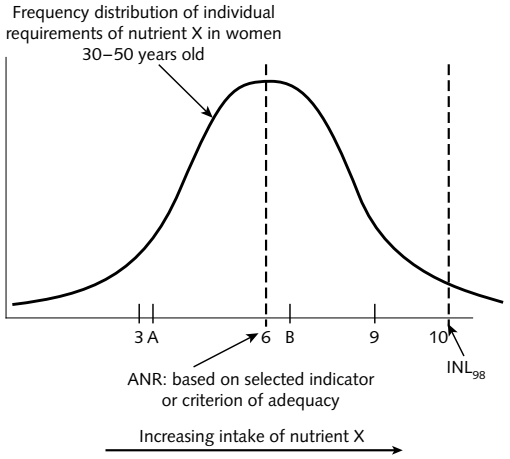


FIG. 2. In this symmetrical distribution of the requirements for a subgroup of the population (assume that it is for apparently healthy, nonpregnant, nonlactating women aged 30 through 50 years, woman A requires approximately 3.5 U of nutrient X (this is the lowest level of intake for her at which the criterion of adequacy chosen is normal or functions normally); other women require less, the same, or more. Woman B requires more, about 7 U of nutrient X, to demonstrate the same level of normal function of the criterion of adequacy selected. The ANR is the average nutrient requirement (in this example, ANR = 6 U) for this group of women; the ANR is specific for the nutrient, the group to which it applies, and the criterion chosen for adequacy. Note that the $INL_x$ is set at 2 SD ($SD_{ANR}$) above the ANR, and in this case, if $SD_{ANR}$ = 2 U, $INL_{98}$ = 10 U. (ANR, average nutrient requirement; INL, individual nutrient level)

convenience sample), or to environmental factors that affect nutrient needs and may differ from those in place during the evaluation of the nutrient requirements in the individuals sampled, as well as a host of other causes.

Often the usual nutrient requirements of all individuals in a population are not known, so data on the specific variation in requirements are not available. In that case, a standard estimate of variance is used. If one assumes that the distribution of requirements is symmetrical, a coefficient of variation (CV) (the standard deviation divided by the mean × 100) can be used to estimate the variation. It is often assumed that nutrient requirements have a symmetrical distribution and a similar CV that is equal to about 10% (i.e., the standard deviation is about 10% of the mean requirement) [8]. This assumption is based on extensive data on the variation in basal metabolic rate [6]; a similar CV of 12.5% was derived from data on the protein requirements of adults [30].

Given the routinely small sample sizes from which ANRs are determined, a default estimate of the variation for nutrients is often adopted for use when there is little information from which to obtain a reliable estimate of the variance or standard deviation of the ANR. The default CV frequently used is 10% ($CV_{ANR}$ = $SD_{ANR}$/ANR). For many nutrients, there are enough data from dose–response curves to determine an ANR, but in many cases the observed variation in response due to the small number of subjects for which the requirements were derived was thought to include errors sizable enough to prevent the observed variation from being used as an estimate. Instead, the default CV, i.e., 10%, is used.

For a few nutrients included in the US/Canadian dietary reference intakes, CVs larger than 10% were applied. In the cases of copper, molybdenum, and niacin, 15% was used on the basis of the variability in response observed in the studies used to determine the ANR [4, 5]; for vitamin A, 20% was used on the basis of the variability in the apparent half-life of vitamin A in liver stores. For iodine, 20% was applied on the basis of iodine turnover studies [5], and for carbohydrate, 15% was used on the basis of observed variability in brain glucose utilization [6].

## Other nutrient intake values derived from an ANR

After the ANR and variance in requirements have been determined, an *individual nutrient level$_x$ ($INL_x$)* may be derived from the ANR and its distribution. The subscript x refers to the percentile chosen for the setting the INL. Often committees add 2 SD to the observed ANR which will cover the needs of most (i.e., 98%) individuals if the distribution of requirements is symmetrical, and it can thus be used as the target for an individual's nutrient intake. This is the approach used in the United Kingdom and the US/Canadian reports, where this model for estimating multiple nutrient references was first applied. In those cases, the $INL_x$ would be an $INL_{98}$; those values are referred to as the reference nutrient intake (RNI) and the recommended dietary allowance (RDA) in the UK and USA/Canadian reports, respectively [3–7, 10]: $INL_{98}$ = ANR + 2 $SD_{ANR}$.

Individuals whose intake is equal to the $INL_{98}$ have a 98% probability that their intake meets their needs. One might also choose to have a recommended intake at a lower level, such as 1.5 SD above the ANR, resulting in a lower target intake for individuals, and a lower probability of adequacy for individuals who achieve this target (in this case, ANR + 1.5 $SD_{ANR}$ = 93%, rather than 98%). One could also set the $INL_x$ at 2.5 SD above the ANR, increasing the probability of adequacy to 99% for individuals whose intakes are at the INL.

Since the $INL_x$ represents a nutrient goal for all individuals in a specific country or region, committees charged with establishing nutrient recommendations for a country or region should choose the specific multiple of the $SD_{ANR}$ they wish to use that is consistent

with the overall nutrient policy.

As an estimate of the between-individual variation, the standard deviation of the ANR ($SD_{ANR}$) is important in developing an INL. If the variation in requirements is small, then an INL that is set at 1.5, 2, or 2.5 $SD_{ANR}$ above the ANR will be relatively close to the ANR; if the variation is broader, then the INL will be larger. This demonstrates that the distribution of requirements is an important determinant of the INL.

Two other NIVs, in addition to the $INL_x$, may also be derived. A "deficient" or lower nutrient intake level may be established at some point below the ANR (e.g., 2 SD below) to serve as an estimated intake that is probably below the needs of practically all individuals. Since the ANR is usually used to estimate the proportion of the population with inadequate intakes by determining the percentage below the ANR [2, 17], a lower nutrient intake level has limited use.

If information is available on the level of intake of a specific nutrient that carries health risks, an upper nutrient intake level (UNL) may also be established. The UNL is usually set where the risk of excessive intakes is very low, close to zero. The UNL is a level of intake that should be avoided on a chronic basis [9]. There is no established benefit to consuming nutrient intakes above the INL set at 2 or 2.5 $SD_{ANR}$.

## Estimating energy requirements

Energy recommendations are set at levels that represent the *average* needs of individuals in the population. In determining energy standards, factors such as age, sex, and level of physical activity should be considered. Energy standards may be expressed as equations that permit the calculation of energy intake requirements based on these factors.

It would not be appropriate to set an energy $INL_x$ as was done for the other nutrients, because adding an increment (e.g., 2 SD) to the average energy requirement would result in an intake goal that would lead to weight gain. If the energy intake is consistently above or below the average requirement for a long enough period of time, changes in body weight will occur and may adversely affect health. As a consequence, recommendations for energy are expressed in terms of energy expenditure rather than energy intake in order to prevent under- or overconsumption. Since nutrient standards are set for healthy individuals, it is assumed that body energy stores are appropriate and that the *average* requirement should be used as the standard for the entire population. Because energy intake and expenditure are not independent, it is not possible to determine the probability that energy intake is adequate.

## Acceptable macronutrient distribution ranges

Recently, several countries or regions have established acceptable ranges for the percentage of energy coming from carbohydrate, protein, and fat in the diet, which are thought to reduce the risk of long-term or chronic disease while providing an adequate intake of essential nutrients. The term used to describe these recommendations is acceptable macronutrient distribution range (AMDR). The AMDR is expressed as a percentage of total energy intake, because the range is not independent of other fuel sources or of the energy requirement of the individual. As mentioned earlier, definitions used for macronutrient intakes as a percentage of energy vary. The AMDRs established by the United States and Canada refer to appropriate ranges of usual intakes of individuals, whereas the WHO standards are population mean intake goals [6, 22]. Thus, the WHO mean intake goal of 15% to 30% of the energy as fat implies that it is acceptable for half of the individuals in a population to have intakes *below* 15%.

Although a range of protein intakes as a percentage of energy has been established, since protein is one of the fuel sources of the diet, there is also a specific minimum need for nitrogen and amino acids that serves as the basis for the protein ANR and INLx. Those values are usually presented as grams or milligrams per day. Since the need for nitrogen or amino acids is likely to be independent of intake, the theoretical statistical model can be used to estimate the probability of inadequacy for an individual and the prevalence of inadequate intakes in a population. When protein NIVs are derived, the committees need to ensure that those NIVs can be acquired within the range of acceptable protein intakes.

## General features of the statistical model for estimating nutrient requirements

There are two important caveats associated with this model for estimating nutrient intake values [31]. First, the estimates of requirements pertain to the maintenance of health in already healthy individuals. Such estimates may or may not pertain to individuals with disease, and they certainly are inappropriate for individuals recovering from nutrient deficiencies. Second, nutrient intake refers to the *usual intake* of an individual or group, i.e., the average intake for weeks or months, not days.

The primary advantage of this general framework is its simplicity. Only the midpoint of the requirement distribution (the ANR) needs to be determined from primary scientific data. Ideally, one would also like information regarding the variance in the requirement in a large, representative population. However,

assuming a coefficient of variation for the $SD_{ANR}$ seems reasonable. Using coefficients of variation ranging from 10% to 20% for the various nutrients is consistent with the variance measured for a number of biological variables [30].

Finally, the underlying biology defining nutrient requirements is likely to be similar across diverse populations. For example, the biological need for absorbed zinc in toddlers to support normal growth is probably similar among diverse populations around the world. However, the amount they need to consume from their food supply probably differs because of the difference in the bioavailability of zinc from various food sources, e.g., cereals versus animal flesh. A description of when and how to make adjustments in the ANR for inefficient use of the nutrient as consumed due to poor absorption or inefficient conversion of a precursor to active forms is given by Gibson in this issue [32]. Since the biological need for absorbed or active forms of nutrients to maintain a specific function probably is similar among diverse populations worldwide, countries should be able to use primary scientific data on nutrient requirements for populations living in other countries or regions, as long as they adjust for specific environmental influences in their country, e.g., nutrient bioavailability, the prevalence of infectious diseases, exposure to sunlight, and altitude.

## Conclusions

A general statistical model for estimating nutrient intake values that permits assessments of the adequacy of nutrient intakes of populations is recommended. The model is based on estimating the average nutrient requirement (ANR) from data on nutrient requirements for a specific function in a well-defined population, determining or assuming a variance in requirements for the general population in an age- and sex-specific group, and calculating an individual nutrient level $INL_x$ that meets the needs of a defined percentage of the population. Most countries and regions use the mean or average energy expenditure levels of a defined population for the energy intake standard, because a higher standard would result in weight gain. It is not possible to assess the probability of energy adequacy, because an individual's energy requirement is related to intake. In addition, many countries and regions are establishing acceptable macronutrient distribution ranges (AMDRs) to define the distribution of dietary fuel sources (carbohydrate, protein, and fat) as a percentage of total energy.

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# Nutrient risk assessment: Setting upper levels and an opportunity for harmonization

Peter J. Aggett

## Abstract

*Upper levels are estimates of the quantity of a nutrient that can be ingested daily over a lifetime without appreciable risk to health. The approach to establishing upper levels for nutrients, nutrient risk assessment, has derived from the risk assessment of foreign chemicals that are deliberately added to foods, or are in food as contaminants. This process of risk assessment is rigorous and transparent, particularly in dealing with the uncertainty arising from the data available and their assessment and extrapolation to human populations. Hazard identification and characterization, i.e., a dose–response pattern, as applied to xenobiotics, are discussed first, and then the difficulties of applying this approach to nutrients are reviewed. Nutrients, in contrast to foreign chemicals, have specific and selective metabolic pathways and homeostasis, as well as specific functions. This is the source of differences in the nutrient risk assessments produced by various national and international advisory bodies. Although the same data are used in such exercises, different judgments are made about identifying adverse effects, the nature of uncertainties in the assessment, and in matching the upper levels with exposure assessments and dietary reference values. The establishment of different upper levels for different national and international communities is a source of confusion in public health policy and practice and a barrier to trade. It is proposed that a basis for harmonizing the existing approaches used in nutrient risk assessment would be the collaborative development of the model for establishing upper levels of intake for nutrients and related substances that has been recently described by a Joint Task Force of the World Health Organization and the Food and Agriculture Organization.*

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**Key words:** Nutrient risk assessment, upper levels, adverse health effects

## Introduction

This contribution addresses the assessment of the risk or safety of high intakes or exposure to nutrients and related substances. I will call this nutritional risk assessment, although, of course, risk and safety assessment, and, perhaps, the approaches described in this paper, could be as readily applied to setting safe lower levels of intake.

For this overview, an upper level of intake (UL) is "the maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans"[1]. This definition, in turn, requires at least two more definitions, setting aside that of defining a nutrient or related substance.

First, an habitual intake is "the long-term average daily intake of the nutrient or substance"; second, an adverse health effect is "a change in morphology, physiology, growth, development, reproduction or lifespan of an organism, system, or (sub) population that results in an impairment of functional capacity, an impairment of capacity to compensate for additional stress, or an increase in susceptibility to other influences" [1].

A hazard is the inherent property of a nutrient or related substance to cause adverse health effects, depending on the level of intake.

These definitions have been derived from the processes and definitions that have been applied to the risk analysis, risk assessment, and regulation of human exposure to xenobiotics as additives to our food, or as natural toxicants or environmental pollutants and contaminants in the food chain.

Since risk assessment approaches to setting ULs for nutrients have drawn on these more established processes for nonessential chemicals, this overview will first describe these processes, and then discuss how such an approach can be applied to nutrient risk assessment.

Most of this discussion will draw on recent considerations of micronutrients [1, 2]. Additionally, there is a current international collaboration exploring systematic safety assessments of intakes of amino acids [3–5].

## Toxicological risk analysis and assessment

The values derived by the risk assessment are estimates of the quantity of a substance that can be ingested daily over a lifetime without appreciable risk to health and are termed health-based guidance values [6–9]. These include the acceptable daily intake (ADI) [6]) and the tolerable intake (TI), which may be weekly (TWI) or daily (TDI) [8]. The ADI is applied for estimates of safe exposure for food additives, i.e., for chemicals that are permitted to be used in foods, and the TDI relates to contaminants and pollutants. The US Environmental Protection Agency has replaced ADI and TDI with the term reference dose (RfD), which has been defined as an estimate of the daily exposure in the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. All these definitions are framed to avoid implying that they are absolutely "safe"; they are advisory (although they might subsequently be translated into regulations) and are the products of a systematic process of risk analysis, of which risk assessment is one element.

Risk analysis is "a detailed examination, including risk assessment, risk evaluation and risk management alternatives, performed to understand the nature of unwanted, negative consequences to human life, health, property or the environment; an analytic process to provide information regarding undesirable events: the process of quantification of the probabilities and expected consequences of identified risks" [10].

The definitions used in the established process vary among the agencies involved, but with the maturation of the discipline and increased international and interagency harmonization, the definitions are very close and common wordings are emerging.

In this process, the definition of a hazard is "the inherent property of a chemical to cause adverse effects depending upon the level of intake." The modification of this to suit the needs of nutritional risk assessment (see above) is straightforward [1]. An adverse effect is "a change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. Decisions on whether or not any effect is adverse require expert judgment" [7]. Again, this is little different from the definition derived for nutritional risk assessment.

A risk is "the probability or likelihood that a hazard will actually cause harm to an individual or population group"

In the model developed by the Food and Agriculture Organization/World Health Organization (FAO/WHO) and the Codex Alimentarius, Risk Analysis comprises three distinct steps: risk assessment, risk management, and risk communication. Although each is a distinct step, collectively they are intended to be a coherent and fluently progressive entity. This is a well-accepted model, because it provides a structure that ensures that any uncertainties, variabilities, or assumptions involved in the assessment can be identified, thereby enabling and encouraging a transparent explanation of the means by which these issues are compensated.

### Risk assessment

In practice, the first step in risk analysis is that of "problem formulation," i.e., setting the key purpose and objective of the exercise. Usually this is done by the risk managers and regulators who will be responsible for managing the risk of any particular exposure and communicating to the public about the risk and the strategy to manage it.. Often, but not necessarily always, problem formulation may involve consultation with those who have the task of assessing the hazards and any attendant risks.

Once the problem has been set, the first phase in risk analysis, risk assessment, can start. Since this involves identifying and prioritizing hazards and the exposures at which they happen, this is the process that is most relevant for this paper. Risk assessment comprises four stages: hazard identification, hazard characterization (sometimes called dose–response assessment), exposure assessment, and risk characterization. Each is briefly described later, but in essence they involve first a full review of all relevant information and a qualitative identification and evaluation of all adverse effects associated with high exposures (hazard identification), followed by a quantitative estimation of risk for each adverse effect (hazard characterization). Assessment of dose–response often includes a modeling exercise to extrapolate from high to low levels of exposure.

**Hazard identification,** as the determination of the relationship between the exposure to the chemical and one or more associated adverse effects, needs a full appraisal of information to characterize the absorption, systemic distribution, metabolism, and elimination (i.e., toxicokinetics) of the chemical and the toxic effects that the chemical, or its metabolites, may have at tissue and cellular functional levels (i.e., toxicodynamics). Both human and animal model data are used. Some of these are systematically acquired through specific studies (**table 1**), and this applies particularly to chemicals that are proposed as food additives. Other data may be acquired more opportunistically, such as from case studies and incident reports. This is more

TABLE 1. Current tests and endpoints used in toxicology[a]

| Study type | Endpoints measured | |
|---|---|---|
| 30-day study | Clinical signs, mortality<br>Body weight, body weight gain, organ weights<br>Food consumption, water consumption<br>Hematology and clinical chemistry<br>Urinalysis<br>Macroscopic/microscopic histopathology | |
| 90-day study | Clinical signs, mortality<br>Body weight, body weight gain, organ weights<br>Food consumption, water consumption<br>Hematology and clinical chemistry<br>Urinalysis<br>Macroscopic/microscopic histopathology | |
| 2-yr/lifetime study | Clinical signs, mortality<br>Body weight, weight gain, organ weights<br>Food consumption, water consumption<br>Hematology and clinical chemistry<br>Urinalysis<br>Macroscopic/microscopic histopathology | |
| Developmental toxicology | Dams | Clinical signs, mortality<br>Body weight, body weight gain<br>Food and water consumption<br>Macroscopic/microscopic histopathology |
| | Fetal data | Numbers of corpora lutea and implantations<br>Numbers of viable fetuses and resorptions<br>Sex ratio, fetal and litter weights<br>Skeletal and visceral examination |
| Reproductive toxicology (multigenerational study) | Parental | Clinical signs, mortality<br>Body weight, body weight gain<br>Fertility<br>Macroscopic pathology<br>Histopathology of reproductive organs |
| | Litter/pup data | Litter size, numbers of live and dead pups<br>Pup sex<br>Pup weight, pup organ weights<br>Pup macroscopic/microscopic pathology |

*a.* In addition to these toxicologic studies, studies of absorption, distribution, metabolism, and excretion are usually undertaken. Furthermore, tests of mutagenicity are carried out both *in vitro* and *in vivo.*

often the case for contaminants and pollutants. As a principle, wherever possible, data from human experimental or observational and epidemiologic studies are preferred. Commonly in toxicology the critical "adverse effect" refers to an unambiguously demonstrable adverse event, rather than a phenomenon that might be regarded as adaptive [8]. This selection is part of the hazard characterization, but in practice there would be some iteration between this and the preceding hazard identification.

The regulatory assessment of applications by producers to add a chemical to a food requires an extensive database. This would be expected to include toxicokinetic information from studies in animals and perhaps in humans, and acute, short-term, repeated-dose studies in two animal species. These are listed in **table 1**. Occasionally, but not usually for food additives, tests relevant to skin sensitivity and allergenicity may be required. Information from metabolic and toxicokinetic studies can help determine whether adverse effects are caused by the parent compound or by its metabolites, and also provide information that would characterize interspecies and interindividual differences in toxicokinetics and toxicodynamics and susceptibility to adverse effects.

It is customary to assess the quality of all published data and to consider their "totality" and coherence. Data that have been peer reviewed and produced in

accordance with benchmark and good-quality control practice are preferred. Systematic reviews that have already been published may help some stages of hazard identification and characterization, but they are not invariably necessary or helpful, because they have not been prepared for risk assessment and because of the judgments that have already been applied in preparing such reviews. For hazard identification and characterization, good practice is to use primary data sources, with access, if possible, to associated quality assurance.

The systematic generation of data for hazard identification and characterization of chemicals that are intended to be added to foods would be resourced and acquired for regulatory review by the producers applying for approval to use that chemical in food. On the other hand, chemicals that are present in food as contaminants have no such commercial sponsor, and many of the data listed in **table 1** would not be available. Nevertheless, risk assessment is required to establish regulatory limits for contaminants in food, but invariably the database will be poor compared with that for additives, and consequently the uncertainty of the assessment of contaminants is increased.

**Hazard characterization** [8] is the determination of the relationship between the exposure to the chemical and the adverse effect or effects; this is a quantitative evaluation of the adverse effects by dose–response evaluation. It includes evaluation of mechanisms of action and of species differences in the responses, if such data are available. This stage should result in the identification of a health-based guidance value or reference dose and an account of the uncertainties inherent in that value.

### Deriving health-based guidance values

The identification and selection of one, or occasionally more than one, adverse effects is crucial to the derivation of a health-based guidance value. This judgment is based on confidence in the effect's characterization, the appropriateness of markers relating to exposure or intake, body burden, and of the adverse effect itself, the quality of the determination of these markers, and their mechanistic relevance. This analysis, ideally, would be able to address toxicokinetic (i.e., ADME; absorption, distribution, metabolism, and excretion) and toxicodynamic information and would describe the dose–response relationship of the critical event or events. Ideally this would involve determining the link between external dose (intake) and the internal or systemic burden associated with the effects, along with the mechanism for the adverse effect. Ideally these data would be supplied from a suite of integrated studies, in one species, but with some further data from other species to enable the detection and characterization of potential species differences (qualitatively and quantitatively) and of sensitive species and strains.

These data are then used to derive a health guidance value or reference dose for human exposure. The derivation of this value is most commonly done by a no observed adverse effect level approach.

### The no observed adverse effect level (NOAEL)

The NOAEL is the highest tested dose or intake at which the chosen adverse effect is not observed. The key notion here is that the dose is below the threshold for a particular health effect. It is, therefore, a conservative estimate.

When a NOAEL cannot be identified, a lowest observed adverse effect level (LOAEL) is identified. The LOAEL is the lowest dose of the chemical of interest that was found to cause an adverse effect. Guidance values are derived from the adverse effect levels by dividing them by factors that represent the risk assessors' objective assessment of the uncertainty in the characterization.

As may be obvious from earlier comments, all risk assessments have an inherent uncertainty and variability. Identifying and allowing for these is an integral part of the data analysis involved in hazard identification and hazard characterization. Variability can be characterized; it relates to interindividual differences arising from, for example, age, gender, absorption efficiency and other metabolic activity, nutrition, and developmental maturation. The roles of functional polymorphisms, programming, and epigenetic effects as contributors to variability are becoming appreciated.

Uncertainty arises from factors that are unknown or imprecise. These include both the quality and the limitations of the database on exposure and on dietary adequacy in model studies, extrapolation of data from animal models to humans, uncertainty about the **representivity** of the animal models, and extrapolation of the available data to human populations, particularly potentially sensitive subpopulations. Other sources of uncertainty include the methodologies used and the measurements made, and the degree of confidence in the selection of the adverse effects and data analyses.

These variabilities and uncertainties are collectively incorporated in an "uncertainty factor," which is defined as a product of several single factors by which the NOAEL or LOAEL of the critical effect is divided to derive a health based guidance value. It is important to note that "these factors account for adequacy of the pivotal study, interspecies extrapolation, interindividual variability in humans, adequacy of the overall database, and nature of toxicity". The term uncertainty factor was considered to be a more appropriate expression than safety factor, since it avoids the notion of absolute safety and because the size of this factor is proportional to the magnitude of uncertainty rather than safety. The choice

of uncertainty factor should be based on the available scientific evidence [7].

Commonly the NOAEL is divided by a default uncertainty factor of 100 to derive a guidance value. This default value comprises two factors of 10: one for interspecies differences, covering extrapolation of data from animal models to humans, and one for interindividual differences (human variability). Uncertainty factors are illustrated in **table 2**.

As a further refinement, the International Programme on Chemical Safety (IPCS) framework [7, 9] apportions the default uncertainty factor [10] for interspecies differences between two factors of 2.5 and 4.0 for toxicodynamics and toxicokinetics, respectively, and that for intraspecies variability into 3.2 and 3.2 for both components. These values represent an initiative to use chemical-specific adjustment factors derived from kinetic and dynamic data to derive expressions of uncertainty that are more definitive and specific than are default values for uncertainty factors.

Sometimes additional factors are used to compensate for the absence of data, such as an inadequate long-term (chronic) study, no discernible NOAEL and the need to use a LOAEL, other gaps in the data, and, sometimes, the severity of the adverse effect.

The preceding outline is the ideal. It describes the application of the NOAEL approach to a systematic set of data. There is usually some such structure in the data for additives that facilitates the use of uncertainty factors, but for contaminants the quality of the data makes their use more challenging, and often several uncertainty factors are applied, resulting in a relatively high overall factor.

## Other approaches to uncertainty and improving risk assessment

The NOAEL approach has been criticized because it may not use all the available data, including dose–response data, and because the dose–response curves are customarily derived by using large, e.g., 10-fold, incremental steps in the intakes of the chemicals under study. Furthermore, the NOAEL approach is deterministic and does not readily allow for flexibility in selecting different levels of risk. There are alternative approaches to hazard characterization, and two, in particular, are seen as being potentially useful. These are the benchmark dose–response and categorical regression. Both allow for more extensive use of available information and for calculation of levels of exposure that can be associated with predetermined levels of risk (e.g., 1%, 2.5%, 5%) within the population. These will be described briefly. Better descriptions of their use and applicability are in the references cited from which I have derived this commentary.

### *Benchmark dose modeling*

Benchmark dose (BMD) modeling [2, 11, 12] fits a dose–response regression to more of the available dose–response data acquired from animal models and human studies (**fig. 1**). This provides an overall estimate of the variance and a way of estimating dose levels at which specific proportions of the population under study would experience an event or effect over and above the base occurrence. A statistical lower bound (BMDL or LBMD) customarily set at 95% is used.

The statistical lower confidence limit of the lower bound is generally used for developing an RfD [12]. Thus, this approach uses all the dose–response data to find the dose associated with a predefined response, whereas the NOAEL usually derives from a single

TABLE 2. Typical uncertainty factors used in toxicology

| Uncertainty factor | Use | Value |
|---|---|---|
| Intraspecies (intrahuman) | When extrapolating long-term studies to provide acceptable daily intakes or short-term studies to produce acute reference doses in the same species | 10 |
| Interspecies | When extrapolating, from one species to another, long-term studies to provide acceptable daily intakes or short-term studies to produce acute reference doses | 10 |
| Subchronic to chronic | Where no adequate chronic study is available | Up to 10 |
| LOAEL to NOAEL | If the critical effect in the critical study is a LOAEL | Up to 10 (often 3 based on dose spacing) |
| Incomplete database | Where the standard data package is not complete | Up to 10 |
| Steep dose–response curve | Where the dose–response curve for a compound is steep, a small error in extrapolation would have dramatic consequences | Judgment, 3–10 |

NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level

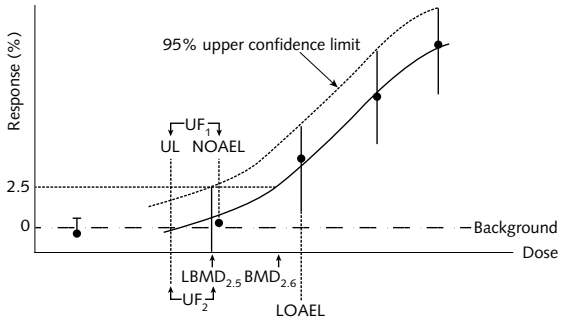


FIG. 1. Benchmark dose (BMD) modeling: A representation of a benchmark dose–response curve of an adverse effect in a population. The 95% confidence interval has been calculated from all the plotted data points, and the horizontal dotted line represents the derivation of an intake, the lower benchmark dose (LBMD), at which there is a 2.5% response or risk of an adverse effect occurring in a population. Hypothetical no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) are indicated, as are the lower bound benchmark dose (BMDL or LBMD), uncertainty factor (UF), and the upper level of intake (UL). Source: International Programme on Chemical Safety [2], modified by Przyrembel for the Joint FAO/WHO Task Force on Nutrient Risk Assessment [1]

level of exposure. Furthermore, the BMD can be used on data from which an NOAEL cannot be derived; however, the dose exposure spacing for the NOAEL approach is not always useful for the BMD. It is thought or hoped by its advocates that the advantages of the BMD would provide an incentive for more rigorous studies that would decrease both scientific and mathematical uncertainty. These advantages have been discussed by a number of investigators [11, 12].

It is possible to apply uncertainty factors as part of the BMD approach, for example, to allow for inter-individual differences. Unfortunately, datasets from studies designed to support the use of the NOAEL approach to setting a UL are not easily used for the BMD approach.

#### *Categorical regression*

Categorical regression [11] can use combined qualitative and quantitative data. It fits ordinal data (i.e., outcomes that are ranked by severity of effect) to a dose–response model that can be applied to a benchmark dose–response approach. It can take into consideration the severity and duration of exposure of effect (i.e., the effects of different periods of exposure to single levels of intakes, which is a common design feature in nutritional studies), use data from multiple studies, and address a variety of endpoints. As with the BMD, it gives equal weight to studies and their data, though it is possible, as with the other approaches, to evaluate the quality of the studies against objective criteria. For example, data amenable to this approach are those from histopathological studies in which the evolution of architectural damage is monitored in relation to the period of exposure. This approach therefore provides information about the evolution and mechanisms of effects at single exposure levels over time; this would be helpful in assessment of prolonged exposure at constant levels of intake.

### Exposure assessment and risk characterization

These are the last two stages of risk assessment.

**Exposure/intake assessment** is the measurement or estimation of exposure to a chemical by any route for the population or its subgroups (e.g., toddlers, children, adults, and ethnic groups). It includes consideration of the pattern, frequency, and duration of exposure and it informs the next step of risk characterization.

**Risk characterization** (sometimes called "advice for decision making") is the integrative consideration of hazard identification, hazard characterization, and exposure assessment to predict whether effects in humans are likely and the nature and severity of such effects. If data permit, it may include identification of the proportion of the population affected and the existence of any vulnerable subpopulations. The product of this exercise is an overall report describing the process, including a description of the nature of the risk, its extent, and all the associated uncertainties [8].

### Risk management and risk communication

Risk management and risk communication are the last two elements of risk analysis. Risk management has two main functions. The first, as has been mentioned, is to frame the question, or set the task for the risk assessors; the second is to act on the risk assessment. This involves option assessment, which is the identification and assessment of possible control options; implementation of management decisions; and monitoring and evaluation of the effectiveness of the risk management measure.

Although risk management and risk assessment are discrete activities, it is accepted that the system works most effectively if there is some interaction between the stages to ensure that the assessment is accurately conveyed between the two stages and is properly understood. However, the prime intention of separating risk assessment and risk management is to protect the integrity of the scientific and objective assessment by the expert risk assessment process, leaving those responsible for risk management to take into account other issues, such as the concerns and interests of other stakeholders.

Finally, risk communication represents the exchange of information taking place throughout the process,

particularly but not exclusively during risk management. It is important that managers, the public, and other stakeholders understand the assessment, and it is envisaged that the entire process of risk analysis should be open and transparent and that the views of stakeholders should be taken into account as part of the process.

## Nutrient risk assessment: Applying the risk assessment model

A joint FAO/WHO Technical Workshop [1] recently reviewed the terms of reference and nutrient risk assessments of three authoritative advisory reports, namely, the European Food Safety Authority [13] for the European Union, the Expert Group on Vitamins and Minerals for the Food Standards Agency in the United Kingdom [14], and the Institute of Medicine of the National Academies for the United States and Canada [15]. It is evident that these nutrient risk assessments did not always find it easy to derive a UL confidently. The groups often used the same data, and there were many commonalities in their risk assessments. However, the data are of an inconsistent and uncertain quality, and there were differences in the use made of the data, the derived ULs, and the expression of the uncertainties. With such a predicament, there is a need, both for public health considerations and for harmonized approaches to regulatory issues in global trade, to have an internationally agreed approach to setting ULs that would serve as a basis for international collaborative nutrient risk assessment, for the identification and prioritization of knowledge needs, and for organizing the necessary research.

The established toxicologic approach when applied systematically to nutrient risk assessment reveals the inherent limitations of the available data. In the United Kingdom, for instance, in some cases the data were felt to be too insecure to set safe ULs, and provisional levels called guidance values (not to be confused with health-based guidance values) were set instead. These uncertainties include difficulties in identifying critical adverse health effects; difficulties in extrapolating between species and population age and gender groups; a lack of confidence in the estimates of intakes (exposures) involved with particular adverse health effects; and no good, if any, dose–response data. Usually nutrient risk assessment or, specifically, nutrient hazard characterization has used the NOAEL and LOAEL approach to develop a UL, and doing this with such poor-quality data has meant using many uncertainty factors. The application of the default values for these uncertainty factors can result in such a large value that when they are applied to a NOAEL or a LOAEL, they produce health-based guidance values that are close to or even below the upper end of the range for reference values for nutrient intakes. This "nutrient paradox" in nutrient safety assessment is well recognized for minerals but can be demonstrated also for organic nutrients (e.g., ascorbic acid) [1]. The reasonable response of risk assessors to this problem has been to reduce the uncertainty factor values applied in deriving the UL. Although this is done openly, the pathophysiological basis of such flexibility is not necessarily easy to explain.

Apart from the paucity of the database, the major source of uncertainty in the risk assessment of nutrients is the nature of the nutrients themselves. For non-nutrient chemicals, the assumptions of risk assessment are that they have no physiological role, have detoxification pathways that are probably not chemical-specific, generally have no interdependence on exposure to other chemicals or nutrients, and do not increase the risk of any adverse health effects at low intakes [1, 11]. Nutrients are different; they have distinctive biochemical and physiological roles, and biological organisms have evolved specific and selective mechanisms to acquire, absorb, distribute systemically, metabolize, and control the body burden of the nutrient itself or its metabolites. These homeostatic mechanisms respond to excursions of intakes above or below physiological requirements. As a result, nutrients have a dual–dose response curve, i.e., there are adverse health effects at levels of intake that, relative to systemic requirements, are inappropriately high and at intakes that are inappropriately low (**fig. 2**) [1, 2, 11]. Furthermore, the shape or inflexions of the dose–response curve at the thresholds of excessive and deficient intakes will be contingent on the efficiency of the mechanisms of homeostasis and, possibly, on the nutrient's interdependence on the supply and metabolism of other nutrients (e.g., the relatively

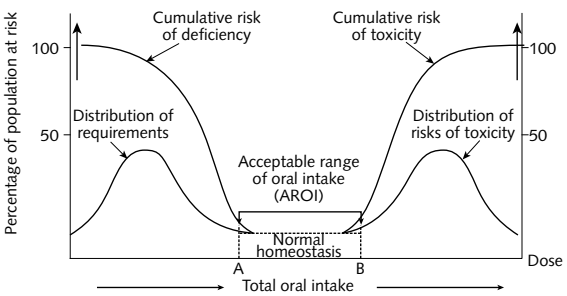


FIG. 2. The dual biological, or U-shaped, response curve to increasing oral intakes of a nutrient as applied to a population. At low intakes, there is a cumulative risk of deficiency that reflects the distribution of requirements, and at the upper range of intakes there is a similar cumulative risk of toxicity. Conceptually, the acceptable range of intakes represents a zone bounded at points A and B within which homeostasis ensures that intakes are used to meet individuals' systemic requirements. Source: International Programme on Chemical Safety [2]

lower limits of toxicity of amino acid supply in zinc deficiency, or the interaction of amino acid metabolism and energy supply). Nutrient risk assessment has to be alert to such confounders.

Between the extremes of adverse health effects, there is a range of intakes within which normal systemic homeostasis is effective (**fig. 2**). This pattern was used by an IPCS task force on "Risk Assessment for Essential Trace Elements" to support the concept of acceptable range of oral intake (AROI) for essential trace elements [2]. Although it is easiest to envisage for minerals and vitamins, the concept can apply equally well to amino acids and to lipids or fatty acids. Extending this idea to the latter nutrients highlights the need for particular consideration in hazard characterization of the duration of any particular intake that is needed to cause an adverse health effect. If, for example, one appreciates the "timeline" of adverse health effects of inappropriately high intakes of a lipid, or a class of lipids in general, then it should be feasible to appreciate the sequential mechanistic pathway and time frame of effects such as disturbances in membrane functioning, cell signaling, cell function, energy metabolism, and vascular intimal integrity and function. Although some of these effects are reversible, early, and minor, they are part of a sequence in which they might be seen to be predictive of later gross effects that would occur if high exposure to the lipid continued. As such, all these effects could be considered as potentially useful to nutrient hazard identification and characterization. This point is revisited later.

Currently, the adverse health effects applied to nutrient risk assessment are clearly associated with intakes that are above the upper adaptive limits of homeostasis. Often the selected adverse health effects either are derived from data from unsystematic observational incident reports and are severe or gross (as would be expected to be inherent in the nature of a case report), or are derived from data from prolonged single-dose studies in humans or in animal models. These points reemphasize that the database available for nutrient risk assessment is much like that available for non-nutrient chemical contaminants.

Although it is conceivable in hazard characterization to work from the marked adverse effects at high exposures back along the responsible pathogenic pathway to identify possible markers of later severe adverse health effects of particular intakes, it is arguably more logical to identify and characterize the hazards of high intakes on the basis of adverse health effects that are known to herald more serious events that would occur at higher or more prolonged intakes. A means of doing this would be to develop a strategy for nutrient risk assessment that is more sympathetic to the dual nature of the nutrient dose–response curve and its regulation. This strategy would involve identifying, at intakes that are nearer those to which consumers might be exposed, events that would be valid markers of potential adverse health effects. These intakes would also be ethically and practically acceptable for studies designed to inform nutrient hazard characterization.

The IPCS task force [2] considered this strategy. It felt that "early" biochemical effects without functional significance should not themselves be regarded as adverse health effects. However, if these could be seen to be part of the pathogenic pathway for an overt effect, then they could be envisaged as biomarkers for subsequent, more serious adverse events. The IPCS task force reexpressed a spectrum involving classes of responses to deficient and excessive intakes: these were biochemical responses without functional significance, subclinical biomarkers of effect with functional impairment, increasing functional disturbances with clinical effects, and death. So although the biochemical changes after short or lower dose exposure are not a threat to health, and may indeed be reversible, the possibility that they herald a potentially more serious effect can be seen to contribute to nutrient hazard identification and characterization.

The recent FAO/WHO Technical Workshop [1] appreciated the distinctiveness of nutrient risk assessment as compared with that of non-nutrient chemicals. It was also sensitive to the differences between the disciplinary vocabularies of nutrition and physiology and that of toxicology. For example, it felt that the terms "dose" and "exposure" caused some confusion and chose to use the term "intake" as a matter of familiarity and consistency within the discipline of nutrition, although the meaning of "intake" is still essentially the same as that of "dose" and "exposure" as they are understood in toxicology. The workshop modified the term "adverse effects" to "adverse health effects" in an attempt to capture the importance it was placing on using a full range of morphological, physiological, and early biochemical responses both as adverse events in nutrient risk assessment and as part of a spectrum including adaptive and beneficial health effects. This provided the opportunity to include, within the range of markers of adverse health effects, mild and reversible effects (such as homeostatic adaptations or, as might be the case with high exposures to complex carbohydrates, phenomena of intestinal fermentation), as well as more serious and potentially life-threatening outcomes, such as hepatic and neurologic damage [1].

This workshop considered that markers indicative of effects of biochemical changes outside the homeostatic range and of minor but reversible clinical symptoms could be used as surrogate adverse health effects [1]. However, it is important for quality assurance and consideration of causation that these markers be properly validated and suitable for systematic use in hazard identification and characterization. The knowledge base for this might come from published literature, but because many published or proposed markers have

not been fully validated, there is probably a need for research to develop and validate markers appropriate for this nutrient risk assessment strategy.

A continuum as a basis for identifying adverse health effects for this strategy in nutrient hazard identification and characterization is illustrated in **figure 3** [16]. Steps 4–7 in the figure reflect the increasing severity of adverse health effects; the workshop [1] agreed that phenomena occurring at level 3, namely, at levels of intake that were assumed to be not far in excess of the homeostatic range, could indeed be surrogates, or predictive markers, of adverse health effects. Furthermore, it concluded that

> when data are available, the optimal endpoint for use in setting a UL would be an effect at step 3 and possibly step 2, with steps 4–7 reflective of clinical phenomena. Step 2 may be applicable in some cases in which sufficient information is available to suggest that changes outside the homeostatic range that occur without known sequelae would be relevant as surrogates for an adverse health effect [1].

This is emphasized in **figure 3** by showing, against the background of the ranked effects, a hypothetical cascade of markers or effects arising from exceeding a safe level, or conceptual UL, of intake. These figures show how a critical control point analytical approach to available information could be used in nutrient hazard identification and characterization; it provides a strategic structure for an evidence-based systematic review and categorization of the data and potential markers and for the determination of gaps in insight of the pathophysiology and uncertainty.

The use of such markers would be an important

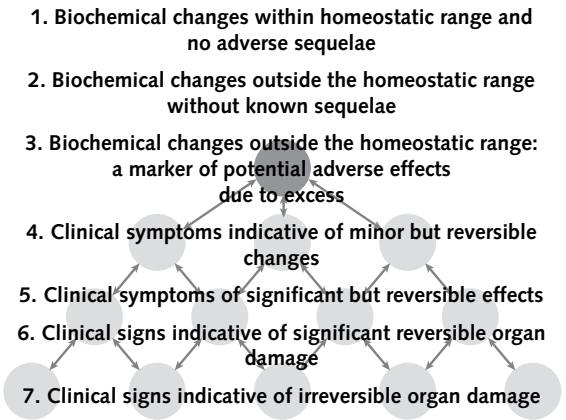


FIG. 3. The range and cascade of effects and of markers: opportunities for identifying critical markers of adverse health effects. Each circle represents a hypothetical marker, and the dark circle represents the marker at a critical point for the subsequent cascade of adverse health effects. The spectrum of health effects is from Renwick et al. [16]

innovation in nutrient risk assessment. Their introduction would be amenable to the NOAEL, BMD, and categorical regression approaches to hazard characterization. Using markers of earlier effects of excess intakes would be expected to increase the size of the database for hazard identification and characterization. They probably will not improve the quality of the database. In particular, the use of markers at steps 2 and 3 of **figure 3** needs to be backed by confidence in their validity and quality.

With these issues in mind, the workshop emphasized that biomarkers comprised two classes: "factors" that represent "an event…directly involved in the process of interest and are causally associated with the adverse health effect"[1] and "indicators" that represent correlated or associated effects and events that have not been shown to be part of the causal pathway. Thus, a biomarker that is part of the causal pathway can be regarded as being "predictive" of an adverse health effect; however, some "predictive" biomarkers might not be causal. In this regard, the workshop appreciated that biomarkers can be diagnostic in that they indicate adverse health effects relevant to nutrient risk assessment, for example, liver damage, but as such these could still be categorized as factors or indicators according to their perceived role in the pathogeneses involved. Thus, nutrient risk assessors may have available a portfolio of biomarkers that could be used as surrogates for adverse health effects, in that such markers can be typified as being causally associated with the adverse health effect; diagnostic of the adverse health effect; and predictive of, but not causally associated with, the adverse health effect [1].

Overall markers, whether they are functional, chemical, or morphological, would need to meet the quality criteria of being biologically valid and reproducible, of known specificity and sensitivity, and methodologically or analytically valid and reproducible. A recent consideration of the use of markers as surrogate endpoints in the justification of claims of reduced risk of disease is particularly relevant to these issues [17] and emphasizes the need for markers to be ethically and practically feasible if they are to be used in systematic studies in populations. The workshop appreciated that these criteria provided a basis for characterizing the uncertainty and variability associated with markers at any stage in the above ranking, but particularly those at stages 2 and 3, where the best chances of improving the database through human research exist.

Theoretically, a biologically based or metabolic dose–response model would be applicable to all nutrients and should or could derive from the compilation and acquisition of new data on absorption, distribution, metabolism, and excretion as the basis of information on biokinetics and biodynamics. In essence, this resembles the use and derivation of chemical-specific adjustment factors (CSAFs) to improve the specificity

of uncertainty factors. Furthermore, ADME data could be used to explore the dose–response curve at the lower extreme of intakes to set safe lower levels as part of a risk–benefit analysis [16]. The construction of biological models for dose–response curves and CSAFs would need different gradations of intakes from those traditionally used for toxicologic studies.

In some instances, high nutrient intakes have already been associated with phenomena that correspond with stage 2 or 3 markers for adverse health effects. These are metabolic interactions among nutrients (for example, those among iron, zinc, and copper) in situations in which imbalanced intakes compromise the specificity of individual metabolic pathways [1].

The value of using markers in nutrient risk assessment would be enhanced if the totality of the evidence supporting the biological validity for each marker could be explicitly evaluated in the context of overall causation, incorporating the strength of the association; consistency across all lines of evidence; specificity; temporal relationship; a demonstrable relationship between intake and a functional or health effect response for the marker (i.e., for a homeostatic stage 2 marker, an indication of an adaptive phenomenon, rather than a linear response that might reflect exposure rather than a specific adaptive health effect [5]); and plausibility, coherence, and experimental support from other sources (e.g., animal models [17]). It is doubtful whether much information is available to support potential markers for nutrient risk assessment.

These principles are also relevant to the early detection of inadequate intakes. Some initiatives have considered whether it would be possible to have a common approach to assessing nutrient deficiency and excess. This has been explored for essential trace elements [2] and as a risk–benefit analysis for micronutrients in general [16]. Recently a human health dose–response risk assessment has been used to explore the dual response curve risk assessment for copper [11], and a spectrum from copper deficiency to copper toxicity has been compiled with the use of data from studies on humans and animal models. The exercise provided an opportunity to explore several approaches to dose– or intake–response modeling, including the benchmark dose and categorical regression. Existing data allowed for these approaches, but the development of a biologically based dose–response risk assessment and of CSAFs was limited by the quality and amount of the data [11]. Many of the individual studies that were reviewed during hazard identification and characterization in this exercise had been designed to demonstrate the effects of prolonged exposures to single measured and usually very high or very low concentrations of copper in diets. These studies were not designed to generate intake–response curves or to examine risk. Most just reported the copper contents of diets fed to animals and gave no indication of actual intakes; these had to be estimated, albeit imperfectly, from knowledge of animal weights and data from other reports on animal food consumption. After such reports were excluded during the systematic literature search, the residual database was very scant. This experience cautions against having high expectations of being able soon to address nutrient risk assessment through a biologically based response approach. The situation for amino acids, where systematic metabolic studies using tracers are improving the generic understanding of the application of kinetic and dynamic studies to homeostasis, may be more encouraging [3–5].

## Summary and conclusions

There is a need for a transparent model for nutrient risk assessment that would enable key elements of nutrient metabolism and function, and gastrointestinal and systemic adaptive phenomena in response to excess intakes (i.e., above the "physiological requirements"), to be identified and used as markers of excessive exposure. Such a biologically based dose–response model to determine ULs for nutrients could also be used to explore lower levels of intake and thereby enable the setting of lower levels of reference intakes.

Nutrient hazard identification and characterization is an iterative process. It needs to be supported by a complete compilation and review of the available literature and data, i.e., an evidence-based systematic review with predefined search and summary strategies and transparent criteria for rating, including and excluding individual studies and their data. As with risk assessment of non-nutrient chemicals, published systematic reviews may be useful. However, they should provide a means to access primary data and to rate their quality, and the bases of their systematization should not be allowed to prejudice the nutrient hazard identification and characterization. The critical intermediate outcome of this process is the agreed selection of an adverse health effect from which a UL, as a health-based guidance value, can be derived for the protection of the public's health. The approach proposed in this paper for identification of the adverse health effect, namely, the use of health effects and markers that occur relatively earlier or at lower intakes on the pathogenic response curve than classic toxicologically adverse effects, necessitates specific data search strategies that make greater use of ADME, biokinetic, and biodynamic data and that will probably need specific research. The advantage of using adverse health effects, or markers thereof, that occur at such lower intakes is that such research should be feasible in human participants. The disadvantages at the moment are the overall paucity of data, the nonsystematic and opportunistic nature of most of the relevant data, and the fact that most systematic data are derived from animal models.

The proposals outlined here are much more fully developed in the Report of the Joint FAO/WHO Technical Workshop [1], as are their implications for international nutrient risk assessment and health policy. The report highlighted five key issues: (1) Scientific judgment is a key aspect of nutrient risk assessment, and the bases for decision-making should be well documented to enhance its transparency. (2) Because data are limited, the model is designed to carefully take into account the identification and evaluation of data uncertainties so that users of the derived ULs would be unlikely to devise additional corrections. (3) Because nutrient risk managers need a UL, a UL should be set whenever possible, despite the limitations of the data; the risk assessor should be able to clarify the degree and nature of the uncertainty so that the risk manager can take this into account in decision-making. (4) The absence of evidence of an adverse health effect is not equivalent to evidence of the absence of an adverse health effect. (5) It is inappropriate to base conclusions on the risk or lack of risk for nutrients in risk assessment on the basis of studies that were designed for purposes other than assessing risk. Points 1 and 2 pick up on the benefit of a harmonized approach to use biologically based dose–response modeling in nutrient risk assessment. Point 3 is sensitive to the pressures on risk managers, who at least want a UL with a transparent and harmonized basis for expressing uncertainty; this would be achievable against a biologically based model. Point 4 is a universal truism that is too often overlooked, perhaps because of overdependence on studies such as those one is warned about in point 5. Collectively, these points show the potential of other agencies to further explore, develop, and apply the model advanced by the Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment [1].

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# Using criteria to establish nutrient intake values (NIVs)

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## Abstract

*One of the most important of the nutrient intake values (NIVs) is the average nutrient requirement (ANR). The ANR is defined as an intake value that will be adequate for half of the individuals in a group of people with similar characteristics. It is used to estimate the prevalence of adequacy, and it serves as the basis for the individual nutrient level ($INL_x$). The determination of adequacy is a complex process, with the resulting value of the ANR dependent on the criterion or functional outcome chosen to define nutrient adequacy. Because nutrients have multiple sites of action in human metabolism, it is possible to demonstrate abnormal function in one parameter measured or observed as a result of inadequate intake of a nutrient, while other parameters requiring the same nutrient appear normal or within normal ranges. Thus, depending on the criterion of adequacy selected, the requirement for a given nutrient may be at a lower or a higher intake amount. In harmonizing development of NIVs, it is important to clearly identify the criterion of adequacy selected and the rationale for its selection. Rarely are available data sufficient to provide dose–response information from which to select a level of intake at which half of the individuals demonstrate adequacy and half appear to demonstrate inadequacy. Three levels of intake, of which at least one level of intake is below the requirement for most of the individuals in the sample, and one level of intake is above their requirement, are useful for establishing a level at which half of the group might be considered to demonstrate adequacy. Types of human nutrient studies that may be used to obtain data are discussed, as well as characteristics of the sample size needed to demonstrate adequacy. The variation in requirements is also an important aspect in predicting levels of intake that will have defined probabilities of adequacy for groups (to develop the $INL_x$, where x is the defined probability chosen). An analysis of the origins of different types of variability is presented. When estimating energy requirements, a special case of NIVs, important issues must be considered. Additionally, an example of evaluating data used to establish an ANR for vitamin A, and the effect of variability in requirements for vitamin A, is provided.*

**Key words:** Average nutrient level, criterion of adequacy, nutrient requirements, sample size

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## Introduction

The framework for nutrient intake values, as discussed in this issue by King et al. [1], and the uses of quantitative nutrient standards, as discussed by Murphy and Vorster [2] and Vorster et al. [3], are well understood by those who work in nutrition policy and program areas; it is less well understood how human nutrition research regarding nutrients and bioactive food components is actually translated into establishing a nutrient intake value, and whether such a reference value is a level of intake thought to be adequate or a level thought to not increase the risks associated with excess intakes. This article outlines the steps in applying criteria to establish nutrient intake values, describes the importance of identifying and substantiating the criterion or criteria selected to establish adequacy, and reviews how data typically available are assembled and compared in the process of establishing nutrient intake values.

