1. Concepts, definitions and approaches used to define nutritional needs and recommendations

1.1 Introduction

The dietary requirement for a micronutrient is defined as an intake level which meets a specified criteria for adequacy, thereby minimizing risk of nutrient deficit or excess. These criteria cover a gradient of biological effects related to a range of nutrient intakes which, at the extremes, include the intake required to prevent death associated with nutrient deficit or excess. However, for nutrients where insufficient data on mortality are available, which is the case for most micronutrients discussed in this report, other biological responses must be defined. These include clinical disease as determined by signs and symptoms of nutrient deficiency, and subclinical conditions identified by specific biochemical and functional measures. Measures of nutrient stores or critical tissue pools may also be used to determine nutrient adequacy.

Functional assays are presently the most relevant indices of subclinical conditions related to vitamin and mineral intakes. Ideally, these biomarkers should be sensitive to changes in nutritional state while at the same time be specific to the nutrient responsible for the subclinical deficiency. Often, the most sensitive indicators are not the most specific; for example, plasma ferritin, a sensitive indicator of iron status, may change not only in response to iron supply, but also as a result of acute infection or chronic inflammatory processes. Similarly anaemia, the defining marker of dietary iron deficiency, may also result from, among other things, deficiencies in folate, vitamin B_{12} or copper.

The choice of criteria used to define requirements is of critical importance, since the recommended nutrient intake to meet the defined requirement will clearly vary, depending, among other factors, on the criterion used to define nutrient adequacy (*1*, *2*, *3*). Unfortunately, the information base to scientifically support the definition of nutritional needs across age ranges, sex and physiologic states is limited for many nutrients. Where relevant and possible, requirement estimates presented here include an allowance for variations in micronutrient bioavailability and utilization. The use of nutrient balance to define requirements has been avoided whenever possible, since it is now generally recognized that balance can be reached over a wide range of nutrient intakes. However, requirement levels defined using nutrient balance have been used if no other suitable data are available.

1.2 Definition of terms

The following definitions relate to the micronutrient intake from food and water required to promote optimal health, that is, prevent vitamin and mineral deficiency and avoid the consequences of excess. Upper limits of nutrient intake are defined for specific vitamins and minerals where there is a potential problem with excess either from food or from food in combination with nutrient supplements.

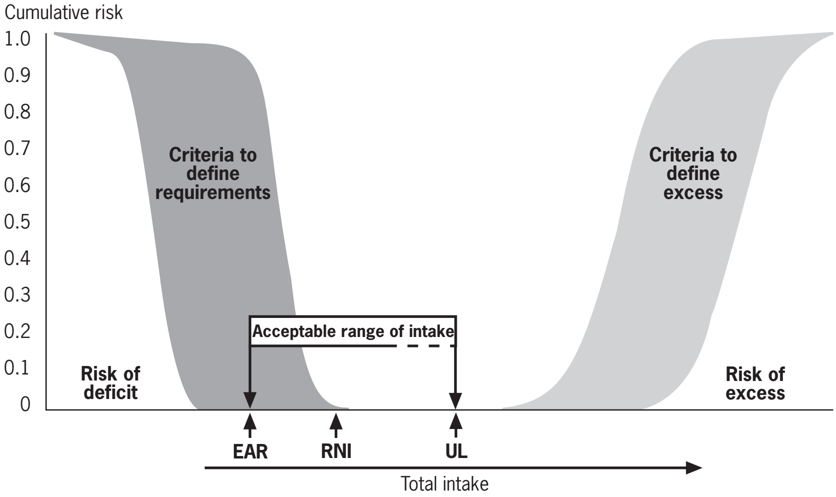
1.2.1 Estimated average requirement

Estimated average requirement (EAR) is the average daily nutrient intake level that meets the needs of 50% of the "healthy" individuals in a particular age and gender group. It is based on a given criteria of adequacy which will vary depending on the specified nutrient. Therefore, estimation of requirement starts by stating the criteria that will be used to define adequacy and then establishing the necessary corrections for physiological and dietary factors. Once a mean requirement value is obtained from a group of subjects, the nutrient intake is adjusted for interindividual variability to arrive at a recommendation (*4*, *5*, *6*).

1.2.2 Recommended nutrient intake

Recommended nutrient intake (RNI) is the daily intake, set at the EAR plus 2 standard deviations (SD), which meets the nutrient requirements of almost all apparently healthy individuals in an age- and sex-specific population group. If the distribution of requirement values is not known, a Gaussian or normal distribution can be assumed, and from this it is expected that the mean requirement plus 2 SD will cover the nutrient needs of 97.5% of the population. If the SD is not known, a value based on each nutrient's physiology can be used and in most cases a variation in the range of 10–12.5% can be assumed (exceptions are noted within relevant chapters). Because of the considerable daily variation in micronutrient intake, daily requirement refers to the average intake over a period of time. The cumulative risk function for deficiency and toxicity is defined in Figure 1.1, which illustrates that as nutrient intake increases the risk of deficit drops and at higher intakes the risk of toxicity increases. The definition of RNI used in this report is equivalent to that of the recommended dietary allowance (RDA) as used by the Food and Nutrition Board of the United States National Academy of Sciences (*4*, *