### Steps in applying criteria to establish nutrient requirements

There are four general steps to determining nutrient intake values (NIVs) based on scientific data where nutrient intake has been estimated and various data

have been gathered on responses to various levels of intake (see **box 1**). This paper covers the first two of these steps in detail.

## Step 1: Determine average nutrient requirement (ANR)

The first step in developing an NIV is to determine the average nutrient requirement (ANR) for the target population group. Although this sounds simple, it is very complex and is a critical step in developing usable nutrient intake values. It requires that the available data fit the model described by King et al. [1] and Murphy and Vorster [2] in this issue.

As described by King et al. [1], the model for setting NIVs has been adopted over the last two decades in whole or in part by a number of countries and regions for use in nutrition policy development and programs. The framework described in King et al. [1] proposes harmonizing the terms used for the various values as well as their definitions in order to move toward adoption, where appropriate, of common NIVs. The model requires the determination of an ANR for a defined subset of the apparently healthy population. From information that relates meeting an individual's requirement to the level of nutrient intake consumed, an individual nutrient level ($INL_x$) is derived that should meet the needs of a defined percentage (which is represented by the subunit x) of that subgroup in the population. More information on the model and its statistical basis and assumptions is provided by Murphy and Vorster [2].

If it were possible to know each person's usual requirement based on a chosen criterion for adequacy, it would be possible to identify an intake amount that is the *average* (*median)* requirement of the group of interest. With this information, and with information on each person's usual intake, it is also possible to use the ANR as the cutoff for the percentage of the population whose diet is inadequate for a given nutrient [5] in order to establish the prevalence of inadequacy for the population group of interest (see Murphy and Vorster [2] for more information).

BOX 1. Steps in applying criteria to establish an individual nutrient level (INL)

1. Determine average (median) nutrient requirements (ANR) for target population subgroup
   a. Evaluate possible requirement datasets
   b. Determine usable dataset based on chosen level of adequacy
2. Estimate variation in requirements in target population group
3. Increase the ANR by a factor based on the variation observed or assumed (e.g., 2 x $CV_{ANR}$) to obtain $INL_x$, the nutrient intake to be used as a goal for individuals (see Murphy and Vorster [2])
4. Determine the nutrient intake values (NIVs) for other population groups with missing data (see Atkinson and Koletzko [4] in this issue)

### Adequate for what?

A number of assumptions must be made when estimating an ANR. Since the ANR plays a key role both in assessing adequacy of the nutrient intakes of groups and in serving as the basis for a recommended nutrient intake for individuals ($INL_x$) (see Murphy and Vorster [2]), it becomes very important to carefully examine the data used to establish the ANR to ensure that the ANR is both applicable to the group and based on the best indicator of adequacy that can be applied.

The effects of inadequacy become apparent when diets are low in or lack a nutrient required for normal physiological function, or when dietary intakes result in increasing the risk of onset of a chronic disease such as cardiovascular disease; with greater deprivation, more serious effects are frequently demonstrated. Correspondingly, after a period of depletion, when small amounts of a nutrient are then provided, some functions may be restored while others remain abnormal and are not reversed. For many nutrients, this results in a continuum of responses observed as levels of intake increase, and may range from easily observed deficiency signs and symptoms when the diet lacks the nutrient (such as is seen in scurvy with chronic and severe lack of vitamin C) to subtle changes indicating suboptimal levels of intake, such as a decrease in the ability to respond to oxidative stress as measured by leukocyte ascorbate concentrations. **Table 1** provides a list of the types of research studies and criteria that can and have been used to determine what is "adequate" for various nutrients.

The possible types of criteria and their indicators can be grouped on the basis of their origins, as biochemical measures (e.g., red blood cell folate), physiological measures (e.g., blood pressure), functional measures (e.g., dark adaptation), equilibrium maintenance (e.g., factorial estimates of iron), disease incidence (e.g., cardiovascular disease), or animal models of inadequacy (e.g., hemolytic anemia in rats).

### Model indicators or criteria for adequacy

To serve as a model indicator or criterion of adequacy, an indicator should meet the following criteria:

» It can be measured without compromising the health or well-being of the individual (thus somewhat noninvasive, particularly for infants and children);
» It does not fluctuate rapidly or markedly when intake is increased or reduced, so that changes in it reflect the gradual nature of a change in availability of the

TABLE 1. Possible criteria that may serve as the basis for adequacy

| Type of study | Measurement | Examples[a] |
|---|---|---|
| Nutrition intervention studies (randomized, placebo-controlled studies) | Functional outcome | Calcium fracture rate with increased calcium intake via supplements or placebo |
| | Biochemical measurements | Red blood cell folate response to varying levels of folate |
| Depletion/repletion studies | Biochemical measurements | Leukocyte ascorbate concentrations for vitamin C<br>Urinary excretion of 4-pyridoxic acid for vitamin $B_6$ |
| Balance studies | Controlled intake and excretion | Protein requirements |
| Animal studies | Biochemical measurements | Vitamin E and red blood cell hemolysis |
| Factorial estimation | Measure losses + bioavailability | Iron requirements |
| Epidemiologic observational studies | Estimate intake and measure losses | Iodine intake and excretion |
| | Functional outcome | Vitamin A and night-blindness |
| Observed intakes in healthy populations | Dietary intake data | Vitamin K |

*a.* Source: Dietary reference intake reports [6–10].

nutrient at the cellular level;

» It has been validated as representative of the functional level present of the nutrient and not easily altered by other environmental changes, including the dietary content of other nutrients.

In the case of chronic disease relationships or developmental abnormalities, a surrogate marker of adequacy is useful because while the initiation of the disease process or abnormality may occur as a result of an alteration in nutrient intake (either not enough or too much) at an early age, the presence of the chronic disease may not be evident functionally for many years via observable clinical signs or symptoms. The surrogate marker, then, must be validated through strong correlation with both the dietary constituent and with subsequent development of the disease in order to be considered a true indicator of adequacy. The generally accepted example of a validated surrogate marker is serum cholesterol and subsequent development of cardiovascular disease.

None of the possible criteria used in establishing nutrient requirements and listed in **table 1** is considered a perfect example of a model indicator of adequacy, although some are better choices, when available, than others.

## Selecting criteria for setting nutrient standards

The values of the ANR and thus the $INL_x$ depend on which criterion is selected to establish adequacy. Nutrient adequacy is not well defined, and in some situations it may become a political decision. It is well known that 10 mg of vitamin C or less per day will prevent scurvy. If the criterion chosen for vitamin C *adequacy* is prevention of scurvy, then the ANR will be at a value less than 10 mg; however, if the criterion chosen is the amount of vitamin C at which ascorbate is first excreted in urine when increasing doses are consumed, assumed to represent a more complete saturation of body stores and thus a purported mechanism to handle oxidative stress [6], then the ANR will be at a much higher value. *It is thus possible to have multiple ANRs, each corresponding to a different indicator or criterion of adequacy* (**fig. 1**). It is then up to nutrition and public health policy planners to determine which level of adequacy is desirable or possibly attainable in the population group of interest. The choice of the criterion, assuming it has a strong scientific basis, is a policy–risk management decision.

## Steps in determining the ANR for a specific group

The applicability of the ANR to the population for which it is developed is improved if the data used meet certain standards. Some important factors are listed in **box 2**. Whatever data are finally selected, it is important to be transparent by identifying the data used, their source, and the rationale for their choice.

## Nutrient research methods that evaluate human nutrient needs

Methods used to establish nutrient requirements include those where specific biochemical measures or functional changes can be specifically attributed to the level of intake and followed as intake varies.

*Factorial estimation.* For many nutrients in normal metabolism, nutrient turnover occurs such that the amount of the nutrient (or its by-products) excreted roughly approximates the amount that needs to be

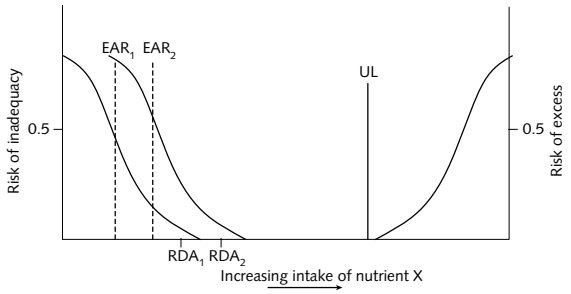


FIG. 1. Model for impact of different criteria used to establish adequacy. $EAR_1$ is the amount of a nutrient X needed that would result in a chosen criterion ($C_1$) to appear to be adequate—defined as within a normal range, functioning normally, or considered as an indicator of health—in half of the individuals in a subgroup consuming that level of the nutrient on a usual intake basis; at this level of intake, $C_2$ (a second criterion, which could also be chosen) would still be abnormal or not functioning normally and thus inadequate for almost all individuals. The level of the intake needed for $C_2$ to be normal in half the individuals in the group is $EAR_2$. Note the corresponding RDA (recommended dietary allowance) that would occur, assuming that the distribution of requirements for $C_1$ is the same as that for $C_2$. (EAR, estimated average requirement)

replaced. Typically, the amounts of a nutrient excreted via the urine, feces, and skin and by miscellaneous losses are measured, and they may be increased by an additional amount to take into account differences in assumed bioavailability (particularly for nutrients for which absorption varies greatly; see Gibson [11] in this issue) and any expected increase in metabolism thought to occur when utilizing a dietary source (the additional amounts needed to allow for nutrient absorption, excretion, etc.). These measurements may not be obtained from the same study or the same subjects, but they represent expected losses in general for the population group of interest. These factors are added together to estimate the ANR.

BOX 2. Factors important in determining the average nutrient requirement (ANR)

1. Dataset should be from a representative sample of the target group
2. Data should
   a. Be from the same individuals consuming a range of intakes
   b. Be an indicator of adequacy measured or evaluated that establishes a level of intake at which half of the individuals demonstrate abnormal responses or inadequacy
3. The sample size needs to be large enough to provide confidence in the data obtained
4. Data should be collected for a long enough period of time to include an estimate of intraindividual variation in requirement

*Nutrient balance studies.* Similar in many respects to the factorial approach, nutrient balance studies also involve collecting and measuring losses of a nutrient in individuals while carefully estimating nutrient intake. Although nutrient balance (nutrient intake = nutrient excreted) will occur at levels above the requirement after equilibrium (usually 5 to 6 days, depending on the nutrient under study), at levels below the individual's requirement metabolism continues to the extent possible in response to the partial lack of the substrate. This results in loss of body stores or breakdown of tissue to meet the more critical demands for the nutrient; the amount of the nutrient or its metabolic by-product excreted is more than the intake when inadequate amounts are consumed, resulting in a state known as negative balance. The lowest intake level at which balance occurs, in which the amount consumed is equivalent to the amount excreted, is used to determine an adequate intake.

With this type of experimental study, a group of similar subjects is initially adapted to a low level of the nutrient of interest while all other dietary components are provided at levels thought to be adequate. Graded levels of the nutrient are then provided (usually in random order) to determine the level of intake that provides a balance between excretion and intake. At this steady-state or equilibrium condition, the nutrient requirement is considered to be met. The most useful data from this approach are derived when the levels of intake tested are below and just above that required by the individual, and in which at least three levels of intake are tested; it is necessary that at least one level of intake be below the individual's requirement. The amount of the nutrient consumed can then be compared against the difference between intake and excretion. The data can form a regression equation that allows the determination of where equilibrium (intake = output) would occur for the group. This point of equilibrium represents the average requirement or ANR for the individuals included in the study.

This method, although it is frequently applied to nutrients such as trace elements for which metabolism does not alter the basic chemical identity, has significant limitations; considered a black-box approach, it is justly criticized for not taking into account or measuring the extent of metabolic adaptation required to maintain homeostasis occurring as the body maximally conserves the nutrient at marginal levels of intake to prevent the adverse effects of inadequacy. Newer methods, such as the use of isotopic tracers, have provided better and more in-depth understanding of changes in response to varying intake levels occurring, rather than solely depending on estimating nutrient balance.

*Depletion/repletion study.* Another similar metabolic study design is depletion/repletion. In this type of experimental study, all nutrients are provided in amounts thought to be adequate except for the nutri-

ent under study; a diet may have to be artificially constructed to attain a goal of negligible amounts of the study nutrient. The effects of the diet lacking the nutrient are followed in a group of subjects until observable signs or symptoms of inadequacy appear; early signs may be abnormal biochemical or neurological measures indicating abnormal metabolism. Once the effects of mild depletion are ascertained, the nutrient is given to the group of individuals under study in incremental amounts to see at what dose (intake) the abnormal signs or measures return to normal. With this type of study, it is possible to identify candidates for the appropriate indicator or criterion of adequacy, as well as see how responsive the criterion is to repletion of the nutrient. This type of study is useful for nutrients for which it is not possible to collect excretion products that would represent turnover of the nutrient.

*Animal studies*. For a few nutrients, there may be few quantitative data available that relate dietary intake in human subjects to specific functional outcomes, whereas significant data from animal models may be available. Such data are particularly useful in establishing the probable mechanism of action and resulting adverse effects that will be experienced in humans as a result of inadequacy; however, without human correlates, such data have not been considered sufficient to define a quantitative relationship between intake and a noted criterion of adequacy. Because evidence of inadequacy for such a nutrient in humans consuming typical diets is lacking, there has been a hesitation to use animal studies to establish quantitative reference intakes. This is the case for nutrients such as silicon and possibly boron in the dietary reference intake (DRI) reviews [7]; animal models have been used to identify the essential role of a nutrient in metabolism for one or more species, but the lack of demonstrated deficiency or inadequacy in humans due to the ubiquitous nature of the nutrient has precluded the establishment of a reference standard for adequacy by extrapolation to estimate human requirements. However, in the case of potential adverse effects due to overconsumption, animal data have been used when human data are not available, with appropriate uncertainty factors to extrapolate from animals to humans (see Aggett [12] in this issue).

*Clinical signs and symptoms.* Functional outcomes of inadequacy of a nutrient, such as a deficiency disease like scurvy, are easy to detect at advanced stages and even at early stages if they present with unique and well-known symptoms; such symptoms can be considered good indicators of adequacy, since symptoms such as bleeding gums and loose teeth are observable and taken together are not associated with other nutrient deficiencies. Other functional outcomes and their relationship to nutrients are less well understood and characterized, such as risk of fracture and calcium intake or potassium intake and hypertension. Although there is a relationship, it is not well understood due either to lack of data or to multiple causative factors that prevent the demonstration of a direct dose–response relationship between intake and outcome.

Although the most valuable endpoints may be observable events, such as fracture, stroke, or myocardial infarction, studies of this type can take much longer and require many more subjects than studies in which surrogate markers are evaluated. The use of biomarkers or surrogate markers must be validated as clinically useful [13], in which mechanisms of action are established that support the relationship between the nutrient and the functional outcome [14].

*Epidemiologic associations*. In 1971 Hill identified six important factors useful in establishing relationships between diet and disease [15] (**table 2**); although they have been modified slightly by others, they continue to aptly describe the required components of the relationship desired between a nutrient or bioactive food component and functional or disease outcome. Although they were developed for epidemiologic associations to assist in determining causation, they are equally applicable to experimental studies in which outcomes are evaluated when the level of nutrients is varied in diets. This type of information is particularly useful in evaluating the role of diet in chronic disease onset.

*Observations in healthy populations.* Nutrient intakes of populations in which individuals show no signs, symptoms, or indicators of inadequacy can also be used as the basis for setting nutrient standards; the nutrient intakes of a representative sample of individuals in the population are estimated and then assumed to be typical of all those in the population. Since there is no evidence of inadequacy, the amount consumed on average is assumed to be adequate for all. This is the basis for establishing most of the default recommended intake values in the DRI process, termed adequate intakes (AIs), a recommended intake reference standard used when it was not possible to determine a level at which half of a population group would have their needs met [7].

It is possible to set a nutrient reference standard using observational information from a representative sample of a healthy population group if the following conditions are met:

» There is a comprehensive food and supplement database that includes the nutrient content of all the

TABLE 2. Diet and disease relationships

| |
|---|
| Strength of association |
| Dose–response relationship |
| Temporally correct association |
| Consistency of association |
| Specificity of association |
| Biological plausibility |

Source: Hill [15].

foods and supplements consumed by the population of interest;

» Very accurate intake data are captured for a long enough period of time to make the sample of days truly represent *usual* intake. For some nutrients, such as vitamin A, for which most of the nutrient intake comes from a few specific foods, the variation in daily intake is quite large [5].

*Intervention trials*. Double-blind, randomized trials of nutrient requirements are considered by many to be of major importance in ascertaining individual nutrient requirements. To be most useful, this type of study provides three or more test levels of a nutrient in a typical diet to a group of subjects in a random or varied order; randomization avoids bias due to time (season) or duration of the study, which can be confounding variables. Keeping the study double-blinded, i.e., neither the study volunteers nor the personnel administering the diet know which level of intake is being provided, prevents both the subjects' and the researchers' possible expectations about requirements from biasing the outcomes.

*Lack of dose–response data directly linking dietary intake with measured response.* For many nutrients, few data are available that systematically tie the level of nutrient intake to an accepted and validated quantitative measure or metabolic criterion of adequacy. In these cases, other data described above have been used to establish nutrient standards that are associated with and thus imply adequacy. These include data from nutrient balance studies, epidemiologic observations of the prevalence of disease associated with lack of the nutrient, and intakes of apparently healthy population groups. Dose–response data may not be available for many important nutrients; in these situations, a default INL such as the AI in the DRI process is provided; this default value, given a different name, represents a judgment of adequacy, and is not tied to an ANR.

Frequently, data from other population groups (perhaps from other countries or cultures) can be used if the ANR is based on metabolic requirements, and adjustments made to take into account known differences in utilization (perhaps due to energy or climatic conditions) or known differences in metabolism (such as is frequently done when establishing ANRs for older age groups or for women when only data from men are available).

## Step 2: Estimate the variation in requirements

Although much effort must be put into deciding on the best criterion or indicator of adequacy to use (the one that is most reflective of meeting nutrient needs), it is also necessary to estimate the variation in requirements for the indicator chosen in each subgroup of the population in order to develop goals for intake—a frequent policy and program need (see Vorster et al. [3] in this issue). This is an even more difficult task, and statistically small errors in its estimation can lead to great uncertainty in the resulting $INL_x$.

Responses of individuals to three or more levels of intake (the dose–response) in the chosen indicators or criteria of adequacy are evaluated to determine which is the lowest level of intake that corresponds to the indicator or criterion of adequacy being classified as "within the normal range" for each individual. The ANR is identified as the level of intake at which half of the individuals tested or observed are in the normal range and half are still in the abnormal range for the selected criterion of adequacy. The variation in this response, the standard deviation of the ANR ($SD_{ANR}$), represents the variation in requirements seen in the sample of the group evaluated at the multiple levels of intake. As described in Murphy and Vorster [2] in this issue, the $SD_{ANR}$ is used to increase the ANR to obtain the $INL_x$ at a desired level of coverage in the population group (where x = percentage of the population group covered).

### Factors that affect variation in requirements

*How many should be sampled?* The type of experiment that is most useful in determining requirements depends on the nutrient of concern and how it is related to health, the methods available to determine its adequacy, and the resources that can be allocated to its evaluation. Although it would be ideal to establish an ANR based on requirement data obtained from each person in the population group of interest, this clearly is not feasible or really necessary. Appropriate sampling of the population, or of a very similar population, can provide data that are applicable to and descriptive of the overall group's requirements. What sample size, then, is necessary to establish a nutrient intake value for adequacy?

The size of the sample needed to estimate nutrient requirements depends on the type of study contemplated and its study design.

*Value based on epidemiologic evidence of adequacy.* Epidemiologic studies that relate nutrient intake to associated behaviors or markers of adequacy in which there is little variability in the distribution of nutrient intakes result in little variation in the risk of inadequacy within the population, and therefore more individuals (a larger sample size) are needed to detect a significant association between intake and risk of disease.

For example, in situations demonstrated by **figure 2**, where there is less variation in intake than in requirements, it is not possible to use the ANR to estimate the prevalence of inadequacy in this group, since one of the statistical assumptions for such a use of the ANR as a cutoff for prevalence of adequacy is that the variation in

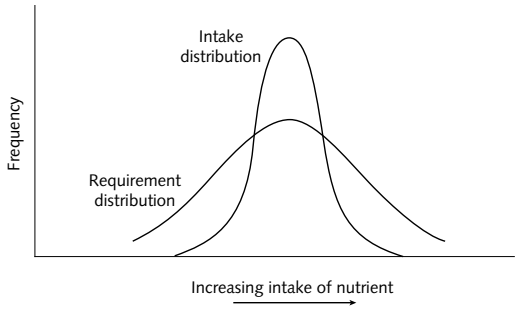


FIG. 2. Situation in which the ANR cannot be used to estimate prevalence of inadequacy. When the variability in the usual intakes of a group is less than the variability in requirements for a nutrient, the cut-point method, using the ANR to estimate the prevalence of inadequacy, cannot be applied. Other methods to estimate prevalence of inadequacy can be employed [5]. In order to detect rare but widely diverse requirements in a given population, much larger sample sizes are needed. (ANR, average nutrient requirement)

intake is greater than the variation in requirements (see Murphy and Vorster [2] in this issue). Low variability in intake therefore leads to the need for a larger sample size [16] in order to obtain good estimates of the true variation that exists within the population.

This need for very large sample sizes to determine normal small variation in responses and to differentiate normal variation from abnormal deviation due to differences in intake may be demonstrated when diet and disease relationships are observed in large population groups but are not subsequently statistically supported in clinical trials. Frequently such trials are said to be "underpowered," since the sample size is not adequate to demonstrate a relatively rare effect that, although it may occur, will not show up in a random selection of a small group of individuals (the group sampled) to the extent that it would be considered as not occurring by chance. This has been given as a major reason that relationships evaluated in intervention trials of different dietary patterns and the subsequent incidence of cancers are less than conclusive, although large sets of observational data from population groups appear to demonstrate significant associations [13].

When epidemiologic information is used as the basis for determination of nutrient adequacy, the data or criteria chosen must be representative of the group of interest and adequately powered (by a large enough sample size) to detect relatively small differences in response (**table 3**). In order to have adequate power ($1 - \beta$) when small changes in chronic disease outcomes due to diet are evaluated, sample sizes need to be large.

*Values based on clinical trials or intervention studies.* Statistics are available to estimate the sample size necessary to determine the ANR as well as the $SD_{ANR}$ in a population group of interest. Such methods require

- Specifying the minimum difference needed for a clinically relevant or important indicator of adequacy ($\mu_1 - \mu_0$);
- Determining the level of confidence ($1 - \alpha$) that the difference should represent between the response due to the variable tested versus a random occurrence;
- The probability ($1 - \beta$) that if the specified minimum difference is not attained, then there truly is not a meaningful difference in response; and
- An estimate of the probable variance ($\sigma_T$) in the overall population.

If all these are determined, are available, or can be estimated, then this general formula can be applied to obtain the relevant sample size:

$$n = [2\,(t_\alpha + t_\beta)\,\sigma/(\mu_1 - \mu_0)]^2.$$

Although components such as $(\mu_1 - \mu_0)$, $\alpha$, and $\beta$ can be set or defined by the researcher, the expected variance ($\sigma$) of the population can only be estimated, and this is not always possible to do well. A possible source of estimation is from other similar studies or previous work.

Given the typical lack of data on human nutrient requirements that include usable estimates of variation, pilot studies or validation studies are frequently conducted prior to initiating expensive larger studies to get an idea of the variation in response present, thus providing an estimate of $\sigma_T$. The costs of such pilot studies may make it impossible, though, to get estimates of the variation expected for use in determining the needed sample size. However, without determining the size of the sample needed to adequately evaluate a requirement, the study may be underpowered and not provide meaningful conclusions.

Many studies of nutrient requirements must limit the number of subjects studied because of limitations in space, the number of samples, and the measurements that can be analyzed with available funds. The goal when testing multiple levels of intake is to see a nutritionally relevant difference in response; often this is not specifically set in advance, with the assumption that the statistical tests performed will indicate what is statistically significant. It is then up to the researcher

TABLE 3. Resulting $\beta$ values for various sample sizes[a]

| N | 100 | 200 | 300 | 400 | 500 | 600 | 700 |
|---|---|---|---|---|---|---|---|
| $\beta$ | 0.71 | 0.48 | 0.31 | 0.19 | 0.11 | 0.06 | 0.04 |

*a.* Example for a given value of $\alpha$ (0.05), predicted mean (0.2), and clinically significant difference of 50% (0.10), $\beta$ values are the probability of making a false negative conclusion (type II error).
Source: Matthews and Farewell [17].

in analyzing the data to identify among statistically significant differences what is nutritionally relevant. For example, this difference might be the reduction in rate of fracture compared at each level of calcium intake. The null hypothesis, that there will be no difference, must be rejected if a difference is demonstrated in one or more treatments.

**Table 4** provides the theoretical framework of sample size calculations for correct and erroneous conclusions and the resulting probabilities of correct and erroneous conclusions. In two of the four cases identified in **table 4**, the researcher reaches a correct conclusion. However, in the remaining two cases, the researcher is wrong, having reached either a false positive or a false negative conclusion. A larger sample will provide more information about the response to the level tested and hence lead to a more precise estimate of the average requirement of the study population. Therefore, by increasing the sample size, the ability to detect any real difference in responses to the level of intake is increased. Since $\alpha$ and $\beta$ both represent probabilities of making an erroneous decision, in the best of all possible worlds the closer each is to zero, the better. However, if $\alpha$ is decreased (from, say, 0.05 to 0.01) without changing the total sample size, *n*, then $\beta$ will necessarily increase (meaning that there is a greater chance of accepting the null hypothesis where there is truly a difference). Conversely, if $\beta$ must decrease without changing *n*, then $\alpha$ necessarily increases. Only by increasing the sample size can a simultaneous reduction in both $\alpha$ and $\beta$ be achieved [17].

Other factors also influence the determination of an adequate sample size. They include the relative sizes of the treatment groups, possible dropout rates in these groups, and rates of treatment noncompliance.

*Variability in requirements.* The variation measured in studies where multiple levels of intake are tested and the chosen criteria or indicators of adequacy are measured is the combined result of the following:

» Biological differences in response to the level provided between individuals in the sample studied—termed interindividual variation;
» Day-to-day variation in the response by a given individual due to differences in that person's metabolism, activity, etc. on one day compared with another, which cause one day's results to differ from another with no definable difference in diet—termed intraindividual variation;
» Possible variation due to environmental factors that may be different for different individuals in the sample, depending on the extent that they are controlled for in the study;
» Variation in the ability to accurately measure or estimate the criterion or indicator used—termed measurement error.

In order to compensate for the very small sample sizes characteristic of most human experimental studies, well-controlled studies evaluating nutrient requirements are designed to minimize as many of these sources of variance as possible, with the exception of the variance resulting from biological differences. This then provides a more robust estimate of the variation due to differences in the actual requirements within a population group rather than to extraneous factors.

In spite of the desirability of the inclusion in nutrient requirement studies of methods to control for other sources of variation, because of cost and availability of resources small sample sizes in the range of 6 to 12 predominate, resulting in uncomfortably large variance in responses ($SD_{ANR}$). Since NIVs are also to provide guidance for use with individuals about an appropriate goal for intake, a recommended intake (such as the $INL_x$, or the RDA [recommended dietary allowance] or AI in the DRI series) is provided. However, in the DRI process, when the variation in response was inaccurately broad and an estimate of the variability in requirements was needed to establish the RDA, a default coefficient of variation ($CV_{ANR}$) (Murphy and Vorster [2]) of 10% was used.

Although the 10% value was used for many nutrients following its initial use with the first group of nutrients reviewed [8], to date the coefficient of variation of 10% has not been examined in any specific way in validation studies to ascertain its closeness to variability in requirements for the nutrients whose RDAs were developed using the default coefficient of variance (vitamin C, vitamin E, thiamin, riboflavin, vitamin $B_6$, folate, vitamin $B_{12}$, copper, magnesium, phosphorus, selenium, and zinc [6, 7, 9]). Developing consensus for the scientific basis for the development and application of such default assumptions is an important issue in moving toward harmonizing reference standards.

TABLE 4. Validity of conclusions: role of size of $\alpha$ and $\beta$

| | Conclusions of study | |
|---|---|---|
| True situation | $H_o$ true | $H_o$ false |
| Conclusions are | | |
| $H_o$ true | Correct | False positive (type I error) |
| $H_o$ false | False negative (type II error) | Correct |
| Probability of conclusions | | |
| $H_o$ true | $1 - \alpha$ | $\alpha$ |
| $H_o$ false | $\beta$ | $1 - \beta$ |

Source: Matthews and Farewell [17].

## Other factors in experimental design

### *Number of treatments*

The number of levels of intake tested in a nutrient intervention or balance study also affects the ability to estimate requirements. Many studies may include two

levels of intake, comparing responses to determine which results in a more "adequate" diet relative to the criterion or indicator of adequacy chosen. Although it is possible to compare two levels of intake, given the types of variation that are independent of the level of nutrient consumed (see variability in requirements section, above), at least three levels of intake of a nutrient should be evaluated in the same individual.

Data can then be evaluated for the parameters that best fit the data [18]; for example, two models currently used for individual amino acid requirements include fitting a smooth regression curve and estimating where it intersects the zero-balance line [19], and fitting a regression curve with an inflection point and estimating where that point lies [20]. Unfortunately, other than protein [21] and some of the amino acids [22], there are few nutrients for which such data are available for analysis in this way.

In evaluating protein requirements for children in the DRI process [10], it was necessary to group data for those aged 9 months through 14 years, since only seven studies could be identified in which individual data were provided and that could be used to obtain a regression curve. These studies were published from 1980 through 1992, and no studies published since then were available. This analysis included a total of 53 subjects from the seven studies from which data on multiple levels were available, plus an additional three studies with a total of 42 subjects for whom only group data were provided. It is of interest that only 16 of the total of 95 children were over the age of 5 years, and almost all were boys (based on the data in the individual publications included, the number of girls could have been as high as 11 or as low as 2). The diets tested in these studies were not standardized:Some were animal-based (milk, egg), some were mixed (rice and fish; beans, wheat, milk), and some were vegetable (soybeans, rice and beans), and they came from geographically diverse areas: China, Chile, Guatemala, and the Philippines. Differentiation between boys and girls, particularly during puberty, was only possible relative to the amount of protein estimated for growth, and not for maintenance [10].

Protein is one of the most studied nutrients, given its importance in normal growth in infants and young children and its frequent lack in the traditional diets of many developing countries. The above example demonstrates that, in spite of this, useful data to obtain requirement estimates, let alone estimates of the amount needed for growth in children, are woefully inadequate.

#### *Publication bias*

Although estimates of human requirements are based on published data, among the assumptions made in reviewing such studies is that the published literature reflects all the data that are worth reviewing. Particularly where equivocal results occur, the tendency of journals to publish papers only if the results reported are significant at the conventional level of $p < .05$ [22] means that there may be a tendency of journals to reject research studies as underpowered when differences do not appear statistically significant. Meta-analyses of studies related to cancer that have been published versus all studies related to cancer have demonstrated a preference for reporting only endpoints that produce the best results [23]; whether this is true of nutrient requirements is unknown.

#### *Nutrient–nutrient interactions*

Although it is not usually evaluated in carefully controlled double-blind studies, the influence of other nutrients on absorption and metabolism of the nutrient under study is an important factor to consider in evaluating data from different studies. Bioavailability issues (see Gibson [11]) can also influence the data and need to be carefully considered.

## Energy requirements

As briefly described by Murphy and Vorster [2] in this issue, estimating energy requirements represents a special situation when NIVs are being developed. Unlike the case with other nutrients, the adequacy (and excess) of energy needs are readily assessed by individuals from changes in body weight. Also unlike the case with other nutrients, physiological mechanisms provide immediate information to individuals that intake should be increased or curtailed; thus, energy intake is not independent of energy requirements, one of the statistical assumptions upon which the use of the ANR is based (see Murphy and Vorster [2]). Since estimates of energy requirements are needed for program development and for planning diets, somewhat different steps are followed both to estimate energy requirements and to then apply them to individuals and groups.

### Energy requirement criteria

As with nutrients, the body can metabolically adapt to lower levels of energy intake on a chronic basis. This has been studied recently in some detail [24]. The definition accepted by international working groups of the amount needed to meet energy requirements can be used as defining what is "adequate" for humans: the amount needed to balance energy expenditure in order to maintain a body size, body composition, and level of physical activity consistent with good health [24]. This includes the energy needed for optimal growth and development in children, deposition of tissue during pregnancy, and normal lactation.

The recommended levels of energy intake for individuals are the levels that provide adequate energy to

each person in a population group. With the growing problem of obesity and overweight, it is more difficult to determine what the mean energy intake of a group should be to meet the requirements of all members of the group, yet not result in overconsumption by some. Unlike the use of average population intakes for nutrients in apparently healthy populations as surrogates for an $INL_x$ where dose–response data are not available, average energy intakes cannot be used, as most populations in both developed and developing countries are now experiencing adverse effects associated with excess consumption of energy, inadequate physical activity, or both.

### Methods of estimating energy requirements

Similar to nutrients, energy requirements measured in individuals with similar characteristics, such as age, body size, sex, and physical activity levels, vary; the variation seen in observed requirements assumes a normal distribution. When energy expenditure over time (e.g., 2 to 3 weeks) is measured carefully in a sample of individuals whose characteristics are known, it is possible to develop estimates of energy requirements for the group, along with a measure of the variance in those requirements among the individuals in the sample. These requirement distributions have been used to predict the requirements for other individuals with similar characteristics. One of the advances in the ability to develop good predictions of energy requirements is the ability now to use tracer methodology, such as doubly labeled water, to obtain independent estimates of energy expenditure without perturbing the environment of the individual [10]. Older methods estimated expenditure indirectly (e.g., metabolic hoods) or estimated food intake and derived the amount of energy consumed, equating it with the amount expended.

#### *Components of energy requirements*

As outlined in the definition of energy requirements, the energy required for five general components makes up the total human energy requirement: basal metabolism, metabolic response to food, physical activity, growth, and needs during pregnancy and lactation. Early studies of energy requirements, in which the method used to estimate energy expenditure itself influenced the energy requirement, often involved estimating each of these individually and summing the total to get the total energy required. With the advent of doubly labeled water, prediction equations that take into account all of these energy components have been developed. Recently, doubly labeled water has been used as well to validate other methods to estimate energy requirements that are less expensive and more adaptable to different settings, such as heart-rate monitoring [24].

Equations that are derived from these data depend on the accuracy of the method employed and the extent to which variables in energy expenditure are included as identified factors that influence energy requirements. Sex, age, body weight (or body mass index [BMI]), and level and types of physical activity have been found to have the greatest effect on total energy requirements [10, 24].

Prediction equations now available are useful as initial estimates of energy requirements for other similar population groups, assuming that the information about the population subgroup (i.e., age, body weight [BMI], and activity level) is known. Prediction (regression) equations developed to date have estimates of variation as well, and these provide the ranges (representing expected variation) that would be predicted in actual requirements as compared with the point estimates obtained from the regression equations [10, 24].

#### *Use of prediction equations as NIVs*

Tables have been constructed that translate the prediction equations into estimates of energy requirements by age, sex, physical activity, and/or body weight; these can be applied to other population groups after adjustment for known differences in physical activity, body size, and other variables that may not be included in the tables. Information on how best to use the data and information is provided in the reports [10, 24] that have developed the prediction equations and should be used as a starting point for group-specific estimates of energy requirements.

## Summary

In establishing nutrient intake values (NIVs) to meet requirements (and usually for excess intakes), it is important to specify the criterion or indicator of adequacy or excess that is selected and to be transparent in the dataset used to set the NIV. For almost all nutrients studied to date, there exists a continuum of definable functions, most of which are impaired at low levels of intake, whereas at high levels of intake all appear to function normally or to be within the normal range. Choice of the indicator will dictate the amount of the nutrient that meets an individual's requirement, as defined by that indicator. Datasets from dose–response studies in which nutrients at multiple levels (at least three) are given to the same individuals and in which at least one level results in measured abnormal responses or functions are important to serve as the basis for determining average nutrient requirements (ANRs). Of equal if not greater importance is the distribution of requirements in a group; variation in requirements can be due to differences in metabolism, but also to differences in environment and diet. A key component

in evaluating data of potential use is the sample size needed to provide a high degree of confidence in the level of intake determined to be required. In moving toward harmonizing NIVs, consensus and agreement on what data should be used to establish NIVs (which of the possible criteria should be chosen, what datasets are the most appropriate, and how best to adjust when data are missing) represent a major obstacle to overcome.

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# *Annex*

## Example of deriving an average nutrient requirement (ANR): vitamin A

Vitamin A functions in many biological systems: 11-*cis*-retinal, the active aldehyde form of vitamin A, is part of rhodopsin, required for vision in the brain, for normal growth and maintenance of the cornea and conjunctival membranes of the eye (to prevent xerophthalmia), and as a regulator of genes that form structural proteins, enzymes, and retinol-binding proteins in the blood, including those involved in normal embryonic development and immune function related to cytokines and T and B lymphocyte production [7]. It is not surprising that some of these functions continue normally at comparatively low levels of intake, while others are depressed or inactive, resulting in detectable changes in indicators that denote vitamin A inadequacy.

In selecting the indicators or functional outcomes to use to establish an ANR and an $NIV_x$ for vitamin A, a number of choices are available (see Annex **table 1**). In reviewing research and studies available in the year 2000, the DRI process selected as the main criterion to establish adequacy for vitamin A in Canada and the United States *adequate liver stores*, with adequacy defined as at least 20 µg retinol/g liver tissue. This resulted in an EAR (estimated average requirement, equivalent to an ANR) of 625 µg/day for men and 500 µg/day for women 19 years of age and older [7].

However, it is recognized that significant liver retinol stores are not required for normal function. Thus, dark adaptation was identified as the most sensitive criterion for which adequate data were available to establish an average requirement. There were data supporting the ability of dark adaptation to identify individuals with inadequate intakes of vitamin A, and there were data from individuals inadequate in vitamin A whose dark adaptation was measured and who then were given various levels of vitamin A and underwent additional dark adaptation tests to ascertain at what level of intake the function returned to normal. The level at which dark adaptation returned to normal (defined as at least $-4.6 \pm 0.3$ log candela/$m^2$) in half of those studied (while dark adaptation in the other half remained abnormal) was 300 µg/day. This could be considered the ANR for vitamin A *based on dark adaptation*. One of the primary limitations of selecting this criterion was that there were only 13 individuals studied in this way.

According to this example, when the selected criterion or indicator of adequacy is dark adaptation, the ANR is 300 µg; this is less than half the amount identified if liver stores at the level of 20 µg/g are chosen (650 µg) [7]. This demonstrates that the important question that must be asked in establishing nutrient requirements, and a potential barrier to harmonizing such

TABLE 1. Possible indicators for evaluating vitamin A adequacy

| Function | Test | Definition of adequacy |
|---|---|---|
| Dark adaptation | Dark adaptometry: final dark-adapted threshold after 35–40 min | $\geq -4.6 \pm 0.3$ log candela/$m^2$ |
| | Pupillary response test | A high threshold in response to incremental pulses of light = low retinal sensitivity due to vitamin A deficiency |
| Circulating levels of vitamin A | Serum or plasma retinol concentration; retinol-binding protein concentration | Considered insensitive indicators and thus not used |
| Total liver reserves (TLR) | Isotope dilution using TLR = F × dose × [(H:D) – 1]; F = efficiency of storage of early administered dose; dose = dose of labeled retinol; H:D = ratio of H to $^2$H-retinol in plasma after equilibration | TLR ≥ 20 µg/g retinol |
| Relative dose–response (RDR) | Change in plasma retinol concentration in response to a test dose after 5 h | RDR < 20% |
| Modified relative dose–response (MRDR) | Amount of test dose vitamin $A_2$ (dehydroretinol) appearing in plasma after 5 h | MRDR < 20% |
| Conjunctival impression cytology (CIC) | Microscopic examination of periodic acid Schiff–hematoxylin-stained epithelial cells | Lack of abnormal cells |
| Immune function | Humoral antibody responses and cell-mediated immunity | Normal number of natural killer cells and normal cytotoxic activity |

Source: Institute of Medicine [7].

requirements, is "adequate for what?" What criterion or outcome is selected?

Given the small number of subjects (13) whose data were used in the DRI process to determine the EAR for dark adaptation for vitamin A, it was also difficult to estimate the variation in requirements based on this criterion. Therefore, other data were used—the half-life of labeled vitamin A in individuals with the first clinical signs of vitamin A deficiency—to determine the variance to use for vitamin A. This half-life was estimated to have a coefficient of variation of 21%. Thus, 20% was used in establishing the RDA for vitamin A from the EAR, resulting in an RDA of 900 μg/day (650 μg + 2 × 20% = 900 μg) [7].

The RDA might well have been 420 μg (300 μg + 2 × 20%) if the criterion chosen as the basis for the recommendation had been prevention of night-blindness. This becomes a risk management question for policy makers: given the continuum of nutrient-related functions that demonstrate increasingly severe symptoms or functioning with decreasing intake, what should be the achievable goal? In the DRI process for Canada and the United States, a small amount of liver storage of vitamin A was selected.

# Methods for using nutrient intake values (NIVs) to assess or plan nutrient intakes

Suzanne P. Murphy and Hester H. Vorster

## Abstract

*This article describes the methods for using nutrient intake values (NIVs) to plan and assess intakes of both individuals and population groups. The advantages of the more recent standards, which use an average nutrient requirement (ANR) and its standard deviation to describe the distribution of nutrient requirements, are highlighted. The goal of assessing the intake of an individual is to determine the probability that the person's usual diet is meeting his or her nutrient needs and whether the person is at risk for adverse effects from excessive intakes, whereas the goal of planning an individual's intake is to ensure that the probability of inadequate intake and the likelihood of excessive intake are both small. The goal of assessing intakes of groups is to determine the prevalence of inadequate intakes and the prevalence of potentially excessive intakes, whereas the goal of planning nutrient intakes for groups is to minimize the prevalence of inadequate intakes and also to minimize the prevalence of potentially excessive intakes. For all of these goals, it is important to utilize appropriate food-composition tables and accurate dietary assessment methods. To fully utilize the new paradigm, it will be necessary for the professional nutrition community to identify ways to implement these new procedures in nutrition research and nutrition programs, to describe the strengths and weaknesses of the results, and to contribute to the evolution of both the theory and the application of the NIVs when planning and assessing diets.*

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**Key words:** Assessing diets, average nutrient requirement, individual nutrient level, nutrient intake values, planning diets, upper nutrient level

## Introduction

In the last 20 years, new knowledge and approaches have led to many new ways to use nutrient intake values (NIVs). The purpose of this article is to describe the appropriate uses of nutrient-based dietary recommendations and to illustrate how they can be implemented for both individuals and population groups. The concepts and terminology for NIVs as described by King et al. [1] in this issue will be used: the average nutrient requirement (ANR), the individual nutrient level ($INL_x$), and the upper nutrient level (UNL).

The uses of NIVs fall into two broad categories: they may be used to *assess* dietary intakes and to *plan* dietary intakes [2, 3]. Assessment involves determining the probable adequacy or inadequacy of intakes for individuals and the prevalence of adequate (or inadequate) intakes for population groups. Planning involves setting intake targets for individuals and determining desirable intake distributions for groups. Assessment and planning are often used iteratively, first to assess a nutritional situation, then to plan an intervention to improve the situation, and finally, to assess the impact of the intervention. Several hypothetical examples of the correct uses of NIVs are given at the end of this article.

There are many specific applications that involve either assessing or planning diets. Assessment applications include evaluating the adequacy of a person's diet and determining the prevalence of inadequacy using national surveys. Planning applications are numerous and include dietary counseling and the design of feeding programs. Vorster et al. [4] in this issue discuss these applications in more detail.

## A new paradigm for calculating the probability of nutrient adequacy

Because the ANR and its standard deviation describe a distribution of requirements, it is possible to calculate the *probability* that usual long-term intake at a given level is adequate. Such probability estimates require knowledge of the distribution of requirements and are not possible if the requirement is stated as a single number. The calculation is based on the assumption that intake is independent of requirement. That is, a person with a higher requirement for a nutrient does not necessarily select a diet that is higher in the nutrient. This assumption is clearly violated for energy intake, but there is no persuasive evidence that intakes are related to requirements for vitamins and minerals.

On the assumption of independence of intake and requirement, the probability that a person's usual intake is adequate can be statistically determined from the distribution of requirements that is described by the ANR and its variation (**fig. 1**). For example, if a person's usual intake is equal to the ANR, the probability of adequacy (using the chosen criterion of adequacy) is, by definition, 50%, because it meets the needs of half of all the people who were studied. If a person's usual intake is equal to the ANR plus 2 SD of the requirement, the probability of adequacy is about 98%, because only 2% of individuals in the group would have a higher requirement. If an individual's usual intake is equal to the ANR minus 2 SD, then the probability of inadequacy is only 2%, because almost everyone would have a higher requirement. A simple statistical algorithm can calculate the probability of adequacy for any given intake based on the ANR and its distribution (by calculating the area under the requirement curve, such as the one in **figure 1**, which is to the left of the intake value) [5]. Note that it is possible to calculate either the probability of adequacy (the area to the left of the intake value) or the probability of inadequacy (the area to the right of the intake value). The probability of inadequacy is 100 minus the probability of adequacy. For a group of individuals, the prevalence of adequacy (or inadequacy) can be estimated as the average probability of adequacy (or inadequacy) within the group.

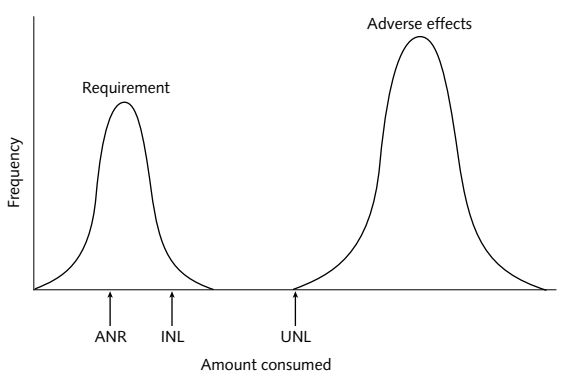


FIG. 1. Graph of a hypothetical distribution of nutrient requirements and adverse effects as the amount of a nutrient consumed increases

Although it is necessary to know (or estimate) the distribution of requirements to calculate the probability of adequacy (for individuals) or the prevalence of adequacy (for groups), requirements do not have to be normally distributed. For example, iron requirements for menstruating women are skewed, but a requirement distribution can be described in a tabular format, as was done for the dietary reference intakes (DRIs) for the United States and Canada [6]. For such nutrients, an individual's probability of adequacy can be determined simply by locating the correct table (for the person's age and sex) and selecting the probability of adequacy that corresponds to the person's usual intake. However, as discussed in more detail below, a shortcut to the probability approach for estimating the prevalence of adequacy for groups (the "cutpoint" method) cannot be used if the requirement distribution is not symmetrical.

## Using NIVs for assessment and planning for individuals

### Assessing nutrient intakes of individuals

#### *Goal*

The goal of assessing the intake of an individual is to determine the probability that the person's usual diet is meeting his or her nutrient needs and whether the person is potentially at risk for adverse effects from excessive intake [2, 7, 8]. However, it is difficult to know if an individual's nutrient intake is adequate, because the person's actual nutrient requirements are usually unknown, and an accurate measure of the person's usual, long-term nutrient intake is almost never available. Although the probability of adequacy can be calculated, as described above, the result is only meaningful if the usual nutrient intake for a person is known. Because of day-to-day variation in intakes, it is usually necessary to observe a person's diet over many days when an accurate estimate of usual intake is needed. This is seldom feasible, so calculation of the probability of inadequacy for an individual may not be meaningful. Indeed, it may be more appropriate to monitor physiological measures (such as blood markers of anemia) rather than rely on intakes that were observed for a small number of days.

#### *Calculating the confidence of adequacy*

Another approach to assessing an individual's intake is to calculate the *confidence* that the usual intake is adequate, which considers the number of days on which the intake was observed, as well as how far the

observed intake is above (or below) the ANR and the observed day-to-day variation in intake of that nutrient. For example, a usual intake that is 2 SD above the ANR should have a 98% probability of adequacy, but if intake was observed on only 3 days, the confidence of adequacy would be lowered to about 85% if the day-to-day variation in intake was about 40%. This calculation is described in detail in Appendix B of the Institute of Medicine report on using the DRIs for dietary assessment [2]. If the day-to-day variation is typically very high for a nutrient (as is the case for vitamins A and $B_{12}$ in the United States), it is not possible to calculate the confidence of adequacy, because it is likely that the assumption of normality is violated (the calculation of confidence of adequacy requires that the distribution of intakes across multiple days for the person must be normally distributed).

#### *Using the AI*

For nutrients for which an ANR cannot be established and only a safe intake or "adequate" intake (AI) is defined, a probability of adequacy cannot be calculated, because the distribution of requirements is unknown. In this case, it is only possible to make a qualitative evaluation of the person's usual intake. If the AI is based on the mean intake of a healthy population, then the usual intake of an individual above the AI is assumed to have a *low probability of inadequacy.* The adequacy of a usual intake below the AI cannot be evaluated. It is also possible to calculate the *confidence* that the usual intake is above the AI when intake is observed for only a few days [2]. This calculation is similar to the one described above for calculating the confidence of adequacy for nutrients with an ANR. However, for nutrients with an AI, the result does not refer to adequacy in terms of meeting requirements, but only to the likelihood that the usual intake is above the AI.

#### *Assessing the risk of excessive intakes*

The possibility of excessive nutrient intakes may also be assessed qualitatively by comparing an individual's usual intake with the UNL. Usual intake below the UNL has a low probability of being excessive, while usual intake above the UNL places a person at potential risk for adverse effects. As for the AI, it is possible to calculate the confidence that the usual intake is below the UL when intake is observed for only a few days [2]. However, a low confidence that the usual intake is below the UL does not mean that the adverse effect is likely to occur, but only that more days of intake should be observed before concluding that the risk of adverse effects is low.

#### *Assessing energy intakes*

Energy intakes for individuals may be assessed if an NIV for an average energy requirement has been established. The usual approach is to compare an individual's usual energy intake to the NIV for energy. If the average energy requirement is accurately calculated (usually considering a person's age, sex, and physical activity), then intakes above the mean would result in weight gain and intakes below the mean would result in weight loss. No probability of adequacy can be determined, because energy intake is almost always related to energy requirement, which violates one of the assumptions of the probability calculation.

#### *Assessing macronutrient intakes*

An individual's macronutrient intakes (for percentage of energy from protein, fat, and carbohydrate) may also be compared with acceptable macronutrient distribution ranges (AMDRs) if this type of NIV has been set. Usual macronutrient intakes for an individual should fall within these ranges.

### Planning nutrient intakes of individuals

#### *Goal*

The goal of planning an individual's intake is to ensure that the probability of inadequacy and the likelihood of excessive intake are both small [3, 9]. The goal for an individual's usual nutrient intake is normally the $INL_x$. If x = 98, then intake at $INL_{98}$ should be adequate for almost all (98%) individuals. Other values of x could be considered, such as 95 or 90. An $INL_{95}$ would be adequate for 95% of individuals, whereas an $INL_{90}$ would be adequate for 90%. The lower the value of x, the lower the probability that intake at that level will be adequate for an individual. The ANR is usually not an appropriate goal for an individual's intake, because it would be adequate for only 50% of individuals based on the chosen criterion of adequacy.

#### *Limitations of the $INL_x$*

Setting an $INL_x$ requires knowledge about the distribution of requirements for the nutrient. Because these data are often sparse, an assumption is frequently made about the coefficient of variation of the requirement, because it is needed to adjust the ANR to obtain the $INL_x$. For this reason, it has been argued that setting an $INL_x$ is not scientifically justified [10]. However, although more accurate data on distributions are needed, the $INL_x$ remains the best target for an individual's nutrient intake [11].

#### *Considering upper levels*

Intakes of individuals should not exceed the UNL, since intakes above the UNL have a potential risk of adverse effects. Thus, to minimize the risk of inadequacy and the risk of excessive intakes, individuals should choose diets with usual nutrient intakes that are between the $INL_x$ and the UNL (**fig. 1**). For nutrients with an AI, nutrient intakes should be between the AI and the UL.

#### *Planning energy and macronutrient intakes*

Energy intakes for individuals may be planned using the NIV for the average energy requirement as a target. An $INL_x$ is not usually set for energy intake, because intakes above the mean requirement would result in weight gain, which is usually undesirable. The calculation of the planned mean energy intake should consider both the person's body size and his or her activity level. An example of this approach is provided in the DRI report on macronutrient intakes [12]. If AMDRs have been set for percentages of energy from fat, protein, and carbohydrate, then the goal for planning an individual's diet is to ensure that the usual intakes of these macronutrients by the individual fall within the ranges.

## Using NIVs for assessment and planning for groups

### Assessing nutrient intakes of groups

#### *Goal*

The goal of assessing intakes of groups is to determine the *prevalence of inadequacy* and the *prevalence of potentially excessive intakes* in the groups [2, 7, 8].

#### *Prevalence of inadequacy*

The *prevalence of inadequacy* is the proportion of the group whose intakes *do not* meet their requirements. Alternatively, the *prevalence of adequacy* can be calculated as the proportion of the group who *do* meet their requirements based on a defined criterion of adequacy. The prevalence of adequacy (expressed as a percentage) is equal to 100 minus the prevalence of inadequacy. Such prevalences should correspond to the proportion of the group that exhibits the functional outcome (or criterion) that was used to set the requirement distribution. If, for example, the vitamin C requirement was set at a level to prevent scurvy, then the prevalence of inadequacy should correspond to the prevalence of scurvy within the group. If it was set at a higher level with the goal of maintaining near-maximal neutrophil concentrations with minimal urinary loss, as was done in the United States and Canada [13], then the prevalence of inadequacy should correspond to the proportion of the population that does not exhibit near-maximal concentrations in their neutrophils.

There are two ways to determine the prevalence of inadequacy (or adequacy) for a group: the full probability approach and the cutpoint method. The full probability approach involves calculating the probability of inadequacy for each person within the group and then taking the average [14]. The average probability of inadequacy is then equal to the prevalence of inadequacy. The cutpoint method is a shortcut of the full probability approach. The prevalence of inadequacy is estimated as the proportion of the group with usual intakes below the ANR. Neither of these methods requires that *intakes* be normally (or even symmetrically) distributed, but other assumptions must be met, as described below.

#### *Full probability approach*

The full probability approach uses the same calculation that was described for estimating the probability of inadequacy for an individual. This probability calculation is performed by using the usual nutrient intake for each individual in the group and then determining the average probability. As noted above, the probability calculations assume that intake and requirements are independent, and of course, the distribution of requirements must be known.

#### *Cutpoint approach*

The cutpoint approach does not require the calculation of any probabilities and thus is easier to implement. The prevalence of inadequacy is simply estimated as the proportion of the group with usual intakes below the ANR (**fig. 2**). For example, if 35% of the group has usual intakes below the ANR, then the prevalence of inadequacy would be approximately 35%. However, the cutpoint approach still requires that intakes and requirements be independent. Although the requirement distribution does not have to be known for this method to be used, it must be approximately symmetrical. Therefore, the cutpoint approach cannot be used to determine the prevalence of inadequate iron intakes for menstruating women, because the distribution of requirements is skewed. Finally, this approach works best if the actual prevalence of inadequacy is neither very high nor very low, and if the variability in intakes among individuals in the group is greater than the variability in requirements of the individuals. This last assumption is met by almost all intake distributions, but it is possible that the variability of intakes could be very low for groups who are eating similar amounts of

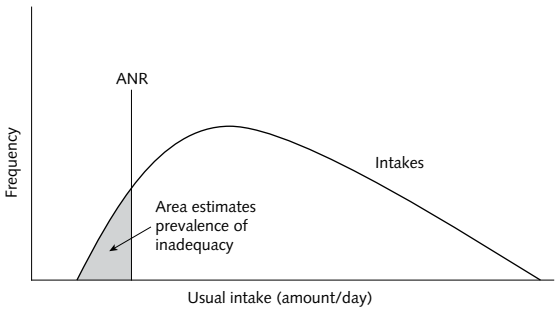


FIG. 2. Graph of a hypothetical distribution of usual nutrient intakes for a group of people. The area to the left of the average nutrient requirement (ANR) represents an estimate of the proportion of the group with inadequate intakes (the prevalence of inadequacy)

similar foods. In this case, the full probability approach should be used.

As explained in more detail elsewhere [2], the cutpoint approach works for statistical reasons. It does not mean that the ANR is an appropriate cutpoint for screening *individuals.* That is, it cannot be assumed that the individuals with intakes below the ANR are the ones who should be targeted for dietary interventions. Although it is true that intakes below the ANR have at least a 50% probability of inadequacy, it is not true that all of these individuals have intakes below their requirements. Even at the ANR, an intake still has a 50% probability of adequacy. Furthermore, some individuals with intakes above the ANR are still not meeting their requirements. As noted above, screening at the individual level is difficult unless usual long-term intakes have been measured. At the group level, however, the prevalence of inadequacy could be reduced by shifting the distribution of intakes so that fewer intakes fall below the ANR; this approach is described below in the section on Planning Nutrient Intakes for Groups.

#### *Adjusting intake distributions*

Both the full probability approach and the cutpoint approach require that the distribution of intakes represents usual intakes for the group of interest. Usual intakes could be obtained by having many days of observation for each individual, but this type of data is seldom available. Alternatively, the distribution can be statistically adjusted to remove the effect of day-to-day variation (**fig. 3**). Several methods are available for making these adjustments [2, 14–17], but all require an estimate of the day-to-day variation in nutrient intakes for the group. These methods may be used even if data from only one day are available for each person, as long as a second day of dietary data is collected for at least a representative subset of the group of interest. Alternatively, an estimate of day-to-day variation may be obtained from multiday data for a similar population. The ability to *statistically* adjust intake distribution to remove the effect of day-to-day variation in intakes, rather than collecting many days of data for each person, makes the assessment of groups more feasible than the assessment of individuals.

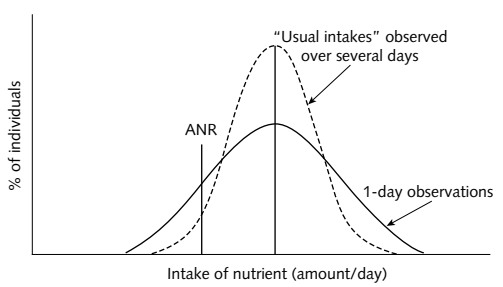


FIG. 3. Comparison of a distribution of one-day nutrient intakes with a distribution of usual nutrient intakes. The proportion of the group with intakes below the average nutrient requirement (ANR) is greater for the one-day distribution

#### *Assessing intakes with an AI*

For nutrients without an ANR, it is possible to evaluate the intake of a group if an adequate intake (AI) has been set based on the mean intake of a healthy population. If the group's mean intake approximates the AI, then the group can be assumed to have a *low prevalence of inadequacy.* However, no such qualitative judgment can be made if mean intake is below the AI. Because the distribution of requirements is not known, it is possible that mean intakes below the AI are also associated with a low prevalence of inadequacy. For this type of assessment, the intake distribution does not need to be adjusted for day-to-day variation, because only the mean intake is being examined.

#### *Assessing the prevalence of potentially excessive intakes*

To assess the *prevalence* of potentially excessive intakes, the proportion of the group with usual intakes above the UNL should be estimated. Because the UNL includes an uncertainty factor, it should not be assumed that everyone with an intake above the UNL is at risk for adverse effects. However, as the prevalence of intakes above the UNL increases, the likelihood that the group is at risk also increases. Prior to this evaluation, the intake distributions must be adjusted to remove the effect of day-to-day variation, as described above.

#### *Assessing energy intakes*

As was the case for assessing individuals, the probability approach cannot be used to evaluate the energy intakes of a group. Instead, the average energy intake of the group can be compared with the estimated average energy requirement for the group, taking into consideration the ages, sexes, and physical activity levels of the individuals within the group. If the average energy intake exceeds the estimated average requirement, then the group, on average, is likely to be gaining weight, assuming that the requirements and intakes have been accurately calculated. If the reverse is true, then the group may be, on average, losing weight. This type of comparison does not require adjusting the intake distribution for day-to-day variation, since only the mean is being evaluated. Energy balance can also be measured by weight changes, which can assist in assessing whether a group's energy intake is inadequate or excessive.

#### *Assessing macronutrient intakes*

If AMDRs have been set, then the group's macronutrient intakes may be assessed using these standards. The proportion of the group with intakes outside the AMDR is a measure of how well the group is meeting these standards. These would be interpreted as the prevalence of intakes that are too high or too low. If

the prevalence of intakes outside the range is high, then some type of intervention might be considered. The prevalence that is a cause for concern could vary among countries or regions, but prevalences greater than 2% to 3% would be consistent with the use of values like the $INL_{98}$ for other nutrients. Because this type of assessment is using the AMDR to define cutpoints, it is essential that the intake distribution be adjusted for day-to-day variation first.

#### *Pitfalls to avoid*

When NIVs are used to assess the intakes of groups, there are several errors to avoid [18].

In the past, the intakes of groups were evaluated by comparing the mean intake with the $INL_{98}$ and assuming that the prevalence of inadequacy was low if the mean was equal to or greater than the INL. However, it can be shown that this assumption was almost always incorrect due to the large variation in nutrient *intakes*. Because intakes are more variable than requirements, a substantial proportion of the group still has intakes below the ANR, even if the mean intake is equal to the $INL_{98}$. Typically, a prevalence of inadequacy of 25% or more may be observed in this case. For the prevalence of inadequacy to be low (less than 5% to 10%), the mean intake must usually be well above the $INL_{98}$. For this reason, it is preferable to use either the probability approach or the cutpoint method to calculate a more accurate estimate of the prevalence of inadequacy for the group.

Likewise, it is not helpful to compare the mean intake of a group with the ANR. If the mean intake is equal to the ANR, then the prevalence of inadequacy is 50%.

Group intake distributions must be adjusted to remove the effect of day-to-day variation in intakes. Unless such an adjustment is made, the prevalence of inadequacy, the prevalence of excessive intakes, and the prevalence of intakes outside the AMDR will not be correctly estimated.

Intakes must be accurately assessed, and the conversion of food intakes to nutrient intakes must use appropriate food-composition tables (both of these topics are covered in more detail below).

### Planning nutrient intakes for groups

#### *Goal*

The goal of planning the nutrient intakes for groups is to minimize the prevalence of inadequate intakes and also to minimize the prevalence of potentially excessive intakes [3, 19].

#### *Using the cutpoint approach to plan intakes*

The cutpoint approach, described above for assessing the intakes of groups, is also useful for planning the intakes of groups. To minimize the prevalence of inadequate intakes, the planner would try to minimize the proportion of the group with intakes below the ANR. As noted earlier, the cutpoint approach may be used for all nutrients with an ANR and a symmetrical requirement distribution. It also assumes that the distribution of intakes is greater than the distribution of requirements, although this assumption is seldom violated among free-living groups. To minimize the prevalence of excessive intakes, the planner would try to minimize the proportion of the group with intakes above the UNL.

#### *Steps in planning intakes*

Planning diets for groups using the cutpoint approach consists of several steps [3, 19].

1. Decide the exact goals for the planning process. This involves deciding what prevalences of inadequacy and prevalences of usual intakes above the UNL are acceptable. Although it might be considered ideal to have only 2% to 3% of the group below the ANR or above the UNL, these may not be practical goals in some situations.
2. Select the target distribution of usual intakes that meets these goals. In order to select this target distribution, it is usually necessary to know the current intake distribution so that the need for change can be assessed. In some cases, the intake distribution may already be acceptable (i.e., very few intakes are below the ANR or above the UNL). In other situations, it may be necessary to increase intakes (i.e., move the current distribution to the right) so that fewer people would fall below the ANR, or it may be necessary to decrease intakes (move the current distribution to the left) so that fewer people are above the UNL.
3. Plan menus or food patterns that achieve these goals for all of the nutrients of concern. This will usually require the use of dietary assessment software that will use an appropriate food-composition table to calculate the nutrient content of a menu. The goal might be for the menu to provide nutrient levels at the midpoint of the target distributions that were chosen for each nutrient. The foods and amounts that will actually be consumed, not just those provided by the menus, should also be considered.
4. Because there are many uncertainties in the planning process, it is usually essential that the results be assessed after the menus or food patterns are implemented. If the planning goals are not met, then it may be necessary to repeat these steps until the results are satisfactory.

#### *Planning using the AI*

For nutrients with an NIV, such as an AI, intakes should be planned so that the mean amount consumed by the group is equal to the AI. The distribution of intakes does not need to be planned for these nutrients.

#### *Planning energy and macronutrient intakes*

The mean amount of energy provided should be approximately equal to the estimated mean energy requirement for the group. The distribution of intakes does not need to be planned for energy intake. For macronutrients with AMDRs, the planning goal is to minimize the prevalence of intakes outside this range. It may be useful to examine the current distribution of the percentage of energy from each macronutrient and decide if these distributions should be changed.

#### *Planning for heterogeneous groups*

The steps outlined above assume that the individuals within the group are similar in age and sex, so a single set of ANRs and UNLs can be used for the planning process. Planning for groups that are not homogeneous is more difficult. One approach for such groups is to identify the subgroup with the greatest nutrient requirement per 1,000 kcal (i.e., the subgroup that requires the most nutrient-dense diet) and then use the procedures described above to plan intakes for each nutrient. The assumption is made that a diet that is adequate for this subgroup will also be adequate for the other subgroups if they meet their energy requirements. The possibility of potentially excessive intakes by some subgroups should also be considered. Finally, it is particularly important to check the actual nutrient intake distributions after the new menus or food patterns have been implemented, to ensure that all subgroups have a low prevalence of inadequacy and a low prevalence of potentially excessive intakes. An alternative procedure is to estimate the nutrient density distribution for the whole group and to plan using this distribution. Although this approach is theoretically preferable, the methods are still under development [3].

## Examples of assessing and planning intakes using NIVs

Following are several examples of appropriate uses of the NIVs for assessing and planning nutrient intakes. The first two illustrate how the intakes of a hypothetical 30-year-old man might be assessed (**table 1**) and what changes might be recommended in planning his intake (**table 2**). Intakes of thiamin and folate were well above the ANR and had a 98% probability of adequacy if observed intake was the same as usual intake. However, the confidence of adequacy for usual intake was reduced to 70% and 80%, respectively, because intake was observed for only three days. Riboflavin intake had only a 50% probability of adequacy because intake was equal to the ANR. Observed phosphorus intake was very high and had a 100% probability of adequacy. However, because observed intake was close to the UNL of 4,000 mg/d, the confidence that usual intake was below the UNL was only 80%. Assuming that the three days of intake reflected his usual intake, the individual would be advised to increase his riboflavin and zinc intakes (**table 2**). If these days were not typical, then it would be appropriate to collect additional days of intake data in order to better evaluate thiamin, folate, and phosphorus intakes. To make these recommendations meaningful to a consumer, food-based

TABLE 1. Assessing the nutrient intakes of a hypothetical individual (30-year-old man; intakes observed for 3 days)[a]

| Nutrient | Intake | ANR | Probability of adequacy | Confidence of adequacy | UNL | Confidence intake is < UNL |
|---|---|---|---|---|---|---|
| Thiamin (mg/day) | 1.3 | 1.0 | 98 | 70 | None | N/A |
| Riboflavin (mg/day) | 1.1 | 1.1 | 50 | 50 | None | N/A |
| Folate (μg/day DFE) | 400 | 320 | 98 | 80 | 1,000 | 100 |
| Zinc (mg/day) | 10.3 | 9.4 | 86 | 65 | 40 | 100 |
| Phosphorus (mg/day) | 3,800 | 580 | 100 | 100 | 4,000 | 80 |

DFE, dietary folate equivalent; ANR, average nutrient requirement; UNL, upper nutrient level

*a.* Using the dietary reference intakes (DRIs) for the United States and Canada; average nutrient requirement (ANR) = estimated average requirement (EAR); upper nutrient level (UNL) = tolerable upper intake level (UL). Adapted from Institute of Medicine [2].

TABLE 2. Planning the nutrient intakes of the same hypothetical 30-year-old individual

| Nutrient | Current intake | $INL_{98}$ | UNL | Recommended change in intake[a] |
|---|---|---|---|---|
| Thiamin (mg/day) | 1.3 | 1.2 | None | None |
| Riboflavin (mg/day) | 1.1 | 1.3 | None | Increase |
| Folate (μg/day DFE) | 400 | 400 | 1,000 | None |
| Zinc (mg/day) | 10.3 | 11.0 | 40 | Increase slightly |
| Phosphorus (mg/day) | 3,800 | 700 | 4,000 | None |

DFE, dietary folate equivalent; INL, individual nutrient level; UNL, upper nutrient level

*a.* Assumes 3-day intake reflects usual intake.

guidance would be appropriate. Ideally, a reassessment would be performed after the recommendations were implemented.

The remaining two examples illustrate an approach to assessing (**table 3**) and planning (**table 4**) the nutrient intakes of a group of women 19–30 years of age. After adjusting the intake distributions to remove the effect of day-to-day variation in intakes, the prevalence of inadequacy ranged from 4% to 14% for the five nutrients shown in **table 3**, and the prevalence of potentially excessive intakes was low for the three nutrients with a UNL. If the goal is to reduce the prevalence of inadequacy for this group of women to 3%, then intakes would need to increase for each of the five nutrients so that only 3% of the group had intakes below the ANR (**table 4**). The necessary shift in the median intake of the group can be calculated, assuming that the shape of the distribution does not change [3]. For example, folate intake would need to increase from 491 μg of dietary folate equivalents (DFE)/d to 581 μg DFE/d for the prevalence of inadequacy to decline from 14% to 3%. To achieve these changes, nutrition education or intervention programs might be implemented. After the programs are in place, their effect on prevalence of adequacy should then be assessed.

## The impact of food-composition tables on uses of the NIVs

It is not possible to plan or assess diets with the NIVs unless food intakes can be correctly converted into nutrient intakes. This conversion is accomplished with the aid of a food-composition table that contains nutrient profiles of foods, usually on a 100-gram basis. Such tables are frequently embedded in computer programs that may be used to both assess and plan food intakes.

Although valid estimates of nutrient intakes require accurate and current food-composition data, use of the correct units for the nutrients is also crucial. The units for the nutrients in the composition table should match the units that are used for the NIVs. NIVs may evolve quickly, and there can be a delay while food-composition methods and analyses catch up. In the United States and Canada, the disconnect between the DRIs and the national food-composition tables was particularly great for vitamin A, vitamin E, and folate [20–22]. The units for vitamin A changed from micrograms (μg) of retinol equivalents (RE) to micrograms of retinol activity equivalents (RAE) to reflect a reduced bioavailability of carotenoids as vitamin A precursors; the units for vitamin E changed from milligrams of α-tocopherol equivalents to milligrams of α-tocopherol, because other tocopherols are no longer believed to have vitamin E activity; and the units for folate changed from micrograms of folate to micrograms of dietary folate equivalents (DFE) to reflect an increased bioactivity of synthetic folic acid. These new units are now available in the national food-composition tables, but considerable time and effort was involved to accomplish these changes.

TABLE 3. Assessing the nutrient intakes of a group of US women aged 19–30 years[a]

| Nutrient | Median intake from food | ANR | Prevalence of inadequacy (%) | UNL | Prevalence of excessive intakes (%) |
|---|---|---|---|---|---|
| Thiamin (mg/day) | 1.4 | 0.9 | 8 | None | N/A |
| Riboflavin (mg/day) | 1.8 | 0.9 | 5 | None | N/A |
| Folate (μg/day DFE) | 491 | 320 | 14 | 1,000 | < 3 |
| Zinc (mg/day) | 9.8 | 6.8 | 13 | 40 | < 3 |
| Phosphorus (mg/day) | 1,136 | 580 | 4 | 4,000 | < 3 |

DFE, dietary folate equivalent; ANR, average nutrient requirement; UNL, upper nutrient level

*a.* Using the DRIs for the United States and Canada; ANR=EAR; UNL=UL. Adapted from Moshfegh et al. [28].

TABLE 4. Planning the nutrient intakes of the same group of US women aged 19–20 years

| Nutrient | Current median intake from food | Current prevention of inadequacy (%) | Desired prevalence of inadequacy (%) | Change in median intake from food[a] | New prevalence of excessive intakes (%) |
|---|---|---|---|---|---|
| Thiamin (mg/day) | 1.4 | 8 | 3 | + 1 mg/day | N/A |
| Riboflavin (mg/day) | 1.8 | 5 | 3 | + 0.1 mg/day | N/A |
| Folate (μg/day DFE) | 491 | 14 | 3 | + 90 μg/day DFE | < 3 |
| Zinc (mg/day) | 9.8 | 13 | 3 | + 1.5 mg/day | < 3 |
| Phosphorus (mg/day) | 1,136 | 4 | 3 | +10 mg/day | < 3 |

DFE, dietary folate equivalent

*a.* Assumes that everyone increases intake by this amount so that the shape of the distribution does not change.

## The impact of dietary assessment methods on uses of the NIVs

The accuracy of the assessment of diets for either individuals or groups is limited by the accuracy of the dietary assessment methods. Also, because assessment is often the first step in planning diets, an inaccurate assessment can lead to incorrect planning as well. Although a complete evaluation of various dietary assessment methods is beyond the scope of this article, other publications provide more details [2, 23]. Some general considerations include the following:

Assessment methods that depend on self-reported intakes over a specific period of time, such as 24-hour recalls or one-day food records, tend to result in underreporting of intakes of energy and probably of other nutrients as well [24, 25]. If food intakes are underreported, then nutrient intakes may be underestimated, and the probability of inadequacy may be *overestimated* for individuals and the prevalence of inadequacy *overestimated* for groups. Likewise, the prevalence of excessive intakes may be *underestimated*.

Food-frequency questionnaires have been useful dietary assessment instruments for ranking intakes within a group, but their accuracy in estimating actual nutrient intake has been questioned [26]. Some food-frequency questionnaires underestimate intake even more than recalls and records [27].

None of the commonly used dietary assessment instruments captures usual long-term intake accurately. Even food-frequency questionnaires exhibit relatively high variation when administered to the same person at two different points in time [23]. For this reason, it is very difficult to accurately assess the *usual* nutrient intakes of individuals unless intakes are measured repeatedly. However, this concern can be addressed when assessing group intakes by using statistical techniques to remove the effect of day-to-day variation from the distribution of one-day intakes. These techniques require that an estimate of day-to-day variation be obtained for the nutrients of interest, either by collecting a second day of intake data or by using an estimate obtained from a similar group.

An accurate assessment of total nutrient intake must consider nutrients consumed in dietary supplements such as multivitamin/mineral supplements. These products are widely used in developed countries, and their use may be increasing in some segments of the population in developing countries as well.

## Summary and recommendations

Nutrient intake values (NIVs) are now being developed by using a new paradigm. Whenever possible, average nutrient requirements (ANRs) are being set, and a standard deviation of the ANR is being assumed or specified. As a result, it is possible to better assess and plan diets for both individuals and groups. Furthermore, the availability of an upper nutrient level (UNL) permits consideration of nutrient intakes that may result in a possible risk of adverse effects. Although the new paradigm has resulted in more meaningful methods for using the NIVs to plan and assess intakes, the potential for misinterpretation has also increased. It is important to ensure that users of the new NIVs are informed and educated in the potential uses as well as the responsible interpretation and application of these standards. To date, there have been few attempts to apply these uses to actual public health problems. The many possibilities are discussed by Vorster et al. [4] in this issue. To fully utilize the new paradigm, it will be necessary for the professional nutrition community to identify ways to implement these procedures in nutrition research and nutrition programs, to describe the strengths and weaknesses of the results, and to contribute to the evolution of both the theory and the application of the NIVs when planning and assessing diets.

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# Determining life-stage groups and extrapolating nutrient intake values (NIVs)

Stephanie A. Atkinson and Berthold Koletzko

## Abstract

*The derivation of reference values in 11 current dietary reference standards is often based on methods of extrapolation or interpolation, but these are not consistent across reports. Such methods are frequently employed to derive nutrient intake values (NIVs) for infants and children owing to the paucity of relevant research data available. The most common method is to extrapolate values for children down from those of adults, employing a weight or metabolic factor and adjusting for growth. In some instances, values for young children are extrapolated up from infants, values for adults are extrapolated up from children, or values for older adults are extrapolated up from young adults. Extrapolation is employed to estimate not only nutrient requirement or adequate intake but also the upper tolerable levels of intake. Extrapolation methods may also form the basis of estimates of tissue deposition of nutrients during growth in children and for the maternal/fetal dyad in pregnancy with adjustments for metabolic efficiency. Likewise, recommended intakes during lactation are extrapolated from known secretion of the nutrient in milk with adjustments for bioavailability. For future dietary standards, a first priority is to obtain relevant scientific data using current methodology, such as stable isotope tracers, body composition analysis, and appropriate biomarkers, from which NIVs for each age group can be derived. Extrapolation to derive an NIV is only acceptable in the sheer absence of sound scientific data and must be modeled with a consistent approach. For the purpose of harmonization of dietary standards, we recommend the following approaches that should be clearly described in reports: standardization of age groups on a biological basis (growth and pubertal stages) with consideration of relevant developmental milestones throughout childhood; application of internationally accepted standards for growth, body size, body composition, fetal and maternal nutrient accretion in pregnancy, and milk composition; and inclusion of appropriate adjustments (metabolic efficiency, weight change, or physical activity).*

**Key words:** Extrapolation, interpolation, lactation, milk composition, nutrient intake values, pregnancy, recommended nutrient intakes, reference body weights and heights

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## Introduction

The approach to determining nutrient requirements based on specific criteria of nutritional adequacy demands availability of data for each defined life-stage group as well as for specific physiological states such as pregnancy and lactation. In addition, the influence of environmental factors such as physical activity, smoking, and food sources may need to be accounted for in setting quantitative nutrient-based recommendations. For example, across all age groups, the influence of environmental variables or stresses has been recognized in the estimation of energy needs as a function of physical activity level.

Growth and development are central characteristics of infancy, childhood, and adolescence and lead to relatively large substrate requirements. Meeting the nutritional needs of children and adolescents is of utmost importance to support their growth and development and their short- and long-term health, well-being, and performance [1]. For children and youth, current reference values for nutrient intakes vary widely, in part due to severe limitations in the available scientific

knowledge of nutrient requirements in childhood, and in part due to major differences in underlying concepts, definitions, and terminology [2]. Relevant scientific data that are age-specific are unfortunately unavailable for many nutrients, especially for infants over 6 months of age and young children, as well as for older age groups. Until appropriate data become available, the general approach to estimating nutrient needs of such populations has been to extrapolate values from one life stage to another using weighting for body size, energy requirement, and other metabolic differences.

In a recent European concerted action, the methodological approaches and current nutritional recommendations in children and adolescents aged 2 to 18 years have been reviewed [3]. An expert working group obtained information for 29 of 39 countries in Europe, and a comprehensive compilation was made of the dietary recommendations current up to September 2002 and the concepts of dietary reference values and the methodological approaches used in each country [4]. Brief critiques were included to indicate the scientific foundations of the reference values for children and to offer, where possible, an explanation for the wide differences that exist between countries. This compilation demonstrated that there are considerable disparities in the perceived nutritional requirements of children and adolescents. Although some of this diversity may be attributed to real physiological and environmental differences, most is due to differences in concepts about the best methodological approach to use, and in the way the theoretical approaches are applied.

For physiological states such as pregnancy and lactation, metabolic data upon which estimates of requirements are based are often lacking due to practical difficulties or ethical limitations on conducting research in women during these reproductive stages. When data specific to physiological state are not available, methods employing a factorial approach or extrapolation from other growth states, with various adjustments for physical activity, weight loss, or metabolic efficiency, have been used instead to derive nutrient requirements.

## Grouping by age or physiological state to derive NIVs

Great inconsistency exists between various countries and agencies in definitions of life-stage groups used to establish nutrient standards, and often clear explanation of the rationale for the age groups is lacking. Having reviewed the approaches used, we recommend that the following factors be considered when establishing age groups: biological patterns related to physical age; use of a functional characteristic such as growth or puberty, both of which put increased demands on nutrient needs, or aging that mark changes in nutrient handling due to functional losses; and the potential applications such as guidelines for duration of breastfeeding or complementary feeding programs.

For pregnancy and lactation, graded increases in NIV during stages of pregnancy and lactation do not appear to be based on strong science. Recent evidence supports the concept that physiological adjustments in nutrient utilization occur during pregnancy and lactation that generally compensate for the shifts in nutrient requirements with stages of gestation or lactation. Furthermore, having more than one NIV for pregnancy and lactation is essentially impossible to implement; advising a women to eat one diet during early pregnancy and another in late pregnancy is not practical. This combination of factors leads us to recommend that pregnancy and lactation not be divided into different stages such as trimesters of pregnancy or early and late lactation.

## Reference weights and heights for application in deriving NIVs

Population reference weights and heights are applied in setting recommended nutrient intakes in several situations. The adoption of reference body weights and heights should be specific for countries or geographic regions, since populations can vary significantly in stature and weight.

For the dietary reference intake (DRI) reports for Canada and the United States, the initial report [5] used data on heights and body mass index (BMI) collected between 1988 and 1994 for the Third National Health and Nutrition Examination Survey (NHANES III) in the United States. Using the reported median heights for life stage and sex groups up to age 30, the median weights were computed from the reported median BMI for the same groups. The reference weights of adults aged 19 to 30 were applied to all adult age groups on the assumption that weight should not change at the older ages if activity is maintained. Beginning with the DRI report on macronutrients [6], the reference weights and heights were updated on the basis of new data on median BMI and height-for-age data from the Centers for Disease Control and Prevention/National Center for Health Statistics (CDC/NCHS) growth charts [7].

The D-A-CH (Germany, Austria, Switzerland, and Slovenia) report [8] used body weight data for adults from the 1985 Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) report [9] and for children from the US National Center for Health Statistics Growth Charts [7].

The report on "Nutrient and Energy Intakes for the European Community" [10] based its calculations of intakes of energy and selected nutrients on rounded values for mean body weights and heights of children

at different ages, based on pooling national datasets from Belgium, Denmark, France, Germany, Greece, Italy, Spain, the Netherlands, and the United Kingdom, weighted on the basis of each country's population at any given age. It is noteworthy that the data for mean body weights and heights of children in the European Union report and the US National Center for Health Statistics Growth Charts [7] correspond closely. Assessments for pregnant women were based on ranges of prepregnancy weight and desirable weight gains during pregnancy assessed by the US National Academy of Science [11].

In the Finnish report of 1999 [12], a reference weight of 70 kg for men and 60 kg for women was adopted, but mean body weights for children and adolescents were taken from the 1996 Nordic report [13]. Similarly, the French report in 2001 [14] adopted the standard 70-kg and 60-kg values for adult men and women when reporting estimated energy intakes for groups of subjects, but also provided values for energy expenditure by 5-kg intervals at various age intervals for adults, although these do not appear to represent population reference weights.

In the most recent Mexican dietary standard [15], it was recognized that the Mexican population is shorter than the average of some other populations, and thus data from several representative National Surveys in Mexico were adopted for use as reference weights and heights. These include the National Nutrition Survey (1999), the National Chronic Diseases Survey (2000), and the Federal District Survey for men and women over 60 years of age (as cited by Bourges [15]). Using the height data from the Mexican population, the desirable body weight was then derived from the 50th percentile of weight-for-height in the American reference population from the National Center for Health Statistics for ages 0 to 9 years, and the body weight to reach a BMI at the 50th percentile of each height from the same database for 10 to 18 years. For adults, the Mexican report adopted data from the FAO/WHO/UNU report [9] to calculate a body weight to reach a BMI of 22 in women and 21 in men. The Caribbean report [16] used reference weights and heights updated from the 1976 report, but the source of the data was not identified.

The Nordic report [13] used rounded body weight values for adults based on mean population weights in Denmark, Sweden, and Finland, with adjustments for individuals outside a BMI range of 18.5 to 25. The values used are thus estimates, assuming that all individuals are of normal weight. Values for body weight of children aged 0 to 5 years were based on the mean of reference values from Denmark, Finland, Norway, and Sweden, whereas values for the age group 6 to 17 years were based on mean values from 1973–1977 because of a gradual increase in weight-for-height and in overweight prevalence in recent years [13]. The UK report in 1992 [17] used weight and height data compiled from different surveys of the UK population.

The reference values for body weights and heights are applied in a number of situations. For some nutrients, such as protein, nutrient requirements may be expressed on a body weight basis, but such values are more applicable to end users if they are expressed as total nutrient intake per day. In this case, an average weight-for-age and sex is applied to compute nutrient intake per day from the value established for nutrient intake per kilogram of body weight.

Another application for reference weights and heights is in the computation of estimated energy expenditure for a group of subjects of given sex and age. A third application is the use of reference weights when biological data for a specific criterion of adequacy are not available for a specific age or sex category. In this instance, weights may be used to extrapolate values from older or younger individuals using an appropriate mathematical model, as described below.

After reviewing the above-noted reports, we recommend that for infants and children between 0 and 5 years of age, the new WHO growth standards [18] be used as the basis for normalizing NIV when such adjustments based on weight are appropriate. For all other age groups, data from NCHS/WHO can be used to derive a standard weight and height [9]. For adults, it is recommended that the average weight of men and women at 18 years of age be used throughout the adult years rather than reflecting the typical secular increase in body weight with age. It is uncertain whether this secular increase is consistent with good health. It is important to adjust energy NIVs for overweight or obese individuals with a BMI greater than 25. For all other nutrients, standard body weight uncorrected for overweight status is appropriate for estimating NIVs.

## Nutrient recommendations established using an extrapolation model among age groups

For many nutrients, sufficient research is not available that allows for derivation of requirements for every age group and for both genders. This is particularly true for older infants (7 months to 2 years) and for children of preschool and school age. In contrast to adult organisms, the nutrient supply for children and adolescents needs to cover not only the requirements for maintenance metabolism, but also obligate losses and physical activity. In addition, children have high and specific substrate needs for growth, which are particularly large during the phases of rapid growth in infancy, during the preschool growth spurt, and during the pubertal growth spurt [1]. In contrast to their high metabolic demands, children tend to have a more limited ability than adults to compensate for unbalanced nutrient

supplies by homeostatic adaptation, primarily because of their smaller body stores of nutrients. Moreover, the metabolic handling of various substrates differs with age and body size.

Documentation of the paucity of existing data from which to derive nutrient requirements for children was exemplified by an electronic literature search performed in August 2003 in PubMed, National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) [3]. The search term "nutrient requirement" limited to "clinical trial" and "human" yielded 176 hits for adults and 53 for newborn infants, but only 21 hits for preschool children aged 2 to 5 years, 29 for children aged 6 to 12 years, and 45 for children and adolescents aged 13 to 18 years. Such a lack of relevant data from which to derive evidence-based population reference intakes for children and youth leads to the application of linear models of extrapolation for requirements and upper intakes from values established for adults and for young infants, usually based on average data for body weight or body surface area [2, 10]. These extrapolation approaches have several limitations. Major concerns about these shortcomings were recently emphasized by the Scientific Committee on Food of the European Community with regard to upper levels of intake:

> The Committee recognises limitations in the methods available and in the approach it has used to extrapolate tolerable upper intake levels (UL) of nutrients from those established for adult populations to children, based solely either on body weight or on body surface area. For some nutrients, the Committee concluded that an extrapolation on the basis of body weight or body surface area would yield UL for children that were incompatible with known nutrient requirements and refrained from recommending UL for children. Physiological differences between adults and young children, especially at a young age, are both quantitative and qualitative in nature. Existing differences in substrate absorption, metabolism, deposition in tissues during growth, and renal or other excretion that may affect UL of nutrient intakes are not always closely related to body size. The Committee recommends this issue be reviewed in order to establish whether further refinements in the approach or further research are needed. [19]

That being said, until appropriate data are available, extrapolation models will have to be considered for predicting NIVs for some nutrients.

With regard to adults, the major need for extrapolation is in setting nutrient recommendations for older adults. Although the approach used is generally based on adjustment for reference body weights between the two age groups, this would not account for differences in absorption or excretion of nutrients owing to degenerative processes, metabolic rate, or activity levels. To address these biological variables, metabolic studies in age-specific populations are much needed.

## Approaches used for extrapolations between ages to derive nutrient recommendations

Extrapolation based on indicators of average body size or metabolic turnover is the easiest way to calculate nutrient reference values for different age groups but obviously will result in marked errors, particularly for those nutrients which are deposited in significant amounts in tissues during growth, such as amino acids, calcium, iron, and others.

### *Extrapolation based on body size*

If there is no evidence of an association between metabolic rate and nutrient requirement, the nutrient requirement is often estimated as directly proportional to total body weight using age- and sex-appropriate reference body weights. Between countries, the population-based reference weights employed in such calculations will vary, thus leading to one significant source of variation between derived nutrient recommendations.

A generic mathematical model based on body weight is

$$\text{Calculated reference intake}_{\text{age}} = \text{adult reference intake} \times \frac{\text{representative weight}_{\text{age}}}{\text{representative weight}_{\text{adult}}}$$

Such a direct extrapolation based on body weight alone does not take into account intermediary metabolic rates, energy intake, and basal metabolic rates and will result in consistently lower values than values based on body surface area or metabolic body weight.

### *Extrapolation based on metabolic turnover*

When appropriate data are not available to determine a nutrient requirement or upper level of intake for children or adolescents, estimates have been derived by extrapolating down by taking the values derived for adults to the 0.75 power of body mass, which adjusts for metabolic differences related to body weight [20, 21]. This method assumes that the maintenance needs of the nutrient, expressed with respect to metabolic weight, are similar for adults and children.

Calculations may be related to metabolic turnover. Nutrient reference intakes might be estimated on the basis of relative body surface area, which shows some correlation with basal metabolic rate (BMR), following the formula

$$\text{Calculated reference intake}_{\text{age}} = \text{adult reference intake} \times \frac{\text{representative body surface area}_{\text{age}}}{\text{representative body surface area}_{\text{adult}}}$$

This method of calculation will result in higher relative

nutrient reference intake values than those based on body weight, as exemplified in **table 1**.

It was first proposed in the 19th century that BMR can also be roughly estimated by calculating metabolic body mass as body mass$^{0.66}$ [23], whereas an estimation as body mass$^{0.75}$ was proposed in the first half of the 20th century [20, 24]. The controversy about the adequate choice of the exponent to be used in the calculation of metabolic body mass has not been resolved today, and values in the range from 0.6 to 0.8 are currently proposed [21, 25–27]. With these considerations taken into account, nutrient reference intake values can be estimated based on different approaches to estimating metabolic body mass, such as

$$\text{Calculated reference intake}_{\text{age}} = \text{adult reference intake} \times \left( \frac{\text{representative weight}_{\text{age}}}{\text{representative weight}_{\text{adult}}} \right)^{0.66}$$

or

$$\text{Calculated reference intake}_{\text{age}} = \text{adult reference intake} \times \left( \frac{\text{representative weight}_{\text{age}}}{\text{representative weight}_{\text{adult}}} \right)^{0.75}$$

Either of these models will produce higher reference intake values for children than those based on body mass.

It cannot be stressed enough that these approaches to calculation of nutrient requirements cannot account for age-related and maturational differences in absorption, metabolism, deposition, excretion, and homeostatic mechanisms, which are known to vary considerably for different nutrients. Thus, a science-based approach should take into account the specific conditions of turnover for each nutrient.

### Approaches to extrapolation employed in dietary standards

For the DRI reports adopted in Canada and the United States, the specific equations employed are summarized in **table 2**. For adults, the estimated average requirement reflects maintenance needs only. When such data are extrapolated down to growing children, a factor for growth must be added. The factor for growth applied for all nutrients was the approximate proportional increase in protein requirements for growth [9], as indicated under (2) in **table 2**. In situations where an average intake (AI) was set for adults (not an estimated average requirement [EAR]), the value for the AI for adults was substituted for EAR in the equations, an AI was calculated, and no recommended dietary allowance (RDA) was set.

TABLE 1. Relative nutrient intake reference values obtained by extrapolation from adult reference values based on a comparison of body weights or body surface areas, respectively[a]

| Age | Relative nutrient intake based on child/adult weight | Relative nutrient intake based on child/adult body surface area |
|---|---|---|
| Newborn | 0.05 | 0.11 |
| 0.5 yr | 0.10 | 0.19 |
| 1 yr | 0.14 | 0.23 |
| 10 yr | 0.46 | 0.59 |

Source: calculated from data of Przyrembel [22].

The vitamins and minerals for which extrapolation models were employed in the DRI reports of the United States and Canada [28–31], the age groups, and the model employed for extrapolation are summarized in **table 3**. For most nutrients, the model for extrapolation included an adjustment for body weight whether the value was being extrapolated from younger to older age groups or vice versa. A growth factor was added if the extrapolation was from adults to children. The exception to the adjustment to body weight was for sodium in older adults, for which an adequate intake was extrapolated from that for younger adults based on the combined average of median energy intakes for men and women according to the NHANES III data, since energy intake declines with age.

The macronutrients for which extrapolations were employed to set the EAR or AI are summarized in **table 4**. For the DRI report on macronutrients [6], the recommendations for carbohydrate and fiber intake in children over 1 year of age are all extrapolated from adult values. For energy, the values for energy deposition during growth were derived from the calculated energy content of tissue deposition, using various reference growth rates, depending on age category. Similarly, for protein intakes beyond 6 months, the value for protein deposition was derived from data for protein accumulation in children fitted to a polynomial equation.

In other reports reviewed, there was meager specific information as to methods of extrapolation employed in deriving recommendations for macronutrient intakes, and often the method was adopted from another country report. The dietary allowances for the Caribbean [16] rely heavily on recommendations from the WHO report [9] or countries such as Canada. For example, recommendations for energy intake were based on BMR from body weight using equations found in the WHO report of 1985 [9]. Total energy expenditure (TEE) was then calculated by multiplying the BMR by a factor for physical activity level (PAL), which was classified as light, moderate, or heavy. For children, assuming that physical activity might be greater than in developed countries, 5% was added to the TEE to

TABLE 2. Extrapolation equations used in the DRI reports of Canada and the United States [5, 6, 28–31[a]]

1. Extrapolation up from the AI for younger infants (0–6 mo) to older infants (7–12 mo):

$$AI_{7-12\ mo} = AI_{0-6\ mo} \times (F)$$

$$F = (weight_{7-12\ mo}/weight_{0-5\ mo})^{0.75}$$

2. Extrapolation from AI or the EAR for adults to an AI or EAR for children for all nutrients except potassium and sodium:

$$(AI)\ EAR_{child} = (AI)\ EA_{adult} \times (F)$$

$$F = (weight_{child}/weight_{adult})^{0.75}\ (1 + growth\ factor^{*})$$

*Growth factors (proportional increase in protein requirements for growth from FAO/WHO/UNU [9])

| Age group | Growth factor |
|---|---|
| 7 mo–3 yr | 0.30 |
| 4–8 yr | 0.15 |
| 9–13 yr | 0.15 |
| 14–18 yr (males) | 0.15 (0 for females) |

3. For water, potassium, and sodium [31], extrapolation from AI for adults to an AI for children:

$$AI_{child} = AI_{adult} \times (F)$$

Where $F = (energy\ intake_{child}/energy\ intake_{adult})$

Energy intakes rather than body weights were used in the extrapolation equations because high levels of physical activity are associated with increased losses of electrolytes in sweat. The energy intake values were based on the average of median energy intakes for both sexes for each age group based on data from NHANES III.

4. Extrapolation of EAR/AI up from younger adults for older adults vice versa was accomplished using reference body weights, except in the case of sodium:

$$EAR/AI_{younger\ adults} = EAR/AI_{older\ adults} \times (F)$$

$$F = weight_{younger\ adults}/weight_{older\ adults}$$

For sodium [31], the extrapolation equation employed was adjusted for combined median energy intakes for men and women:

$$AI_{older\ adults} = AI_{younger\ adults} \times energy\ intake_{older\ adults}/energy\ intake_{younger\ adults}$$

5. In situations in which data were not available to set the UL for children (which occurred frequently), the UL for adults was extrapolated down using reference body weights for all nutrients except sodium, and in this case median energy intake was used in the adjustment equations:

$$UL_{child} = UL_{adult} \times weight_{child}/weight_{adult}$$

For sodium the extrapolation equation was:

$$UL_{child} = UL_{adult} \times energy\ \ intake_{child}/energy\ intake_{adult}$$

DRI, dietary reference intake; AI, average intake; EAR, estimated average requirement; UL, upper tolerable limit of nutrient intake.

*a.* Median reference weights for children, adolescents, and adults were derived from reference weights for children and adults in the United States (NHANES III, 1988-94) in DRI reports [5, 28–30] published prior to 2002. After that the time, in reports published for macronutrients [6] and water and electrolytes [31], updated data that provided median reference heights and weights for the US population were used [7].

allow for desirable physical activity, as described in the WHO report [9]. Reference body weights were adopted from the NCHS data from 1977. The report did not contain any further examples of extrapolations or interpolations.

In the D-A-CH reference values for nutrient intakes [8], the method of extrapolation or interpolation could not be discerned from the statement, "In those frequent cases in which recommendations could not be made for every age group, values for intermediate age groups had to be interpolated."

In the population reference intake (PRI) report for the European Community provided by the Scientific Committee for Food [10], PRIs for children aged 1 year or more have generally been derived, in the absence of reliable data, by extrapolation from the PRI of young adults on the basis of energy expenditure (e.g., thiamin, riboflavin, niacin, folate, vitamin $B_{12}$, and iodine), unless specific other statements were made. In the case of some nutrients, recommended intake values for children were derived on the basis of further considerations. The specific considerations for each nutrient are summarized in **table 5**. For infants aged 6 to 11 months, the values were usually derived by interpolation between those for infants under 6 months, which are often derived from intakes of

TABLE 3. Summary of vitamins and minerals for which AI/EAR was derived from extrapolations in the Canada/USA DRI reports [28–31]

| Age group | Vitamins | Macro minerals | Micro minerals |
|---|---|---|---|
| 7–12 mo | Vitamin A[a]<br>Vitamin K[a]<br>Thiamin[b]<br>Riboflavin[a,b]<br>Niacin[b]<br>Vitamin $B_6$[a,b]<br>Folate[a,b]<br>Pantothenate [a,b]<br>Biotin[a]<br>Choline[a] | | Iodine[a]<br>Manganese[b]<br>Molybdenum[a] |
| 1–3 yr | Vitamin A[b]<br>Thiamin[b]<br>Riboflavin[b]<br>Niacin[b]<br>Vitamin $B_6$[b]<br>Folate[b]<br>Pantothenate [b]<br>Biotin[a]<br>Choline[b]<br>Vitamin C[c]<br>Vitamin E[c] | Calcium[e]<br>Magnesium[c]<br>Potassium[f]<br>Sodium[f] | Chromium[b]<br>Copper[c]<br>Molybdenum[c]<br>Selenium[c] |
| 4–8 yr | As for 1–3 yr | Magnesium[c]<br>Potassium[f]<br>Sodium[f] | Chromium[b]<br>Copper[c]<br>Molybdenum[c]<br>Selenium[c] |
| 9–13 yr | As for 4–8 yr | Potassium[f]<br>Sodium[f] | Chromium[b]<br>Copper[c]<br>Molybdenum[c]<br>Selenium[c] |
| 14–18 yr | As for 4–8 yr | Potassium[f]<br>Sodium[f] | Chromium[b]<br>Copper[c]<br>Iodine[c]<br>Molybdenum[c]<br>Selenium[d] |
| 19–30 yr | | | |
| 31–50 yr | Vitamin C[d]<br>Vitamin E[d] | | Selenium[d] |
| 51–70 yr | Vitamin C[d]<br>Vitamin E[d] | Phosphorus[d]<br>Sodium[g] | Iodine[d]<br>Copper[d]<br>Molybdenum[d]<br>Zinc[d]<br>Selenium[d] |
| > 70 yr | Vitamin C[d]<br>Vitamin E[d] | Calcium[h]<br>Phosphorus[d]<br>Sodium[g] | Iodine[d]<br>Iron[d]<br>Copper[d]<br>Molybdenum[d]<br>Zinc[d]<br>Selenium[d] |

*a.* Average intake (AI) extrapolated up from AI for infants 0–6 months.
*b.* AI extrapolated down from adults to children adjusted for body weight.
*c.* Estimated average requirement (EAR) extrapolated down from adults or older children to young children adjusted for body weight.
*d.* EAR extrapolated up from younger adults to older adults.
*e.* AI extrapolated down from older children to younger children adjusted for body weight.
*f.* AI extrapolated down from adult values adjusted for median energy intakes.
*g.* AI extrapolated up from younger adults based on median energy intakes.
*h.* AI extrapolated up from younger adults.

TABLE 4. Summary of macronutrients for which AI/EAR was derived in all or part from extrapolations in the Canada/USA DRI report [6]

| Age group | Carbohydrates | Fiber | Protein | Energy |
|---|---|---|---|---|
| 0–6 mo | | | | Value for energy deposition during growth was derived from the calculated energy content of tissue deposition[c] adjusted to the 50th percentile of weight gain[d] |
| 7–12 mo | | | Value for protein deposition during growth was derived from data for protein accumulation in children [a,b] that were fitted to polynomial equations | Same as for 0–6 mo |
| 1–3 yr | Extrapolated from adult values (EAR = 100 g/day; RDA = 130 g/day) without adjustment | Extrapolated from adult AI of 14 g fiber/1,000 kcal × median energy intake level (kcal/1000 kcal/day) | Same as for 7–12 mo | Same as for 0–6 mo |
| 4–8 yr | Same as for 1–3 yr | Same as for 1–3 yr | Same as for 7–12 mo | Value for energy deposition during growth was derived from the calculated rates of weight gain of children in the FELS longitudinal study[e] and rates of protein and fat deposition for children[f] |
| 9–13 yr | Same as for 1–3 yr | Same as for 1–3 yr | Same as for 7–12 mo | Same as for 3–8 yr |
| 14–18 yr | Same as for 1–3 yr | Same as for 1–3 yr | Same as for 7–12 mo | Same as for 3–8 yr |

AI, average intake; EAR, estimated average requirement; DRI, dietary reference intake; RDA, recommended dietary allowance
*a*. Butte et al. [32]. *c*. Butte et al. [34]. *e*. Baumgartner et al. [36].
*b*. Ellis et al. [33]. *d*. Guo et al. [35]. *f*. Fomon et al. [37].

TABLE 5. Population reference intakes for children in the European Community aged 1 year and over derived, in the absence of adequate data, by extrapolation from the population reference intakes (PRIs) of young adults on the basis of energy expenditure or other criteria by the Scientific Committee for Food [10]

| Nutrient | Basis for extrapolation |
|---|---|
| Thiamin, riboflavin, niacin, folate, vitamin $B_{12}$, iodine | Energy expenditure |
| Protein | Amount of high-quality egg or milk protein needed for achieving nitrogen balance plus the additional need for growth |
| Polyunsaturated fatty acids | Estimated needs for tissue deposition |
| Retinol | Smooth transition (interpolation) from infant values, based on provision with breastmilk, to adult values |
| Vitamin $B_6$ | Reference intake for protein |
| Vitamin C | Gradual increase (interpolation) from infant to adult reference values |
| Vitamin E | Dietary intake of polyunsaturated fatty acids |
| Calcium | Estimated deposition for skeletal development |
| Magnesium | Body weight with additional adjustments based on physiological considerations |
| Potassium | Factorial approach |
| Iron | Estimated tissue deposition and losses |
| Zinc | Factorial approach |
| Copper | Estimated tissue deposition and losses |
| Selenium | Body weight |

breastfed infants, and those calculated for the group from 1 to 3 years of age.

The Finnish Nutrition Recommendations [12] rely on the Nordic Nutrition Recommendations [13] and the WHO report [9]. For example, the energy recommendations are based on BMR corrected to a PAL; however, estimates were based on a reference man or woman rather than reference weights for age, as was done in the DRIs of the United States and Canada and the Caribbean reports. This report did not contain any examples of application of extrapolations or interpolations in the derivation of the nutrient recommendations.

The report from France [14] does not appear to employ extrapolations in the development of nutrient recommendations. The values provided in this report represent recommended intakes for populations, not individuals.

The Mexican report [15] employed reference weights, derived as outlined above, in developing some of the nutrient recommendations. No information was provided as to whether extrapolations were employed across age groups. For the dietary reference values (DRVs) from the United Kingdom, it was not possible to determine the basis of the DRVs for children from the statement, "Some DRVs are also related to body weight."

### Extrapolations of upper safe levels of nutrient intakes for children

In the DRI reports of Canada and the United States, extrapolations based on body weight were used to establish tolerable upper levels for children from adult values when scientific data were not available.

In Europe, the upper safe levels (ULs) of nutrient intake established by the Scientific Committee for Food and the European Food Safety Authority [19] were estimated for children, in the absence of adequate data, on the basis of criteria specific for each nutrient. For instance, relative body weight (using reference weights), was used for vitamin $B_6$, niacin, folate, copper, selenium, and molybdenum; or body surface area (body weight$^{0.75}$) was used for zinc and boron. In some instances, the upper level was based on age-specific outcomes, such as for fluoride, where dental fluorosis in young children and bone health in older children were the outcomes used to set the UL. In the case of magnesium, the same UL was set for adults and children over 4 years of age, but the available data did not allow the definition of a UL for younger children. However, the committee expressed severe concerns regarding the limitations in the methods available and in the approach it has used to extrapolate ULs for children, and it recommended that this issue "be reviewed in order to establish whether further refinements in the approach or further research are needed" [19].

## Nutrient recommendations established using an extrapolation model for physiological states of pregnancy and lactation

Adequate research results from which to derive the requirements for human subjects during pregnancy and lactation are not available for a great number of nutrients. For pregnancy, often a factorial model is applied using knowledge of fetal accretion of nutrients and addition of nutrients in the expanded fluid volume of a woman during pregnancy [10]. Insensible losses of nutrients and altered efficiency of absorption of nutrients during pregnancy are applied to adjust the nutrient accretion value, if such information is known. If the basis for the estimate of nutrient accretion is not normally distributed, then modeling of the data is done for some nutrients, such as iron.

For example, a factorial estimate of nutrient intake during pregnancy is calculated as the sum of the following:

» Nutrient intake for age in the nonpregnant state;
» Fetal accretion of nutrients (or glucose/free fatty acid utilization);
» Nutrients to supply expanded maternal tissue, metabolic activity, and/or fluid volume;
» Adjustment for change in insensible losses, physical activity, or efficiency of absorption, if known;

If the values used as the basis of this estimate are not normally distributed, then modeling of data is required, for example, for nutrients such as iron.

For lactation, the general approach to the factorial model is simply to sum the nutrient needs for a woman of similar periconceptional age who is not lactating with the amount of nutrient delivered into an average volume of breastmilk, as used in the Canada/USA DRI reports [5, 6, 28–31] and the PRI report for the European Community provided by the Scientific Committee for Food [10]. However, the average daily intake of breastmilk has been reported to vary between studies [38], as does milk composition [39]. Whereas average milk volume might vary between populations in regions with different temperatures or population-based body size, the composition of human milk should be relatively stable in well-nourished mothers. In addition, adjustments are sometimes applied in estimating needs for energy, protein, or carbohydrate to account for utilization (e.g., protein), conversion efficiency (e.g., energy), or maternal weight loss during lactation.

The approaches to derivation of estimates of recommended intakes in pregnancy and lactation across agencies and countries are summarized in **table 6** for macronutrients and in **table 7** for vitamins. Since many reports provide nutrient recommendations for different stages of pregnancy and lactation, these are individually noted. Although the general method of extrapolation might be similar across reports, the

TABLE 6. Summary of extrapolations used to determine recommended macronutrient intakes for pregnancy and lactation for all reports reviewed

| Agency | Pregnancy | Lactation |
|---|---|---|
| | Protein | |
| Canada/USA [6][a] | Only for 2nd half of pregnancy: the EAR for age plus the amount for tissue deposition in mother and fetus and maintenance of the expanded protein tissue pool<br>RDA is the EAR plus an estimated CV of 12% | The EAR for age plus the amount of protein secreted in breastmilk adjusted for dietary protein utilization. RDA is the EAR plus an estimated CV of 12% |
| Caribbean [16] | Adopted from WHO (1985) [9]: an increase of 6 g/day to nonpregnant allowance | Addition of the amount of protein secreted in breastmilk (no adjustment for efficiency of conversion of dietary protein to milk protein owing to 25% nonprotein nitrogen in breastmilk) |
| D-A-CH [8] | From 4th month additional * 10 g (= 58 g/day) or 6.3 g/MJ<br>(general: upper limit for adults of protein intake at 2.0 g/kg/day)<br>(p32) | Recommended protein intake is derived from the amount of protein secreted in the milk and given a 70% protein utilization:<br>+ 15 g (= 63 g/day) or 5.8 g/MJ (p32) |
| European Community [10] | Based on FAO/WHO/UNU (1985) [9], body compositional changes, increased efficiency of utilization of amino acids:<br>Pregnancy + 10 g/day | Individual variation depending on amount of breastmilk provided:<br>1st 6 mo + 16 g/day<br>2nd 6 mo + 12 g/day |
| Finland [12][b] | | |
| France [14][c] | | |
| Mexico [15][a] | | |
| Netherlands [40][d] | Derived from amount of nitrogen lost and additional nitrogen required for pregnancy and lactation<br>EAR 0.7 g/kg/day<br>RDA 0.9 g/kg/day<br>UL 25%<br>(p65 + 75) | EAR 0.8 g/kg/day<br>RDA 1.0 g/kg/day<br>UL 25%<br>(p75) |
| New Zealand/ Australia [41][e] | | |
| Nordic [13] | 10%–20% of energy intake, based on physiological considerations | 10%-20% of energy intake, based on physiological considerations |
| United Kingdom [17][f] | Requirements were calculated to allow for protein retention in the products of conception and in the maternal tissue associated with the birth of a 3.3-kg infant<br>DRV: + 6 g/day<br>(for 100% digestibility) | Requirements are based on protein content of milk added to value for nonpregnancy<br>RNI:<br>0-4 mo: +11 g/day<br>4 + mo: + 8 g/day |
| | Carbohydrates | |
| Canada/USA [6][a] | EAR for age plus fetal brain glucose utilization | EAR for age plus average human milk content of carbohydrate |
| Caribbean [16] | Proportion of additional energy as carbohydrate calculated as 55%–60% of energy | Same as for pregnancy |

| | | |
|---|---|---|
| D-A-CH [8] | A guiding value of > 50% of dietary energy from carbohydrates is substantiated by epidemiologic findings. No special recommendations for pregnant or lactating women | |
| European Union [10] | No recommendation given | No recommendation given |
| Finland [12][b] | | |
| France [14][c] | | |
| Mexico [15][a] | | |
| Netherlands [40][d] | Digestible carbohydrates: RDA 40%, based on the 97.5 percentile of endogenous glucose production. This remains unchanged during pregnancy; RDA is the same for pregnant and nonpregnant women (p137 + 145) | RDA 40% |
| New Zealand/ Australia [40][e] | | |
| Nordic [13] | No specific recommendation | No specific recommendation |
| United Kingdom [17][f] | | |
| | Energy | |
| Canada/USA [6][a] | EER for age plus change in TEE plus pregnancy energy deposition (different for each of 3 trimesters) | EER for age plus milk energy output minus weight loss |
| Caribbean [16] | Additional energy suggested by WHO [9] (285 kcal/day) | Energy for age plus milk energy, the energy cost of producing the milk and an assumed 80% efficiency of conversion of food energy to milk minus energy from weight loss |
| D-A-CH [8][f] | Energy requirement is calculated by considering the BMR, physical activity, thermogenesis after food intake, and needs for growth, pregnancy and lactation. Pregnant women should receive an additional 1.1 MJ (255 kcal)/day for the duration of pregnancy, independent of PAL | Considering the energy contents of breastmilk, energy needs for milk synthesis and utilization of maternal endogenous fat stores during lactation, the recommended additional energy intake independent of PAL is:<br>Up to completion of 4th month: + 2.7 MJ (635 kcal)/day<br>After the 4th month:<br>Full breastfeeding + 2.2 MJ (525 kcal)/day, partial breastfeeding + 1.2 MJ (285 kcal)/day |
| European Community [10] | EER estimated with BMR (WHO/FAO/UNU, 1985) [9] and PAL<br>Thin women: 1.7 MJ/day<br>Overweight and normal-weight women: 0.75 MJ/day from 10th week of pregnancy to reach lowest neonatal and postnatal mortality | Energy requirements proportional to quantity of milk produced, considering the conversion efficiency of dietary energy to milk energy (now proposed 97%) and average weight loss following delivery |
| Netherlands [40][d] | EAR + 1.2 MJ/day<br>Based on increased metabolic rate and reduced level of physical activity during pregnancy (method used: factorial and $^{2}H_{2}0$ $^{18}O$)<br>(p57 + 58) | EAR + 2.1 MJ/day<br>Based on energy required for the production of human milk and an average metabolic combustion of 0.5 kg of body fat/mo (method used: factorial)<br>(p58 + 59) |

*continued*

TABLE 6. Summary of extrapolations used to determine recommended macronutrient intakes for pregnancy and lactation for all reports reviewed *(continued)*

| Agency | Pregnancy | Lactation |
|---|---|---|
| Nordic [13] | Increased daily energy needs estimated as 1,500 kJ (350 kcal) in the 2nd trimester and 2,100 kJ (350 kcal) in the 3rd trimester, which can be at least partly covered by reduced PAL. Increased dietary energy need acknowledged but not specified | Based on a factorial approach, an extra energy intake of 2.0 MJ (280 kcal)/day is recommended |
| United Kingdom [17][d] | Increase of tissue mass and metabolic activity (resulting in greater BMR and energy cost of movement):<br>EAR + 0.8 MJ/day or 200 kcal/day only during the 3rd trimester | Values for milk output derived from combined data from British and Swedish women, including a 4% correction to allow for insensible water losses. These values are considered representative of well-nourished women and differ from those in the FAO/WHO/UNU) [9] by less than 10% in the early months.<br>Gross energy supplied by the mother, conversion from maternal diet to gross milk energy (80%), maternal weight loss (500 g/mo), and maternal EAR<br>EAR:<br>1 mo: + 1.90 MJ or<br>450 kcal/day<br>2 mo: + 2.2 MJ or<br>530 kcal/day<br>3 mo: + 2.40 MJ or<br>570 kcal/day<br>EARs recognize 2 distinct groups of breastfeeding women:<br>Group 1: exclusive or almost exclusive breastfeeders who progressively introduce weaning food<br>Group 2: women who introduce only limited complementary food after 3–4 mo and intend that breastfeeding provide primary source for 6 or more months |

EAR, estimated average requirement; RDA, recommended dietary allowance; D-A-CH, Germany-Austria-Switzerland-Slovenia; CV, coefficient of variation; UL, upper tolerable limit of nutrient intake; DRV, dietary reference value; RNI, reference nutrient intake; EER, estimated energy requirement; TEE, total energy expenditure; BMR, basal metabolic rate; PAL, physical activity level

*a*. The reports from Canada/USA [6] and Mexico [15] assumed a milk volume of 780 mL/day during lactation for the first 6 months and 640 mL/day after 6 months.

*b*. The Finnish recommendations [12] for some nutrients are higher for pregnant and lactating women than for women of periconceptual age, but no information is provided in the report as to the basis of these special recommendations. The recommended intakes are intended for use in diet planning for groups, but it is noted that the individual requirements are often lower than the recommended intake for populations.

*c*. In the report from France [14], the average volume of milk produced on which the recommended intake was derived for lactation was 750 to 850 mL/day.

*d*. A committee of the Health Council has drawn up these recommendations, and the Standing Committee on Nutrition and the Standing Committee on Medicine have reviewed them [40].

*e*. As an interim measure, Australian Dietary Intakes [41] were adopted, and adapted where necessary for use in New Zealand.

*f*. The Panel on Dietary Reference Values (DRVs) was set up by 1987 by the Committee on Medical Aspects of Food Policy (COMA) to review the RDAs, set in 1979. Four expert working groups reported their considerations and conclusions to the panel [17].

TABLE 7. Summary of extrapolations used to determine recommended vitamin intakes for pregnancy and lactation for all reports reviewed

| Agency | Pregnancy | Lactation |
|---|---|---|
| Vitamin A | | |
| Canada/USA [28][a] | EAR for age plus estimated daily accumulation by fetus | EAR for age plus amount secreted in human milk |
| Caribbean [16] | Add amount for maternal stores and fetal growth to nonpregnant value | Add amount secreted in human milk (400 μg RE/day) in addition to nonpregnant value |
| European Community [10] | Plus 100 μg RE/day (= total 700 μg RE/day) to enhance maternal storage and provide for fetal supply.<br>Intakes > 6,000 μg/day have been associated with birth defects | Plus 350 μg RE/day (= total 950 μg RE/day) based on amount secreted with human milk |
| Nordic [13] | An additional 50 μg/day would be needed to achieve adequate accretion in fetal liver; to cover individual variation an additional 100 μg/day (total 800 μg/day) is recommended (p4 + 6) | An additional 400 μg/day (total 1,100 μg/day) is recommended to cover average milk excretion reported in Western countries as about 350–450 μg/day |
| United Kingdom [17] | Additional vitamin A supply to support fetal growth and maternal tissue growth, in line with FAO/WHO recommendations based on body pool size<br>RNI: + 100 μg/day | To cover amounts secreted with milk RNI: 0–4 mo and<br>4+ mo: + 350 μg/day |
| Vitamin E | | |
| Canada/USA [28][a] | | Add amount secreted in milk |
| Caribbean [16] | Add amount for fetal growth adjusted for incomplete absorption | Add amount for vitamin E secreted in milk adjusted for incomplete absorption |
| D-A-CH [8] | Additional intake + 1 mg TE/day (total 13 mg TE/day) based on increased intakes of energy and polyunsaturated fatty acids | Additional intake + 5 mg TE/day (total 17 mg TE/day) based on increased intakes of energy and polyunsaturated fatty acids |
| European Community[10] | No specific recommendation | No specific recommendation |
| Nordic [13] | + 2 mg TE/day (2nd and 3rd trimesters) to cover additional needs | + 2 mg TE/day to cover secretion with milk |
| United Kingdom [17] | No specific recommendation | No specific recommendation |
| Vitamin C | | |
| Canada/USA [28][a] | | EAR for age plus amount secreted in human milk |
| Caribbean [16] | NA | |
| D-A-CH [8] | Additional intake + 10 mg/day (total 110 mg/day), considering reduced plasma concentration and decreased body reserves during pregnancy, and bioavailability | Additional intake + 50 mg/day (total 150 mg/day), to cover excretion with breastmilk |
| European Community [10] | Additional intake + 10 mg/day (total 55 mg/day) to allow for the 50% higher fetal plasma levels and higher catabolic rate of the fetus | Additional intake + 25 mg/day (total 70 mg/day) to cover at least 20 mg/day excreted with breastmilk, assuming 85% bioavailability |
| Nordic [13] | Additional intake + 10 mg/day (total 85 mg/day) to cover increased needs for fetal growth and catabolized vitamin C | Additional intake + 25 mg/day (total 100 mg/day) to cover excretion with breastmilk |
| United Kingdom [17] | Additional intake + 10 mg/day (total 50 mg/day) during the 3rd trimester to allow for the 50% higher fetal plasma levels and higher catabolic rate of the fetus | Additional intake + 30 mg/day (total 70 mg/day) to ensure that maternal stores are maintained and breastmilk levels are in the upper half of physiological range |

EAR, estimated average requirement; RE, retinol equivalent; RNI, reference nutrient intake; D-A-CH, Germany-Austria-Switzerland-Slovenia; TE, alpha-tocopherol equivalent

*a.* The reports from Canada/USA [5, 6, 28-31] assumed a milk volume of 780 mL/day during lactation for the first 6 months and 640 mL/day after 6 months.

reference values applied for fetal accretion of nutrients or nutrient composition of milk were often different; thus, variation between reports in the recommended intakes is likely. Unless geographic or racial differences can be proven for fetal accretion of nutrients or breastmilk composition, a future goal would be to standardize reference values across individual dietary standard reports. For fetal accretion of nutrients, it is important that consistent source evidence be used. Most commonly, such information is derived from the body composition of fetuses aborted or dying in the third trimester of pregnancy [42]. We recommend that when NIVs are developed for lactating women, the values for average daily milk volume provided by the WHO report on complementary feeding of young children in developing countries [38] should be used, and for milk nutrient composition the values cited in the DRI reports [5, 6, 29–32] for well-nourished women should be adopted.

## Future development of NIVs based on human research

It is evident that further systematic scientific research is urgently required to provide adequate data on physiological nutrient requirements, especially for children and adolescents [2], pregnant and lactating women, and older adults. The opportunity is there, because methodological progress has made less invasive or noninvasive approaches available that allow ethical investigations of a number of these issues in healthy children, for example, and also in pregnant and lactating women. Therefore, it would be feasible today to narrow many of the existing gaps in knowledge in this respect, if such research were given sufficient priority both by academic institutions and by funding agencies.

A clear priority for the future development of NIVs is to conduct research that will provide for internationally applicable physiological data (e.g., on absorption, distribution, deposition, metabolism, and excretion of nutrients for different sex and life-stage groups) using state-of-the-science techniques. Opportunity exists with noninvasive measurements using stable isotope tracer methodology for energy expenditure, amino acid oxidation using stable isotope breath tests for the estimation of amino acid requirements [43], and turnover of macrominerals (e.g., calcium and magnesium) and trace elements (e.g., iron and zinc). Body composition measurements to obtain data on nutrient accretion can be obtained longitudinally by methods such as dual-energy x-ray absorptiometry, total body electrical conductivity (TOBEC), and magnetic resonance imaging (MRI).

## Summary

This review of dietary standards revealed that the approach of extrapolation to obtain nutrient recommendations is used frequently and that a great amount of inconsistency exists in the factors considered in extrapolation. The use of original research data is the preferable way to estimate nutrient requirements for different life-stage groups. However, because of the paucity of research data for some subgroups, it is often necessary to extrapolate information from other groups in order to derive estimated requirements. Extrapolation should always be a second choice, and scientists are encouraged to develop new, innovative, noninvasive methods or use existing methods, e.g., stable isotopes, to specifically determine the nutrient requirements of understudied groups, such as pregnant and lactating women, infants, children, and the elderly.

Until data are available for all life-stage groups, extrapolation from one group to another is necessary. Frequently, this involves extrapolation from adults to children and adolescents and from younger adults to older adults. The rationale or scientific basis for the method chosen should be completely transparent and thoroughly described for each nutrient and life-stage group. It is likely that different approaches will be used for different nutrients and different extrapolations for a single nutrient. There is no one "correct" method of extrapolation and scientific judgment will probably be part of the process. Examples of approaches to extrapolation models include adjustments for body size (weight or metabolic weight), energy intakes for age, or application of factorial estimates of requirements for growth, pregnancy, and lactation. However, extreme caution is advised if the extrapolation is based on energy intakes, median body weights, or activity levels, which may vary widely among populations.

## Note

The following reports were reviewed but, because they do not constitute nutrient-based dietary recommendations, were not included in this review: Swedish National aims and strategies for nutrition 1999–2004 [44]; National Institute of Nutrition, Hyderabad, India, Annual Report 1998–99 [45]; Socialist Republic of Vietnam, National Plan of Action for nutrition 1995–2000 [46]; Warsaw, Poland: Programme of health improvement of the Polish population through improvement of health quality of food and modification of pattern of nutrition (1996) [47]; and South African food-based dietary guidelines (2001) [48].

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# The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates

Rosalind S. Gibson

## Abstract

*To convert physiological requirements into dietary requirements, adjustments are needed for some nutrients that take into account certain diet- and host-related factors specific to a country or region. Nutrients whose requirements should be adjusted in this way include calcium, magnesium, iron, zinc, protein, folate, vitamin A, and carotenoids. The diet-related factors that must be considered depend on the nature of the habitual diet and may include the chemical form of the nutrient and the nature of the dietary matrix, interactions between nutrients and/or organic components, and food preparation and processing practices within the country or region. The host-related factors can be further subdivided into intestinal and systemic factors. Reductions in the secretion of hydrochloric acid, gastric acid, and/or intrinsic factor, together with alterations in the permeability of the intestinal mucosa, are all examples of intestinal factors that can markedly influence the absorption of certain nutrients, but that are often ignored when setting dietary requirements. Systemic factors that should also be considered include nutrient status of the host, age, sex, ethnicity, genotype, and physiological state (e.g., pregnancy or lactation), and chronic and acute infectious disease states. Algorithms can estimate the bioavailability of iron, zinc, protein, folate, vitamin A, and carotenoids, although their accuracy is limited by the complex interactions among the absorption modifiers in the whole diet. For calcium and magnesium, the amount available for absorption is still estimated from their major food sources in the habitual diet. Currently, there are often large differences in the adjustments employed to convert physiological requirements to dietary requirements, even among countries consuming diets of similar patterns.*

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**Key words:** Dietary requirements, bioavailability, bioefficacy, diet-related factors, intestinal factors, systemic factors, algorithms

## Introduction

Variations exist among countries in estimates of nutrient requirements, even for a specified class of individuals. There are several reasons for the differences observed. This paper focuses on the factors that must be taken into account when the *physiological requirements* of a nutrient for an individual are translated into *dietary requirement* estimates. It is at this stage that adjustments are made to the dietary requirement to take into account particular types of diets consumed by individuals and certain host-related factors. The magnitude of these adjustments and their relative importance varies with the nutrient, life-stage group, and setting (e.g., environment). In this paper the following operational definitions will be used:

*Physiological requirement* is the requirement of absorbable and utilizable nutrient. In environments where the nature of the diet ingested and/or the intestinal or systemic conditions of the host affect either the absorption or the utilization of an ingested nutrient, the dietary requirements will be higher than the physiological requirements.

*Dietary requirement* is the requirement of the nutrient *as ingested* in a specified type of dietary pattern and under specified conditions of the host. Hence, this requirement takes into account both dietary and host-related factors that affect the absorption or utilization of the nutrient or both. The host-related factors include systemic factors (e.g., nutrient status, age, and physiological status) and the possible coexistence of intestinal factors (e.g., atrophic gastritis) known to influence the efficiency of intestinal digestion and absorption. Because these factors vary markedly according to the nature of the habitual dietary pattern and the conditions of the host, it follows that the dietary requirement may differ among countries, even if there is approxi-

mate agreement on the physiological requirements.

*Bioavailability* is the proportion of the ingested nutrient that is absorbed and utilized through normal metabolic pathways [1]. It is influenced by diet- and host-related factors.

*Bioefficacy* is the efficiency with which ingested nutrients are absorbed and converted to the active form of the nutrient [2].

The objective of this paper is to examine the diet- and host-related factors that may influence the bioavailability of certain nutrients and hence the adjustments required to translate physiological requirements of these nutrients to dietary requirement estimates. For this review, the diet-related factors have been classified into the chemical form of the nutrient and the nature of the dietary matrix; interactions occurring among nutrients themselves or with other components of the diet; and pretreatment of food as a result of processing and/or preparation practices. The host-related factors considered in this paper include intestinal factors that influence the efficiency of luminal and mucosal digestion and absorption, and systemic factors such as nutrient status of the host, age, physiological status, and illness due to chronic or acute infections. The final step in setting dietary requirement estimates takes into account the variations in requirements among individuals, according to life stage and sex.

Most of the literature cited in this review encompasses results of *in vivo* human isotope studies, where possible and relevant, although some results based on *in vitro* methods and epidemiologic studies have also been included.

## Dietary factors affecting the bioavailability of nutrients

Several factors in the diet can influence the bioavailability of nutrients. The magnitude depends on inhibitors and promoters in any one meal, and hence the composite diet. Increasingly, the influence on bioavailability of fortificants or supplements as well as intrinsic components of the diet must be considered.

There are some concerns about the predictive value of certain methods (e.g., *in vitro* assays) used to investigate the dietary factors affecting the bioavailability of inorganic nutrients in foods, and inconsistent results have been reported. Moreover, most of the *in vivo* isotope studies have been based on single meals. However, there is some evidence that the effects of dietary modifiers on the absorption of iron, and possibly other trace elements, from single-meal studies differ from those based on a total diet of similar composition as the single test meal [3, 4]. Therefore, caution must be used when interpreting the results in the literature on diet-related factors affecting nutrient bioavailability. In general, diet-related factors have less influence on the bioavailability of macronutrients than micronutrients. Of the latter, the bioavailability of iron and zinc is especially affected. For some micronutrients (e.g., iodine, vitamin C, thiamin), the effect of dietary factors on bioavailability appears to be very limited, and for micronutrients such as riboflavin [5], vitamin $B_{12}$ [6], magnesium [7], and chromium [8] bioavailability data in humans are sparse. In some cases (e.g., vitamin $B_6$), existing bioavailability data are difficult to interpret because of methodological difficulties [9]. The three groups of dietary factors known to influence nutrient bioavailability are discussed in turn below.

### Chemical form of the nutrient and nature of the dietary matrix

The absorption and/or utilization of several micronutrients are affected by the chemical form of the nutrient (i.e., speciation); some examples are given in **table 1**. Of these, the bioavailability of intrinsic iron in indigenous diets as well as the form in supplements and fortificants

TABLE 1. Examples of nutrients for which bioavailability is affected by chemical form

| Nutrient | Forms |
|---|---|
| Iron | Heme iron (bound in a porphyrin ring) in hemoglobin and myoglobin from meat, poultry, and fish is more readily absorbed than nonheme iron found in foods of plant and animal origin. Bioavailability of iron from fortificants or supplements depends on their chemical form |
| Selenium | Main food sources of selenium are the organic forms, selenocysteine and selenomethionine. These tend to be better absorbed than the inorganic form of selenium, selenite |
| Zinc | Organic zinc complexes (e.g., from oysters) are more readily absorbed than inorganic zinc salts |
| Folate | Polyglutamates (mainly 5-methyl tetrahydrofolate [5MeTHF] in fresh food) are less well absorbed than synthetic monoglutamate form (i.e., folic acid used as fortificant and supplements) |
| Vitamin $B_6$ | Free pyridoxine, pyridoxamine (plus phosphorylated forms) in plants and pyridoxal (plus phosphorylated forms in animal foods) are better absorbed than pyridoxine β-D-glucoside in heat-processed milk products |
| Niacin | Niacin in mature maize is present as niacytin (nicotinic acid esterified to polysaccharides), which is unavailable for absorption |

has been most extensively studied. Two forms of iron exist in foods: heme iron and nonheme iron. They are absorbed by separate pathways, but once inside the mucosal cells of the small intestine all iron enters a common pool. Heme iron, bound in a porphyrin ring, is derived mainly from hemoglobin and myoglobin in meat, poultry, and fish, whereas nonheme iron is found primarily as iron salts in a wide variety of foods of both plant and animal origin, and possibly as contaminant iron introduced during processing, preparation, and storage and by contamination from the soil [10]. Heme iron is absorbed as the intact moiety and nonheme iron from the common pool within the gastrointestinal tract [11]. Of the two forms, heme iron is much more readily absorbed than nonheme iron. Absorption of heme iron depends on the iron status of the individual, whereas absorption of nonheme iron depends not only on the individual's iron status, but also on the content of absorption modifiers consumed during the same meal. In general, for any given individual, the higher the nonheme iron content of the diet, the lower the absorption efficiency [12].

Hurrell [13] reviewed the bioavailability of the forms of iron used as fortificants. Their bioavailability is usually expressed as relative bioavailability value (RBV), i.e., bioavailability relative to that of ferrous sulfate, the latter being assigned a standard RBV of 100%. Absorption is highest for those iron compounds that are soluble in water or dilute acids (e.g., ferrous sulfate, ferrous fumurate, ferric saccharate). However, such compounds often cause oxidative reactions when added as fortificants to food unless they are manufactured in encapsulated forms. Consequently, compounds that are less likely to interact with food components (e.g., ferric pyrophosphate, ferric orthophosphate, and elemental iron) are often used, despite their lower RBV. Note that the composition of the native diet can be a more important determinant of iron absorption than the type of fortificant itself, especially in plant-based diets.

Absorption of zinc and selenium is also influenced by chemical form. In both cases, the organic forms tend to be more readily absorbed than the inorganic forms. For zinc, the organic form (in oysters) appears to be less affected by absorption modifiers than the inorganic forms [14]. Selenomethionine, the organic form of selenium found in most plant tissues (and selenized yeast) is absorbed more efficiently than the inorganic form (e.g., selenite) used in supplements [15], although absorption rates for all forms of selenium are high (70% to 95%).

Chemical form also affects the bioavailability of some vitamins. For example, the bioavailability of carotenoids varies depending on their isomeric form [16]. In contrast, there is some evidence that the bioavailability of polyglutamyl and monoglutamyl forms of native folate in foods is probably similar [17]. Nevertheless, some inconsistencies have been reported among studies that have been associated with differences in the study design and protocols used, variation among individuals in folate digestion, absorption, and metabolism, and analytic difficulties [18]. In contrast, the native polyglutamyl forms of food folate have a much lower bioavailability than does the synthetic form, folic acid, a monoglutamate used as a supplement or fortificant [6, 19, 20]. To take into account some of these differences, the Institute of Medicine [6] has introduced a new term: dietary folate equivalent (DFE). Certain forms of vitamin $B_6$ found in heat-processed milk products (e.g., pyridoxine β-D-glucoside) are also less available than those normally found in foods [9]. Likewise, niacin in cereals such as maize is present as niacytin, an unavailable form of which only a small amount is hydrolyzed by gastric acid in the gastrointestinal tract. Coffee beans also contain an unavailable form of nicotinic acid (trigonellin, 1-methyl nicotinic acid) that becomes available after roasting [21]. In contrast, for thiamin, chemical form has little effect on its bioavailability [22].

The food matrix probably has the greatest effect on the absorption of provitamin A carotenoids [23] and folates [17, 24]. Both of these micronutrients may be entrapped in the insoluble matrix or cellular structure of certain plant foods, reducing their bioavailability. For example, β-carotene is bound to proteins in the chloroplasts in dark-green leafy vegetables, whereas in orange and yellow fruits (mango, papaya, etc.) and pumpkin and sweet potato, carotenoids are dissolved in oil droplets in chromoplasts and are more readily extracted during digestion, so that their bioefficacy is fourfold higher than that from dark-green leafy vegetables [23]. In spinach, bioavailability of folate is higher after the leaves have been chopped, minced, or enzymatically liquefied than for the same amount of whole spinach leaves [25]. Vitamin $B_{12}$ is also bound to enzymes or other carrier proteins in food and must be released prior to absorption.

### Interactions among nutrients themselves and with other components in the whole diet

The mechanisms whereby components in the diet influence the bioavailability of nutrients may involve noncompetitive interactions between nutrients and organic components in diets whereby insoluble or soluble complexes are formed in the acid pH of the stomach and proximal duodenum, and direct competitive interactions between two (or more) inorganic nutrients with chemical similarities that share the same absorptive pathways.

Note that the bioavailability of nutrients from supplements or fortificants when taken together with a meal is affected by the same factors as those present in a food [26]. Their net effect depends on the balance between those factors inhibiting and those factors

enhancing nutrient absorption and/or utilization in the diet. In addition, some of the isotope studies are based on single meals, which tend to exaggerate the results of nutrient interactions involving iron absorption compared with studies based on whole diets [3, 4]. Hence, caution must be used when interpreting some of the bioavailability results reported in the literature based on single-meal studies.

#### *Noncompetitive interactions*

Several organic dietary components have the capacity to form insoluble or soluble complexes with certain nutrients in the gut, thus inhibiting or facilitating absorption. In some cases, reabsorption of certain inorganic nutrients that are excreted endogenously in the gastrointestinal tract (e.g., calcium, zinc, copper) may also be affected [27, 28]. **Table 2** lists these naturally occurring organic substances, their major food sources, and summarizes their nutritional consequences. Some occur in large amounts in many of the plant-based diets in developing countries; they are discussed briefly below. Note that the effects of these organic components on bioavailability can often be modulated by pretreatment of the food with commercial and/or traditional food processing and preparation methods.

*Phytate* refers to phytic acid (*myo*-inositol hexaphosphate) as well as the salts: magnesium, calcium, or potassium phytate. Phytate is the major storage form of phosphorus in cereals, legumes, and oleaginous seeds, so that diets based on unrefined, unfermented cereals are especially high in phytate, whereas those based on starchy roots and tubers are low [29]. Phytate chelates metal ions, especially zinc, iron, and calcium, but not copper [30], in the gastrointestinal tract, making them unavailable for absorption. It also complexes endogenously secreted minerals such as zinc [26, 28] and calcium [31], making them unavailable for reabsorption into the body.

*Myo*-inositol hexaphosphate can be hydrolyzed by certain food processing and preparation methods to lower *myo*-inositol phosphates, which do not form insoluble complexes with iron and zinc. For example, *myo*-inositol phosphates with fewer than five phosphate groups (i.e., IP-1 to IP-4) do not have a negative effect on zinc absorption [32], whereas those with fewer than three phosphate groups do not inhibit nonheme iron absorption [33]. There appears to be no adaptation to the inhibitory effect of a high-phytate diet on iron absorption in long-term vegetarians [34]; whether the same is true for zinc is uncertain.

High amounts of calcium may exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium-zinc-phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone [35]. In general, because the calcium content of most plant-based diets is low, such an effect is probably rare. Indeed, calcium did not add significant predictive power in a model developed by the International Zinc Nutrition Consultative Group (IZiNCG) [36] to predict zinc absorption. Nevertheless, some diets may be exceptions, notably diets in Latin America based on lime-soaked maize, diets of some lacto-ovo vegetarians, diets in regions where betel nut is chewed with lime [31], and cases of geophagia involving calcareous soils [37].

*Soybean protein* inhibits absorption of zinc as a result of its phytate content, although the magnitude of its effect may depend on the processing method. The effect of soybean protein on iron absorption depends on the processing method and food source. Some fractions of soybean protein inhibit iron absorption even when completely dephytinized (e.g., conglycinin) [38], whereas ferritin present in nodulating soybeans may be a bioavailable source of iron for persons with low iron stores [39].

*Polyphenols* are almost ubiquitous in plant foods. Some examples of foods and beverages that contain high levels are shown in **table 2**. Genetic and environmental factors, as well as germination, processing and storage, and degree of ripeness, influence the polyphenol content of plant foods; polyphenols are not denatured by heat [40].

Polyphenols, like phytate, form insoluble complexes with metal cations that inhibit intestinal absorption of nonheme iron [41], perhaps copper [42] and zinc [43], but not calcium [44] or manganese [45]. The active compounds are the galloyl group found in tannin and gallic acid [41]. The effect of polyphenols is independent of that of phytate [46]. Foods and beverages containing these compounds are shown in **table 2**. Several *in vivo* intervention studies based on radioiron isotopes have confirmed the inhibitory effect of tea on iron absorption [47]. The effect can be partly counteracted by simultaneous consumption of ascorbic acid [48]. Some epidemiologic studies of adults have shown that a high consumption of tea [49] or coffee [50] is associated with low iron stores. The inhibitory effect of polyphenol-containing beverages can be significant even during infancy in countries where tea (e.g., Egypt and Israel) or coffee (e.g., Guatemala) is fed to infants [51, 52].

Certain polyphenols, such as caffeic acid, chlorogenic acid, and tannins, also exhibit thiamin-inactivating properties by oxidizing the thiazole ring to the disulfide, which in turn reduces thiamin absorption [53]. Some polyphenols can also influence the digestibility of macronutrients by binding endogenous proteins such as salivary enzymes and digestive enzymes in the intestinal tract. Hence, they can reduce the digestibility of starch, protein, and lipids [54–56]. Tannins can also interfere with protein digestibility by enhancing excretion of endogenous protein, and they increase fecal fat excretion [40, 57].

*Dietary fiber* is composed of nonstarch polysaccha-

TABLE 2. Effects of noncompetitive interactions involving organic substances on nutrient bioavailability: inhibiting and enhancing factors

| Dietary component | Food sources | Main technical influences | Nutritional consequences |
|---|---|---|---|
| Phytate (*myo*-inositol hexaphosphate) plus magnesium, calcium, or potassium phytate[a] | Unrefined cereals, legumes, nuts, oil seeds | Binds certain cations to form insoluble complexes in gut | Zinc, iron, calcium, and probably magnesium are poorly absorbed |
| Soybean protein | Soybeans and unfermented soy products (e.g., textured vegetable protein) | Contain phytate<br>In nodulating soybeans, ferritin is main source of iron | Inhibits zinc absorption<br>Nodulating soybeans may be a bioavailable iron source when iron stores are low |
| Polyphenols | Certain cereals (red sorghum), legumes (red kidney beans, black beans, black grams), spinach, betel leaves, oregano<br>Beverages: tea, coffee, cocoa, red wine | Form insoluble complexes with iron<br>Some polyphenols inactivate thiamin<br>Bind certain salivary and digestive enzymes<br>Enhance excretion of endogenous protein | Inhibit nonheme iron absorption<br>Reduce thiamin absorption<br>Reduce digestibility of starch, protein, and lipids<br>Interfere with protein digestibility |
| Dietary fiber | Unrefined cereals, legumes, nuts, oilseeds, fruits, and vegetables | Lignin and pectin bind bile acids<br>Pectins/psyllium/gums retain water and form viscous solutions in the gut<br>Dietary fiber is fermented in large intestine by microflora | Reduces absorption of fats, fat-soluble vitamins, and carotenoids<br>Slows gastric emptying and digestion and absorption of nutrients<br>Short-chain fatty acids that are produced enhance calcium solubility |
| Oxalic acid | Amaranth, spinach, rhubarb, yam, taro, sweet potato, sorrel, sesame seeds, black tea | Oxalates form insoluble complexes with calcium and possibly iron | Reduce absorption of calcium and possibly iron<br>Increase urinary calcium |
| Organic acids (citric, lactic, acetic, butyric, propionic, formic acids) | Fermented milk products (e.g., yogurt), vegetables (e.g., sauerkraut), soy sauce, cereals (e.g., tobwa) | May form soluble ligands with some trace minerals in the gut | May enhance absorption of zinc and iron |
| Ascorbic acid | Citrus fruits and juices, other fruits (e.g., guava, mango, papaya, kiwi, strawberry), vegetables (e.g., tomato, asparagus, Brussels sprouts) | Reduces ferric iron to more soluble ferrous iron; forms iron–ascorbate chelate | Enhances nonheme iron absorption; may counteract inhibitory effect of phytate<br>May enhance selenium and chromium absorption |
| Protein | | Amount and type (e.g., animal protein) enhance bioavailability of zinc, iron, and copper, but not calcium | Enhance absorption, possibly by formation of soluble ligands<br>Increase urinary calcium excretion |
| Fat | Fats and oils, animal adipose tissue, milk and milk products, vegetables, seeds, nuts | Products of fat digestion (fatty acids, monoglycerides, cholesterol, and phospholipids) plus bile salts solubilize fat-soluble vitamins and carotenoids in intestinal milieu | Enhance absorption of fat-soluble vitamins and provitamin A carotenoids |

*a. Myo*-inositol phosphates with fewer than five phosphate groups (i.e., IP-1 to IP-4) do not inhibit zinc absorption [32], and those with fewer than three phosphate groups do not inhibit nonheme iron absorption [33].

rides, which include cellulose, mixed-linkage β-glucans, hemicelluloses, pectins, and gums. These constituents are found especially in unrefined cereals, legumes, nuts, and fruits and vegetables. The effect of dietary fiber on nutrient bioavailability is linked to some of its physical properties in the gastrointestinal tract, such as cation exchange capacity, bile acid binding, water-holding capacity, viscosity, and its ability to act as a substrate for fermentative microorganisms [58].

In general, dietary fiber alone does not have a major effect on the absorption of minerals (e.g., calcium or magnesium) [59] or trace elements [26, 60], assessed *in vivo* using isotope techniques, although α-cellulose may affect the utilization or endogenous losses of copper [61]. Note that these findings are in contrast to earlier *in vivo* results in which pure fiber fractions were not used [62].

The effect of dietary fiber on the absorption of most water-soluble vitamins appears to be minimal [63]. Possible exceptions may be an interference with the bioavailability of naturally occurring vitamin $B_6$ in wheat, rice, and corn bran in humans, although *in vitro* studies with isolated forms of fiber have not confirmed this [9].

Absorption of fat-soluble vitamins (e.g., vitamin E) and carotenoids [23] may also be impaired by diets high in pectin and ligin through their capacity to bind bile acids *in vivo* [64] at an acidic pH and affect micelle formation in the small intestine. A high-fiber diet may also lead to enhanced elimination of vitamin D [65], probably as a result of a reduction in transit time.

Absorption of nutrients such as fatty acids is also affected by the water-holding capacity of dietary fibers such as pectins, psyllium, and various gums, which retain water within their matrix, forming highly viscous solutions in the small intestine. These can in turn alter gastric emptying time and slow the rate of nutrient digestion and absorption, although the total amount absorbed will be unaffected [58].

Fermentation of dietary fibers by the microflora in the large intestine may lead to an increase in short-chain fatty acids (SCFA) (acetate, propionate, and butyrate), depending on the type of fiber. These SCFAs are absorbed across the colonic mucosa and can serve as a source of energy (150 to 200 kcal/day) that would otherwise be lost in the stool. In addition, the acidic pH in the colon created by these SCFAs enhances the solubility of calcium and in turn calcium absorption [66].

*Oxalic acid* is present in many plants, the level varying with the cultivar, growing conditions, and distribution within the plant [67]; examples of food plants that contain high levels are shown in **table 2**. Some animal species (e.g., snails and certain other mollusks) also contain high levels of oxalate [68]. Oxalates form insoluble complexes with calcium, thus reducing absorption [69, 70]. Their inhibitory effect can be reduced by soaking and boiling, both of which reduce the oxalate content of foods. Whether oxalic acid inhibits the bioavailability of trace minerals such as iron and zinc is less clear. Some early human studies reported that the addition of 1 g of oxalic acid to a cabbage meal significantly reduced iron absorption [71], and foods rich in both fiber and oxalic acid, such as spinach, were reported to decrease zinc balance [72].

*Vitamin C* has a strong enhancing effect on absorption of nonheme iron when it is consumed in the same meal. This effect is now attributed largely to the formation of an iron–ascorbate chelate in the acid milieu of the stomach, which prevents it from forming a complex with phytate or tannin; Teucher et al. have presented a detailed review [73]. The magnitude of the enhancing effect depends on the level of ascorbic acid and the composition and properties of the meal; the effect is greater for meals that contain inhibitors of iron absorption. Vitamin C appears to enhance chromium absorption [74] and may influence the bioavailability of selenium [75]. Whether vitamin C affects copper absorption is still uncertain [76]. Note that the bioavailability of ascorbic acid from food sources is similar to its bioavailability from supplements and is not affected by the type of food consumed [77].

*Organic acids* (citric, lactic, acetic, butyric, propionic, and formic acids) produced during fermentation of cereals (e.g., tobwa) [78], vegetables (e.g., sauerkraut) [79], and some soy sauces [80], have the potential to form soluble ligands with trace minerals in the gastrointestinal tract [73] and thus may facilitate absorption of nonheme iron [71] and zinc [26]. Their effect on iron absorption appears to depend on the type of organic acid, the molar ratio of organic acid to iron, and the iron source, based on the results of a study in human Caco-2 cells [81]. The effect is not as consistent as that of ascorbic acid [82].

*Protein,* both the type and the amount, influences the bioavailability of nonheme iron, zinc, and copper. The enhancing effect of cellular animal protein on nonheme iron absorption is well documented [83]. The mechanism is not clear, but some "meat factor" may be implicated, perhaps through the release of certain amino acids, oligosaccharides, or possibly cysteine-containing peptides, during the digestion of cellular animal protein. Other proteins from eggs and from milk and dairy products (especially casein) impair iron absorption [84]. Hence, it is not surprising that some epidemiologic studies have shown positive correlations of serum ferritin with meat and fish intake but negative correlations with dairy products in adults [85, 86].

In contrast, increasing the amount of total protein enhances zinc absorption, and if the protein is from cellular animal sources, the enhancing effect is even greater [87]. Animal protein may also enhance copper absorption [88]. In contrast, diets high in protein increase the urinary excretion of calcium (i.e., hypercalciurea), which is not compensated by increased calcium absorption. However, this negative effect of

protein is probably significant only when calcium intakes are low. It also depends on other constituents, such as potassium and phosphorus, both of which blunt the hypercalciuric response [89]. Weaver et al. [70] provide data on calcium intakes required to offset urinary losses in adults consuming various amounts of dietary protein.

The presence of protein in the small intestine also helps to stabilize fat emulsions and enhances micelle formation and thus uptake of carotenoids [23] and vitamin A [90].

*Flesh foods* (meat, poultry, fish, and seafood) enhance the absorption of nonheme iron and zinc from plant-based foods. In meals containing meat, fish, or chicken, nonheme iron absorption is about four times greater than that from a meal containing equivalent portions of milk, cheese, or eggs [12]. The relative enhancing effect of animal muscle proteins on nonheme iron absorption varies: beef apparently has the highest effect, followed by lamb, pork, liver, chicken, and fish [91]. No comparable data exist for zinc. The precise mechanism is unclear, as noted earlier. The enhancing effect of flesh foods on nonheme iron absorption is evident even in the presence of phytic acid [91], but its magnitude appears less when a meal is already high in ascorbic acid [82].

*Carbohydrates* can stimulate bacterial fermentation in the intestine and enhance absorption of certain minerals by increasing their solubility in the ileum. Examples include lactose, known to enhance both calcium and magnesium absorption [7].

*Fat*, both the type and the amount, influences the absorption of fat-soluble vitamins (e.g., retinol) and provitamin A carotenoids. Hence, in the low-fat, plant-based diets characteristic of developing countries, absorption of fat-soluble vitamins and carotenoids may be impaired. The minimum amount of fat required for optimal absorption of provitamin A carotenoids ranges from 5 to 10 g per day [92]. Further, there is no dose–response relationship above the threshold value. Such low levels of fat have been reported in diets of children living in rural Malawi [93]. The type of dietary fat may also be important for absorption of β-carotene: absorption may be higher with dietary fats rich in polyunsaturated fatty acids than with dietary fats rich in monounsaturated fatty acids [23].

#### *Competitive interactions between two or more inorganic nutrients*

Inorganic nutrients with similar physicochemical properties that share the same absorptive pathways are known to interact with one another by several mechanisms, only some of which are understood. They may compete for carrier sites on transport proteins. In most indigenous diets, such competitive interactions are unlikely to modify bioavailability, because the intrinsic levels of inorganic nutrients are not high enough to induce antagonistic interactions. Even if plant foods or diets are fortified with micronutrients, the risk of antagonistic interactions appears to be low, because the micronutrients become chelated to dietary ligands resulting from the digestion of food and thus are absorbed by different pathways [94]. However, these interactions could become important if high doses of micronutrient supplements are consumed without food, as may occur among certain population groups with high micronutrient requirements, such as infants, adolescents, and pregnant and lactating women. Excess zinc (25 or 50 mg/day) has been shown to decrease biochemical indices of copper status [95, 96] and in some cases also of iron status [96] in adults. In a stable isotope study of fasting pregnant Peruvian women, zinc absorption in the third trimester of pregnancy was significantly less in women receiving daily supplementation with a combination of iron (60 mg) and folate (250 μg) than in their unsupplemented counterparts [97]. Among adult ileostomy subjects, iron supplements inhibited zinc (but not copper) absorption [98]. An adverse effect of supplemental iron on biochemical zinc status [99, 100] has also been observed among infants. The mechanism for this adverse effect of supplemental iron on zinc absorption is not clear, but studies suggest that iron may inhibit both the uptake and the transfer of zinc through the intestinal cell [101].

*Calcium* has an acute inhibitory effect on iron absorption [102], although the mechanism for this effect is uncertain and its impact is controversial. The adverse effects have been noted in single-meal [102] and short-term [103] intervention studies; long-term studies have failed to demonstrate any significant inhibitory effect [104–106]. For example, a recent year-long study of calcium supplementation (500 mg calcium/day) in adolescent girls failed to show any adverse effect on biochemical iron indices [106]. Nevertheless, a cross-sectional study in six European countries has demonstrated a weak but inverse association between calcium and serum ferritin, but the effect was not dependent on simultaneous ingestion of calcium and iron, and no dose–response relationship was noted [107].

In some emerging countries, where pollution is a problem and where controls for landfills in mining operations are inadequate, antagonistic interactions between zinc and cadmium, iron and lead, chromium and zinc, copper and cadmium, and selenium and mercury may be of concern [27].

### Influence of food processing and/or preparation practices on nutrient bioavailability

Increasingly, research has emphasized that prior treatment of food before consumption may have a marked effect on the bioavailability of nutrients and hence must be taken into account when formulating nutrient-based

dietary requirements. Such treatments may involve commercial and/or traditional household food preparation and processing practices. The treatments may involve thermal processing (including canning, extrusion, baking, and boiling), milling or home pounding, malting, fermentation, and soaking at the commercial

TABLE 3. Examples of the influence of food processing and preparation practices on nutrient bioavailability

| Processing method | Main technical influences | Nutritional consequences |
|---|---|---|
| Thermal processing | Destroys heat-labile vitamins such as thiamin, vitamin C, and riboflavin | Reduces amount in final product |
| | Releases some vitamins from poorly digested complexes | Enhances bioavailability of vitamin $B_6$, niacin, folate, certain carotenoids |
| | Inactivates heat-labile antinutritional factors | May enhance bioavailability of vitamin $B_1$, iodine, biotin, etc., depending on food item |
| | May degrade phytate, depending on temperature, but losses are modest | Possibly small improvements in bioavailability of zinc, iron, calcium |
| | Gelatinizes starch | Enhances starch digestibility |
| Baking | Induces Maillard browning in foods containing reducing sugars | Destroys basic essential amino acids: lysine, arginine, methionine; reduces protein quality and protein digestibility (specific to baking) |
| Boiling | Reduces oxalate content | Enhances calcium and possibly iron bioavailability |
| | Some leaching of water-soluble components | Some loss of water-soluble vitamins and inorganic nutrients (specific to boiling) |
| Extrusion | May degrade phytic acid, causing modest losses | Possibly small improvements in bioavailability of zinc, iron, calcium |
| | Induces starch gelatinization | Enhances starch digestibility |
| | Induces Maillard browning | Destroys basic essential amino acids; reduces protein quality (specific to extrusion) |
| Milling or home pounding | Reduces phytate content of those cereals with phytate localized in outer aleurone layer: (rice, wheat, sorghum) or in germ (maize) | May enhance bioavailability of zinc, iron, and calcium, although mineral content simultaneously reduced |
| | Reduces B-vitamin content | |
| Malting, also known as germination | Increases phytase activity via *de novo* synthesis or activation of endogenous phytases | Induces hydrolysis of phytate to lower inositol phosphates and hence may increase zinc, iron, and calcium bioavailability |
| | Reduces polyphenol content of some legumes *(Vicia faba)* | May enhance nonheme iron absorption |
| | Increases α-amylase content of cereals: sorghum and millet | Facilitates starch digestion; may increase nonheme iron absorption through a change in consistency |
| Microbial fermentation | Induces hydrolysis of phytate by microbial phytase | May enhance bioavailability of zinc, iron, calcium |
| | Increases content of organic acids | May form soluble ligands with iron and zinc and enhance bioavailability |
| | Microbial enzymes may destroy protein inhibitors that interfere with nitrogen digestibility | May improve protein quality in maize, legumes, groundnuts, pumpkin, millet seeds |
| Soaking in water and decanting | Passive diffusion of water-soluble sodium and potassium phytates in cereal and legume flours | May enhance bioavailability of zinc, iron, calcium, but some loss of water-soluble vitamins |
| | May activate some endogenous phytases in cereals and legumes | Some phytate hydrolysis and thus enhanced bioavailability of zinc, iron, and calcium |
| | Soaking maize with lime releases niacin from niacytin | Enhances bioavailability of niacin |

or household levels; they are summarized in **table 3** and discussed in turn below. When a combination of strategies is used, such as soaking, germination, and fermentation, phytate can be almost completely removed. This is important, because phytic acid is a potent inhibitor of iron absorption at low concentrations [38]. During commercial food processing, the use of certain food additives, as well as some inadvertent or intentional contaminants, may also influence the bioavailability of some nutrients.

#### *Thermal processing*

Thermal processing generally enhances the digestibility of proteins and carbohydrates and may improve the bioavailability of iodine and certain vitamins (e.g., niacin, thiamin, vitamin $B_6$, and some carotenoids) (**table 3**). In some cases, these improvements in nutrient bioavailability arise because of the destruction of some heat-labile antinutrients summarized in **table 4**. The digestibility of protein, for example, is enhanced by the destruction of protease inhibitors found in many legumes, grains, and other foods. Protease inhibitors block the activity of pancreatic enzymes such as trypsin and chymotrypsin but are destroyed during roasting and toasting, although boiling may not fully deactivate them. Similarly, the harmful effects of lectins (agglutinins) found in certain legumes, including soybeans and peanuts, can also be removed by heat treatment. Lectins can agglutinate red blood cells, and they also have the capacity to adhere to glycoproteins of the intestinal mucosal membrane surface, leading to a decrease in digestive and absorptive capacity as well as symptoms of nausea and diarrhea [108].

Goitrogens can also be inactivated by thermal processing. The major goitrogens in plant foods are sulfur-containing glucosides (glucosinolates) which can block the absorption or utilization of iodine, and thus its uptake into the thyroid gland. More details are given by Gaitan [109]. Their action is especially important when iodine intake is low [110]. Neonates, and to a lesser extent pregnant women, are more sensitive to the antithyroid action of dietary goitrogens than are infants and children.

Reports on the effects of thermal processing on phytate degradation are inconsistent and depend on the plant species, temperature, and/or pH. Thermal processing involving high temperatures, such as those used in canning, has been reported to reduce the phytate content of beans by 70% to 91% [111]. An *in vivo* study by Hurrell and co-workers [112], however, concluded that the extent of phytate degradation in industrially thermally processed cereal porridges or home-prepared pancakes or chappattis made from the same cereal flours (i.e., unrefined and refined wheat flour) was not sufficient to improve iron absorption.

Thermal processing can also enhance the bioavailability of some vitamins. Heat-labile thiaminases in brussels sprouts and red cabbage are destroyed (**table 4**), whereas any thiamin, vitamin $B_6$, niacin, or folate entrapped in the cellular structure or insoluble matrix of certain foods may be released. For example, significantly greater increases in serum β-carotene levels after consumption of cooked carrots and spinach [113] and in serum lycopene levels after consumption of cooked tomatoes [114] have been reported than after consumption of the same amounts raw. This effect is attributed to softening or disruption of plant cell walls and disruption of carotenoid–protein complexes.

In contrast, as noted in **table 3**, thermal processing, especially when it involves baking or extrusion cooking, can reduce the biological value of some proteins. This is caused by the induction of Maillard browning, which results in the destruction of certain essential amino acids, especially lysine, and to a lesser extent arginine and methionine. Oxidation of sulfur-containing amino acids can also occur. Losses of heat-labile (thiamin, vitamin C, and riboflavin) and water-soluble vitamins also occur, the extent of the losses depending on the temperature, pH, oxygen, light, and amount of water used [108]. Extrusion may also inhibit degradation of phytic acid through inactivation of phytase, resulting in less efficient apparent absorption of zinc compared

TABLE 4. Heat-labile antinutritional factors that have the potential to influence nutrient bioavailability

| Antinutritional factor | Common food sources | Effects of antinutritional factor |
|---|---|---|
| Avidin | Egg whites | Binds biotin, making it biologically unavailable |
| Protease inhibitors | Legumes, grains, egg white, potatoes, sweet potatoes, soy products | Inhibit activity of digestive enzymes trypsin and chymotrypsin |
| Lectins (agglutinins) | Legumes: red kidney beans, black beans, yellow wax beans, soybeans, peanuts | Can agglutinate red blood cells and may also decrease digestive and absorptive capacity of GI tract |
| Goitrogens | Sweet potato, cassava, millet, beans, cabbage, Brussels sprouts, turnip | Cause goiter by interfering with absorption or utilization of iodine |
| α-Amylase inhibitors | Cereals (wheat, barley, maize, rice), peas, beans | Slow starch digestion |
| Thiaminases | Fish, shellfish, Brussels sprouts, red cabbage | Destroy thiamin |

Source: modified from Erdman and Poneros-Schneier [108].

with that from similar but nonextruded cereal products [79, 115], although not all studies have confirmed these findings [116]. At the same time, extrusion induces starch gelatinization, rendering it more accessible to enzymatic digestion.

#### *Milling or household pounding*

Milling or household pounding is used to remove the bran and/or germ from cereal grains such as rice, maize, sorghum, and wheat. These processes reduce the phytate content of those cereals in which the phytate is localized in the outer aleurone layer (e.g., rice, sorghum, and wheat) or in the germ (i.e., maize) [117] and thus may enhance mineral bioavailability, although the content of minerals and certain B vitamins in the milled cereals is simultaneously reduced. As a result, in some countries, milled cereal flours are sometimes enriched to compensate for the nutrients lost. Sieving unrefined maize grits, as practiced in the Philippines, can potentially enhance the bioavailability of iron and zinc in the sieved maize by removing the germ, which contains as much as 90% of the phytic acid [117].

#### *Malting or germination*

Malting, also called germination, involves soaking cereal grains or legumes in water until sprouting occurs. This leads to an increase in phytase activity through *de novo* synthesis, activation of endogenous phytase, or both, and as a result some reduction in the IP-5 and IP-6 content of the germinated whole cereal grains, most legume seeds, and most oil seeds [38, 118]. The rate of hydrolysis via phytases (*myo*-inositol hexakisphosphate 3-phosphohyrolase) (EC 3.1.3.8) varies with the species and variety, as well as stage of germination, pH, moisture content, temperature (optimal range, 45° to 57°C), solubility of phytate, and presence of certain inhibitors [119, 120]. Egli et al. [118] observed that during germination, rice, millet, and mung beans had the largest reductions in phytate content. Some loss of water-soluble sodium and potassium phytates may also occur during germination.

Germination may also reduce the content of tannins and other polyphenols in some legumes (e.g., *Vicia faba)* and red sorghum by complexing with proteins [121].

α-Amylase activity is also increased during germination of cereals, especially sorghum and millet. Because these enzymes hydrolyze amylase and amylopectin to dextrins and maltose, the viscosity of thick cereal porridges is reduced to an easy-to-swallow, semiliquid consistency, which may facilitate iron absorption. A threefold increase in iron absorption has been reported in amylase-treated, roller-dried rice cereal compared with untreated roller-dried cereal, which Hurrell et al. [112] attributed to the viscosity changes induced by α-amylase.

#### *Microbial fermentation*

Microbial fermentation also results in some hydrolysis of IP-5 and IP-6 to lower inositol phosphates through the action of microbial phytase enzymes [79]. The extent of the reduction depends on the type of fermentation; sometimes as much as 90% or more of phytate can be reduced by fermentation of maize, soybeans, sorghum, cassava, cocoyam, cowpeas, and lima beans [79, 122, 123]. The action of microbial phytase is important, because there is no phytase activity in the human intestine [124]. Such reductions in phytate can have a major impact on enhancing calcium, iron, and zinc bioavailability, as shown by *in vivo* isotope studies in which adults have been fed tortillas or polenta made from low-phytate maize hybrids compared with wild-type unmodified maize [125–127]. This effect is important, since fermented maize, sorghum, and soybean products are widely consumed in Africa and Asia. Fermentation of bread dough with yeast also induces phytate hydrolysis, although if calcium is added as a fortificant, phytase activity in yeast is inhibited.

Organic acids produced during fermentation also have the potential to enhance iron and zinc absorption via the formation of soluble ligands with iron and zinc, as noted earlier [73], and also create the low pH that is optimal for the native phytases. Improvements in protein quality have also been documented after fermenting blends of maize and legume flours [128] and groundnuts, pumpkin, and millet seeds [129], possibly associated with the destruction of protein inhibitors by microbial enzymes that interfere with nitrogen digestibility [128].

#### *Soaking*

Soaking can also reduce the IP-5 and IP-6 content of unrefined cereal (and most legume) flours by passive diffusion of water-soluble sodium and potassium phytate [130–132]. Reported reductions in IP-5 and IP-6 after soaking white rice, maize, and legume flours (e.g., mung bean flour) range from 57% for maize flour [133, 134] to more than 90% for white rice flour [135]. Note that only modest losses occur after soaking whole legume seeds and cereal grains, with the exception of rice [135]. This has important implications for diets in Southeast Asia based on glutinous rice, which is often soaked overnight and then steamed, because these practices result in a substantial loss of water soluble phytate (the author, personal communication). The extent of the losses depends on the species, pH, moisture content, temperature, and solubility of phytate, the presence of certain inhibitors, and the length and conditions of soaking. Soaking under optimal conditions may also activate endogenous phytases and result in some phytate hydrolysis, as well as some reduction in the content of other antinutrients, such as saponins and polyphenols [79]. Note that some losses of water-soluble B vitamins, such as thiamin, riboflavin, and niacin,

may occur during soaking, so that alternative dietary sources of these vitamins must be considered.

The polyphenol content of some legumes (e.g., *Vicia faba)* and red sorghum may also be reduced by germination as a result of the complexation with proteins and gradual degradation of oligosaccharides [121]. Naturally occurring polyphenol oxidase extracted from banana or avocado can also been used to reduce the polyphenol content of red sorghum [136].

#### *Food additives and contaminants*

Some food additives used in processed foods can also influence nutrient bioavailability. An example is the practice of nixtamalization or liming used in Central America for processing maize. This increases the calcium content of tortilla-based diets to a level that may potentiate the inhibitory effect of phytate on zinc absorption [137]. This practice, however, also releases niacin from niacytin in maize [138].

In industrialized countries, erythorbic acid (also termed isoascorbic acid, D-arabo-ascorbic acid) is widely used as a preservative in processed foods. Erythorbic acid is a steroisomer of ascorbic acid that does not have any antiscorbutic activity but is a potent enhancer of nonheme iron absorption [139]. US diets can provide as much as 200 mg of erythorbic acid per day, which could enhance nonheme iron bioavailability.

*Contaminants* in the food supply arising, for example, from plant-based foods grown in soils contaminated with sewage sludge, phosphate fertilizers, or pesticides may influence nutrient bioavailability. Heavy metal contaminants (e.g., cadmium, lead) can cause antagonistic interactions between cadmium and zinc, cadmium and iron, lead and zinc, lead and iron, and lead and calcium, resulting in reduced bioavailability of zinc, iron, and calcium. Environmental contaminants can also accumulate in aquatic food chains, the most well-known example being methyl mercury in fish [140], which is known to bind selenium in foods and reduce its bioavailability.

Inadvertent or intentional ingestion of soil (geophagia) may also have an impact on mineral bioavailability. Indeed, geophagia was implicated in the etiology of the first cases of human zinc and iron deficiency reported in the Middle East [141, 142]. Although soils are a rich source of minerals, their ingestion does not necessarily provide a source of absorbable minerals. Absorption of minerals depends on the soil type and the timing of the consumption of soil in relation to the consumption of foods. Calcareous soils may provide a source of absorbable calcium [37], but others, such as clay, contain inhibiting constituents (e.g., silicates) that may prevent the absorption both of inorganic nutrients derived from the soil and of those intrinsic to food [143]. A detailed review of the possible impact of nonfood sources of iron on iron status is available [10].

In contrast to contaminants from the soil, trace element contaminants in food from cooking equipment, cooking pots, or storage conditions, acquired either during commercial food processing or in the household, may be readily absorbed [10]. Examples include sources of iron or zinc in foods cooked in iron or galvanized pots. A study in Ethiopia reported lower rates of anemia and higher serum ferritin concentrations among children whose food was cooked in iron pots than among those whose food was cooked in aluminum pots [144], suggesting that contaminant iron from cast-iron cookware may be sufficiently bioavailable to influence iron status under certain conditions. Note that the bioavailability of trace elements from the cooking utensils will be subject to the same interactions with components in the whole diet as the trace elements intrinsic to the food. Thus bioavailability of iron and zinc will be low in high-phytate foods.

## Influence of host-related factors on bioavailability of nutrients

Several host-related factors are known to influence the bioavailability of nutrients. They can be classified as intestinal or systemic factors. Because information on these host-related factors is often limited, their effects on nutrient bioavailability are often ignored when dietary requirement estimates are set. This is unfortunate, because in some developing countries they might have a further modifying influence on nutrient bioavailability over and above that of the dietary factors discussed earlier. The extent to which they influence absorption and/or utilization varies with the nutrient, life-stage group, and environment.

### Intestinal factors

Both luminal and mucosal factors can influence intestinal digestion and absorption of nutrients; some examples are summarized briefly below.

Atrophic gastritis is probably one of the most significant luminal factors influencing nutrient bioavailability, predominantly through its association with hypochlorhydria. The latter is a condition in which there is a progressive decrease in the capacity of the parietal cells of the stomach to secrete hydrochloric acid [145]. Hypochlorhydria is linked to infection with the bacterium *Helicobacter pylori,* an infection that is especially prevalent among children in developing countries, where it is typically acquired in childhood and persists throughout life. Even in Western countries, infection with *H. pylor*i has been linked to the hypochlorhydria that may affect as many as 10% to 30% of persons over 60 years of age.

Absorption of several vitamins and minerals that are

dependent on pH for absorption can be impaired as a result of the pH changes that accompany hypochlorhydria. For example, low acid conditions of the stomach can impair the release of protein-bound vitamin $B_{12}$ contained in food [146] and the absorption of β-carotene [147] and folate [148]. Alterations in the pH of the jejunum may also impair deconjugation of folate and thus folate absorption [24].

Absorption of iron, calcium, and possibly zinc may also be affected by reduced gastric acid secretion, because gastric acid affects the solubilization of these inorganic nutrients [149–151]. Hence, reduced gastric output arising from infection with *H. pylori* could limit the absorption of iron, calcium, and possibly zinc and may be an important factor in the etiology of iron deficiency and iron-deficiency anemia, especially among children in developing countries. However, in a recent study of *H. pylori*-infected Bangladeshi children, no increase in iron absorption was observed, despite an improvement in gastric acid output and hemoglobin concentration after treatment with antibiotics [152].

Other physiological consequences of atrophic gastritis include decreased acid-pepsin digestion in the stomach and decreased secretion of intrinsic factor, both of which result in malabsorption of vitamin $B_{12}$. Bacterial overgrowth in the stomach and proximal small intestine is also often associated with atrophic gastritis and may exacerbate vitamin $B_{12}$ deficiency because the bacteria take up any freed vitamin $B_{12}$ from food [153].

Bacterial overgrowth and infection with *Giardia lamblia*, *Ascaris lumbricoides*, rotavirus, and salmonella, as well as malaria and iron deficiency [154], can alter the integrity of the intestinal mucosal, causing increases in intestinal permeability and reductions in nutrient absorption. Such perturbations in the morphology and function of the intestine may even arise in normal healthy persons residing in tropical areas [155] and are known to reduce the absorption of vitamin A [156], folate [157], and probably those nutrients for which secretion and absorption of endogenous sources in the intestine are key homeostatic mechanisms (e.g., zinc, copper, calcium). For example, the large endogenous fecal losses of zinc that perturbed zinc homeostasis in young, apparently healthy Malawian children aged 2 to 5 years [28] may have resulted from abnormal intestinal permeability [158]. Alternatively, the high phytate content of the maize-based diets of the children may have bound endogenously secreted zinc, making it unavailable for reabsorption [26]. Alterations in the structure of the intestinal mucosa will also compromise iron homeostasis, because iron is regulated by uptake and transfer by the intestinal mucosa. The proportion of bioavailable carotene converted to retinol (bioconversion) is probably also affected, because the enzyme (15-15'-carotenoid dioxygenase) responsible for this cleavage is present in the intestinal mucosal brush border [23]. In addition, reductions in transit time are often associated with many of these infections, which will in turn decrease the time required for extensive solubilization of nutrients in the intestinal tract and again further compromise intestinal nutrient absorption.

### Systemic factors

Systemic factors that can influence absorption and utilization of nutrients include age, sex, possibly ethnicity, genotype, physiological state (e.g., pregnancy, lactation), and the nutrient status of the host, together with the presence of coexisting gastrointestinal disorders or disease such as chronic and acute infections [159]. For example, during the first 6 months of infancy, maturation of both the gastrointestinal tract and the digestive and absorptive processes are known to affect bioavailability of macronutrients and probably of micronutrients as well, although no data are available at the present time [160].

Potential reductions in nutrient absorption with advancing age are especially important in those countries with an aging population. Alterations in the secretory and absorptive capacity of the intestine occur with advancing age, which in turn affect the bioavailability of certain micronutrients; macronutrients are unaffected. The micronutrients most affected are vitamin $B_{12}$, as noted earlier, as well as calcium, vitamin D, and possibly vitamin $B_6$ [153]. Decreased absorption of calcium is linked to age-related changes in vitamin D metabolism involving a reduction in the number of vitamin D receptors in the intestinal mucosa, and thus diminished capacity to absorb vitamin D [161]. Additional problems with vitamin D metabolism in the elderly include decreased ability of the kidneys to dihydroxylate 25-hydroxyvitamin D to the active 1,25-dihydroxy form and decreased ability of the skin to synthesize vitamin D after exposure to ultraviolet light [162]. Such disturbances may lead to malabsorption of both vitamin D and calcium; decreases in dietary intakes of vitamin D and sun exposure among the elderly may be exacerbating factors. Whether changes also occur during aging in the bioconversion of provitamin A carotenoids to vitamin A is unknown.

Increases in vitamin $B_6$ requirements with age have also been reported, although they have not been related to malabsorption, but instead to problems with cellular uptake or metabolism of the vitamin [151, 163]. Similar disturbances may also occur with thiamin during aging, but this question requires more investigation [22]. In contrast, body stores of vitamin A in the elderly do not decline, despite lower intakes. Initially, an increase in the efficiency of absorption with advancing age was said to be responsible [164], but later research confirmed that such well-maintained vitamin A stores were due to decreased clearance of the vitamin from the circulation into peripheral tissues [165]. Whether

decreased clearance from the circulation occurs with other fat-soluble substances with advancing age is not clear. Renal function is also known to decline with advancing age [166], which could influence the bioavailability of those nutrients (or nutrient metabolites) which are excreted mainly via the kidney (e.g., selenium, iodine, chromium) and for which renal conservation plays a key role in homeostasis.

Certain physiological adaptations may occur that alter nutrient bioavailability in circumstances when nutrient needs are high (e.g., during periods of rapid growth, pregnancy, and lactation) or habitual dietary intakes are low. These adaptations may include increased gastrointestinal absorption of nutrients from the diet, enhanced renal conservation of nutrients, enhanced utilization of absorbed nutrients by tissues, and/or bone resorption [160, 167]. Conversely, when nutrient needs are low, intestinal absorption may be reduced and/or the mechanisms for renal clearance increased. Note that the extent to which these physiological adaptations compensate for the increased or decreased needs for these inorganic nutrients is not always known. Likewise, the time scale over which such homeostatic mechanisms occur is uncertain.

**Table 5** summarizes the homeostatic adaptations that may occur for certain inorganic nutrients to meet these increased nutrient needs during pregnancy and lactation and in response to changes in dietary intake [27, 160, 167–169]. There is also some evidence that absorption of certain nutrients (e.g., calcium) may vary with ethnicity [170, 171]. Whether the absorption of vitamins is modified by the nutrient status of the host is uncertain; limited data are available. Some data for vitamins A and D suggest there is little or no evidence that vitamin A deficiency up-regulates conversion of β-carotene in humans [172], or that absorption of vitamin A [90] or vitamin D [173] is influenced by the nutrient status of the host. In contrast, data based on animal studies have led others to postulate that absorption of β-carotene and canthaxanthin [23, 174] and bioconversion of β-carotene to retinol [175] may be dependent on the vitamin A status of the individual. More studies are needed to confirm these suggestions.

Other highly significant determinants of nutrient requirements are chronic and acute infections. Chronic and acute infections are of marked concern when endemic and highly prevalent or when acute infectious illnesses occur frequently, especially when affected populations are poorly nourished. The latter is of particular concern because of the adverse synergy between poor nutrition and infectious illness [176]. These considerations are especially relevant to coun-

TABLE 5. Examples of homeostatic adaptations for selected inorganic nutrients in response to increased needs and changes in dietary intakes based on results from *in vivo* human studies

| Nutrient | Homeostatic adaptations during pregnancy and lactation | Homeostatic adaptations in response to changes in dietary intake |
|---|---|---|
| Calcium | Pregnancy: increased absorption<br>Lactation: increased renal conservation and bone resorption | Increase in fractional absorption from the diet when intake or status is low, and decrease in fractional absorption when intake or status is high. This adaptive response is reduced among the elderly |
| Magnesium | No data | Increase in fractional absorption from the diet when intake or status is low, and decrease in fractional absorption when intake or status is high. |
| Zinc | Pregnancy and lactation:<br>» increase in zinc absorption but not in renal conservation<br>» no release of maternal tissue zinc in pregnancy | Decrease in endogenous zinc excretion provides "fine control" to maintain zinc balance when intake or status is low; increase or decrease in fractional absorption from the diet may provide "coarse control" of body zinc when intake or status is low or high, respectively; renal conservation when intake is very low. |
| Iron | Pregnancy: increased absorption, especially in 3rd trimester | Increase in fractional absorption from the diet when intake or status is low, and decrease in fractional absorption when intake or status is high. |
| Chromium | Pregnancy: unknown<br>Lactation: do not appear to reduce urinary chromium excretion | Possibly a reduction in urinary excretion when intake or status is low |
| Copper | Pregnancy: increased absorption likely<br>Lactation: no data on copper absorption available | Increase in fractional absorption from the diet when intake or status is low, and decrease in fractional absorption when intake or status is high; reduction in urinary excretion when intake is very low |

tries with developing economies. Such countries generally have relatively high proportions of infants and children and other high-risk populations, poor disease prevention infrastructures, and inadequate availability and accessibility of health care systems able to provide prompt and efficacious treatment. These conditions are not uncommon and exacerbate the adverse synergy between poor nutrition and infection, thus it is surprising that data assessing the impact of infectious illness on energy and macro-, micro-, and trace nutrient needs remain inadequate [177–182]. Despite the inadequacy of available data, what is available often is sufficient to calculate semi-quantitative estimates of the impact of illness on nutrient needs. Impacts of illness are not uniform, however. Among the factors that should be considered in assessments of specific nutrient needs are the offending agents, illness severity, age of hosts, stage of illness (e.g., acute or convalescent phase), hosts' nutritional status, and other underlying health conditions. In assessing the impact of infections on populations, it is essential to know relatively well the prevalence of conditions of interest.

Infectious illness impacts nutrient needs by one or a combination of various mechanisms. These include decreased absorption through direct and indirect effects on gastrointestinal function, increased losses through the gastrointestinal tract or other routes (e.g. renal system), heightened metabolic activity, or the sequestration of nutrients in the liver and other sites. These mechanisms have been investigated for bacterial, parasitic, and viral infections [e.g., 183–185]. Nutrient status also influences tissue repair mechanisms and the susceptibility to disease [186]. Relationships between nutritional status and the progression of illness have been investigated most recently and intensively for HIV [187].

In the future, several other factors may also be taken into account when setting dietary requirement estimates. These may include race or ethnicity, lifestyle (e.g., smokers, oral contraceptive users), the existence of chronic disease (e.g., asthma, diabetes), environment (e.g., lead pollution), family history, and genetic predisposition to disease [188].

## Implications for adjusting physiological requirements to dietary requirements

At present, the diet- and host-related factors influencing the bioavailability of many nutrients are not well established, limiting the development of algorithms to predict nutrient bioavailability. Notable exceptions are the algorithms available for iron, zinc, protein, folate, vitamin A, and carotenoids. These mathematical models attempt to predict bioavailability, taking into account the amount and form of the nutrient (where applicable), the presence of dietary enhancers and inhibitors, and, when necessary, the nutrient status of the individual. The models then apply certain general principles to the complex whole-diet matrix. However, the accuracy of the algorithms is limited by interactions known to occur between the enhancing and inhibiting factors in the whole diet, as discussed earlier. For example, when the absorption modifiers are contained in the same meal, their effects are probably not additive [189]. Furthermore, because most of the effects of the dietary modifiers on micronutrient absorption have been calculated from the results of single test meals, their effects may be exaggerated in comparison with the extent of the enhancement or inhibition measured over several days [3]. Furthermore, the magnitude of the effect of the absorption modifiers depends on the background dietary matrix [82]. These findings emphasize that as new research findings emerge, algorithms must be modified on an ongoing basis.

### Algorithms for available iron

The first algorithm for estimating available iron intakes was developed by Monsen et al. [190] and can be used when intakes of flesh foods, vitamin C, and total iron at each meal are known. In this model, 40% of the total iron found in meat, poultry, and fish is assumed to be heme iron. Nonheme iron is calculated as the difference between total iron and heme iron intakes.

Absorption of heme iron was assumed to be 25% in the initial model but 23% in a later model [191]. The absorption of nonheme iron was assumed to be lower and to vary according to the amounts of meat, poultry, and fish and of ascorbic acid in each meal, as well as the level of iron stores of the individual. Total available iron intakes per day can be derived from the sum of available iron from each meal and snack. Note that this model does not take into account the amounts of any absorption inhibitors in a meal or any possible synergistic effects of absorption enhancers that are present together in the same meal.

FAO/WHO [192] has also developed a semiquantitative classification system for estimating iron bioavailability based on measures of iron absorption from typical meals in Asia, India, Latin America, and Western countries. In this model, diets are classified into three broad categories of low (iron absorption approximately 5%), intermediate (iron absorption approximately 10%), and high (iron absorption approximately 15%) bioavailability depending on their content of flesh- versus plant-based foods together with their content of ascorbic acid–rich foods. The estimates of absorption are for nonanemic persons (i.e., those with normal hemoglobin levels) with normal iron transport but no iron stores. In cases of iron-deficiency anemia (i.e., low hemoglobin levels), each absorption value is assumed to be increased by 50% [192]. Currently, it is difficult to clearly distinguish between a low- and

intermediate-bioavailability diet with this classification system.

FAO/WHO [182] still recommends this classification system but has proposed the use of two categories of bioavailability for diets in developing countries (5% and 10%) and another two categories for more Western-type diets (12% and 15%), depending on the meat content of the diet. As more comprehensive data become available on the absorption of iron from meals of differing composition, this classification system should be refined.

Murphy et al. [193] adapted the algorithms of Monsen et al. [190] and FAO/WHO [192] to estimate iron bioavailability in diets from developing countries. To use this algorithm, quantitative data on the intake of iron and of two enhancers—ascorbic acid and protein from meat, fish, and poultry—are required. The cutoffs applied to these two enhancers are expressed per 4.18 MJ (1000 kcal), so that the same algorithm can be used for males and females across all age groups. A tea or coffee factor can also be applied, depending on the average number of cups of tea or coffee per day, to account for the inhibitory effects of usual tea or coffee consumption on nonheme iron absorption. The effect of phytate on iron absorption is not considered.

Murphy's model [193], like that of Monsen [190], also assumes that heme iron constitutes 40% of the iron in meat, poultry, and fish and assumes 25% absorption. The computer program supplied with the WorldFood Dietary Assessment System calculates intake of available iron by using Murphy's model [193]. This program is available from http://www.fao.org/infoods/.

Several alternative algorithms have been developed for calculating available iron, each of which takes into account differing numbers of absorption modifiers. For example, Tseng et al. [194] have refined Murphy's model so that nonheme iron absorption can be adjusted for the enhancing effect of meat, poultry, fish, and vitamin C. Separate adjustments can be made for the inhibitory effects of tea and phytates in the diet. However, this model does not account for the combined effect of enhancers and inhibitors on iron absorption and has not had extensive use.

Du et al. [195] compared the use of the algorithm developed by Tseng et al. [194] with the algorithms of Monsen et al. [190] and FAO/WHO [192] for estimating iron bioavailability in the diets of Chinese adults, based on 24-h recalls collected over 3 consecutive days. Hemoglobin as an indicator of iron status was also measured. None of the algorithms appeared to be appropriate for estimating iron bioavailability in these Chinese diets. These investigators emphasized that for vegetarian diets it is important to consider the combined effect of multiple dietary factors on iron bioavailability.

Two additional algorithms are available for estimating dietary iron absorption. Reddy et al. [189] studied iron status (serum ferritin) and iron absorption (via extrinsic radioiron labeling) from 25 different single meals eaten by 86 subjects. An algorithm was then developed, using multiple regression analysis, to predict iron absorption after adjustment for each individual's iron status and including dietary modifiers as independent variables. It is of interest that only 16.4% of the total variance in iron absorption was accounted for by the amount of animal tissue, phytic acid, and ascorbic acid in the typical Western diets studied, with the major portion being explained by the animal tissue and phytic acid contents of the meals. Nonheme iron, calcium, and polyphenols were not significant predictors of iron absorption. These results emphasize the relatively small influence of diet on the amount of iron absorbed in comparison with the more important but unknown physiological factors.

Of all the algorithms available to date, the model of Hallberg and Hulthén [196] is the most detailed, taking into account the affects of all the known enhancing and inhibiting factors on nonheme iron absorption, as well as interactions among the different factors. Application of this more detailed model is limited at the present time by the paucity of food-composition data for the content of both phytate and iron-binding polyphenols in foods. However, this situation is changing.

### Algorithms for available zinc

The bioavailability of dietary zinc, like that of iron, is affected by the presence of several absorption enhancers and inhibitors in the whole diet, as well as the total zinc content.

Three algorithms have been developed for calculating available zinc. The first algorithm was developed by WHO [37] and takes into account the impact of one absorption enhancer (protein from meat, fish, and poultry) and two absorption inhibitors (the proportion of phytic acid to zinc, and high levels of calcium). In this algorithm, diets are classified as having low (15% absorption), moderate (30% or 35% absorption), or high (50% or 55% absorption) zinc bioavailability. Of the two inhibitors considered in this algorithm, phytate is the major determinant of zinc absorption, especially for diets in developing countries with a low content of flesh foods. Calcium is unlikely to have any adverse effect because its levels are low in plant-based diets. The inhibitory effect of phytate on zinc absorption follows a dose-dependent response [197], and the molar ratio of phytate to zinc in the diet can be used to estimate the proportion of absorbable zinc [198]. Because *myo*-inositol phosphates with fewer than five phosphate groups do not inhibit zinc absorption, any food processing or preparation method (e.g., fermentation or germination) that might hydrolyze phytate must also be considered; details are given by WHO [37]. This algorithm has been adopted by FAO/WHO [182],

although only the absorption estimates for the normative zinc requirements (i.e., level of intake that maintains a reserve adaptive capacity) are now included.

The second algorithm for available zinc was developed by Murphy et al. [193] and is based on that of WHO [37]. It again takes into account the content of animal protein and the content of the same two inhibitory factors, phytate and calcium, in the whole diet. For this algorithm, the phytate:zinc molar ratio of the whole diet is calculated; details are given by Hotz and Brown [36]. The bioavailability of diets with phytate: zinc molar ratios of 0 to 5, 5 to 15, 15 to 30, and > 30 is 55%, 35%, 15%, and 10%, respectively. These bioavailability estimates are then further modified, depending on the animal protein and calcium content of the diet; details are given by Murphy et al. [193]. In most plant-based diets in developing countries, however, intakes of animal protein and calcium are generally too low to influence zinc absorption. Even in Latin American countries where calcium intakes are often above 1 g/day, phytate intakes are so high that any further reduction in zinc absorption is assumed to be unlikely.

The International Zinc Nutrition Consultative Group (IZiNCG) has also developed an algorithm for the bioavailability of zinc based on measurements of zinc absorption in adults using only total diet studies; studies using a semipurified diet or exogenous sources of zinc in the form of zinc salts were excluded. Details are given in Hotz and Brown [36]. A logit regression model was used to describe the relationship between four dietary factors (zinc, phytate, protein, and calcium) and the percentage of the zinc intake absorbed. However, in the final model only zinc and the phytate:zinc molar ratio were shown to be significant predictors of the percentage of zinc absorption in adults. Neither calcium nor protein added significant predictive power.

The bioavailability figures for zinc calculated by IZiNCG are 26% for men and 34% for women consuming mixed or refined vegetarian diets with phytate: zinc molar ratios of 4 to 18, and 18% for men and 25% for women consuming unrefined, cereal-based diets with phytate:zinc molar ratios >18. Whether these bioavailability factors are appropriate for children, pregnant or lactating women, or the elderly has not been established [36].

### Algorithms for other nutrients

So far, steps have been taken to quantify the bioavailability of protein, folate, carotenoids, and vitamin A in human diets.

*Protein* intakes can be adjusted for both protein quality and digestibility by using the FAO/WHO/UNU guidelines [199]. Such adjustments are especially necessary in countries where habitual diets are plant-based, because the safe levels of protein intake were calculated from studies based on animal protein [199]. Further, large intakes of dietary fiber, especially insoluble fiber, are known to increase fecal nitrogen excretion, resulting in a reduction in apparent protein digestibility of approximately 10%. Note that the World Dietary Assessment program computes utilizable protein by adjusting intakes to account for both digestibility and amino acid score, using these FAO/WHO/UNU procedures.

In 1990, FAO/WHO recommended the use of a protein digestibility–corrected amino acid score (PDCAAS) [200]. This is based on comparison of the concentration of the first limiting amino acid in the habitual mixed diet (calculated from food-composition values) with the concentration of that amino acid in a reference pattern of essential amino acids; details are given by Schaafsma [201]. Once the amino acid score has been derived in this way, it is then corrected for true fecal digestibility, preferably by weighted summation of the individual protein sources, as specified by FAO/WHO/UNU [199].

*Folate* is a naturally occurring vitamin found in foods. However, the term is also used to embrace synthetic folic acid found in fortified foods and supplements. In recognition of the known differences in bioavailability between naturally occurring polyglutamate forms in foods and synthetic folic acid found in fortified foods and supplements, the Institute of Medicine [6] has introduced a new term, dietary folate equivalent (DFE), to take into account the differences in the bioavailability of all sources of ingested folate. The dietary folate equivalent content of a food is defined as

$$\mu g \text{ food folate} + (1.7 \times \mu g \text{ synthetic folic acid}).$$

This equation is based on the assumption that the bioavailability of food folate is about 50%, whereas that of folic acid taken with food is 85% (i.e., folic acid is 85/50 = 1.7 times more available) [19, 20].

Many countries are now fortifying foods such as breads and grains with the synthetic monoglutamate form, but most current food-composition tables do not distinguish folate found naturally in foods from folic acid added to foods. Work is under way in some countries to provide this information [202].

*Vitamin A* in the diets of most industrialized countries occurs mainly as preformed vitamin A derived from animal products. In contrast, in most tropical countries, the main sources of vitamin A are the provitamin A carotenoids from dark-green leafy vegetables and certain yellow- and orange-colored fruits and vegetables [203]. Provitamin A carotenoids include β-carotene, α-carotene, and α- and β-cryptoxanthins.

Currently there is debate about the bioefficacy of ingested provitamin A carotenoids [181, 203, 204]. For example, dietary vitamin A levels may be expressed in terms of micrograms of retinol equivalents (RE), calculated using either the FAO/WHO [192] or the West [203] conversion factors for the bioefficacy of

ingested provitamin A carotenoids to that of retinol. An alternative term developed by the Institute of Medicine [204] and adopted by the International Vitamin A Consultative Group [205] is retinol activity equivalent (RAE). The latter is based on different conversion factors, which are discussed in detail in Institute of Medicine [204].

FAO/WHO [181] still recommends use of the 1988 FAO/WHO [192] conversion factors to calculate RE until more definitive data are available. These conversion factors do not distinguish between synthetic sources of provitamin A compounds and natural sources in plants. Increasingly, synthetic sources of retinol and provitamin A compounds (mainly β-carotene) are being added to foods or used as dietary supplements in both industrialized and developing countries.

For other nutrients, such as calcium and magnesium, the amount available for absorption is generally estimated from the major food sources of these minerals in the diet.

### Alternative methods for estimating bioavailability of iron and zinc from regional or national diets

Application of the algorithms described above requires detailed food-consumption data, preferably at the national level. Unfortunately, such data are often not available, especially in developing countries. Instead, alternative approaches are needed, possibly using food-balance sheet data. Food-balance sheets, published by FAO, provide data on the annual supply of 95 individual food commodities and 15 major food groups per capita that are available for human consumption in 176 countries. The major food groups are cereals, roots and tubers, sugars and honey, pulses, nuts and oilseeds, vegetables, fruits, meat and offal, eggs, fish and seafood, milk, oils and fats, spices, stimulants, and alcoholic beverages; details are given in FAO/WHO [206].

The FAO food-balance sheets also provide data on the daily per capita availability of energy (kilocalories per day), fat (grams per day), and protein (grams per day) for each commodity and each food group, calculated from regional food-composition tables. Hence, the mean daily per capita availability of the proportion of energy from each food commodity and food group can be calculated. This may provide a useful approach for estimating the bioavailability of selected nutrients at the national level. For example, to estimate the bioavailability of iron at the national level, the mean daily availability per capita of the percentage of energy from cellular animal protein (meat, fish, poultry) may be useful, whereas for calcium, the percentage of energy from dairy products is probably more appropriate. Alternatively, if data on the mean daily per capita availability of additional nutrients such as iron, zinc, and calcium become available, then the percentage of iron or zinc from cellular animal protein, or the percentage of calcium from dairy products, could be calculated. Caution must be used, however, in the interpretation of these data if staple foods are fortified with iron, zinc, and/or calcium at the national level.

Calculation of mean daily per capita availability of phytate:zinc molar ratios from food-balance sheet data could provide a useful assessment of the bioavailability of zinc at the national level. As an example, **table 6** shows the per capita phytate:zinc molar ratios and proportion of energy from animal-source foods for selected countries calculated from FAO food-balance sheet data; further details are given by Hotz and Brown [36]. Depending on the phytate:zinc molar ratios, countries could be classified into those with diets of moderate (phytate:zinc molar ratios of 4 to 18) or low (phytate:zinc molar ratios > 18) zinc bioavailability by using the corresponding absorption estimates [36].

At present, national food-balance sheet data cannot be used to estimate the bioefficacy of provitamin A carotenoids. The food commodities itemized in the vegetable and fruit group are not comprehensive enough to compile a separate food group for green leafy vegetables, orange/yellow vegetables, or orange/yellow fruits.

### Comparison of adjustments for diet- and host-related factors influencing iron and zinc bioavailability across countries

In view of the uncertainty about the bioavailability of iron and zinc in diets of differing compositions, it is not surprising that there are large differences in the adjustments employed to convert the physiological requirements for iron and zinc to yield dietary require-

TABLE 6. Phytate:zinc molar ratios and proportion of energy from animal-source foods for selected countries, based on FAO food-balance sheet data

| Country | Phytate:zinc molar ratio | Energy from animal-source foods (%) |
|---|---|---|
| Mongolia | 3.5 | 45.3 |
| Egypt | 27.5 | 7.0 |
| United Kingdom | 8.6 | 31.6 |
| United States | 10.6 | 27.8 |
| Armenia | 12.8 | 16.3 |
| Cambodia | 24.0 | 8.0 |
| Kenya | 26.7 | 12.6 |
| Mexico | 27.7 | 16.9 |
| Bangladesh | 27.7 | 3.1 |
| Guatemala | 36.3 | 8.6 |
| Malawi | 37.3 | 2.7 |

Source: data compiled from Hotz and Brown [36].

ments, even among countries where omnivorous diets are habitually consumed. For example, in UK mixed diets, iron is assumed to have a fixed bioavailability of 15%, irrespective of age and life-stage group [207], whereas in the United States and Canada, a factor of 18% is used for the bioavailability of iron in the mixed diets of both children and nonpregnant adults, but 25% is used for women in the second and third trimesters of pregnancy and 10% for those consuming vegetarian diets [204]. Similarly, the United Kingdom uses a fixed factor of 30% for zinc, irrespective of age and life-stage group [207], but the United States and Canada apply a factor of 41% and 48% for the bioavailability of zinc from diets of adult (over 19 years) males and females, respectively, and 30% for preadolescent children [204]. Australia and New Zealand have adopted the US and Canadian bioavailability adjustments for iron for children 4 years of age or older, nonpregnant adults, and vegetarians, but the IZiNCG adjustments for zinc [36, 208]. Other expert groups, such as FAO/WHO [181] and IZiNCG [36], employ several factors to adjust for the bioavailability of iron and zinc, depending on the composition of the habitual diet, as noted earlier.

## Conclusions

Adjustments are needed to translate physiological requirements into dietary requirements for certain nutrients, notably calcium, magnesium, iron, zinc, protein, folate, and vitamin A. The magnitude of the adjustments depends on the nutrient and will vary according to the nature of the habitual diet and a variety of host-related factors. However, information on some of the host-related factors, especially those that influence the efficiency of luminal and mucosal digestion and absorption, is often limited, so that their effects on nutrient bioavailability are often ignored when setting dietary requirement estimates. At present, several algorithms have been developed to predict the bioavailability of iron, zinc, protein, folate, vitamin A, and carotenoids, but there is still no consensus among countries about which are the best algorithms to use. In some countries, fixed bioavailability factors are still used for certain nutrients, even though their efficiency of absorption may vary with the dietary level of the nutrient or the life-stage group.

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# Human nutrition and genetic variation

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## Abstract

*Human genetic variation is a determinant of nutrient efficacy and of tolerances and intolerances and has the potential to influence nutrient intake values (NIVs). Knowledge derived from the comprehensive identification of human genetic variation offers the potential to predict the physiological and pathological consequences of individual genetic differences and prevent and/or manage adverse outcomes through diet. Nutrients and genomes interact reciprocally; genomes confer differences in nutrient utilization, whereas nutrients effectively modify genome expression, stability, and viability. Understanding the interactions that occur among human genes, including all genetic variants thereof, and environmental exposures is enabling the development of genotype-specific nutritional regimens that prevent disease and promote wellness for individuals and populations throughout the life cycle. Genomic technologies may provide new criteria for establishing NIVs. The impact of a gene variant on NIVs will be dependent on its penetrance and prevalence within a population. Recent experiences indicate that few gene variants are anticipated to be sufficiently penetrant to affect average requirement (AR) values to a greater degree than environmental factors. If highly penetrant gene variants are identified that affect nutrient requirements, the prevalence of the variant in that country or region will determine the feasibility and necessity of deriving more than one AR or upper limit (UL) for affected genetic subgroups.*

**Key words:** Genome, nutrients, nutrition, polymorphism, requirements, variation

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## Introduction

Knowledge derived from the comprehensive identification of human genetic variation offers the potential to predict, prevent, and/or manage physiological and pathological consequences of individual genetic differences. Genetic variation contributes to human disease susceptibility, optimal nutritional requirements, food tolerance, drug efficacy, inflammatory responses, longevity, and virtually every human phenotype [1–8]. Nutrients and pharmaceuticals are two exposures that are proven to be effective in modifying genome function and stability for benefit, but whose utilization and efficacy are modified by human genetic variation.

Dietary guidelines, both food- and nutrient-based, are established to assist individuals and populations achieve adequate dietary patterns to maintain health. Their derivation and goals evolve continuously in response to new knowledge [9, 10]. When possible, guidelines for single nutrients and other food components are scientifically and quantitatively derived, and these numeric standards are essential to validate the efficacy of food-based guidelines [10]. Nutrient requirements vary within all human populations and can be modified by age, sex, and life stage, among other factors. Therefore, numeric standards are often derived separately for population subgroups. It is established that genetic variation can modify the efficacy, dosage, and safety of pharmaceutical agents [5] and tolerance for certain foods [11]. However, the contribution of genetics to optimal nutrient requirements within and among human populations remains to be evaluated rigorously. This report focuses on advancements in our understanding of the human genome and the emerging application of the genome sciences to identify genetic variation that affects optimal nutrient requirements and food tolerance within and among populations, understand the role of nutrients and dietary components in modifying genome function for benefit and the intake levels required to do so, understand both the benefits and the risks of population-based nutrition policies to subgroups, and develop genome-based

outcomes for assessing nutrient adequacy and risk for disease prevention.

## Human genetic variation

The primary sequence of the human genome was determined from 5 to 10 individuals of diverse ancestry and geographic history. The human genome is composed of approximately 3.1 billion nucleotide base pairs that are organized into 24 nuclear chromosomes [12]. There are an estimated 30,000 genes within the human genome that encode information required for the synthesis of all cellular proteins and functional RNA molecules, although less than half of human genes have been assigned known or putative functions. Only about 2% of the total human DNA primary sequence encodes genes. Most nuclear DNA is termed noncoding and has structural or regulatory roles or no known roles. The biological complexity of the mammalian cell is not limited by the number of genes encoded by its genome. A single gene can encode more than one RNA or protein product through posttranscriptional and posttranslational processing reactions, including RNA editing, alternative splicing, and other modifications including differential phosphorylation or methylation. Therefore, human cells contain more than 100,000 proteins with distinct primary sequences as a result of these processing and modification reactions [13].

The primary nucleotide sequence of the human genome varies by approximately 0.2% to 0.4% among humans [14, 15]. Sequence variations are referred to as polymorphism and constitute a primary molecular basis for human phenotypic variation. There are several distinct classes of polymorphism, including single nucleotide polymorphisms (SNPs), micro- and macrosatellite repeat sequences, and viral insertions. SNPs are defined as common nucleotide base pair differences in the primary sequence of DNA and are the most common variation in human DNA.

### SNPs and haplotypes

There are estimated to be more than 10 million SNPs in the human genome; over 4.5 million SNPs were validated as of 2004 [15]. SNPs can be single base pair insertions, deletions, or substitutions of one base pair for another. Nucleotide substitutions are the most common polymorphisms, whereas insertion/deletion mutations occur at 1/10 the frequency [16]. SNPs differ from DNA mutations in two regards: they are present in the germ line and therefore are heritable, and they must have a prevalence of at least 1% in humans. The generation of high-density SNP maps of the human genome facilitates the identification of human disease alleles, including low-penetrant alleles that may make relatively small contributions to the initiation and/or progression of complex disease [12].

DNA sequence is inherited in "blocks" that average 25,000 base pairs during meiotic recombination [13]. Therefore, SNPs that are physically close with respect to DNA primary sequence segregate rarely and are inherited together [13, 17]. SNPs captured within these blocks are said to be in linkage disequilibrium, which is defined as the nonrandom association of alleles at a nearby locus. Linkage disequilibrium is usually correlated with physical distance between loci but is also influenced by distance from the centromere and recombination frequency, which can vary throughout the genome. Inherited blocks of genetic variation are referred to as haplotypes, and the size of the haplotype blocks decays as the number of meiotic recombination events increases within a population. Ancestral populations that maintain a high effective population size for long periods are expected to have smaller haplotype sizes and therefore decreased linkage disequilibrium because of the increased number of historical recombination and mutation events, both of which cause linkage disequilibrium decay [12]. As predicted from evolutionary theory, African populations display higher levels of genetic diversity than all other human populations whose founder groups probably exhibited less genetic variation than the population from which they emerged and had less time to respond to their new environments. African linkage disequilibrium patterns exhibit a greater number of haplotypes and more divergent patterns of linkage disequilibrium than non-African populations [12]. Linkage disequilibrium in the Nigerian population extends an average distance of 5 kilobases, whereas European linkage disequilibrium can extend nearly 60 kilobases, a finding consistent with the increased number of recombination events that have occurred in ancestral populations [12]. Haplotype maps of human genetic variation offer advantages for disease associational studies because of their reduced complexity compared with SNP maps [18], but their utility may be limited because of the variability in haplotype diversity across candidate genes [19]. Furthermore, haplotype associations do not identify disease-causing mutations due to genetic hitchhiking [12] (polymorphisms that are in linkage disequilibrium with a mutation that is under selection will change in frequency along with the site undergoing selection). Because otherwise rare disease alleles can be enriched in geographically or culturally isolated populations, full characterization of SNP diversity and haplotype structure from ethnically diverse populations is critical for the identification of risk alleles that may be specific to small but identifiable subpopulations.

### Transposable elements

Genetic variation can also result from the integration and/or transposition of viral DNA. Approximately half

of noncoding human DNA originates from the insertion of highly mobile and repetitive sequences termed transposable elements. There are two types of transposable elements, retrotransposons and DNA transposons [20–22]. Retrotransposons are classified by size and include long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). About 10% of the human genomic sequence consists of 280 base pair *Alu* SINE elements; there are an estimated 1.4 million in the human genome. *Alu* elements display promoter activity, but their transcripts lack an open reading frame and therefore are not translated. *Alu* elements are rich in the dinucleotide sequence CpG, which is also common in promoter regions of mammalian genes and is recognized by DNA methylases that convert CpG sequences to $^{me}$CpG. Methylation of CpG sequences within *Alu* elements usually silences their promoter activity. Transposable elements are mutagenic; they can integrate within and disrupt a gene and can also serve as nucleation sites for unequal intrachromosomal and interchromosomal homologous recombination events that lead to chromosomal aberrations, including deletion and translocation events. New *Alu* insertions have been associated with 0.1% of human genetic disorders, including Apert syndrome, cholinesterase deficiency, and breast cancer. Other inherited disorders, including type 2 insulin-resistant diabetes and familial hypercholesterolemia, comprise part of the 0.3% of human genetic disease that results from *Alu*-mediated unequal homologous recombination events [12, 22]. Such events are rare, because *Alu*-mediated unequal homologous recombination events are usually inhibited by CpG methylation of the insertion.

Human populations are polymorphic for viral insertions [12]. More than 1,200 *Alu* elements integrated into the human genome following early human migrations; a new *Alu* insertion event occurs every 200 births [22]. *Alu* element insertions can alter gene function and stability around their region of integration and are thought to be catalysts for organismal evolution [22, 23]. Transposition events that occur near or within a gene can alter its expression or create a new gene. *Alu* elements can function as transcriptional silencers or activators; some *Alu* elements have retinoic acid response elements and therefore can confer new types of transcriptional regulation to genes neighboring the insertion site. *Alu* insertions near gene promoters can also confer transcriptional regulation by DNA methylation to that locus because they contain CpG sequences. The degree of transcriptional silencing is modifiable by diet [24]. For example, embryonic CpG methylation density can vary proportionately with folate status at defined loci during development [25, 26]. Maternal folate and other methyl donor supplementation alters the methylation status of targeted alleles in the mouse embryo, and these methylation patterns and subsequent effects on gene expression are retained throughout adulthood [24]. This type of epigenetic phenomenon may provide mechanistic insight into the many observational studies that associate risks of adult chronic diseases with maternal nutrition and embryonic nutrient exposures [27].

## Nutrition and the origin of human genetic variation

Organismal evolution is driven in part by complex and reciprocal interactions among genomes and environmental exposures that result in adaptive phenotypes. Modern human genetic variation is, in part, a product of such historical interactions and is manifest through the formation and propagation of primary sequence differences in DNA. Changes in DNA primary sequence constitute the molecular basis for human evolution and for the generation of adaptive genes that alter an organism's response to environmental challenges and hence to its fitness. Genomic polymorphism arises through the sequential processes of genetic mutation, followed by expansion of the mutation within a population; environment influences both of these processes.

### Mutation

Mutation is a consequence of the inherent chemical instability of DNA bases, stochastic error associated with DNA replication and recombination, and exposure to chemical radicals generated during oxidative metabolism as well as by environmental toxins. Therefore, a significant portion of mutations are not modifiable, although DNA repair systems detect and correct most mutation events. Environmental exposures and cellular oxidative stress can accelerate DNA mutation rates by inducing DNA modification reactions and/or accelerating DNA polymerase error rates. For example, nutrient deficiencies of iron or B vitamins impair nucleotide biosynthesis and thereby enhance polymerase error rates. Folate deficiency inhibits dTMP synthesis, which increases the incorporation of dUTP into DNA, resulting in increased rates of single point mutations as well as increased frequency of DNA strand breaks [28–32]. Mutation rates are also accelerated by radiation, cellular oxidative stress, and natural and synthetic genotoxic xenobiotics that are present in the food supply. Certain aflatoxins, a common class of natural xenobiotics, increase DNA mutation rates, leading to the transformation of somatic cells and localized cancer epidemics [33]. Furthermore, deficiencies of dietary antioxidants that scavenge chemical radicals, or excesses of prooxidant nutrients, including iron, may increase mutation rates [34–36]. However, only mutations that occur in the germ line contribute to a species' heritable genetic variation.

DNA mutation rates and polymorphism frequencies vary throughout the human genome. These differences have been attributed to the region-specific differences in DNA recombination rates (sites of more frequent recombination may exhibit elevated mutation rates), the mutagenic potential of specific nucleotide sequences, and local chromatin structure [12, 37]. For example, the sequence CpG is underrepresented in the human genome and its frequency has decayed throughout evolution because of its inherent instability [38]. Methylation of CpG sequences increases mutation rates, because methylcytosine ($^{me}$C) deaminates spontaneously to thymidine (T), whereas cytosine (C) deaminates to uracil (U), which is recognized as foreign to DNA and excised by the DNA repair enzymes [16].

## Selection

Mutation of the germ line is necessary but not sufficient for the creation of genetic variation. Germ line mutation that does not affect or confer function is assumed to be both phenotypically silent and selectively neutral, and therefore its frequency is exclusively a function of the DNA mutation rate (estimated to be $2.5 \times 10^{-8}$ on average for autosomes in regions of the genome presumed to be nonfunctional, including intronic and intergenic regions) [12, 16]. Only mutations that expand and become fixed within a population contribute to human genetic variation. Mutations that become fixed within a population contribute to genetic variation as polymorphisms, and this expansion is the basis for the molecular evolution of genomes. Fixation is a function of effective population size, population demographic history, and the effect of the mutation on an organism's fitness [12]. Polymorphisms expand within a population through the processes of genetic drift and natural selection. Drift is a stochastic process that results from random assortment of chromosomes at meiosis, because only a fraction of all possible zygotes are generated or survive to reproduce [12]. Therefore, mutations can expand from one generation to the next through the random sampling of gametes in the absence of selection. Drift generally has a greater impact on allele frequencies in small populations that are expanding rapidly. Drift in static large populations is not usually as significant because of the greater dilutional effect of such populations. Genetic drift can have a greater than expected impact in large populations when they undergo bottlenecks (massive reductions in population) or founding events that have occurred during human migrations, e.g., in population groups that include the Old Order Amish, Hutterite, and Ashkenazi Jewish [12]. In these populations, rare disease alleles can expand rapidly and increase the incidence of disease, including breast cancer, Tay-Sachs, Gaucher, Niemann-Pick, and familial hypercholesterolemia [12]. It is assumed that the majority of human genetic variation arose as a result of the neutral processes of mutation and genetic drift and rarely has physiological consequences.

The neutral theory of evolution does not account for the proportion of amino acid substitutions observed in mammalian genomes [6, 37, 39, 40]. Although protein-coding sequences are conserved among mammals in general, rates of amino acid substitution vary markedly among proteins compared with rates of synonymous substitution among genes (changes in the coding region of genes that do not affect protein sequence) [37]. Whereas patterns of genetic variation across the entire human genome are affected by the demographic histories of populations, variation at particular genetic loci is influenced by the effects of natural selection, mutation, and recombination [12]. Mutations that alter amino acid sequence may influence protein structure and function, and the resulting physiological consequences may be beneficial, deleterious, or neutral and thereby may influence an organism's fitness in specific environmental contexts. Likewise, mutations that affect protein expression level can alter metabolism and other physiological processes and therefore are also under constraint and subject to positive, balancing, or negative selection. Natural selection, which is the differential contribution of genetic variants to future generations, is the only evolutionary force that has adaptive consequences [41]. Darwinian selection favors the maintenance and expansion of favorable mutations (by positive or balancing selection) and the elimination of mutations that are deleterious (referred to as negative or purifying selection). Positive selection increases the rates of fixation at defined loci within the genome, indicating that not all genes are expected to evolve at the same rate. Adaptive mutations expand within populations at accelerated rates relative to neutral mutations and replace a population's preexisting variation. The proportion of amino acid substitutions that result from positive selection is estimated to be 35% to 45% [37].

Comparison of genomic sequence divergence among mammalian species (to identify ancient selection) and comparison of the diversity of genomic sequences among human populations (to identify more recent selection following human migrations out of Africa) are complementary approaches that have permitted the identification of genes that have undergone accelerated or adaptive evolution (**table 1**) [6, 42]. Rapidly evolving genes are inferred to have enabled adaptation and thus became fixed in populations by positive or balancing selection. Genes that have been subject to positive selection exhibit specific genomic signatures, which include an excess of rare variants within a population (which can be indicative of a selective sweep), large allele frequency differences among populations, and

TABLE 1. Diet-related genes and pathways that display genomic signatures of adaptive evolution

| Gene | Species/function | Reference |
|---|---|---|
| Lysozyme | Langur monkey | 37, 117, 118 |
| Ribonuclease | Langur monkey | 37, 118 |
| *Cox4* | Primates | 119 |
| *LCT* | Human lactose metabolism | 52 |
| *ADH1B* | Human ethanol metabolism | 59 |
| *ALDH2* | Human ethanol metabolism | 61 |
| *HFE* | Human iron homeostasis | 55 |
| *PPARg* | Human nuclear receptor | 41 |
| *PTC* | Human bitter-taste receptor | 72 |
| *KEL* | Human protein metabolism | 42 |
| *TRPV5* | Human calcium transport | 42 |
| *TRPV6* | Human calcium transport | 42 |
| *ABO* | Human protein metabolism | 42 |
| *ACE2* | Human protein metabolism | 42 |
| *CYP1A2* | Human arylamine metabolism | 120 |
| *G6PD* | Human NADP metabolism | 65 |
| Pathway | | |
| Amino acid metabolism | Human, chimpanzee | 6 |
| Amino acid transport | Chimpanzee | 6 |
| Purine metabolism | Chimpanzee | 6 |

a common haplotype that remains intact over long distances [23, 41–43]. These genomic signatures are expected to arise from region-specific selective factors and therefore concentrate in specific geographic regions where the selection occurred [12]. Signatures of positive selection can be used to predict the geographic origins of an individual to the degree that different selective pressures are operative across populations, but they do not always correspond to specific ethnic or racial groups because races are not homogeneous [15, 44].

Understanding the historical selection of gene variants enables the identification of human disease alleles that arose from nutritional challenges, including single-gene as well as low-penetrant complex disease alleles [12, 42]. The common disease-common variant hypothesis predicts that disease-susceptibility alleles will exist at high frequency across all human populations and therefore arose prior to human migrations out of Africa [45, 46]. However, both single-gene disorders, including cystic fibrosis and hemochromatosis, and complex diseases are also associated with less common and geographically restricted alleles that arose after migrations out of Africa [15, 23, 42, 47–49]. Therefore, although 85% to 90% of all human genetic variation is found within populations, some of the 10% to 15% of variation among populations arose from recent selective pressures that contribute to both simple and complex disease [50, 51]. In fact, many of the human alleles known to affect metabolism, food tolerance, or optimal nutrient intake display signatures of positive selection (**table 1**); some examples are described below.

#### *Lactose and calcium metabolism*

The metabolism of lactose requires expression of the enzyme lactase-phlorizin hydrolase encoded by the *LCT* gene. *LCT* expression declines after weaning in most mammals, including most humans, resulting in primary lactose intolerance. The expression of *LCT* persists into adulthood for some human populations, including humans of northwest European descent and nomads of the Afro-Arabian desert region. An SNP was identified 14 kilobases upstream of the *LCT* transcriptional initiation site in a *cis*-acting transcriptional element that is enriched in individuals of northern European descent and displays genomic signatures of positive selection [11, 52, 53]. Its prevalence correlates with, but does not account fully for, the persistence of *LCT* expression and resistance to primary lactose intolerance throughout adulthood [53]. The benefits of milk consumption in cattle-herding populations, both as a source of liquid in arid regions and for prevention of rickets and osteomalacia in regions of low solar irradiation, may have driven the fixation of this polymorphism [43, 52, 54]. The need for efficient calcium absorption may also have driven alleles for *TRPV5* and *TRPV6* to fixation in the same populations (**table 1**) [42].

#### *Iron metabolism*

Hereditary hemochromatosis is a recessive iron-storage disease prevalent in populations of European descent with an incidence of 1 in 300 persons. A common polymorphism in the *HFE* gene (C282Y), which encodes a protein that regulates iron homeostasis, is associated with the disease phenotype in 60% to 100% of Europeans and arose approximately 138 generations ago [23, 55, 56]. The *HFE* C282Y allele is not present in Asian and African populations where iron-storage diseases exist; mutations in other genes are also associated with the phenotype. The penetrance of the C282Y *HFE* allele for the iron overload phenotype varies widely among homozygotes, with some individuals being asymptomatic, indicating the presence of modifying alleles. The recent expansion of this polymorphism may have conferred selective advantages in iron-poor environments [55, 56] or resistance to microbial infection [57].

#### *Alcohol metabolism*

The efficiency of ethanol metabolism varies widely among human ethnic populations [58]. *ADH* encodes the enzyme alcohol dehydrogenase, which oxidizes

ethanol to acetaldehyde; acetaldehyde is subsequently oxidized to acetic acid by the enzyme aldehyde dehydrogenase encoded by *ALDH2*. Seven *ADH* genes have been identified and cluster on chromosome 4; all encoded proteins display distinct catalytic properties and tissue-specific expression patterns. Two of the genes encoding class I enzymes (*ADH1B* and *ADH1C*) are expressed in liver, function in systemic ethanol clearance, and display functional polymorphism. A variant *ADH1B* 47His* allele predominates in Japanese and Chinese populations but is rare in European and northern African populations [59]. The variant allele encodes an enzyme with elevated enzyme activity leading to more rapid formation of acetaldehyde. The *ADH1C*349Ile* variant is found in Europeans, whereas the *ADH1B*369Arg* variant is mostly restricted to individuals of African descent. *ALDH2* is also highly polymorphic; members of Asian populations carry a common dominant null allelic variant (E487K) and when consuming alcohol develop a characteristic "flush" reaction resulting from acetaldehyde accumulation [60]. *ADH* and *ALDH* alleles that predominate in east Asian populations display signatures of positive selection, and the expression of these variant alleles results in elevated acetaldehyde concentrations following alcohol consumption, which may have conferred advantage by protecting against parasite infection [61].

#### *Energy metabolism*

The "thrifty gene" hypothesis was first proposed over 40 years ago to account for the epidemic of type 2 diabetes observed in non-Western cultures that adopt Western-style diets and lifestyles [62, 63]. The hypothesis states that exposure to frequent famine selected for gene variants that enabled the more efficient conversion of food into energy and fat deposition during periods of unpredictable and sometimes scant food supplies. The putative adaptations also may have resulted in more efficient adaptations to fasting conditions (e.g., more rapid decreases in basal metabolism) and/or physiological responses that facilitate excessive intakes in times of plenty. Conclusive genomic data have not yet supported this hypothesis [63, 64].

#### *Oxidative metabolism*

Variations that impact human nutrition and metabolism may have arisen independently of direct nutritional challenges. The enzyme glucose-6-phosphate dehydrogenase is solely responsible for the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in red blood cells and therefore is required to prevent oxidative damage. Variants with low activity resulting from amino acid substitutions, including the *G6PD-202A* allele, are enriched in sub-Saharan African populations and arose 2,500 to 6,500 years ago [65]. Presumably, this allelic variant became enriched in populations as a result of balancing selection because it conferred resistance to malarial disease in heterozygous females and hemizygous males [66, 67].

These examples illustrate the role of environmental exposures, including pathogens and dietary components, as selective forces that facilitated the fixation of alleles that alter the utilization and metabolism of dietary components. Adaptive alleles may become recessive disease alleles, or disease alleles even in heterozygote individuals, when the environmental conditions change profoundly, such as those brought about by the advent of civilization and agriculture, including alterations in the nature and abundance of the food supply [6, 37, 41, 43, 68–72]. Adaptive alleles may be responsible for the generation of metabolic disease alleles both within and across ethnically diverse human populations and therefore are strong, nonbiased candidate genes for disease association studies; the interacting and modifying environmental factors can be inferred from the nutrients and/or metabolites that are known to interact with the gene product [12].

## Functional consequences of human genetic variation

Polymorphisms that affect nutrient utilization or metabolism probably arose from historical adaptation and can be identified now by "blinded" computational approaches. However, prior to the advent of whole genome approaches, most functional polymorphisms were identified as highly penetrant disease alleles from epidemiologic or clinical studies. Candidate genes were selected for analyses of variation based on knowledge of metabolic pathways and predictions that their impairment could result in metabolic phenotypes that either mirror a particular disease state or affect the concentration of a biomarker associated with the disease. Genetically modifiable organisms, including yeast, *Drosophila*, *Caenorhabditis elegans*, and mice, are also excellent resources to identify candidate genes and serve as models to confirm gene function. Candidate gene approaches have been successful in identifying many disease susceptibility alleles (**table 2**) [73, 74], but they are limited by incomplete knowledge of gene function, incomplete knowledge of transcriptional and metabolic networks that suggest candidate genes for analyses, and inconsistent findings among epidemiologic studies, especially for low-penetrant alleles. Once candidate genes are identified, establishing alleles as disease-causing is equally challenging. Because many SNPs are in linkage disequilibrium, it is not always possible to determine with certainty whether an individual SNP or allele is functional. Furthermore, SNP penetrance cannot always be inferred from *in vitro* studies of proteins or studies of model organisms. Metabolic

TABLE 2. Human gene variants that affect the uptake or metabolism of dietary components

| Food component | Gene | Polymorphic allele | Reference |
|---|---|---|---|
| Vitamins | | | |
| Folate | *MTHFR* | A222V | 1, 121 |
| | *CBS* | 844ins68 | 122 |
| | *GCPII* | H475Y | 123, 124 |
| Vitamin $B_{12}$ | *MTR* | N919G | 121 |
| | *MTRR* | I22M | 121 |
| Vitamin D | *VDR* | Many | 125 |
| Minerals | | | |
| Iron | *HFE* | C282Y | 126, 127 |
| Sodium | CIC-Kb | T481S | 128, 129 |
| Lipids | *APOB* | Many | 130, 131 |
| | *APOC3* | Many | 132 |
| | *APOE* | Many | 83 |
| Alcohol | *ADH*/*ALDH2* | Many | 58, 60 |
| Carbohydrates | | | |
| Lactose | *LCT* | Promoter | 53 |
| Fructose | Aldolase B | Many | 82 |
| Detoxification/ oxidative stress | *NAT1*/*NAT2* | Many | 133, 134 |
| | *PON1* | Q192R;L55M | 135 |
| | *Mn-SOD* | Ala(-9)Val | 136, 137 |

networks are robust and maintain flux through redundancy, degeneracy, and/or compensatory alterations in epigenetic programming, gene expression, and protein regulation, among others, which serve to maintain homeostasis by masking the effects of otherwise deleterious genetic mutations [27, 75, 76].

Regardless of the methodology used to identify alleles that alter nutrient metabolism or utilization, knowledge of the functional consequences of genetic variation is essential for the rational design of nutritional interventions that seek to modify the penetrance of the deleterious alleles. Genetic variation that influences gene expression or changes amino acid sequence encoded within an mRNA transcript can influence cellular metabolism and/or other physiological processes. Metabolic alterations may confer advantage or risk to the organism in specific environmental contexts, and basic knowledge of enzyme function and metabolic flux theory predicts that both the risk and the benefit are modifiable by nutrients or nutrient intake levels. The rate of an enzyme-catalyzed reaction or nutrient transport is a function of the concentrations of enzyme (E) and substrate (S) and will be reflected by the proportion of enzyme present as an ES complex, as shown in equation 1:

$$E + S <\rightarrow ES \leftarrow> E + P \quad (1)$$

The rate of ES complex formation is dependent upon productive collisions between the enzyme and substrate(s), as stated by the law of mass action. Therefore, the rate of an enzyme-catalyzed reaction is usually directly proportional to the molecular concentrations of the reacting substances (both E and S) and to the affinity of E for S. The Michaelis-Menten constant $K_m$ refers to the substrate concentration required for half-maximal velocity of E and indicates the affinity of E for S. Genetic variation influences the formation of the ES complex by affecting the cellular concentration of E (as occurs with expression variants, e.g., *LCT*) or by altering $K_m$ (as seen with ethanol metabolizing enzyme variants). The concentration of E is influenced by alterations in its expression or turnover; the expression of E can be affected at the level of transcription, translation, mRNA splicing, mRNA stability, or post-translational processing and modification. Specific nutrients can regulate the expression and processing of many genes. Therefore, interventions or therapies can be designed rationally to affect the concentration of E for health benefit. Genetic variation in protein coding sequence can alter $K_m$ and thereby affect the concentration of substrate required to drive the formation of the ES complex and/or result in the accumulation of metabolic intermediates in cells. Therefore, alterations in $K_m$ can indicate the minimal level of nutrient intake that is required to maintain flux through a metabolic pathway, or potentially alter the safe upper level of nutrient intake to avoid the accumulation of toxic intermediates.

The rate of product formation is also a function of the Michaelis-Menten constant $k_{cat}$, which refers to the

maximal rate of catalysis and usually represents the rate of breakdown of the ES complex to product (P) at infinite substrate concentration (all enzyme is present as an ES complex). Genetic variation that alters amino acid coding sequence can influence $k_{cat}$ and thereby influence rates of nutrient uptake or clearance of metabolic intermediates and overall net flux through a metabolic pathway in a substrate-independent manner. Severe alterations in $k_{cat}$ can indicate food or nutrient intolerances. Therefore, a detailed understanding of the functional consequences of allelic variants can be used to predict nutrient intolerance and may indicate genotype-specific variation in nutritional requirements, and can lead to the development of tailored nutritional interventions and therapies for populations and individuals. Specific examples are discussed below.

#### One-carbon metabolism

Folate-mediated one-carbon metabolism is required for purine, thymidylate, and methionine biosynthesis and affects genome synthesis, genome stability, and gene expression [77]. Several polymorphic alleles have been identified to be associated with metabolic perturbations that can confer both protection and risk for specific pathologies and developmental anomalies [78]. SNPs in *MTHFR* (A222V) and *MTHFD1* (R653Q) [79], which encode folate-dependent enzymes, are associated with increased risk of neural tube defects; *MTHFR* (A222V) is protective against colon cancer in folate-replete subjects [80]. The MTHFR A222V variant protein has reduced affinity for riboflavin cofactors and is thermolabile, resulting in reduced cellular MTHFR activity; its stability is increased when folate is bound [81]. Although the biochemical role of these polymorphisms in the etiology of neural tube defects and cancer is unknown, it has been demonstrated that some carriers of *MTHFR* variants require higher folate intakes than others in order to stabilize the MTHFR protein, lower the concentration of the metabolic intermediate homocysteine, and decrease women's risk of bearing children with developmental anomalies [1]. The *MTHFR* variant is prevalent in Caucasian and Asian populations but is nearly absent in African populations [1]. Fortification of the food supply with folic acid, as practiced in many countries, targets women of childbearing age for prevention of birth defects, with genetically identifiable subgroups receiving the most benefit.

#### Fructose metabolism

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder of fructose metabolism caused by low fructose-1,6-aldolase activity, which results in an accumulation of the toxic metabolic intermediate fructose-1-phosphate. Twenty-five allelic variants of the human liver isozyme aldolase B have been identified that impair enzyme activity by altering $K_m$, $k_{cat}$, and/or protein stability [82]. The prevalence of these variants differs throughout Europe; the L288 delta C frameshift mutation is restricted to Sicilian subjects. The accumulation of fructose-1-phosphate inhibits glycogen breakdown and glucose synthesis, resulting in severe hypoglycemia following ingestion of fructose. Prolonged fructose ingestion in infants leads ultimately to hepatic and/or renal failure and death. Affected individuals are asymptomatic in the absence of fructose or sucrose consumption and can avoid the recurrence of symptoms by remaining on a fructose- and sucrose-free diet. The incidence of HFI intolerance has increased since the widespread use of sucrose and fructose as nutrients and sweeteners, providing an excellent example whereby an environmental shift resulted in the apparent conversion of normally nonpenetrant "silent" aldolase B alleles into HFI disease alleles.

#### Lipid metabolism

Apolipoprotein E (apoE) is a polymorphic protein that functions in lipid metabolism and cholesterol transport [83]. The three common allelic variants, ε2, ε3, and ε4, encode proteins that differ in their affinity both for lipoprotein particles and for low-density lipoprotein receptors. All human populations display apoE polymorphism, but the relative distribution varies among populations; the frequency of the ε4 allele declines from northern to southern Europe. The ε4 allele increases the risk of late-onset Alzheimer's disease and arteriosclerosis with low penetrance. Carriers of the ε2 allele tend to display lower levels of plasma total cholesterol, whereas carriers of the ε4 allele, which may be ancestral, display higher cholesterol levels. Therefore, serum cholesterol levels are likely to be more responsive to low-fat and low-cholesterol diets in carriers of the ε4 allele [84, 85].

## Genetic variation and human nutrition

Nutrients and the genome interact reciprocally; genomes confer differences in food tolerances and nutrient requirements, and nutrients can influence genome expression, stability, and viability [77]. Characterization of gene variants that modify optimal nutrient requirements has diagnostic value; it enables the classification of genetic subgroups for which generalized nutritional requirements may not apply. Parallel advancements in understanding the interactions among human genes, including all genetic variants thereof, and environmental exposures is enabling the development of genotype-specific nutritional regimens that prevent disease and promote wellness for individuals and populations throughout the life cycle. Current challenges associated with the incorporation

of genomic information into the nutritional sciences are described below.

### Genetic variation and nutritional requirements

Human genotypes that do not support basic physiological processes will be selected against in large part because of fetal loss or the failure to survive to reproduce. Allelic variants that confer nutrient requirements that cannot be met by the mother or that result in severe metabolic disruptions are expected to be embryonic lethal. Nearly 60% of all human conceptuses are not viable and do not survive to the 12th week of gestation [86, 87]. Human alleles associated with developmental anomalies that encode folate-dependent metabolic enzymes are not in Hardy-Weinberg equilibrium (alleles are not inherited at the expected frequency), consistent with evidence that elevated homocysteine and the *MTHFR* A222V polymorphism are risk factors for spontaneous miscarriage and decreased fetal viability [77, 79, 88–90]. Nutrition, unlike pharmaceuticals, is an *in utero* and lifelong exposure that can serve as a selective pressure to eliminate genomes that are not compatible with the nutrient environment. Therefore, genotype is not anticipated to confer extreme variations in optimal nutrient requirements among individuals and populations. Alleles that confer more subtle differences in nutrient requirements or food tolerances are expected to be enriched in subgroups or populations and to contribute to disease in certain environmental contexts (**tables 1 and 2**).

The concept of generalized nutrient requirements within and among populations is nullified only when a level of nutrient intake that represents minimal nutrient adequacy for one genetic subgroup exceeds a safe intake level for another group, assuming that nutrient deficiency and avoidance of harm or toxicity avoidance are the primary criteria for requirement. For example, optimal folate intakes may differ among identifiable genetic subgroups. However, it is not established that the magnitude of the genetic contribution to variations in adequate dietary folate intake warrants genotype-specific recommendations, especially considering that folic acid intakes up to 1 mg/day are not associated with known toxicities [91]. Iron is another candidate nutrient for which genotype-specific nutrient requirements have been considered [57, 92–94]. For these and other cases, the *penetrance* (contribution of the individual allele to variation in nutrient requirements) and the *prevalence* of these functional gene variants must be elucidated both within and among human populations to validate the concept of generalized nutrient requirements for all genotypes. Unlike the effects of sex and life cycle, no common allelic variant has been shown to be sufficiently penetrant and to warrant genotype-specific numeric standards for nutrient adequacy or upper levels of intake associated with harm or toxicity. At this time, genetic variation is known only to influence nutrient and food intolerance. However, genetic variation has not been characterized in many geographically and culturally isolated populations that have existed in nutrient-poor or otherwise unique nutritional environments for many generations, and therefore the presence of adaptive alleles should be expected in such populations. Recent experiences have demonstrated the severe adverse health consequences that result from rapid alterations in dietary patterns among certain populations [95].

Finally, to achieve dietary guidelines that optimize health for all individuals and populations, the many functions of individual genes and their regulation within metabolic and transcriptional networks must be understood comprehensively. Recently, it was shown that the *LCT* gene also encodes the enzyme pyridoxine-5'-β-D-glucoside as a result of differential processing of the *LCT* transcript [96]. Pyridoxine-5'-β-D-glucoside activity is necessary for the bioavailability of pyridoxine-5'-β-D-glucoside, the major form of vitamin $B_6$ in plant-derived foods [97]. Therefore, *LCT* variation predicts both lactose tolerance and preferred dietary sources of vitamin $B_6$ in adulthood.

### Genetic variation and benefits and risks of using food as medicine

Nutrients can be effective agents to modify genome expression and stability for benefit. Designer diets and nutritional supplements can compensate for deleterious alleles, as illustrated by the use of phenylalanine-restricted diets to avoid severe cognitive deficits associated with phenylketonuria and the use of folate supplements and/or fortified food to prevent the occurrence and recurrence of neural tube defects. High-dose vitamin therapy is advocated to rescue impaired metabolic reactions that result from mutations and polymorphisms that decrease the affinity of substrates and cofactors for the encoded enzyme [73]. ω-3 fatty acids and tocopherols are suggested to promote healthy aging and longevity by modulating the inflammatory response by altering gene transcription [8]. Other genes and allelic variants that influence longevity (a trait that is not likely to be adaptive) are being identified [7], and their penetrance can be modified by the rational design of nutrition-based interventions and therapies. Repression of energy metabolism through caloric restriction or transcriptional regulation of metabolic enzymes reduces oxidative stress and promotes longevity in many experimental model systems. Manipulation of these transcriptional or metabolic networks by diet may promote healthy aging. However, caution is warranted. Genes encoding virtually all physiological process are not adapted to excessive nutrient intake exposures that exceed what has been achieved in historical and healthful food-based diets. Therefore, new risks and toxicities

should be anticipated in populations or population subgroups when nutrients are administered at pharmacological levels, as illustrated by the introduction of high fructose into the food supply. Other genomic consequences may also result, including permanent alterations in genome-wide methylation patterns, as observed in mouse embryos whose mothers received elevated doses of folic acid and one-carbon donors during gestation [24]. Methylation patterns that are established *in utero* can be metastable and influence gene expression and potentially mutation rates into adulthood [24]. The effect of diet on DNA methylation and genome programming in adult stem cells is unknown. Although antioxidants can decrease mutation rates, they also function as prooxidants *in vivo* [98] and may be cancer-promoting at elevated intakes by inhibiting cellular death programs in transformed cells [99]. In conclusion, elucidation of robust gene-by-nutrient interactions will inform dietary approaches for individuals and for population-based interventions that prevent and/or manage rare inborn errors of metabolism as well as complex metabolic disease. Furthermore, these and other examples indicate that rigorous hazard identification is essential prior to the establishment of policies that result in pharmacological intakes of nutrients and other food components.

## Genomic criteria for setting requirements and toxicities

Genomic technologies may provide new criteria for establishing numeric standards for adequate levels of nutrient intake by targeting the molecular antecedents of disease. Mutation increases the risks of developmental anomalies, degenerative diseases, and cancers and can be quantified in controlled experimental settings, indicating that the effects of key minerals and vitamins on DNA mutation rates should be considered when establishing RDAs (recommended dietary allowances) [30]. Marginal deficiencies in folate, vitamin $B_{12}$, niacin, and zinc can influence genome stability, and antioxidants, including carotenoids, vitamin C, and vitamin E, may prevent damage resulting from oxidative stress. Validation of these protective effects on DNA mutation rates in controlled human trials may indicate benefits and lead to increased recommended intake levels, perhaps at levels not normally achievable from a natural food-based diet. Similarly, the use of functional genomic approaches, including expression profiling and proteomics to quantify gene expression and metabolomics to quantify metabolic pathway flux, provides a comprehensive set of quantitative and physiologically relevant "biomarkers" to model and assess nutrient efficacy in the context of optimal network function [100].

Other genomic outcomes are emerging as criteria for hazard identification and may influence the establishment of tolerable upper levels of nutrient intake during pregnancy. Studies of animal models are revealing that nutrients can rescue deleterious genetic mutations, leading to the concept that "good diet hides genetic mutations" [101]. Individual nutrients can rescue severe genetic lesions in mice when administered in supraphysiologic levels during critical developmental windows. Maternal retinoic acid administration between 7.5 and 9.5 days postconception rescued deafness and inner ear development in *Hoxa1*$^{-/-}$ mice [102], and folic acid can rescue skeletal defects associated with deletion of a *Hox* gene, as well as neural tube defects in mice that have no evidence of disrupted folate metabolism [101]. This rescue phenomenon is not established in humans, but animal studies indicate that nutrients can modify the viability of genomes, including genomes that confer atypical nutrient requirements on the surviving fetus [103].

There is increasing evidence that maternal nutrition can induce epigenetic changes in the fetal genome that may program increased risks of metabolic disease and nutrient requirements throughout the lifespan of the offspring. The effects of fetal glucocorticoid exposure on adult chronic disease risk provide some of the strongest evidence for the fetal origins of disease hypothesis [104–107]. Fetal glucocorticoid levels are maintained at low concentrations relative to maternal concentrations primarily through the action of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which catalyzes the oxidative inactivation of cortisol and corticosterone [108]. Elevated fetal glucocorticoid exposures during late gestation, which can result from 11β-HSD2 inhibitors, rare mutations in the human 11β-HSD2 gene, or large existing variation in placental 11β-HSD2 activity among humans, can have lifelong consequences for the fetus, including low birthweight, elevated plasma glucocorticoid, hypertension, hyperglycemia, insulin resistance, hyperinsulinemia, and anxiety [107]. Low maternal dietary protein intake during gestation causes a specific loss of placental 11β-HSD2 expression and similar outcomes resulting from elevated fetal glucocorticoid exposure [109]. Similarly, obstetric glucocorticoid therapy to accelerate lung development prior to anticipated preterm deliveries also increases the risk of reduced fetal birthweight and long-term susceptibility to hypertension, hyperglycemia, cardiovascular disease, and increased hypothalamic-pituitary-adrenal axis activity. These disorders persist not only into adulthood, but also into the next generation [110]. Lifelong consequences associated with fetal glucocorticoid exposure may result from premature glucocorticoid receptor-mediated chromatin remodeling in the hippocampus [107]. Prenatal glucocorticoid exposure decreases fetal glucocorticoid receptor expression which remains reduced through adulthood. Maternal undernutrition can elicit the same effect, presumably by decreasing placental 11β-HSD2 levels. Maternal GC exposure also affects glucose and

insulin homeostasis [111] by programming hepatic PEPCK levels through changes in PEPCK gene methylation with effects that persist into adulthood [112, 113]. The modification of glucocorticoid-mediated fetal programming by maternal folate status has not been investigated, nor have the effects of fetal programming on adult nutritional requirements.

## Conclusions: effects of human genetic variation on NIVs

Human genetic variation is a determinant of nutrient efficacy and of tolerances and intolerances and has the potential to influence nutrient intake values (NIVs). Historically, the nature and abundance of the food supply has been one of several environmental selective pressures that governed the evolution of humans by facilitating the expansion of polymorphisms within human populations. Genetic variants that enable survival in challenging nutrient environments become enriched in populations through the process of natural selection. This process has been shown to create variation in the utilization of lactose, iron, and alcohol and the associated food intolerances. Genetic determinants of nutrient tolerances display genomic signatures of positive selection, indicating that these variants offered survival advantage in specific geographic regions. Recent history has also revealed that rapid and severe alterations in the food supply can unmask previously silent genetic variation and create new or more prevalent food intolerances, as occurred with the infusion of large quantities of fructose in the food supply [82, 114, 115]. To date, no gene variant has been demonstrated to affect nutritional requirements sufficiently to warrant genotype-specific recommendations, although the affect of the MTHFR A222V variants on folate requirements has been considered. However, because many human populations have existed for many generations in unique, isolated, and challenging nutrient environments, relatively rare gene variants that influence NIVs may be highly prevalent in historically isolated, stable human populations. All human genetic variation is expected to be identified in the near future. Linking specific gene variants to known nutrient sensitivity in ethnic or geographic human populations, such as salt sensitivity in African Americans, may enable population-specific recommendations for genetic subgroups [116]. Because polymorphisms can confer both health benefits and risks, depending on the outcome of interest, and these outcomes may respond differentially to nutrient intake levels, it may important to consider the effects of genetic-specific recommendations on all known health outcomes.

The impact of a gene variant on nutritional requirements will be dependent on its prevalence and penetrance. Penetrance, which is the probability that a gene variant will express a phenotype from a given genotype at a given time, usually varies inversely with prevalence. Recent experiences indicate that few gene variants are anticipated to be sufficiently penetrant to affect variation of an average requirement (AR) to a greater degree than environmental factors. Once highly penetrant gene variants are identified, the prevalence of the variant in that country or region will determine the feasibility and necessity of deriving more than one AR or upper limit (UL) for genetic subgroups. For example, it is unlikely that gene–gene interactions will be a major consideration in the determination of NIVs because of the very low prevalence associated with highly penetrant gene–gene interactions. Likewise, because chronic diseases are polygenic complex traits, a single SNP is unlikely to have an impact on NIVs that target long-term chronic disease prevention.

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# Application of nutrient intake values (NIVs)

Hester H. Vorster, Suzanne P. Murphy, Lindsay H. Allen, and Janet C. King

## Abstract

*The process of applying nutrient intake values (NIVs) for dietary assessment, planning, and implementing programs is discussed in this paper. In addition to assessing, monitoring, and evaluating nutritional situations, applications include planning food policies, strategies, and programs for promotion of optimal nutrition and preventing and treating malnutrition (both over- and undernutrition). Other applications include nutrition education, food and nutrient legislation, marketing and labeling, research, product development, food procurement and trade (import and export), food aid, and therapeutic (clinical) nutrition. Specific examples of how NIVs are used to develop food labels, fortification policies, and food-based dietary guidelines are described. Applications in both developed and developing countries are also described. In summary, NIVs are the scientific backbone of all aspects of nutrition policy in countries and regions worldwide.*

**Key words:** Diet assessment, diet planning, dietary guidelines, food fortification, food labeling, nutrient recommendations

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## Introduction

As mentioned elsewhere in this supplement to the *Food and Nutrition Bulletin* [1–3], harmonization of the process and methods to establish nutrient intake values (NIVs) provides a common basis for the uses and applications of these values across countries and regions of the world. In the paper by Murphy et al. [3], methods for using NIVs to assess nutritional status, policy planning, and development of strategies and programs contributing to optimal nutritional health of individuals, groups, and populations are described. The main objectives of this paper are to highlight some of the applications of NIVs, to show how the new proposed terminology and methods of derivation improve the ability to develop realistic, achievable nutrient goals in developed *and* developing countries, and to provide specific examples of how the NIVs can be used to establish food labels, make decisions about fortification, and derive food-based dietary guidelines.

The terms used to describe the components of a set of NIVs are as follows. The framework used to derive these values is described in the paper by King et al. [2].

*Nutrient intake values (NIVs)* is the umbrella term for a set of specific nutrient standards. At least three different values should be derived: an average nutrient requirement (ANR), an individual nutrient intake level ($INL_x$), and an upper nutrient level (UNL). Detailed definitions of these terms follow. The US/Canadian set of nutrient standards is called the dietary reference intakes (DRIs); in Britain they are called dietary reference values (DRVs).

The *average, or mean, nutrient requirement (ANR)* is estimated from a statistical distribution of requirements for a specific criterion (e.g., to prevent a deficiency or maintain body stores) for specific life-stage (age) and gender (sex) groups. In the US/Canadian and UK recommendations, this value is called the estimated average requirement (EAR), while the European Communities use the term average requirement intake (ARI).

The *individual nutrient intake level ($INL_x$)* is the

recommended intake for all healthy individuals in a specific subpopulation. If set at 2 SD above the ANR, the $INL_x$ would be $INL_{98}$. It could be set lower if food policies or food supplies in a country or region do not permit achieving an intake at the 98th percentile for all healthy individuals. Other terms used to describe this value include the recommended dietary allowance (RDA) by the United States and Canada, the reference nutrient intake (RNI) by the United Kingdom, and the population reference intake (PRI) by the European Communities.

The *upper nutrient level (UNL)* is the highest level of intake that is likely to pose no risk of adverse health effects for almost all individuals in a specific life-stage and sex group. The USA/Canada developed a comparable term, which was called the upper tolerable nutrient intake level (UL).

## General framework

NIVs are used in many different ways to promote optimal nutrition and to prevent and treat under- and overnutrition. **Figure 1** illustrates the "pathway" of applications, showing how planning of nutritional policies, strategies, programs, regulatory frameworks, legislation, etc. should be based on the results of assessment and surveillance of nutritional status. Policies and planning, usually done by the government, lead to nutritional actions, interventions, or programs. The outcomes of these interventions should be evaluated and monitored on a regular basis to influence adjustments in planning if necessary. NIVs are used for all these steps in the assessment, planning, and evaluation process for both groups and individuals.

## Specific applications of NIVs

A brief overview of the many different applications [4] of NIVs affecting nearly all aspects of food and nutrition policy and practice is described below. Specific descriptions of how the NIVs are used to derive food labels, make decisions about food fortification, and develop food-based dietary guidelines are also provided.

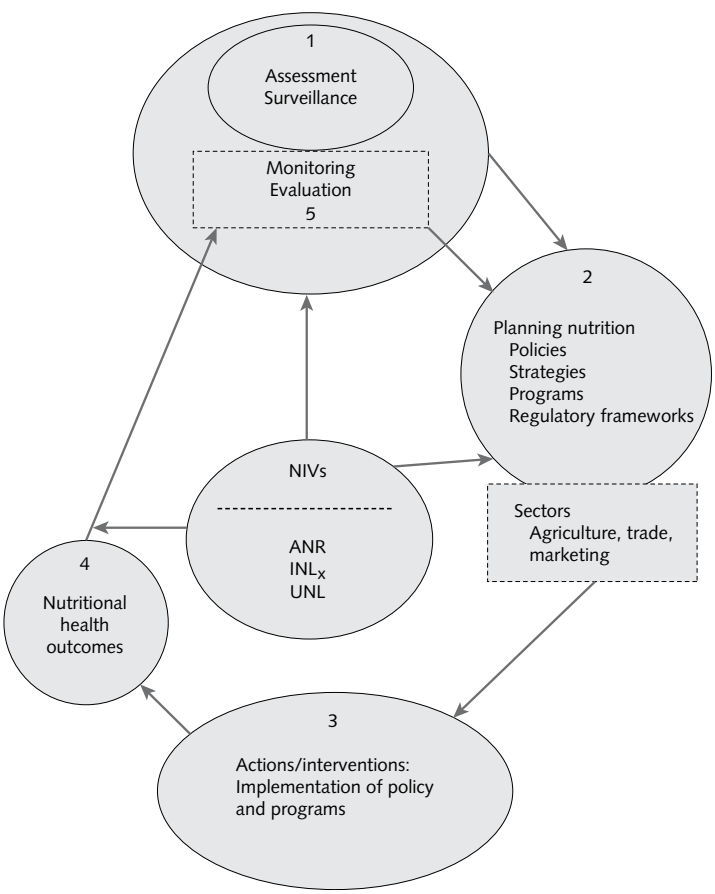


FIG. 1. A framework for a pathway of application of nutrient intake values (NIVs)

### Assessment, surveillance, or monitoring of nutritional situations

Nutritional monitoring programs generally involve an assessment of nutritional risks, identification of the gaps or excesses in nutrient intakes of individuals or groups, planning of appropriate interventions, and monitoring of the results. NIVs are used to identify nutrient inadequacies or excesses of various population groups or individuals; they can also be used to estimate the percentage of the population at risk for inadequate or excessive intakes and to determine the impact of nutrition programs on the prevalence of low or excessive intakes over time in the target populations and/or subgroups. For example, NIVs were used to evaluate the prevalence of low nutrient intakes among the participants in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) in the United States [5]. The NIVs can also be used to evaluate the adequacy of a country's or region's food supply to meet the nutritional needs of the population and to examine trends in nutrient consumption over time.

### Nutrition policy, regulatory frameworks, legislation, strategy, and program planning

Although food and nutrition policy often is a national responsibility, many countries depend on international organizations for guidance in formulating policies. These organizations include the World Bank and several United Nations organizations and agencies such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the United Nations International Children's Fund (UNICEF), the United Nations Educational, Scientific, and Cultural Organization (UNESCO), the United Nations University (UNU), the United Nations Development Program (UNDP), the United Nations World Food Programme (WFP), and others. All of these organizations use NIVs to plan, develop, and define policies that support food and nutrition security and safety at all levels, including individuals, households, communities, vulnerable groups within communities, and whole populations. For example, the program for feeding refugees developed by the FAO is based on NIVs.

### Food and nutrition interventions

NIVs are used to plan and design interventions such as food fortification and supplementation programs, school nutrition programs, changes in the food supply, and other interventions to improve the nutritional status of individuals and groups. NIVs are also frequently used to evaluate the eligibility for specific programs and to monitor the outcomes of interventions. Specific uses of NIVs include planning meals, food purchases, and budgeting decisions for intervention programs. NIVs can be used to determine if the goals of the interventions are reached by comparing postintervention intakes of individuals and groups with values collected at baseline; failure to reach goals provides a basis for modifying the intervention program. Use of the ANR for assessing the impact of a program in specific individuals will provide quantitative data on the number of individuals with improved nutrient intakes. Before having an ANR for estimating prevalence of adequacy, program planners could only make qualitative statements about the effectiveness of the intervention.

### Food and nutrition education

NIVs are used *per se* or translated into food-based dietary guidelines to educate individual consumers, health personnel, or groups of people on how to select optimal diets to meet nutritional requirements, how to interpret nutrition labels on food products, and how to evaluate nutrition advertising in the media. Thus, NIVs should provide the basis for all food and nutrition education programs in a country or region. For example, the first step in deriving any nutrition education program should be an assessment of nutrient shortfalls and excesses among the target population. Most countries and regions develop food-based dietary guidelines (pyramids, plates, etc.) to assist individuals in making good food choices. A detailed description of how to translate NIVs into food-based guidelines follows in the next section. The goal should be to provide guidance for how populations can meet the INLx without exceeding the UNL.

### Nutrition research

NIVs are used to design research studies for determining nutrient functions, the relationships between nutrient intakes and health or disease, nutrient–nutrient interactions, nutrient–gene interactions, and other nutrient issues. For example, NIVs are used to assess the association between the intake of nutrients and/or other dietary components and the risk of cardiovascular disease, cancer, diabetes, and other long-term disorders in clinical and epidemiologic studies. Data from large-scale population studies have been used to set standards of intake for the percentage of energy from carbohydrate, protein, and fat to reduce the risk of chronic disease.

### Product development

Using specific, quantitative information on the effect of specific nutrients or food components on health outcomes, NIVs may be used to design and develop new technologies and new food products with health

benefits. These foods may be called "functional foods" [6]. Studies of functional foods are an active area of research to identify the active component(s) in specific foods associated with health outcomes and the dose–response relationship with this component(s). An ANR and a UNL are needed for these components to derive a safe or acceptable range of intake and to evaluate the safety of the new product.

### Therapeutic (clinical) nutrition/dietetics

Although NIVs are derived for healthy people, these values are frequently used as the basis for planning therapeutic diets for patients suffering from various diseases, since no other standard is available. Adjustments in the values are made whenever possible, using information about the disease process and nutrient metabolism. For example, certain adjustments may be made in the diets of individuals suffering from infections in order to enhance their immune function [7]. Diet manuals of hospitals and professional medical organizations and societies often provide the criteria used to modify the intake of specific nutrients in menus for patients.

### Food procurement for institutions and groups

Institutions such as schools, hostels, nursing homes, the military, prison services, etc., use NIVs for planning menus and procuring foods. The $INL_x$ should be used as the basis for menu planning. However, since individuals and groups fed in institutions vary widely in age, lifestyle habits (e.g., smoking), physical activity, and nutritional and health status, the menu planners will need to select the standard most appropriate for the group as a whole. Ideally, nutrient intakes should be carefully monitored to ensure that practically all of the individuals have intakes above the EAR and very few exceed the UNL.

### Food import, export, and subsidies

Governments, agencies, and businesses (industry) use NIVs to motivate and formulate policies and actions regarding food import, export, and subsidies. The paper by Ramaswamy and Viswanathan [8] in this issue outlines the importance of using a set of standards with a common basis in international trade.

## Specific uses of NIVs for food labeling, food fortification, and derivation of food-based dietary guidelines

### Food labels

Dietary reference standards are used to label and market products by comparing the nutrient composition of the product (usually per 100 g or serving size) with recommended intakes.

In most instances the $INL_{98}$ (RDAs, RNIs, or PRIs) are used to compare the contribution of the particular product with a reference standard, often expressed as a percentage. For example, in the United States the reference standard for vitamin C is 60 mg/day, so a glass of orange juice that provides about 95 mg of vitamin C would be labeled as having 160% of this standard. However, a recent committee of the Institute of Medicine recommended that the EAR (ANR), rather than the RDA, be used as the nutrient standard, because this standard is the best estimate of a consumer's actual requirement [9]. Others have argued that the RDA should continue to be the basis of the standard for food and dietary supplement labels, because this higher standard should be adequate for almost all healthy individuals and is more consistent with the educational objectives of the food label [10].

Food labels also serve as a basis for nutrient content claims and in some products for health claims. For nutrient content claims on the food label, such as a claim that the food is a good source of a particular nutrient, many countries legislate the minimum percentage of the $INL_{98}$ (RDA or other standard) that a portion or serving usually consumed should provide. Likewise, for a health claim, for example one with regard to lowering neural tube defects, the food or product must be a good source of folate.

The food industry often uses food labels to promote the nutritional benefits of products. The challenge is to harmonize the specific nutrient reference standard to be used, the guidelines for classifying a product as a good source of the nutrient, and the circumstances that will allow a health claim. With increasing international trade, developing comparable practices in different countries in a way that will be understood by most consumers will become more urgent.

### Food fortification

Food fortification is a very cost-effective intervention to address micronutrient deficiencies. Types of fortification include *mass* (mandatory) fortification of staples, *market-driven* (voluntary), as in the addition of micronutrients to ready-to-eat cereals, or *targeted* to special groups in the population such as complementary foods for young children. The use of NIVs is necessary to assess the prevalence of inadequate intakes of specific nutrients and to calculate appropriate levels of fortification. Specifically, using food intake data from a relatively small number of individuals from each population group of concern, the prevalence of intakes below the ANR is calculated. Then the effect of adding different levels of fortificant nutrients to one or more staple foods can be simulated, with the opti-

mal level of fortification being that which minimizes the prevalence of intakes below the ANR and above the UNL. An example of this approach is provided by WHO/FAO in its Guidelines on Food Fortification with Micronutrients [11].

### Food-based dietary guidelines

Food-based dietary guidelines translate scientific information on nutrient requirements and dietary characteristics that promote good health into recommendations and advice for the general public. Thus, food-based guidelines are the backbone of nutrition education efforts throughout the country, and they also reflect the nutrition policy of a country.

The NIVs for a country that include the three components (ANR, $INL_x$, and UNL) can be used to develop food-based dietary guidelines and patterns to assist the general public in selecting a diet that meets their nutrient needs. Often food-based dietary guidelines emphasize the importance of consuming a variety of nutrient-dense foods and beverages within and among the basic food groups used in the country, i.e., fruits; vegetables; grains; meat, fish, poultry, beans, and nuts; and fats and oils. Specific food patterns quantify the amounts needed from each food group for individuals in various age and sex groups. A food pattern can be developed using the following five-step process. This process was developed by the US Department of Agriculture and used to develop the new My Pyramid, which shows Americans how to meet their RDAs [12].

1. *Establish nutrition goals for the food pattern.* The nutrient goals should be the $INL_x$ values for vitamins, minerals, electrolytes, and macronutrients, as developed by the country or region.
2. *Establish energy levels.* Modifications of the food pattern can be developed for energy levels that cover the needs of all members of the population above 2 years of age. For example, 12 different food patterns could be developed with energy values ranging from 1,000 to 3,200 kcal/day in 200-kcal increments.
3. *Assign nutrient goals to each specific energy level.* The nutritional values assigned to each energy level are the $INL_x$ values for age and sex groups that most closely match that specific energy level. For example, the 1,800 kcal/day level might be the highest $INL_x$ value recommended for women aged 31 to 50 years, men and women aged 9 to 13 years, and women aged 14 to 18 years.
4. *Assign a nutrient value for each food group and subgroup.* The nutrient values for food groups and subgroups used in My Pyramid were developed in the following way [12]. The nutrient values assigned to each food group (i.e., fruits, milk, meat and beans, whole grains, enriched grains, dark-green leafy vegetables, orange vegetables, legumes, starchy vegetables, and other vegetables) were the weighted *average* nutritional value of foods consumed by Americans within that group based on the results of the nationwide food-consumption surveys (the USDA Continuing Survey of Food Intakes by Individuals, 1994-96). For example, broccoli constitutes 53% of the dark-green leafy vegetables consumed, spinach 20%, and other vegetables the remaining 27%. Therefore, the nutritional value of dark-green vegetables was 0.53 for broccoli, 0.20 for spinach, and 0.27 for other foods. The form of milk and meat with the lowest fat content was used exclusively. Thus, fat-free milk was the single food item used for the dairy group.
5. *Determine the daily intake amounts for each food group or subgroup.* The amounts of each food group or subgroup were increased or decreased in an iterative manner until the pattern for each energy level meet its nutritional goal (i.e., $INL_x$) or came within a reasonable range.

### Use of NIVs in developing countries

NIVs may be particularly useful to policy makers in developing countries for reducing the prevalence of nutrient inadequacies and preventing excessive intakes of other food components. The nutrition transition currently occurring in many developing countries [13] provides evidence that both underconsumption and overconsumption are present within the same population. An advantage of the NIVs, described in the paper by King et al. [1] in this issue, is that individual countries can determine their own specific nutrient goals within the context of the entire harmonization process. For example, if animal source foods are scarce in a developing country, policy makers may decide to set the $INL_x$ for zinc and iron at 1 SD above the average nutrient level ($INL_{67}$) instead of 2 SD ($INL_{98}$).

The general applications of nutrient standards for populations living in developing countries (i.e., dietary assessments, developing targeted intervention programs such as food aid programs, and evaluating or monitoring these programs) parallel similar applications in developed countries, although the scale and scope of the programs may differ. Many uses of the NIVs in feeding and intervention programs require making adjustments in the measurements of nutrient intake to account for day-to-day variation. These adjustments are usually made by using 2 or more days of estimated intakes from the target population. Although diets in developing countries may appear to be monotonous, it cannot be assumed that it is unnecessary to make adjustments for day-to-day variation; the food supply is often less predictable than in wealthier populations. Thus, a similar adjustment protocol should be followed in all populations [4].

The high prevalence of micronutrient deficiencies or the widespread prevalence of disease, such as HIV/AIDS, will probably have an impact on the actual amounts of the NIVs established in some of the developing countries. For example, countries with a high prevalence of HIV/AIDS may want to set different standards for nutrients known to influence immune function. Also, typical foods consumed in these countries may be high in phytate, which reduces the bioavailability of minerals for absorption. Ideally, NIVs should be derived from studies of the nutrient requirements in representative individuals consuming typical diets from the population in each country. If experimental data on nutrient requirements are not available from the country's population, extrapolations can be made from published data of similar populations in other countries or regions.

Thus, the basic uses of NIVs are similar in developed and developing countries, but the specific application may require unique decisions about goals and policies as well as specific adjustments in the actual quantities of nutrients recommended based on the food supply and general health of the population.

### Potential users of NIVs

There is a wide variety of potential users of NIVs, including international organizations such as UN agencies; nongovernmental organizations (NGOs); governments (from local to district to national level); researchers from the disciplines of nutrition, foods, medicine, biochemistry, policy, etc.; health professionals, including doctors, dieticians, nutritionists, nurses, etc.; the food industry; institutions (hostels, homes, schools, the military, prisons, etc.); and caterers and restaurateurs, as well as the public (individual consumers and small community groups). The UN agencies and organizations, NGOs, and governments use the nutrient-based dietary standards to identify nutrient requirements of populations to formulate food and nutrition policy for food aid, supplements, rationing, fortification, education, legislation, export and import of foods, and subsidies for certain foods or for producers of foods [14]. The food industry uses these standards to develop new food products that will respond to consumers' needs for healthy choices and to market foods by using nutrient labeling. Health professionals and researchers may use the nutrient-based reference standards to assess the nutritional adequacy of diets, plan appropriate corrective interventions, and evaluate these interventions [14]. Institutions, caterers, and restaurateurs use the reference standards to assess food requirements, make procurement decisions, and plan menus. Consumers use the standards to interpret the nutrition information provided by the media and on food labels.

It is the responsibility of nutrition scientists involved in establishing NIVs to inform and educate the users about the appropriate interpretation of the NIVs and the appropriate use of the three different values (ANR, $INL_{x,}$ and UNL). It is also the responsibility of those doing nutrition interventions, such as food fortification, to use the NIVs because these interventions have a direct effect on public health.

## Conclusions

Expanding the NIVs to include an average nutrient requirement (ANR), an individual nutrient level ($INL_x$), and an upper nutrient level (UNL) expands the potential uses of these standards. The three components of a set of NIVs are derived from a distribution of requirements for a nutrient for a specific function. The standards can be used for a broad range of functions—from the assessment, surveillance, monitoring, and evaluation of nutritional situations, to informing the formulation of policy and strategies, to planning and designing a large variety of appropriate interventions, and to evaluating the outcomes of these interventions in individuals and groups. A number of potential users, ranging from international organizations, NGOs, governments, industry, health professionals, to institutions and individual consumers, have been identified. All of these users should be targeted in educational efforts to promote the correct use of the NIVs.

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# Trade, development, and regulatory issues in food

Sunder Ramaswamy and Brinda Viswanathan

## Abstract

*Trade in food and animal products has increased several-fold in the past decade, and simultaneously regulations governing the movement of such products across national boundaries have also increased. The present study reviews harmonization in food trade regulation by focusing on nutritional aspects to understand its role in enhancing world trade on the one hand and consumer interest and welfare on the other. Harmonization to a large extent brings in more regulation from the developed world acting through their governments, consumer organizations, and multinational companies; it does not seem to address, in general, the concerns of the large segments of the poor population for whom agriculture and food trade are the main sources of livelihood. There is a lack of quantifiable estimates of the loss in well-being of the disadvantaged. However, there is substantial research focused on the potential harm to developed nations as a result of nonadherence to the rules. Clearly, lack of adequate infrastructure, resource constraints, and weak institutions not only result in poor food safety regulation within developing countries but also remain barriers to realizing the greater potential benefits from increased trade. Harmonization of standards would have some losers and some winners, but to make it more inclusive, scientific knowledge alone may not be adequate; social and cultural aspects also need to be considered, since food systems differ among regions, with varying preferences, local resource availability, and levels of economic development. Improvement in governance in many countries not only would ensure better participation in international rule-making and the negotiation process for fairer trade but also would result in effective domestic legislation to ensure safer health for citizens, resulting in higher overall well-being.*

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**Key words:** Food safety, SPS and TBT agreements, trade in food

## Introduction

At the dawn of this century, the value of international trade in food exceeded US$400 billion per annum (with the estimated total value of international trade being US$6.5 trillion) and accounted for on the order of 500 million tons of food products, and on average the trade has continued to grow significantly since then [1]. The dominant share belongs to the developed countries in terms of both exports and imports. The trade share of developing countries has increased over the years, but developing countries remain mainly exporters of primary products and importers of processed food. The present trade scenario is complicated by several factors: the presence of World Trade Organization (WTO) rules since its inception in 1995 on the one hand, and innovations such as genetically modified foods and food fortification, with lowered immunity of the populations in developed countries on the other. The complication in the trading rules arises from the varying standards set by importing nations. Most often the differences between trade and regulatory standards arise from the lack of concrete scientific evidence on the potential health impacts, and therefore the justification of such standards is questioned. Thus, smooth facilitation of trade calls for a harmonization of food safety standards that are transparent and easy to comply with.

International food safety standards have prevailed for a very long time and so has trade in food; but why the need for harmonization now? The world population will reach 8 billion by 2020, with about 96% of growth concentrated in the developing world. Overall increases in population and, in particular, increases in urban population pose great challenges to the food systems of the world, from production to distribution to consumption. With greater emphasis on trade as an engine of growth and poverty reduction, developing countries around the globe are choosing (or in some cases, are forced) to liberalize their trade regimes. Trade in food

can also have a positive impact on nutritional options in both developed and developing countries, since it tends to lower the price of food in general and often makes a greater variety of foods available throughout the year. Scientific developments have also allowed for a better understanding of the nutritional qualities of diets and their health implications. This has led consumers to become more discriminating in food matters and to demand protection from food of inferior quality and unsafe food. Consumers, at least in the wealthier segments of society, routinely expect that food, regardless of whether it is domestic or imported, will meet certain quality, nutritional, and safety requirements.

Due to this higher awareness and concern to protect human, animal, and plant health among the developed countries, the safety standards set by them quite often overrule the existing international standards. Thus, quite often lowered tariff barriers seem to be replaced by non-tariff barriers based on protective safety considerations, some of which could actually be protectionist measures to shield domestic producers from imports.* Since trade directly impacts the economic situation of both the exporting and the importing countries, these standards set by the trading partner impact the financial status of individual nations, particularly the developing nations. Higher exports bring in foreign currency for the developing nations, generating more resources, whereas imports bring cheaper products to consumers but may also affect domestic producers in both developing and developed nations.

Given this scenario, questions naturally arise about the justification for these rules, which quite often are reflected in the trade disputes registered under the WTO framework between developed and developed, developed and developing, and developing and developing trading partners. Mutual settlement of differences and recognition of two different standards (set to have the same outcome) as equivalent were the predominant ways in which cooperation in trade has been taking place. But increasingly these are being replaced by standards that are integrated or harmonized across all nations of the world. Not only do the standards that are set for food safety have to be adequately backed by scientific evidence, but implementation of those standards may have to be cost effective. The present study is a review of the economic issues concerning the process of harmonization in food safety standards across the world, focusing more on nutritional aspects under a more liberalized trade regime. Governments, aid agencies, and companies routinely use nutrient-based dietary standards to formulate and monitor policies pertaining to exports and imports of food and food products. In this regard, a more harmonized approach to nutrient intake values (NIVs) and other reference standards should benefit trade and development.

This article first discusses the need for food safety regulation from an economic perspective and presents a review of food safety regulation before and after the forming of the WTO. It then looks at the role played by the Codex Alimentarius Commission in the harmonization process. The next sections discuss issues arising from harmonization with regard to food fortification, genetically modified foods, nutrition labeling, and hazard analysis and critical control point (HACCP). A further section looks at the pros and cons of the process of harmonization by looking at the efficiency and equity aspects of this process from both a developed and a developing country perspective. A final section presents the conclusions of the review.

## Economic rationale for food safety regulation

Having crossed over the threshold of subsistence consumption, a large number of people in several developing nations are demanding more variety and better quality of food products. This has increased the flow of trade in food products across countries over the past decade. However, in this scenario, as compared with the developing nations, the developed nations have put into place many regulations—arising mainly from consumers' concerns for safer public health—to ensure safe food standards, particularly in the case of imports. Although there may be a similar concern (or desire) among the developing nations in this regard, constraints on resources often result in their not being able to assess as well as implement these standards. A complex system of market, legal, fiscal, and regulatory measures thus governs the incentives of firms to supply food products that meet, if not exceed, the safety and quality attributes sought after by consumers.

Unlike quality, which is often determined by taste, nutrition, appearance, and organoleptic characteristics, information on food safety is often imperfect, so that safety characteristics may not have a favorable impact on marketing [3, 4]. The food safety issue typically has three elements: risk communication, which involves consumers and their perceptions of risks; risk management, which involves products, firms, and governments**; and risk assessments, which involve science [5]. As Antle [3] indicates, even though the producer may have information on the type of pesticide used in

* Food originating from countries (developing and newly industrialized) in Asia did face significant regulatory barriers in the mid-1990s, making it difficult for them to get easy access to US markets [2].

** The amount of information that consumers have about the safety of an imported food item depends upon risk communication; the provision of that safety by exporting firms depends upon risk management in exporting countries; and the regulatory import barriers imposed by importing country governments depend upon scientific risk assessments either undertaken by them or based on internationally acceptable standards.

producing a crop, the residue in the final output may be unknown to the producer and the consumer may be unaware of the harm that this may cause. Similarly, a meat producer may have less control over possible contaminants during the processing stage and hence their public health implications.

Even though there is an increasingly high emphasis on food hygiene in both developed and developing countries, food safety standards do not automatically become internalized into the production process due to lack of information on where the problem is and how to tackle it. Therefore, interventions by regulators in terms of product standards and risk assessment methods throughout the chain of production have emerged in recent times.* Often there is a potential for differences to exist between trade and regulatory standards by virtue of an externality caused by trade partners. Hypothetically, if the United Kingdom exports beef to the United States, the United States may have fears about the safety of the British beef and may ask for higher standards of safety, including destruction of beef that does not meet such standards. This then may result in the British beef industry destroying its animal stock for the public health of US consumers, thus raising the price of beef in the United Kingdom. Such a separation of costs from the beneficiaries creates divergent incentives to comply or to harmonize in the first place.**

Before the multilateral trading system as envisaged by the WTO came into practice, the various regulations across countries were sorted out by either coordination or mutual recognition. The increasing impact of the WTO in all aspects of trade is paving the way for more stringent forms of regulation to be implemented by all countries exporting food commodities, either processed or raw. Consumers in importing countries felt that since food safety standards are not the same across various countries, and they have to deal with many countries in a multilateral trading regime, a harmonized framework of regulations alone would ensure uniform safety standards. Further, producers from developed countries felt that having different standards for products increased their cost of production and that hence a single unified regulation would be better. To tackle such impediments to trade, international uniform or similar standards across regions have emerged over the years. However, there are different ways in which the regulatory rapprochement facilitates cooperation in this regulated regime [8]:

- Coordination: minimize differences by making use of voluntary international codes of practice;
- Equivalence or mutual recognition: accepting different forms of achieving food safety as it prevails in different countries;
- Harmonization: standardization of food safety regulations through international standards.

The first and second options seem to have been the dominant mechanism for bilateral, regional, or preferential trade. Conceptually, food safety standards are difficult to internalize both because of asymmetric information problems and because of coordination failures. The lack of information is clear, but there is also a lack of private incentive to gather or analyze information, because this imposes costs on industry and because it is costly to adopt controls. The separation of costs from the beneficiaries—a point made earlier—creates divergent incentives.*** The present study looks into some of the recent issues dealing with the last of these rapprochements to facilitate trade with the least trade distortions.****

## Trade and food safety regulations: From the past to the present

The majority of food regulations are social welfare–enhancing measures that may place legitimate restrictions on trade. Nevertheless, food regulations are routinely appearing at the center of trade disputes. One difficulty is that the incidence of risks or available market information varies across countries. Thus the benefits of a regulation may exceed its costs in one country but not elsewhere. Another difficulty is that regulations may rest on comparisons of non-market benefits with market costs and thus are more susceptible to challenge by trading partners. But above all, the most significant problem for the global food system is that the stringency of regulations and the provision of farm support and protection policies tend to increase among the high-income countries, complicating the political economy of how food trade ought to be regulated [9].

A Food Control Act at the national level to a large extent can take care of the problem of regulations and safety standards in food export and import. However, there are those who find it difficult to implement such

* To take care of the market failure, statutory regulation of either process or performance standards alone need not be the only option, since markets do have the capacity to signal quality through indirect policy mechanisms such as liability laws and building of (private) quality reputation by advertising or certification [6, 7].

** The authors wish to thank an anonymous reviewer for this observation.

*** The question then becomes whether an industry will adopt costly controls to make food safer for a foreign population or even the local population if it only raises its prices as compared with rival noncompliers.

**** The impact of a food regulation on trade thus stems from the direct cost of compliance incurred by domestic suppliers, the indirect impacts of the regulation in question on domestic supply and demand, and its impacts on related foreign excess supplies. The incidence of this cost will ultimately depend on market structure, the combination of elasticities of supply and demand, and the scope of the regulation.

acts, due either to a lack of expertise or to the presence of rent-seeking behavior that seems to bypass rules and regulations. More importantly, exporting countries sometimes have to deal with losses or wastage, since the goods may have to be destroyed when the standards are not met. Consequently, for the purpose of importing, countries set up independent regulatory measures with differing requirements, leading to a myriad of standards.

Thus, to put in place a set of common food standards that would ensure that healthier products were traded around the globe, the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1963 established the Codex Alimentarius Commission.* The Codex Alimentarius prescribes food standards, codes of practice, and recommendations that national governments are expected to take into account when formulating their food laws and practices. This would ensure safe food not only for export but also for domestic consumers. However, the Codex Alimentarius Commission has no legal authority to impose its codes on any country. The Codex standards deal not only with processed food but also with raw food (primary agricultural products) in prescribing standards for storing and packaging as well as pesticide residues in food.

The General Agreement on Tariffs and Trade (GATT), introduced in 1947, on the one hand had provisions for individual countries to have their own laws to protect animal, plant, and human health, while on the other hand it also made it clear that countries should not restrict trade or discriminate against countries that had similar standards under the guise of protecting consumers [10]. An increasing number of instruments are available to governments for implementing food regulations: quantitative restrictions (such as temporal restrictions including quarantine periods and export prohibitions), technical specifications (product and process standards), and informational requirements (mandatory disclosure and controls on voluntary claims). However, by the time the Uruguay Round of agreements began in 1986 (the Punta del Este agreements), many of the country-level food safety measures seemed to have turned into trade barriers, some intentional and others unintentional. Thus, toward the conclusion of this round in 1994 (the Marrakesh agreement), the earlier GATT agreement was modified to make it more effective. Countries were still allowed to set their own standards while keeping in mind the Codex Alimentarius Commission standards, and they had to justify them with scientific backing in case they were perceived to be too restrictive or discriminatory vis-à-vis trade.**

## WTO and food safety

Since the setting up of the WTO in 1995, three agreements have been made concerning trade in food. The first is the Agreement on Agriculture, whose aim was to increase free trade by improving market access and export competition in agricultural commodities. This was to be achieved through tariff reduction for imported goods and reduction in domestic support and export subsidies. These economic instruments, however, did not seem to facilitate smooth trade, mainly due to a lack of complete information on the quality of products. Other issues emerged in terms of countries setting their own safety and quality norms. Therefore, two other non-tariff agreements emerged: the Sanitary (human and animal health protection) and Phytosanitary (plant health protection) agreement (SPS) and the Technical Barriers to Trade (TBT) agreement (non-health concerns addressing food quality, nutrition etc.).

The SPS agreement was negotiated on the premises that domestic sanitary and phytosanitary standards based on international norms could reduce trade conflicts and lower transaction costs and that requiring scientific justification for norms would make it more difficult for countries to shelter domestic industries behind restrictive health and safety regulations. The SPS measures deal with the risks caused by toxins, additives, disease-causing organisms and contaminants in food products, and so forth to human, animal, and plant life. Some of the measures include inspection of food products for microbial contaminants, setting limits on pesticide residues in agricultural products, mandating fumigation, and declaring areas free from pests or disease. The spirit of the WTO is echoed well in the SPS agreement, according to which countries are expected to follow international standards to the extent possible but are also allowed to set their own regulations, provided they are necessary for the protection of health and are backed by scientific evidence and are nondiscriminatory.

The TBT measures cover not only food items but also a wide range of commodities and include "technical regulations, standards and conformity assessment procedures as applicable to process, product or production methods." Most measures related to human disease control are under the TBT Agreement, as are food labeling requirements dealing with nutrition claims,

* The purpose of the Codex Alimentarius ("food law" or "food code" in Latin) was to ensure that traded food commodities were not harmful to the consumer [10]. The application of risk analysis consisting of risk assessment, risk management, and risk communication to food safety has been the subject of consultations carried out by FAO and WHO.

** The GATT also had another provision to minimize restrictive trade practices through the agreement on technical barriers to trade (TBT) established in Tokyo in 1970. The negotiation was mainly to ensure that countries did not use technical regulations citing national security or fraudulent practices to restrict trade and covered a wide range of food and nonfood products.

quality, and packaging regulations.

The three major differences between the SPS and TBT measures are that the former concerns only health protection while the latter deals with aspects such as improving product standards for quality, providing more information to the consumer to avoid deception about product content, and national security as well as health concerns; the SPS is bound by scientific standards when a measure is being adopted, whereas it may not be possible for all TBT measures to have a technical or scientific justification; and the SPS relates to food safety but the TBT is applied to a wide range of nonfood products as well. Although there are overlapping aspects, the distinctions are often quite clear. For instance, food labeling dealing with health warnings, use, and dosage are SPS measures, whereas the label's position, lettering, composition, nutrient content, and quality are TBT measures [11]. Consequently, settlement of a dispute if one should arise would depend on under which measure it was registered. Interestingly, though, whereas TBT measures give more scope for discriminatory trade practices, given that in many instances scientific and technical regulations are not very binding (so that proving a case against them is difficult), there have been no disputes against a TBT measure for food safety as yet, whereas a few have been registered, and some resolved, against SPS. Unlike the Codex Alimentarius, the TBT and SPS agreements are legally binding on the country, but only for the internationally traded segment of the commodity, such as fresh fruits and vegetables, and need not ensure that the same standard be followed at the national level for domestic consumers [12]. Therefore one may observe that in a developing country the products may be sold in the domestic market, with export quality products, which are mainly accessible to the richer sections of the population, having higher standards, whereas a lower standard would be applicable for the larger domestic market. However, in some Latin American countries, the lack of public safety standards or poor implementation has resulted in private safety standards "seeping" into the supermarkets. This issue will be discussed in a later section.

At the time of its writing (March 2006), the Doha round of the WTO has been hailed as the "development" round for its marked emphasis on economic development issues. Given the Agreement on Agriculture and the importance of agriculture to developing countries in general, the issue of food safety poses some vexing concerns. Developing countries signal frustration with the increasingly stringent standards faced by their exports, the new obligations to justify their regulatory mechanisms, or both. On the other hand, many developed countries question the adequacy of the WTO rules in light of new disease outbreaks, new production technologies, and new demands from concerned consumers about food safety [9].

## Harmonization and the Codex Alimentarius Commission

The SPS agreement recognizes in particular three international standard-setting bodies: the Codex Alimentarius Commission, which establishes food safety measures, standards, guidelines, and recommendations; the Office Internationale des Epizooties, which addresses animal health measures; and the Secretariat of the International Plant Protection Convention, which sets norms for plant health measures. Since the focus of this review is mainly on human protection concerning nutrition, most of the subsequent discussion will be based on the Codex standards and their linkage to the SPS/TBT agreements. The SPS agreement in particular sets out the following principles to guide trade in food: harmonization (Article 3)—member countries are urged to adopt international standards, and a country that adopts the standards of the Codex is in compliance with WTO standards; science-based risk management (Articles 2 and 5)—in addition to SPS measures deriving sanction from scientific principles, measures ought to be chosen so as to minimize distortions to trade and to be no more restrictive than necessary to achieve a country's appropriate level of protection; equivalence (Article 4)—a WTO member has to accept the SPS measures of another country as equivalent to its own if it is objectively demonstrated that there is equivalence in levels of protection; and regionalization (Article 6)—a country is required to allow imports from subnational regions from abroad that are free of pests and disease. Although the member countries of the WTO (through the SPS and TBT agreements) in principle agree to the Codex standards and guidelines, there is no legal binding to implement them domestically. However, two situations may arise:

» If a particular domestic measure falls below the Codex standards and the country is exporting at the lower food safety or quality level, then the agreement becomes binding on the exporting country to implement it. This consequently has a bearing on the domestic food law as well. Many studies suggest that this last aspect is particularly useful for developing nations not only to increase their trade but also to improve standards within the country [13]. There may of course be resource constraints on implementing these and the country may need to seek help.
» If the domestic measure is more trade restrictive on account of more stringent rules than those prescribed by the Codex Alimentarius Commission, it must be justified by proper scientific assessments and risk analysis.

Under these two situations, there is now a challenge for the Codex Alimentarius Commission. In the case of countries (usually developing countries) with food safety standards lower than Codex standards, the challenge is to raise resources to improve upon the existing

situation. This would not only enable the developing nations to participate regularly in the Codex meetings to discuss their problems and give suggestions, but also help them improve their risk assessment and scientific capacities. In situations where the standards are higher than those prescribed by the Codex, the commission has to play an active role in helping the resolution of disputes either through bilateral discussions or through the WTO dispute settlement mechanism. Apart from these two issues, the increased pace of globalization would also bring in various problems related to property rights and spreading the knowledge about healthy practices and adverse impacts as soon as they are brought to light by scientific experts. For instance, an issue recently taken up by the Codex Alimentarius Commission was to set upper limits for proteins and vitamins beyond which they become toxic for human consumption; this problem arises from the increased number of products introduced into the market that are fortified [14].

### Current issues

There are a number of issues of interest when it comes to the future of trade in food products, which can be grouped as follows [9]: process attributes—genetically modified food, food fortification, and organic foods; product attributes—nutritional labeling for example—and process controls—Hazard Analysis and Critical Control Point (HACCP). Before we turn our attention to a discussion of these pressing issues, it is important to remind ourselves that since the times of Adam Smith and David Ricardo, international trade has been largely assumed to be a global exchange of goods with inherent characteristics that can be revealed in final form regardless of whether an inspection is done preborder or postborder. Now with regard to some of these critical issues facing us, when the process *itself* defines the product (as might be the case with genetically modified foods or fortification of food), countries may have to rethink acceptable conventions of trade policy.

## Process attributes: Harmonization in trade

### Food fortification

Food fortification is the addition of one or more essential nutrients to a food for the purpose of preventing or correcting a deficiency of those nutrients in target populations. It is one approach to achieving the objective of eliminating or substantially reducing important micronutrient deficiencies. A fortification regime should be designed so that the prevalence of intakes of a targeted nutrient below an average nutrient requirement (ANR) or above the upper nutrient level (UNL) is low. Such a design ensures that very few individuals within the population would have either inadequate or excessive intake of a target nutrient. Examples include the addition of iodine to food-grade salt, calcium to fruit juices (to provide this micronutrient to people who do not consume diary products), and iron to breakfast cereals. Fortification is also done to restore minerals lost during packaging or storing (e.g., addition of B vitamins to wheat flour after processing) and to provide for substitute foods to have the same nutrition level as the ordinary food (e.g., addition of vitamins A and D to margarine to produce the same level of the vitamins as in butter).

Availability of alternative dietary or supplementation sources and concern about upper limits being violated resulting in toxicity are reasons put forth to prohibit fortification in the food safety laws of many countries. In such cases, increased access to and availability of the dietary supplement and awareness programs could tend to eliminate the deficiency. However, the fact that this has not happened in a sustained and cost-effective manner makes fortification an important mechanism to address micronutrient deficiencies in particular. Given the impact of fortification on public health, countries sometimes prescribe mandatory fortification. Countries then resort to a ban on the import of non-fortified commodities or products, which may be considered as a non-tariff trade barrier under the WTO regime. In such instances the concerned state is expected to notify the WTO so that settlement of a dispute if one arises can be taken up more effectively. A dispute may arise if the importing country has mandatory fortification laws or the non-fortified imports are cheaper and get market access in the liberalized trade regime; or if the exporting country has mandatory fortification regulation while the importing country may already have reached the upper limits due to its consumption pattern. Alternatively, countries insist on a harmonized mandatory fortification if the problem persists in many parts of the world.

Many of the concerns are usually raised by developed country consumers, whereas concerns in developing countries, though similar, involve certain other issues as well. The two different sets of issues in harmonization are discussed below.

#### *Fortification and developed countries*

The standards (and hence harmonization) of food fortification have to deal with the following issues, which have arisen mainly from developed country experiences:

- The upper and lower levels of vitamins and minerals that can be added before excess amounts lead to toxicity;
- The nature of fortification: i.e., do fortificants have to be mainly vitamins and minerals, and if so what are the permissible fortificants?
- The list of food items that can be fortified: fortifica-

tion of "unhealthy" foods (alcohol or foods high in salt, sugar, or fat) may cause other health problems.

In late 2003, the European Commission proposed common regulations for fortification among European Union countries, which were finally passed in the European Parliament in mid-2005 [15]. The important points to note are a positive list of vitamins and minerals that may be added to food; a recommendation to define daily intakes of specific substances, leading to fortified foods being labeled as such; a ban on fortification of beverages containing more than 1.2% alcohol; and a list of forbidden substances. However, separate European Union legislation has also been formalized for specialized categories such as foods for infants and young children, foods to promote weight loss, and food supplements, such as vitamin pills. This recent setting of standards by the European Union for the addition of vitamins and minerals has caused many other nations within and outside Europe to realign their domestic food fortification standards to facilitate trade.

Two-tailed risk in nutrient consumption is perhaps the most contentious issue for fortification. It is well known that calorie intake within a certain range is healthy and necessary; intakes below a certain level cause chronic energy deficiency, and intakes above a certain value lead to overweight and obesity. Similarly, scientists have recorded that low and high doses of vitamins and minerals affect health status, causing various kinds of impairments and in some cases death. Hence safe limits are prescribed for most vitamins and minerals by most countries across the developed world, with guidelines from WHO as well as their own country-specific research.

For instance, Norway and Iceland had a regulation that no fortification should be allowed for baby foods, whereas several other European Union countries allowed small amounts of vitamin A and D. This non harmonization within the European Union prior to 2002 forced the manufacturers to make two separate products, increasing the cost of production. However, successive negotiations and pressure from the European Union led to the acceptance of the European Union norm by these two countries, although it was shown that these supplements in large amounts could be poisonous.

In 2004 Denmark banned the entry of 18 cereals and cereal bars manufactured by Kellogg because the company wished to add iron, calcium, vitamin $B_6$, and folic acid in amounts higher than permissible safety levels. Although the same products were being consumed by other nations in the European Union, Denmark, which has no recommended daily allowances for vitamins, has longstanding policies that reflect the cultural belief that addition of too many vitamins and supplements can do more harm than good. In 2001, the European Union ordered Denmark to accept enriched food products, and since then Denmark has been allowing enriched foods but with levels lower than those proposed by Kellogg. Consequently, around the same time Denmark allowed fortified drinks, although fortificants were not needed by the population, the products were deemed safe by Denmark's food administration. Kellogg had also applied for permission to enrich the relevant products with other nutrients that would not carry any health risk, and these have been approved by the Danish Veterinary and Food Administration.

According to the Danish Veterinary and Food Administration, this was not a discriminatory policy, since its decision to ban certain Kellogg products was based on a scientific risk assessment produced by the Danish Institute for Food and Veterinary Research. The Danish maximum levels are based on the upper safe limits for the intake of vitamins and minerals determined by the European Commission's Scientific Committee on Food. If the maximum levels are exceeded, the total intake from enriched food, nonenriched food, and dietary supplements can reach a level that exceeds the upper safe limits, and may consequently represent a potential health risk. The Danish Institute for Food and Veterinary Research bases its calculations on large-scale surveys of the diet of Danes and their intake of dietary supplements. These surveys show that around half of the adult population and 70% of children aged 4 to 10 years regularly consume dietary supplements, typically a multivitamin mineral tablet. For a multinational corporation like Kellogg, this could well be a small percentage of their business and might not be a matter of immediate concern, but there may be a concern about the possibility of this ban spreading to other nations.

These two instances of harmonization (in Norway) and nonharmonization (in Denmark) have not been raised within the WTO for discussion, perhaps because they were considered intra-European Union matters, but they do raise the following welfare- and trade-related issues:

- If the dietary habits of the population tend toward higher doses of calories or vitamins and minerals, this needs to be addressed through a national educational campaign. In particular, when the impact of the excessive usage is undiscovered as yet, a similar educational campaign on the possibilities of danger could be initiated.
- The European Union (EU) in 2005 passed a resolution on harmonized rules for the addition of vitamins and minerals in food across the EU, but interestingly enough, Denmark continued to have its own fortification policy while Norway and Iceland were pressured to follow the rule.
- In a globalized world, if the banned product was being manufactured in a developing country site, this might well hamper the local employment due to loss in production.
- There is a trade-off between an individual country's

choice to take precautions against overdoses of nutrients by not permitting excessive fortification and this being considered as a non-tariff barrier in the absence of scientific evidence on the health impact.

» Are firms using fortification as a marketing strategy when there could be cheaper and easier ways of enhancing micronutrient consumption, particularly among developing countries?

» How do private firms raise or bring to WTO notice such nontariff barriers as the dispute settling mechanism set up at the country level?*

There has been a four- or fivefold growth in the processed food industry in the past decade in both developed and developing countries, with the businesses usually privately owned and multinational in character. Consequently, a fortification regulation would affect the business of an individual (multinational corporation) firm for which fortification is the main marketing strategy to increase or sustain consumption.

#### *Mandatory fortification and trade*

Some countries introduce mandatory fortification if there are public health concerns, and in that case nonfortified products would not be traded in the country. As members of the WTO, the countries are obligated to notify the WTO about mandatory fortification in case they are in violation of the existing WTO rules.

Even in developed countries, iodine deficiency and folic acid deficiency are noted among a large segment of the population, and despite awareness campaigns to the target groups there have not been significant changes in the consumption patterns of the people. One issue that emerges out of this is mandatory fortification with folic acid and iodine and its impact on trade.

Mandatory folic acid or folate fortification is a recent food policy issue in many developed countries such as Australia, New Zealand, Canada, the United States, and the United Kingdom, mainly to address the problem of neural tube defects. Mandatory folic acid fortification programs in the United States and Canada aim to lower the rate of neural tube defects by providing additional synthetic folic acid. The two countries introduced this policy at the same time and set a similar deadline of mid-1998 mainly to ensure freer movement of these products across the borders. The impact of this was that though the program had significant impact on folate stores and resulted in a 50% reduction in the risk of open neural tube defects, there was some concern regarding vitamin $B_{12}$ deficiency masking, since folic acid consumption increased among younger and older women during this period [16]. Thus, there is a suggestion to include this vitamin in the fortified program to reduce harm among the elderly women.

A study in Germany found a 50% to 70% increase in folate consumption due to increased intake of folic acid-fortified food between 1990 and 2001 [17]. The study concluded that since the intakes were higher among younger children than older children and adolescents, mandatory folic acid fortification (of basic foods like flour) may not be necessary, given this trend in food-consumption habits. The study found about 1% of the sample exceeding the upper levels, although no cautionary note has been sounded about the increased availability of folic acid-fortified food from cheaper imports from other countries that have mandatory fortification. Similarly, another study in the United States indirectly assessed the impact of universal folic acid enrichment of cereal-grain foods introduced by the US Food and Drug Administration in 1998 [18]. The study indicated that the increase in median intake was twice that predicted and hence that further monitoring and possible reconsideration of folic acid fortification was warranted. In comparison with this, a more recent study based on 10-year data (1988–98) from 10 European countries (in some countries only select regions were surveyed) suggests that since the issuance of the folic acid recommendations in 1992 there has not been a significant decline in neural tube defects, and hence that there is a need for "a reasonable strategy to quickly integrate food fortification with fuller implementation of recommendations on supplements" [19].

This contradicting evidence about the need for mandatory fortification is clearly based on behavioral patterns after science has indicated a way out of micronutrient deficiency in the form of fortification. The implementation of fortification would largely depend on consumer preferences and risk perception and assessment, all of which may be evaluated using a scientific approach but are not based only on science.

An adverse impact on nutritional intakes leading to a poor nutritional profile in the developed countries is blamed on the popularity of convenience foods and the decrease in time available to shop and prepare food with varied nutrition. Although the issues are similar among the richer segments of the population in urban areas of developing countries, micronutrient deficiency is still a cause of high morbidity and mortality among women and children in these countries. Countries such as India, China, and Brazil are faced with the double burden of infectious and chronic diseases, thus nutritional challenges in a liberalized trade regime are even more pronounced. The next section looks at the challenges faced by developing countries in linking fortification and the WTO reforms.

#### *Fortification and developing countries*

The challenges of fortification take on an entirely different character in a developing country. First, there are

* The private firm can raise the issue through their home country provided there are such laws within the country, and hence this issue is also an important aspect of harmonization.

multiple micronutrient deficiencies, and absorption or supplementation of one deficiency would require sufficient complementary or basic macronutrients such as fats or carbohydrates, without which it may not be very effective. At the same time, multiple fortification interactions between certain minerals and vitamins may sometimes enhance and sometimes decrease the absorption of the required amounts of the micronutrients by the body. This poses a problem when it comes to implementation of schemes.

Second, with the emphasis on micronutrient deficiency on the rise, many smaller countries with insufficient production and processing infrastructure may have to depend on imports of fortified food. The cost of such products may be higher, making then unaffordable to a section of the vulnerable population. However, it is important to note recent initiatives, such as the WHO's new Global Alliance for Improved Nutrition (GAIN) that brings the benefits of foods fortified with vitamins and minerals for the poor in developing countries to end micronutrient deficiency and help save millions of lives.

Third, gradations in micronutrient deficiencies among sections of the population are observed, and hence different levels of dosages may be required to improve undernourishment. Consequently, the health impact will be more effective if the fortified products are targeted to the population suffering from deficiencies rather than to the entire population, since universal fortification might lead to toxicity among those who have access to a more balanced diet.

Iodized salt is an example of a food that undergoes mandatory fortification in many countries to prevent iodine-deficiency disorders.* No other dietary source of iodine is available; supplementation may be an alternative but it has not been found to be cost effective and accessible to a large population in a sustained manner. More importantly, international standards on food fortification are available only for food-grade salt, and as a result various fortification standards are observed for different food items across the world. Thus, there is a need for harmonized standards to facilitate least restrictive trade practices and safeguard health concerns.

What if a country has a mandatory fortification law for salt but due to the WTO restrictions may be forced to import cheaper non-iodized salt? Or what if countries do not produce salt locally and hence have to depend on imports? In such cases, if the import of non-iodized salt is prohibited, then the country should be in a position to defend itself if its policy is misconstrued as a non-tariff barrier under the WTO regime. Many of the countries in transition in Europe seem to be facing such a dilemma; Georgia recently banned imports of non-fortified salt [20]. Although the countries that produce salt locally have the option of iodizing and then distributing it, they may also face the situation of lower prices for non-iodized salt from domestic and/or imported sources. For instance, India had a ban on the sale of non-iodized salt that was removed in 2002 under pressure by the salt manufacturers' association (as some media reports indicated), since packaging costs were higher for iodized salt, thereby reducing the profit margin. It was then found that within a year of removal of the ban, cases of iodine-deficiency disorders increased substantially, and the ban was reimposed in 2004 [21].

Because the effects of deficient intakes of folic acid have been known only recently, even in developed countries, assessment of the magnitude of neural tube defects does not seem to have been carried out. Hence, fortification issues do not seem to have been considered at present, although the extent of the problem could be more severe due to higher fertility rates in these countries. How this will affect the harmonization process is still unclear, although the issues may remain broadly the same, as in the case of iodine.

Therefore, in comparison with developed countries, not only are public health problems more widespread and severe in developing countries, but lack of awareness, institutions, and adequate resources to ensure that products with safe standards are available only to the consumers are equally challenging issues to deal with. The process of harmonization in trade standards with increased globalization will have impacts on these aspects as well and hence have far-reaching impacts on efficiency as well as equity.

The TBT is more applicable to food fortification issues in trade than the SPS. As discussed above, there are possibilities that international trade regulations may affect country-level nutrition legislation, but to date no instance of a dispute has been registered under the TBT. The reason may be that the importing nation has a clear public health reason to restrict trade to protect health, so certain trade policies may not be perceived as protectionist. Perhaps consumption of such fortified products is still from domestic production or countries are still giving more importance to public health issues, with limited research on the upper safe limits.

### Genetically modified organisms, trade, and harmonization

There are three interrelated concerns when it comes to the issue of genetically modified food: approval of varieties for use by domestic producers, approval of varieties for sale on the domestic market, and labeling and traceability of genetically modified food and products made from genetically modified ingredients [9]. Lower

* The United States had such a policy in the 1950s and successfully eliminated iodine-deficiency disorders from the population.

than adequate intake of micronutrients causes various types of health problems, including high mortality, and is a major concern among the developing regions where it is referred to as the problem of "silent hunger" or "hidden hunger." In many developing countries, processed foods are not consumed in large amounts, and hence biofortification either through breeding or transgenic processes is also being considered as an option.*

Biofortification is the process of breeding food crops that are rich in bioavailable micronutrients. These crops fortify themselves: they load high levels of minerals and vitamins in their seeds and roots, which are then harvested and eaten [22]. This form of biofortification through breeding is possible only if there are several thousand varieties of the crop with varying levels of micronutrients, so that the best available source is then crossbred with the local variety and the resultant crop is enriched. For instance, zinc and iron contents vary across certain crops, whereas vitamin A in rice shows a constant pattern. In the latter case, transgenic technology is used to add or remove certain genes in order to achieve certain desired properties [23].

Genetically modified crops have been shown to be not only cost effective but also resistant to some of the problems associated with soil degradation or recurrent drought in these regions or regular attack by pests. This in some sense is considered as a second Green Revolution, which could not only raise production and productivity but also take care of nutritional deficiency problems by addressing both quantity (increased production and hence higher consumption) and quality (enrichment with micronutrients) [24]. The major issue concerning biofortification or its nonadoption lies in its transgenic nature, which has already resulted in major debates around the world. The main concern with genetically modified food is the issue of allergens for human consumption and the associated environmental damage. More importantly, the effects of genetically modified foods and crops on human health have not been completely analyzed, and their rejection is based on the precautionary approach in some developed countries in the European Union and in Japan. Whereas more than 10 food and feed crops with transgenic events have been approved for cultivation in developed countries, the developing countries are using a very cautious approach [25]. For instance, very few developing countries have allowed a transgenic event in maize, whereas Mexico, Uruguay, Argentina, and South Africa have done so for soybeans. China, Indonesia, and India have transgenic cotton, which is, however, a nonfood crop. The precautionary approach to using a product seems to arise mainly for trade reasons and, in some instances, a concern for public health of the domestic population as well.

The European Union has taken the toughest stance on genetically modified foods, in that labeling and traceability are important and any possibility of contamination would also lead to the rejection of the product. For developing countries, however, not only is labeling costly but so is the cost of physical segregation of genetically modified and non-genetically modified crops. These issues, coupled with weak scientific and administrative capacities to conduct case-by-case biosafety screening, as insisted by the developed countries, have dissuaded the countries from adopting such technologies. In 2002, the Codex Alimentarius Commission reached agreement on a final draft of "Principles for the risk analysis of foods derived from biotechnology." The principles provide a framework for evaluating the safety and nutritional aspects of genetically modified foods and define the need for a premarket safety assessment of all such foods on a case-by-case basis. Postmarket monitoring would be one of the management options described in the principles. Guidance related to analytic methods and other tools to be used in risk management are also provided. The task force also reached an important agreement concerning the tracing of genetically modified products for the purpose of facilitating withdrawal from the market when a risk to human health has been identified. The agreement is likely to mark a breakthrough in international negotiations concerning the use of tracing systems in relation to food in international trade. The world market for food products, especially for soybeans, corn (maize), and cotton, has been increasingly polarized, with some countries either insisting on non-genetically modified products or suggesting strict inspection norms.

## Organic foods

Organic foods remain one of the fastest-growing segments in the food sector. The potential for profits is considerable and has attracted large entrepreneurs to coexist with small producers. Growth rates of 15% to 20% in Japan, the European Union, and the United States are forecast [9]. Governments typically have used process regulations while defining product characteristics of organic foods, such as "no pesticide use." It is only recently that countries in the European Union and the United States have started to regulate this sector, and regulation has been imperfect. Ultimately, to sustain any linkage between consumption of organic foods and health, such foods would have be tested on the same grounds as conventional foods, which would have implications for how much organic foods will be traded across national boundaries in years to come.

* As is well known, commercial fortification (loading of semiprocessed food with fortificants), for instance with iron, provides higher doses than biofortification, and iron supplementation provides even higher doses.

## Product attributes: Harmonization and trade

### Nutrition labeling

Labeling is a classic case within information economics of the problems that firms confront in a marketplace: *what* product information to convey, and *how* to make that information credible. With the rise in diet-related disease patterns around the world and the increase in consumption of processed and packaged food, nutrition labeling is increasingly demanded. Nutrition labeling is supposed to assist consumers in making an informed decision about the content of the product. It is now customary for the governments in several developed countries such as Canada and the United States and also in newly industrialized countries such as Malaysia to have mandatory labeling norms. For the manufacturers, on the one hand, this makes them aware of the nutritional content of their product, thereby giving them an edge in selling the products, and on the other hand it instills an element of social responsibility to produce and promote healthy food among consumers. Of course, possibilities of misleading the consumers based on health claims are also likely to arise if this is to be used as a strategy to increase sales. Consequently, regulation of labeling format (what to label, how to label, and where to label) is considered important. Several countries follow the Codex labeling pattern, whereas others have their own regional versions and hence tend to vary from one country to another. With the rise in trade in processed food, a common approach to labeling is becoming important, leading to calls for harmonization.

Recently, the issue of harmonization in labeling of *trans* fatty acids (TFA)* in food products emerged as a point of contention in trade between Canada and the United States [26, 27]. The differences in labeling format include the following: *trans* fat is to be declared separately, with a minimum daily value for both saturated and unsaturated fats in the Canadian proposal, whereas the US format requires that *trans* fat be declared separately from saturated fats, but only the saturated fat is accompanied by a minimum daily value; the minimum threshold of *trans* fat declaration is lower in Canada (0.2 g) than in the United States (0.5 g or more); and Canada requires that the label be printed in English and French. Consequently, there is a request for mutual recognition rather than harmonization by the US manufacturers, who seem to have a less stringent labeling format. The welfare impacts of the nutrition labeling have been a significant increase in sales of products marked "no *trans* fat" and increased research on and availability of oils with lower or no *trans* fat content (partly driven by the nutrition labeling deadline set by NAFTA for January 2006).

Similar issues could emerge in trade between two groups of nations, as the countries vary in terms of what they deem necessary to be cited on the label about nutrient content and how it is explained. For instance, Food Standards Australia New Zealand (ANZFA), the joint food code for Australia and New Zealand, has mandatory nutrition labeling, whereas the European Union considers mandatory labeling only for food with nutritional or health claims. It expects the information to be given both per 100 g and per 100 mL and in terms of an average serving, whereas the latter is not an essential requirement in the European Union [28, 29] Similarly, countries in the Southeast Asia region have varying standards, and attempts have been made to bring the countries together for a harmonized nutrition labeling due to increased demand by the consumers as well as their regional trading partners [30].

Although it is widely accepted that nutritional claims cannot be the only approach to change dietary habits, they could have significant impacts on some consumers, and therefore restricting trade on the basis of a different labeling format does not seem to be justified [31]. Thus, guidelines such as those of the Codex Alimentarius Commission may be necessary to increase harmony in the labeling format. Within the Codex Alimentarius Commission, the Codex Committee on Food Labeling gives guidelines for label texts that have four subcategories dealing with prepackaged food, nutritional claims, and nutritional labeling (see Van den Wijngaart [31] for a brief explanation of these guidelines). The important feature of these guidelines seems to be that they allow for flexibility in national policy formulation so that local needs are taken care of. This and the emerging scientific evidence on nutrient content result in variation in labeling formats and regulations around the world, which has an impact on harmony. Greater consistency or harmonization can only be achieved by discussions among trading partners and discussions in the Codex meetings, leading to fewer barriers to international trade with benefits to the consumers and producers.

* TFAs are polyunsaturated fats that are harmful to health, particularly those from partial hydrogenation of vegetable oils. Addition of TFA increases the shelf-life of a product with a stable flavor but also increases the cholesterol level, causing a higher risk of coronary artery disease. Consequently, some countries in the European Union, the United States, and Canada have imposed regulations on the permitted use of such oils and hence have mandatory labeling requirements whose format varies among the countries. In Australia and New Zealand voluntary labeling is practiced.

## Process controls: HACCP and harmonization

The monitoring and enforcement of performance standards for microbial pathogens is costly. There is a WHO/FAO protocol that is recognized and forms the basis for the protocol used in the United States and

other countries [32, 33]. Several developed nations such as the United States, Canada, and the European Union countries imposed mandatory Hazard Analysis and Critical Control Point (HACCP, pronounced hassip) regulations in the early to mid-1990s.* This finally led the Codex Alimentarius Commission in 1993 to recommend the use of this approach, since it is a preventive mechanism to identify and hence avoid health risks while the production process is operating, and it transfers the regulation process, which used to be in the form of end product sampling and testing, away from the government to the private producer. HACCP involves seven principles:

» *Analyze hazards*. Potential hazards associated with a food and measures to control those hazards are identified. The hazard could be biological, such as a microbe; chemical, such as a toxin; or physical, such as ground glass or metal fragments.
» *Identify critical control points*. These are points in the production of a food—from its raw state through processing and shipping to consumption by the consumer—at which the potential hazard can be controlled or eliminated. Examples are cooking, cooling, packaging, and metal detection.
» *Establish preventive measures with critical limits for each control point.* For a cooked food, for example, this might include setting the minimum cooking temperature and time required to ensure the elimination of any harmful microbes.
» *Establish procedures to monitor the critical control points*. Such procedures might include determining how and by whom cooking time and temperature should be monitored.
» *Establish corrective actions to be taken when monitoring shows that a critical limit has not been met*—for example, reprocessing or disposing of food if the minimum cooking temperature is not met.
» *Establish procedures to verify that the system is working properly*—for example, testing time- and temperature-recording devices to verify that a cooking unit is working properly.
» *Establish effective record-keeping to document the HACCP system*. This would include records of hazards and their control methods, monitoring of safety requirements, and actions taken to correct potential problems. Each of these principles must be backed by sound scientific knowledge: for example, published microbiological studies on time and temperature factors for controlling food-borne pathogens.

HACCP offers a number of advantages. Most importantly, HACCP focuses on identifying and preventing hazards from contaminating food; it is based on sound science; it permits more efficient and effective government oversight, primarily because the record-keeping allows investigators to see how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day; it places responsibility for ensuring food safety appropriately on the food manufacturer or distributor; and it helps food companies compete more effectively in the world market and reduces barriers to international trade. However, as Josling et al. [9] note, the push for HAACP to achieve content attributes is ultimately a "process standard" that is typically more difficult to implement internationally than a "product standard."** The aspect of varying approaches to HACCP leads to the issue of mutual recognition or equivalence of HACCP procedures but may eventually end in harmonization.

When the US Food and Drug Administration established mandatory HACCP regulation in the mid-1990s, neither efficiency nor distributional effects were considered.*** The impact of this rule on the productivity of the meat and poultry industry and the variable costs of production were not taken into consideration, nor were the administrative costs of regulation [3]. Hence, the cost of this regulation exceeded the benefits, which was the reverse of what that the government had claimed. In several countries, including developed ones such as Australia, larger firms are in a position to set up HACCP systems in their production process, whereas the smaller and medium-sized firms are unable to do this. There is considerable debate about the impact of HAACP on plant costs and market structure [34].

Ollinger and Mueller [34] in their analysis, however, found no appreciable relation between plant size and ability to absorb sanitation and process control costs. They also found that implementing pathogen reduction/HACCP programs in meat and poultry plants would account for about 1.1% of their total costs, adding about 1.2 cents to the cost of a pound of beef, 0.7 cents to the cost of a pound of pork, and 0.4 cents to the cost of a pound of poultry. The benefits were estimated to range from $1.9 to $171.8 billion annually. This translates into a benefit value (in terms of health cost savings) that is at least twice the cost to the industry.

Many smaller nations, for example, Turkey in its trade with the Netherlands in seafood, meat, and poultry, require that all firms participate in the HACCP procedure, which would involve huge costs [35]. Further,

* Hazard Analysis Critical Control Point (HACCP) is a preventive regulatory procedure to identify points in the production process that are most critical to monitor and control.

** Process standards involve complex verification and enforcement procedures by regulatory institutions in two or more countries. Judgments about the capabilities of the testing and certifying institutions in the exporting countries figure prominently in these regulatory decisions [9].

*** In particular, the HACCP regulation in the meat industry was set to reduce pathogen levels in order to improve food hygiene, as was expected by domestic consumers, and also to raise the trade competitiveness of the product.

trade in such products as seafood involving a developing country exporter and a developed country importer may be costly for the former due to higher marginal costs of implementing HACCP arising from lower sanitary standards and lack of technical expertise [36].

The US ban on importation of frozen fish from Thailand in the 1990s due to salmonella contamination led to revamping of the Thai industry, with HACCP standards being imposed universally for products sold in the market [37]. During this transformation process, the producers, assisted by producer cooperation, established producer organizations or more informal cooperative working arrangements. Governments and private institutions also had to deal with varying standards set by different countries. Bangaldesh's shrimp industry introduced HACCP certification with support from multilateral agencies and government assistance [38]. This was in response to a European Union ban in mid-1997 on processing plants with poor hygienic conditions. Subsequent to all this investment, and because of the perceived potential of huge export earnings, there was an overexploitation of natural shrimp farming; this resulted in declining production from natural reserves, and the fish-processing plants consequently began diversifying manufacturing, with some resultant laxity in food-safety standards, all of which presents a challenge to exporters.

When developing nations trade with any other nation (e.g., Turkey with the European Union, Colombia's free trade agreement with countries in the Andean Community Region, or Thailand with the United States) in poultry or seafood products, and the developed-country importing partner has mandatory HACCP rules where the developing countries do not, the exporting country is faced with the following issues. The larger firms within these countries are in a position to acquire resources and also put into place HACCP systems, whereas the smaller businesses, many of which would be in the informal sector, are not in a position to take advantage of the international trading options. This may result in these businesses either being shut down or continuing to produce for domestic consumption when the population already faces large threats from food-borne diseases. Even the management of the larger firms may not find it worthwhile to invest in safety when there is less demand for such products, thereby losing out on trade or being taken over by multinational corporations [9, 39]

## Efficiency and equity considerations in food safety regulations

The use of pesticides to improve productivity through control of pests and improvement in storage and transport is very high in developing countries. There are also associated health risks due to on-farm ingestion, contamination of water and air by pesticide discharge, and consumption of food with higher doses of pesticide residue. By 2001 all the countries within the European Union had adopted a newer and stricter standard, whereas most of the other importing countries used the Codex Alimentarius Commission standard set in 1989 and revised in 1995. This harmonized regime within the European Union together with nonharmonizaton within the world market seems to have caused, for example, huge losses to many Latin American, Asian, and African countries in banana exports due to regulations against importation of residues of chlorpyrifos pesticide. Further, Wilson and Otsuki [40] showed through simulated models that the most stringent of these standards—0.05 ppm by the European Union as opposed to 2 ppm by the Codex Alimentarius Commission—would cause a decline in the value of exports of about 50%, thus significantly affecting the trade flows for the exporting countries.

Among the three major health risks mentioned above, pesticide residue seems to have the lowest risk, and the risks are still uncertain due to limited scientific evidence. Alar (daminozide) residue, which caused alarm in the early 1990s, carried a negligible lifetime cancer risk of 1 in 1 million, whereas aflatoxin pesticide residue is likely to carry a risk of 1.4 deaths per billion per year in the European Union [41, 42]. Therefore, a regulation not adequately backed by scientific evidence or public health concern would amount to a non-trade barrier for an agricultural commodity. Crop producers in developing countries that overuse pesticides are at risk of health problems from direct exposure to pesticides. On the other hand, there is a resulting loss of income from declining exports and reduced productivity if the pesticides are used less. Either course of action is a disadvantage for developing country farmers and a greater hardship for them.The current stringent measures to restrict pesticide use, however, would have significant health impacts for everybody, but they need to be imposed provided there are alternative ways of improving productivity to replace excess pesticide use.

First, the increase in efficiency due to better quality and safety standards brings in higher costs of compliance and increased cost of the product, thereby undermining the equity considerations, as poorer households would have to pay a higher price per unit. Second, in developing nations, where the increased cost could not be borne by the domestic consumers, the producers would have to make two products of different quality, which would add to their cost. In both of these instances, the government might have to subsidize to reduce the burden on the consumer in the former case or the producer in the latter case. Third, subsidies to the producers to reduce the cost are not permitted under the WTO rules, since they are considered discriminatory. Given this scenario, inspection levels for food

safety would have to be strengthened in order to avoid rejection of export shipments owing to contamination of the higher-quality product with the lower-quality product, which would increase the cost of regulation to the government. Fourth, importing countries may choose to adopt different standards, which are usually higher than those specified by international agencies. The continuous changes in standards and the monitoring of standards would further increase the price of the product, since the cost of production would be a nonlinearly increasing function of higher standards and their effective monitoring. Fifth, if one of the main intentions of promoting trade liberalization is to enhance growth and reduce poverty among the less developed and developing countries, then the higher standards imposed on food safety would increasingly channel the resources from export earnings toward ensuring food safety for the world market, neglecting several other investments (such as health, education, and sanitation) that might have more widespread and long-term benefits for the low-income country.

Arguments emerging from developing countries in favor of harmonizing with international food safety standards do not seem to indicate a loss in equity considerations, for the following reasons [43]. Food safety standards in developing countries are very low, and trying to align with international standards would surely bring large health benefits to the domestic consumers. Low food hygiene standards account for a huge number of deaths in developing countries: up to 70% of diarrhea cases among children under 5 may be due to food-borne contaminants, and several other pathogenic and parasitic infections are carried by food [44]. Better information flow with the possibility of foreign technical collaboration and advice would bring improvements in technical expertise of the producers and an upgrading of their skills. One of the conditions of the WTO is that (developed) exporting countries should assist exporting countries in setting standards so that the higher standards do not appear a barrier to trade. An example is the development of supermarkets in Central America during the phase of increased exports of fresh fruits and vegetables to developed countries. Berdegue et al. [45] note that the supermarkets have begun to raise quality and safety standards, and domestic sales from supermarkets are growing faster than exports from this region. This has happened because of improvements in procurement standards and imposition of higher standards on the suppliers, as well as increasing emphasis on safety aspects along with cosmetic and flavor concerns. However, the effects of these changes have reached only the middle- and upper-income segments of the population and are more marked in those countries where the government takes a stronger hand in enforcing safety standards. Similarly, the trade in fresh vegetables between sub-Saharan Africa and the United Kingdom has grown rapidly with improvements in quality and safety, but this has resulted from the rules imposed by a few large retailers in the United Kingdom and has marginalized the smaller exporters [46]. As Busch and Bain [47] highlight, with the global transformation of agrifood systems in the post-WTO phase, private standards are on the rise with the growing influence of private food retailers who are more global and oligopolistic.

It is clear that improvements in governance will ensure better participation in international rule-making, and since good governance has a strong public health component, will improve overall well-being. But for the developing world in particular, the problems stem not just from weak governance and lack of concrete scientific evidence to justify harmonization (given all other developmental pressures). We also should be aware that in many countries, the diverse local ethnic groups may or may not share the same commitment to national or international institutions or to (Western) scientific methods. Moreover, in the presence of rampant illiteracy, social taboos and discriminatory attitudes that often prevent women in general and ethnic minorities in particular from participating fully in national governance or even in markets all serve to exacerbate the aforementioned issues.

## Conclusions

This article has reviewed the process of harmonization concerning nutritional issues in food trade regulation around the globe to try and understand who the winners and losers are and whether harmonization is an effective mechanism to promote the goal of safer and nutritious food with regular access at the lowest price.

With the formation of the WTO and the ongoing process of globalization, there has been an increase in global trade in food and animal products. The increased exchange of goods around the globe to benefit from a lowered tariff regime, however, seems to have also brought in nontariff barriers to trade in the form of safety and quality standards. Most of the regulations expectedly emanate from the developed nations (in particular the European Union), who have been net importers of food in recent times and where consumer concern about food safety has been rather high. This concern, however, has to be balanced by concern for those in the developing nations for whom earnings from international trade are an opportunity for faster growth in the short run and possibly poverty reduction in the long run. Therefore, lack of access to developed-country markets for food and agricultural products because of stricter regulations, which is a competitive advantage, is a cause for concern.

As has been discussed above, regulations of food safety and quality have existed since the early 1960s, with the setting up of the Codex Alimentarius Com-

mission jointly by FAO and WHO. Despite guidelines given by these world bodies, countries have evolved their own regulations according to their level of development. This has resulted in different standards and regulatory practices, some of which follow the Codex and others of which have either lower or higher standards than the Codex. However, many of these rules are being revised more frequently than has been the case in the past. In general, it is always costly for an exporting country to trade when there are several types of regulations followed by different importing nations, especially when some of these regulations seem to be discriminatory in nature in the absence of sufficient scientific knowledge on how to assess the losses from nonadherence.

In order to ensure smooth trade, a harmonized framework that allows for uniform codes and norms while pooling scientific knowledge has to be pursued by both the WTO and the Codex Alimentarius Commission. Under the WTO there are two agreements, the SPS and the TBT, which address the regulations for food safety; the SPS deals with human, animal, and plant health-related concerns, whereas the TBT deals with non-health-related quality issues. The SPS mainly draws its rules from the Codex, whereas under the TBT technical aspects have evolved through bilateral and multilateral discussions.

Among several food safety concerns, food fortification, the loading of additional micronutrients, directly concerns nutrition, and the problem clearly differs between developed and developing countries. The former are increasingly concerned about higher doses than are required, whereas the latter are still grappling with public health problems due to far lower intakes of micronutrients. Under a situation of universal fortification, mandatory fortification may be justified in some situations, whereas not all countries may impose it. Consequently, the same set of rules for levels of fortificants is not possible, and harmonization could lead to disputes. It should also be noted that the harmful effects of a dosage level are not always clearly known, while only consumer concerns seem to determine the standard. This aspect is more apparent in the trade in genetically modified foods, where the European Union in particular has requested labeling and traceability. In recent times the European Union has approved some genetically modified foods but overall has imposed strict regulations, while countries like the United States contend that in the absence of clear evidence on adverse impacts, there should not be any restrictions on the movement of such goods. Countries like Zambia that suffer from high levels of malnutrition have strongly opposed genetically modified imports, mainly because of fear of contamination of other crops, some of whose products are exported to the European Union. At the same time, several neighboring countries of Zambia have approved the entry of genetically modified crops, either through imports or in the form of food aid.

The issue of nutrition labeling attempts to address public health concerns such as obesity while knowing very well that labeling may have limited impact. This is an issue that concerns the affluent, who have a higher consumption of processed food along with a sedentary lifestyle. Naturally, when a majority of the population is still on subsistence consumption and illiterate, labeling is a nonissue. More importantly, the empirical evidence for the success of labeling in general in educating and modifying consumer behavior is mixed and in the case of nutrition labeling is limited. More interesting is the issue of labeling of genetically modified food, which seems to be the core concern for trade, even though the adverse impact of genetically modified food is also not well known. Therefore, rather than harmonization, equivalence is a preferred option for labeling and is considered efficient by some countries. Similarly, HACCP is another regulation for which harmonization is important in the sense of having a system in place for the food-processing industry. However, confusion prevails as to whether the process itself needs some standardization and whether there should be checks on pathogen levels at the entry point even when HACCP is followed by the exporting country.

If free trade is the goal, then there must obviously be few to no regulations; but if consumer health and protection are the goals, then variations in food and hygiene standards and immunity levels of the population surely require country-specific regulations. Clearly the challenge for a harmonized framework under a liberalized trade regime, particularly for food, lies in the fact that the regulations sometimes cannot be based on scientific evidence alone, since they are also influenced by varying tastes and cultures and differences in the level of economic development that ought to be considered in a comprehensive manner.

In conclusion, there is a lot to be gained in terms of a benefit-cost ratio by participating in international food trade with harmonized regulations; however, more sustained and solid research is needed. Given the limited evidence, based mainly on developed country research, it is clear that the impact varies with the context or the nature of the commodity traded. Quantifiable estimates of developing country impacts are very few, and the emphasis for further research needs to be on this. Nevertheless, it would be worthwhile to follow *equivalence* wherever the trade is still at the regional level to allow some flexibility for an individual nation's choices and pursue foreign aid, technology transfer, and management advice whenever the economically disadvantaged nation is lagging in standards. At the same time, it would be necessary to educate the heads of government and the population in developing countries on the existing scientific practices and evidence on health hazards and the need for effective nutrient-based dietary standards. Implementing stricter rules

within their own nations not only would improve their access to world trade but also would result in reduction in food-borne diseases and related mortality. The increased awareness would not only enable them to effectively (and increasingly) participate in the Codex meetings to raise issues of concern on local food consumption patterns and health (during the formulation of international rules on food safety standards) but also use the dispute settlement mechanism of the WTO by being able to distinguish between a regulatory standard and a nontrade barrier. There is lot to be done in this area, but clearly steps are being taken in the right direction.

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# Beyond recommendations: Implementing food-based dietary guidelines for healthier populations

Suttilak Smitasiri and Ricardo Uauy

## Abstract

*To reduce the increased burden of diet-related disease and promote human potential through food and nutrition globally, harmonization of efforts is urgently needed. This article examines the concept of food-based dietary guidelines (FBDGs) and discusses the possibilities and challenges of harmonizing the process of developing and implementing dietary guidelines. The authors argue that while the development of FBDGs has contributed to the understanding of the role of nutrients and foods in achieving optimal health, the impact of these guidelines on human health has been limited.*

*Science or evidence must be used in FBDG development; nevertheless, there are limitations in current nutrition science. FBDGs should address the health consequences of dietary insufficiency, excess, or imbalance with a broader perspective, considering the totality of the effects of a given dietary pattern, rather than focusing on single nutrients alone. Moreover, the food selection guideline should be seen as complementary to a strategic, comprehensive, and culturally appropriate dietary and health promoting intervention, and not only as a tool for providing nutrition policy and information.*

*Technically, a single unified global set of FBDGs may be desirable and even achievable. This concept, however, presents novel challenges on how to address cultural diversity and the complex social, economic, and political interactions between humans and the food supply, not to mention the complexity of its communication and implementation. Therefore, global harmonized efforts in support of strategic dietary interventions, together with strong global scientific support and facilitation for the development and communication of FBDGs at national or regional levels, are proposed to implement FBDGs for healthier populations.*

**Key words:** Food-based dietary guidelines, nutrient-based dietary guidelines, food selection guides, unified food guidelines

## Introduction

The establishment of food- or nutrient-based dietary guidelines (FBDGs or NBDGs) is a complex issue; this complexity will most likely continue to increase in the future as knowledge of the science base and the need to consider context in the application of FBDGs/NBDGs increase. International agencies and national authorities around the world are faced with the need to inform and educate all constituencies involved in this process. Food and nutrition policy makers, planners, and common citizens need to be able to manage and make food choices at the national, community and individual levels. Several countries, beginning with those in the industrialized world, have developed FBDGs as one of many tools for this purpose. FBDGs are generally developed with the expectation that they will help to improve the effectiveness of nutrition education efforts, directed both to the general public and at the national level [1].

The US Government has developed and tendered official dietary advice for the public since 1894. The first US food guides were promoted in 1916 as conceptual frameworks for selecting the kinds and amounts of a variety of foods, which together provided a nutritionally satisfactory diet [2, 3]. Over the years, the US food guides have changed as nutrition knowledge has advanced and new discoveries relating diet and health have been made. The guides primarily focused on the

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needs of individuals; they were supposed to ensure that people were getting enough nutrients from their diet. This focus has progressively been reoriented by the need to obtain a diet sufficient in energy and specific nutrients and avoiding deficiency as well as excess. In 1992, despite successful promotion of food guides, the US government wanted the public to place greater emphasis on following FBDGs and thus developed the Food Guide Pyramid as a graphic representation of the dietary guidelines [4]. As a result, several countries in both developed and developing regions of the world followed the approach taken by the US Department of Agriculture in establishing dietary guidelines as well as in adopting or applying the Food Guide Pyramid [5].

The greater recognition of how dietary changes affect patterns of disease and the evidence that a nutrition transition was occurring led the Food and Agriculture Organization/World Health Organization (FAO/WHO) to address the need to establish a common approach in the "Preparation and use of food-based dietary guidelines." The corresponding report of the joint Expert Consultation was published in 1996 [6]. The objective of establishing FBDGs was both to ensure the prevention of nutrition-related communicable diseases (NRCDs) and to prevent nutritional deficiencies through proper nutrition education and food-based interventions if necessary. This publication has been of key importance for international agencies, especially FAO and WHO, as well as for countries in the promotion of national FBDGs. FBDGs have served to take nutrient-based recommendations one step closer to practice, particularly in developing countries. They have also served to define nutrition education goals as part of the national plans of actions for improved nutrition [1]. Recently, some fundamental questions regarding the concept and effectiveness of FBDGs have been raised. For instance, evidence indicates that inappropriate diets and lack of physical activity are responsible for approximately 30% of preventable morbidity and mortality in the United States. Given the fact that FBDGs have been developed and implemented in the country for more than 100 years, the success of the food guides in contributing to better health and nutrition of this population is thus open to question [7]. Other critical issues raised include the process utilized in deriving FBDGs and the process used in developing a scientific consensus, insufficient participation of important stakeholders in the developmental process, lack of transparency in both their development and implementation as well as a greater concern about scientific adequacy in examining the relationship between diet and disease. Further, some suggest that the real criticism of the FBDGs is the fact that governments have not yet given adequate attention to promoting them [2, 7–9].

This article aims to examine the concept of FBDGs and explore the possibility of harmonizing the process of developing dietary guidelines. We will also suggest ways and means to enhance effectiveness of future FBDGs by addressing critical aspects of both development and implementation. The specific objectives are to

» Provide a brief background on the evolution of FBDGs in various countries;
» Establish the necessary science-based foundation for the development of FBDGs;
» Specify the necessary processes in the development and the key elements for success in the implementation of FBDGs within countries and regions;
» Discuss weaknesses and strengths in the communication and implementation strategies for FBDGs;
» Recommend ways and means to enhance effectiveness of the FBDGs in the future;
» Analyze whether harmonized approaches in establishing global FBDGs are desirable, possible, and achievable.

## The foundations of FBDG development: Common sense and science

Food selection guides have been proven useful in the past, especially during difficult times when it was necessary to manage national food supplies and food and nutrition security carefully. During World War I, for example, the food guides and related campaigns served to define targets for food production and distribution in an effort to balance the requirements of feeding US troops and populations. They were also helpful in the control and management of food security problems and undernutrition during the Great Depression. The US Government used the food guides to issue monthly ration stamps to citizens and assigned point values for each food item in order to ensure national food security during World War II [2]. As societal dynamics became more complex during times of peace, food selection guides and other food and nutrition policies became closely linked in the country [8].

Thus, it can be said that food selection guides were originally created with the common sense and good will of individuals in a society ready to follow guidelines to avoid hunger and starvation among its citizens. These guides become especially important in convincing the public to sacrifice a more abundant food supply in order to support more urgent priorities, i.e., feeding the troops to win a war. During wartime, mass psychology as a force shaping human behavior operates quite differently than in periods of peace and prosperity. Human beings tend to comply more willingly with authoritative directives when they feel threatened by external agents. Individuals are ready to give up their individual freedoms temporarily in support of the overall well-being of society. This has been a fundamental justification for efforts to support the necessity of food guides, to enhance their credibility,

and to secure compliance with food guides. Moreover, previous experiences indicate that the guidelines are especially effective when the food supply is plentiful and decisions on food choices no longer belong to the government but depend on individual choice. Over the past decades, a science-based approach evaluating the available evidence on diet and health has been adopted to strengthen the validity of FBDG development [10].

A greater interest in establishing a solid science base for food and nutrition recommendations among nutritionists and policy makers has led to significant advances in our knowledge of how food, diets, and nutrition affect health throughout the course of life. However, due to the reductionistic nature of present nutrition, food, and medical sciences, establishing FBDGs serves to better understand the role of nutrients and diet in health but is thus far clearly insufficient to take the knowledge of how diets affect human health and well-being into action. Problems due to lack or excess of specific nutrients remain, and preventive measures such as food fortification or nutrient supplementation programs have been established in both developed and developing countries. These approaches are, nevertheless, insufficient to tap on the knowledge required to modify food intake and physical activity-related behaviors responsible for the present epidemic of obesity and related chronic diseases. Addressing the need for individual and social behavioral development has major implications in defining policies and programs to prevent and control the present epidemic of nutrition-related diseases.

Expert groups or committees developing science-based FBDGs commonly face the problem of overwhelmingly fragmented massive scientific information. The process needed to translate this information into knowledge that allows the categorization of the strength of the evidence in support of relationship between dietary constituents and disease is a key step in the development of nutrient intake goals and the corresponding food-based recommendations for optimal health at all stages of life. Additionally a necessary technical consensus concerning the role of multiple other factors that condition the relevant nutritional problems must be reached. This is commonly difficult, since the role of the factors determining the supply of and access to safe and nutritious foods, as well as the social, economic, and cultural determinants of food choices, are ignored or not clearly identifiable from the published literature. In most cases, fulfillment of these conditions is difficult to achieve. Experiences often indicate that the guides are not based on science alone. In practice, they are usually restricted by the limitations in available scientific knowledge and judgments of selected experts that may have strong biases or are not fully independent of selected interest groups; there is also commonly a strong open or covert pressure from various stakeholders which often succeed in defining the final form taken by FBDGs. For the development of the US 2005 Dietary Guidelines, an evidence-based approach was used for the first time to develop the key messages [11]. Despite the limitations, technical consensus opinion of expert groups is an essential step in establishing food- and nutrient-based dietary guidelines and recommendations [12].

Many countries have followed the methods outlined in this section as a way to define goals for recommended nutrient intakes and foods that supply them; thus guiding the selection of foods for optimal health in a given population. In fact, as will be presented later, there are many commonalities in the key food-based dietary guidelines around the world. The basic premise is that the guides should promote overall health and prevent physical and/or mental disability at all stages of the life course. Consequently, the main target group for FBDGs is people in good health. The guides, in principle, should be based on up-to-date research, focus on the total diet, be useful to the target audience, and meet nutritional goals based on people's usual food and dietary patterns. Moreover, the guides should allow maximum flexibility and practicality. Last but not least, the development of the food guides should be evolutionary, promoting gradual progress and avoiding radical revisions. These considerations are based on the analysis of a study of the evolution of food guides and on a needs assessment of the US professional community conducted in the early 1980s [4].

Despite these sound philosophic goals, it is indeed perplexing to realize that most US citizens are currently facing the consequences of nutrition-related chronic diseases. Perhaps the successful application of FBDGs in countries such as the United States has become extremely complex, and the capacity for science-based FBDGs to affect food choices in that context is limited. They are no longer relevant, unlike the case during difficult times in the past when food guides proved to maximize health benefits given a restricted food supply. We suggest that at present there are basic contradictions between the goals of optimizing nutrition and public health and the prevailing market forces that shape the supply and demand for foods, thus imposing limitations on the effective implementation of FBDGs. Or perhaps FBDG promotion has been ineffective in achieving sustained changes in nutrition-related behavior necessary to affect health conditions. Since no systematic evaluation of these alternative explanations for the limitations of FBDG has been attempted, there is no conclusive answer to this question. Moreover, diet is important but is only one among many interactive factors in the complex causation of nutrition-related chronic disease.

In some European countries, comprehensive and tailor-made strategic nutrition policies and interventions have been important in achieving successful large-scale dietary change with the corresponding

health benefits in curbing the epidemic of chronic disease. A wide range of social measures have been used: legal regulatory frameworks, economic incentives for healthy consumption and disincentives for unhealthy choices, organizational structures at the governmental level that establish national policies and programs to achieve the desired goals, and educational efforts at all levels of formal and nonformal systems. The food selection guides used in these countries serve to support or complement the overall dietary and nutrition interventions [13]. However, experiences thus far in developing countries generally indicate inadequate resources for both the development and the implementation of the food guides.

Therefore, it is indeed timely to consider revisiting the concept of FBDGs and their application in this rapidly changing world. Before they actually adopt the concept of FBDGs, countries and communities should perhaps examine themselves to determine the contextual elements within countries or communities that will enable or restrict the successful development and implementation of FBDGs. For instance, in the United States the dietary guidelines, by law, form the cornerstone of US federal nutrition policy and provide the basis for all federal nutrition education activities [14]. In Canada, dietary guidance has been an important element in the country's comprehensive health promotion efforts. These contexts are enabling factors for the development and implementation of FBDGs in these two countries. However, these positive factors are counteracted by the strong influence of marketing and advertising in shaping food choices of individuals and communities. The resources supporting the marketing of unhealthy diets are between 100- and 1,000-fold greater than the funds available to promote consumption patterns based on FBDGs. These are important common-sense considerations and serve as the foundation that will define the effectiveness of the efforts. It is essential that these be addressed before embarking on scientific exercises necessary for the development of FBDGs.

## The process of defining FBDGs: From nutrients to foods and diets

The approaches used to define nutritional adequacy of diets and dietary recommendations have changed over time in accordance with the scientific understanding of the biochemical and physiological basis of human nutritional requirements in health and disease. The science of modern nutrition provides in most cases a solid underpinning for nutrient-based dietary recommendations but has limited information on the long-term effect of dietary patterns on health. There are obvious limitations to the reductionist nutrient-based approach; people consume foods and not nutrients, and moreover they consume foods in combinations that change over time and are affected by season and climate in addition to social and economic factors. The effect of specific foods and dietary patterns on health goes beyond the biochemistry and metabolism of the essential nutrients a food contains. For example, the availability and utilization of specific micronutrients is dependent on multiple interactions among nutrients themselves and between nutrients and the food matrix that contains them. Unless these are considered we will not assess the true nutritional value of foods. In addition, factors unrelated to diet commonly play a key role in the effect of diet on health; for example, parasitic infections rather than low iron intake may be the cause of anemia in many parts of the world. Similarly, if we ignore or undervalue the key role of physical activity in achieving energy balance, dietary recommendations per se will be of no use in preventing obesity and its consequences [5–7]. Moreover, people eat meals, and knowledge of meals and meal patterns is also critical in deriving the guidelines (see **fig. 1**).

## Methods currently used in establishing nutrient-based recommendations

The *clinical approach* is based on the need to correct or prevent nutrient-specific diseases associated with intake deficiency. This method is highly specific but not very sensitive; for ethical reasons, clinical outcomes are clearly inappropriate to establish nutrient dose–response relationships.

*Biochemical, physiological, or functional approaches* based on indicators of nutritional sufficiency can serve to define the limits of insufficient and excess intake of specific nutrients. This approach requires that we have

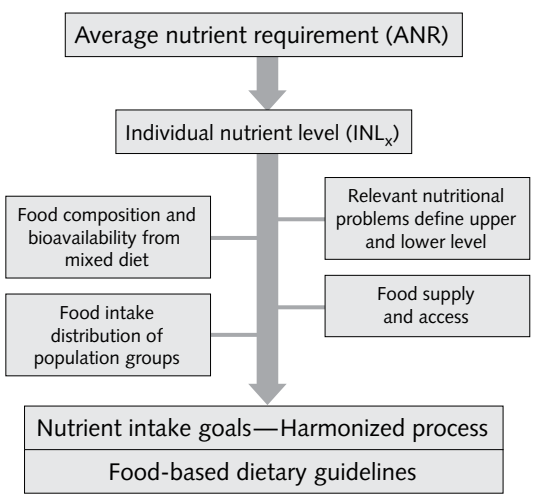


FIG. 1. Proposed process for defining food-based dietary guidelines for healthier populations

a defined set of biomarkers that are sensitive to changes in nutritional state and specific in terms of identifying subclinical deficiency conditions. The use of balance data to define requirements should be avoided whenever possible, since in most cases, observed balance based on input–output measurements is greatly influenced by level of intake. Subjects adapt to high intakes by increasing output; conversely, they lower output when intake is low. Biomarkers that can be used to define requirements include measures of blood levels, nutrient stores, nutrient turnover, and critical tissue or organ pools.

*The habitual consumption levels of "healthy" populations* serve as a basis to establish a range of adequate intakes in the absence of quantitative estimates of requirements. This criterion has important limitations but remains the first approximation to establishing requirements when no other data are available.

The concept of "*optimal nutrient intake*" has been proposed over the past decades. The notion of an optimal nutrient intake is based on the quest for improved functionality in terms of muscle strength, immune function, or intellectual ability. This approach is based on the possibility that diet or specific nutrients may improve or enhance a given function, ameliorate the age-related decline in function, or decrease the burden of illness associated with loss of function. However, the concept of optimal intake is usually unsupported by appropriate population-based controlled studies of sufficient duration. The concept of optimal diet implies that we are looking beyond immediate benefits; the aim is to attain long-term benefits in both the duration and the quality of life [5].

Values of recommended nutrient intakes (RNIs) based on different approaches may differ, so a key issue for harmonization is defining the preferred method to establish the nutrient-based recommendation. This in turn will be used to establish individual nutrient intake goals, which correspond to the desired target intakes that will contribute to achieving better health and nutrition for individuals living within an ecological setting. Their purpose is to promote overall health and/or control specific nutritional diseases induced by excess or deficit, and to reduce health risks, considering the multifactorial nature of disease. For some nutrients, the goal will be to consume at least a given amount, in other cases it may be to consume less than a certain amount, whereas in other cases it will be to consume within a given range with an upper and lower boundary. The nutrient intake goals should represent the intakes of individuals within a population. Thus, if we limit the intake of fat to less than 10% of energy from saturated fat, it means that individuals may obtain from 0% to 10% of their energy from saturated fat. If, on the other hand, we say that between 10% and 20% of total energy should be obtained from protein, it means that individuals should consume at least 10% and not more than 20% of energy from protein. In some cases the figure is given as a single number: for example, obtain at least 2.5% of total energy from essential fatty acids means that individuals should derive 2.5% or more of their total energy from these nutrients. When the chemical characterization of the food component responsible for the health benefit is not fully established, or the analytic methods differ significantly, the goal may be set in terms of type of foods, rather than an amount of nutrient: for example, in the case of fiber the recommendation is to "consume at least 400 g of fruits and vegetables a day." There may be occasions when there is more than one distribution curve of individual nutrient intakes within a given apparently homogeneous population; it is even possible that one subgroup may have an intake that is considered too low whereas another subgroup may have an intake that is close to excess. The goal in this case is to increase in the intake for some, whereas there is a need to reduce the intake in another group. Establishing an optimal range in this case may mean increasing the intake for some individuals while decreasing the intake in others [5–7, 15, 16].

FBDGs can be developed once the relevant nutrient intake goals for long-term health and the actual distribution of nutrient intakes are known. These should take into account the customary dietary intake patterns of individuals and the distribution of nutrient intakes within the population group(s), what foods are available, and the factors that determine the consumption of foods. FBDGs indicate what aspects of the dietary intake pattern should be modified, considering the ecological setting, the socioeconomic and cultural factors, and the biological and physical environment that affect the health, nutrition, and food supply of a given population or community.

Recent international and national expert committees have established nutrient-based recommendations for virtually every known essential human nutrient. The quantitative definitions of nutrient needs and their expression as recommended nutrient intakes (RNIs) have been important instruments for food and nutrition policy in many countries, serving to focus the attention of international agencies on nutritional deficiencies or excesses with health consequences. RNIs are customarily defined as the intakes of energy and specific nutrients necessary to satisfy the requirements of a group of healthy individuals. This nutrient-based approach has served well to advance science but has not always fostered the establishment of nutritional and dietary priorities consistent with broad public health interests at national and international levels. In fact, judged post facto, nutrient-based recommendations may have misguided some efforts to solve key nutritional problems. For instance, the emphasis on both the quantity and the quality of protein derived from studies of single food sources that evaluated the effect

of protein on growth rates of young animals placed a premium on the development of animal foods (meat, eggs, and cow's milk) and failed to include the concept of amino acid complementarities of cereal–legume mixes. In fact, when human infant and adult studies were conducted several decades later, the nutritional value of mixed diets from around the world was similar to that of animal proteins, except for a small increase in nitrogen lost in the stool when mixed vegetable protein sources were consumed. Thus, the protein gap was closed not by global initiatives to produce animal foods, but by a change in the experimental model used to assess human protein needs.

The change in the approach used to evaluate the energy needs of children serves as another illustrative example of the implications of the approaches used to assess nutritional needs. Recently the method has changed from one based on assessing intakes observed in industrialized countries to one based on measuring energy expenditure and evaluating energy stores necessary to maintain health. This change is of importance in addressing the global epidemic of obesity. The recent FAO/WHO/UNU 2004 report [15] established that energy needs of children have been systematically overestimated by 10% to 25%. Present data using energy expenditure estimation from doubly labeled water studies in infants under 1 year of age reveal that for this group the overestimation has been close to 20%; moreover, the estimates for breastfed infants are about 7% below those for formula-fed infants. These changes may appear to be of small magnitude, but if old recommendations are systematically used in the feeding of infants and children today, they can effectively serve to promote obesity in the early years of life. We are just now realizing that definitions of normality cannot be based solely on observations of intakes of apparently healthy populations but rather need to be based on favorable nutrition and health outcomes across the life span. We as nutritional scientists have a responsibility to critically examine not only the data but also the experimental designs used to obtain them. The context in which the experiment is done commonly defines the answer that is obtained; studies to assess nutritional needs and establish recommendations are by no means an exception.

In contrast to nutrient-based recommendations, FBDGs as instruments of policy are more closely linked to the diet–health relationships of relevance to the particular country or region of interest. Several key aspects should be considered in moving from nutrient intake recommendations to food-based dietary guidelines. We will provide a brief roadmap for this process; more detailed information can be obtained from the recently released US Department of Agriculture Dietary Guidelines Advisory Committee Report (www.health.gov/dietaryguidelines/dga2005/report) [16]. The first step in this process is defining the magnitude of the deficit or excess based on the estimates of nutritional needs for long-term health and the distribution of intakes. This serves to assess what nutrients are most likely to be consumed in low enough or in excessive amounts to constitute a potential health risk. The likelihood of nutritional deficiency is based on the probability that subjects or group of subjects within a population consume below the estimated average requirement (EAR), or in the case of excess, that they consume above the defined tolerable upper intake level (UL) of the specific nutrient over time. In order to adequately calculate this probability, we need to know the nutrient intake for at least 2 days but preferably for several days. In general, if the measured usual intake is above the EAR and below the UL, the risk of deficit or excess will be low. If, on the other hand, we find a significant proportion of the population consuming below the EAR or above the UL we need to consider establishing guidelines to correct this situation and prevent the adverse consequences of nutrient excess or deficiency [5, 6].

In developing FBDGs, we need to assess the change in dietary patterns required to achieve the recommended nutrient intakes. This will require that we have appropriate information on the chemical composition of the foods available to the population of interest and the amount of these foods commonly consumed. In some cases we need to increase or reduce the intake for virtually all of the population: for example, to reduce excess sodium intake typically resulting from consumption of salt added to foods during preservation or in cooking. In this case we may need to modify food-processing practices to prevent the health consequences of excess sodium consumption. On the other hand, for example, we will find that most of the population consumes insufficient folate. Thus the guideline in this case leads us to establish the need to have folate-fortified foods available to the general population, since it is quite difficult to meet the folate requirement with foods alone. In order to judge the adequacy of a dietary pattern, we need to assess not only the adequacy of the foods in providing sufficient but not excessive specific nutrients, but also the overall nutrient-to-energy density of the diet. Thus, if in order to meet the nutritional needs of a group the diet needs to be consumed at a level of energy that may prove excessive, given the activity pattern of the population, we may need to revisit our dietary guideline. In this case, we may need to increase the recommended consumption of nutrient-dense foods or possibly avoid energy-dense foods that may provide low intakes of specific nutrients needed for health. Following this reasoning, dark-green vegetables and legumes provide specific nutrients and fiber at relatively low levels of energy [16].

Special dietary patterns may be needed to cover the requirements of population subgroups such as the elderly, who will maintain or increase their needs for specific nutrients but in most cases will require less

energy because of their lower activity level. Similarly, women of childbearing age have an additional need for iron and thus require extra iron and ascorbic acid at the time of the meal in order to enhance absorption of ionic iron in the foods consumed. Meeting vitamin D and $B_{12}$ needs of the elderly also requires special considerations, since because of age-related changes in skin vitamin D metabolism and in gastric acidity, they are unlikely to obtain these nutrients in sufficient amounts unless they receive them as supplements or in fortified foods. In summary, FBDGs need to address the health consequences of dietary insufficiency, excess, or imbalance with a broader perspective, considering the totality of the effects of a given dietary pattern, and not focusing on single nutrients alone [5, 16].

## The complexity of FBDG communication and implementation

As already discussed, the complexity of FBDG development should not be underestimated. A carefully designed process should result in guidelines that are credible within the context of national nutrition public policies, and ultimately the technical consensus should gain support of the various stakeholders. Nevertheless, these are just part of the necessary process to take FBDGs beyond the letter of the recommendations and have them contribute to shaping food-consumption patterns. Another important component is translating a credible policy into effective action. This involves integrating scientific knowledge into a process in which contextual knowledge of local conditions, societal values, and economic and political interests plays a major role [17].

Unfortunately, the knowledge and critical analysis of successes and failures in taking FBDGs into action are not yet well documented in the literature. Of the countries that have developed FBDGs, Canada is one of the few that discusses the importance of communication and implementation of their food guides. Health Canada, in the process of updating the 1990 dietary guidance, appointed two advisory committees: a scientific review committee and a communication and implementation committee. The second committee's mandate was to translate the nutrition recommendations into dietary advice for the public and to recommend implementation strategies to facilitate comprehensive use and integration of the dietary guidelines into the policies, programs, and messages. This second committee designed an inclusive, intersectoral, and multidisciplinary process in fulfilling its mandate. Consultations with stakeholders were designed not only to gather input on strategies for implementation, but also to maximize consensus on one common set of dietary guidelines to be used by all when communicating with the public [18].

It is now well recognized that there is no direct road between knowledge and action. It is also acknowledged that bombarding the public with multiple and often conflicting nutritional messages does not lead to better nutrition and health [19]. Food and nutrition issues are complex by nature; as a consequence, they will require solutions that recognize these complexities in addressing implementation strategies. Based on experiences from nutrition education and health promotion fields thus far, it can be said that awareness or even a high level of comprehension of FBDG messages among the public does not necessarily result in overall improvement of nutrition and health [20]. A strategic nutrition policy and comprehensive as well as sustained culturally appropriate dietary intervention are needed to achieve significant improvement in nutrition and health in a given context [21]. FBDGs thus can be an important complement in the process of overall diet and health promotion interventions. Nonetheless, they cannot and will not bring about dietary changes in a population by themselves. Some considerations in developing future FBDG communication and implementation are discussed below.

One of the recently proposed strategies for improving the FBDG development process in the United States is to consider developing messages that will lead to behavior change among the public [22]. Contrary to this proposal, knowledge of communication clearly indicates that messages alone, regardless of how well they are developed, will not lead to behavior change. Behavior development needs good facilitating processes that can influence both personal and environmental factors necessary to modify the intended behavior(s) [23]. It usually requires a strategic and comprehensive intervention to address and support the expected change. In normal situations, communication is often only an important complement in the development and implementation process, and message design is only one essential element in a communication process. A realistic expectation is needed of what FBDGs as a concept can actually contribute in a given society. Based on this review, we propose that countries or communities should first consider the development and implementation of a strategic and comprehensive culturally appropriate dietary and nutrition intervention. Within this framework, FBDGs should be designed as a complement in the development and implementation process if dietary behavioral change is the ultimate aim. Norway is noted for this successful approach. With a strategic and comprehensive intervention, Norwegians significantly changed their diet-related behaviors and were able to lower prevalence of heart disease and other nutrition-related chronic conditions. This was in fact achieved in slightly over a decade [13, 21]. In Canada, as an important part of overall health promotion efforts, the communication and implementation advisory committee also positioned FBDGs only as

an element in changing conditions to support healthy eating, which went far beyond the provision of information [18].

By nature, FBDGs are a generic nutrition education tool. Strategic implementation accompanying the guidelines is definitely necessary in order to bring about significant changes in a specified period of time. Along this line of thinking, the objectives for FBDG communication and implementation should be clearly stated, measurable, and realistic in order to justify the usefulness of this concept in a given society. If behavior development is expected, it is critical in the communication and implementation process to pinpoint "which consumer(s) and what action(s)." Moreover, a consumer-centered approach will be needed, since consumers themselves are key actors in the change process, with perhaps an exception during wartime and other extremely insecure situations. The bottom line is that people, in general, will not routinely follow expert advice on food selection just to improve health [19, 24].

In developing FBDG communication and implementation, therefore, a few important assumptions should be considered. First, most consumers will be either only mildly interested or not at all interested in FBDGs. Second, it will be important to set up or to utilize environmental support systems in transferring information into appropriate action. And last, careful consideration needs to be given to delineating specific target consumers in terms of their demographic and psychological attributes, their lifestyles, values, and belief systems, as well as their media habits. In most complex societies today, a "one size fits all" communication and implementation approach will probably be ineffective.

To formulate meaningful FBDG messages, consumer factors must be considered as well. For instance, issues such as the public's attitudes about ideas related to FBDGs, other ideas that the public associates with the idea of FBDGs, specific needs that the public expects FBDGs to fulfill, and specific questions in the public's mind regarding FBDGs should, at least, be well understood by those involved in developing FBDG messages. Therefore, consumer research is as critical as scientific reviews if FBDGs are to be more relevant to the public. Development of the US Food Guide Pyramid was a good example of how consumer research helped select a graphic representation of the dietary guidelines, which has become popular in the country as well as many other countries around the world [4, 5]. In addition, it should be noted that the development of a graphic representation is a creative process that should be based on a good understanding of consumer factors. Unlike science, there is no one correct formula for this. To make it work, the visual tool must be meaningful in the mind of the public, not the experts. For example, some suggest that a choice of a familiar graphic might convey the message better for poor illiterate target groups [25]. Only a systematic assessment among the target population can indicate the level of effectiveness of a selected visual tool and enable its modification according to consumer perception.

Also from a communication point of view, FBDGs as a concept to promote national nutrition policy and at the same time to serve as a food selection guide for an individual are challenging exercises. To be effective, very comprehensive and sustained communication activities will be necessary. Without commitments from stakeholders on strategic outcomes of these exercises, it is very likely that a society will only bombard the public with more nutrition messages while an increased prevalence of diet-related diseases is observed.

Communicating and implementing FBDGs to promote the overall health of a population is usually multifaceted in nature due to the complexity of food and nutrition at both the individual and the community levels. It is therefore proposed, especially for developing countries, that FBDGs should be considered only as a complement to a strategic and comprehensive dietary intervention or health-promoting intervention, which has a clear vision of success for a specified period of time. If this is not a case, FBDGs will only be seen as providing nutrition policy and information that is unlikely to result in overall improvement of nutrition and health in a population.

## Harmonized approaches in establishing global FBDGs: possibilities and limitations

Reviews of experiences related to FBDGs around the world indicate that significant numbers of guidelines tend to be similar in their purposes, development, and uses. For instance, a number of guidelines in both developed and developing countries recommend a variety of grains daily (16 guidelines); a variety of fruits and vegetables (19 guidelines); a diet low in saturated fat and cholesterol and with a moderate content of total fat (23 guidelines); moderate intake of free sugars (16 guidelines); limited salt intake (22 guidelines); moderate consumption of alcohol (for those who drink alcohol) (16 guidelines); and maintaining healthy weight or performing physical activity each day (30 guidelines). According to this trend, unified guidelines should be achievable and possible, with some exceptions such as calcium and vitamin D [5].

One important criterion for good FBDGs is the use of science or evidence in their development. To do this, however, it is necessary to have available databases and research to back up the development, which is not normally feasible for developing countries and even some developed countries. Collaboration with countries that have more capacities and available resources in developing and continually updating an appropriate evidence base could be invaluable in preventing dupli-

cation of effort and allowing each country more time and resources to address country-specific issues [26]. For this reason, harmonized approaches are desirable. In sum, a global harmonized effort for scientific reviews for food selection guides should be encouraged.

Nevertheless, it is proposed that a single global food guide as dietary advice for the public should be discouraged, especially if it may be construed as an "authoritative nutrition education tool"—i.e., let the global Pyramid guide your food choices. A complex problem is only rarely solved with a simple solution. Based on experiences thus far, significant dietary changes for better health at a large-scale level can be achieved, but a strategic and comprehensive intervention is necessary. FBDGs can be a good complement in this process, it is believed, if they are designed as an *advisory* nutrition education tool together with a process that encourages all involved, especially related professionals [27], to take the necessary action to improve diet and nutrition within the overall strategy selected. While harmonized approaches might be desirable at the scientific review level, a process that would make it possible for the global community to come up with a consensus on unified food guides is unimaginable, not to mention a process necessary for communicating and implementing them to the global public.

Based on cumulative knowledge of how diet-related diseases can possibly contribute to socio-psycho-economic costs of the global community, countries, communities, families, and individuals, a global effort on nutrition and dietary change is urgently required. In the conceptual representation in **figure 2**, what is needed is a *global facilitation process* that empowers countries and communities to plan and implement culturally appropriate diet-related interventions. Scientific knowledge of nutrition and diet leading to recommendations should be evaluated in the context of the implementation. Moreover, transforming relevant diet and nutrition knowledge into good recommendations should involve not only the biological, medical, and environmental sciences, but also the social, economic, and political sciences. Most importantly, recommendations will turn into action only when stakeholders at the implementation level actively participate in the change process. Achieving public confidence requires partnerships among scientists, policymakers, community leaders, and consumers in both the development and the implementation process [28]. Thus, in terms of giving dietary advice to the public, diversified approaches should be considered rather than unified approaches. Diversity in communication and implementation with a shared purpose is proposed; for example, our global common purpose is to promote food for better health while each country or region is encouraged to use a contextual appropriate approach to offer dietary advice to a specific population. We suggest, for example, that the main message with global coverage be "food for better health"; based on this theme, specific contextually appropriate guidelines can be developed for specific country or regional settings. In summary, success in achieving dietary change will occur not because countries or communities have good or even the best FBDGs, but rather when key stakeholders in countries or communities take sustained action to improve diets and nutrition. To take effective action, knowledge-based food selection guides together with participatory development and implementation as well as culturally appropriate strategic communication are essential.

## Conclusions

The development of FBDGs has stimulated a greater understanding of the role of nutrients and foods in

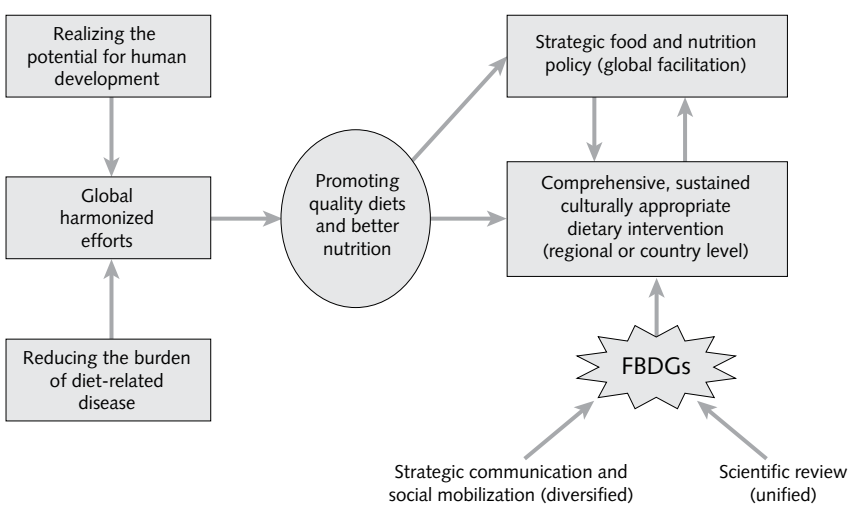


FIG. 2. Proposed framework for global harmonized efforts to promote food-based dietary guidelines

achieving optimal health. However, this valuable knowledge has not yet been translated into a significant improvement of human health and well-being. Harmonization of efforts is needed now to link knowledge and action and to reduce the burden of diet-related disease in both industrialized and developing countries. The food and nutrition community should be ready to move beyond offering recommendations and participate actively in the implementation of the knowledge gained. The possibility of defining one set of dietary guidelines is indeed attractive, considering the need for uniformity in the global village and the potential economic benefits to producers and perhaps to consumers of having a common regulatory framework.

Are unified guidelines desirable? Cultural and/or ethnic differences may determine the selection of population-specific foods to meet human nutritional needs. Yet these differences do not necessarily imply different dietary guidelines or different regulatory frameworks. The optimal diet need not be different from one population to the next. It is obviously unnecessary to have different nutrition labels of sodium and fat quality for different countries. The only justification for national- or ethnic-based dietary guidelines would be if there was a solid genetic basis for nutritional individuality. Present knowledge of the biological basis of nutritional needs, in most cases, does not support genetic or ethnic specific nutritional recommendations, especially if we restrict genetic differences to those of public health relevance.

Are unified guidelines achievable? The answer to this is that for some nutrients, universal guidelines are certainly possible. Dietary guidelines can certainly be harmonized following a unified methodologic approach to define them. However, there must be room to accommodate environmental variables that define nutritional and metabolic characteristics as well as the specific epidemiologic situation of a given society. Universal guidelines may be desirable, but they also present new problems and novel challenges. There is a clear need to ensure that guidelines respond to the ethnic specific, cultural, and social determinants of food choices.

Global guidelines will fail unless they provide the necessary options for individuals and societies to select the foods they prefer and combine them in the way that best suits their taste and other sensory preferences. A single unified global set of FBDGs will fail to address cultural diversity and the complex social, economic, and political interactions between humans and their food supply.

Globalization should promote diversity while sharing the common purpose (harmonization) of securing the right to food and providing a better future for humankind, especially the most vulnerable. Presently, there is a real need, in all countries and regions, to reduce the increased burden of diet-related disease and promote quality diets and better nutrition to realize the potential for human development. This requires determined global harmonized efforts, both in terms of sustained commitments and reasonable investments in support of key strategic dietary and health promoting interventions. The development and creative implementation of FBDGs, nationally or regionally, with a strong global scientific support and facilitation will definitely leverage the much-needed effectiveness of nutritional development around the world.

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# International harmonization of approaches for developing nutrient-based dietary standards: Reviewers

The guest editors would like to thank the following distinguished scholars for their willingness to review the manuscripts in this supplement and for their valuable time spent working on this project.

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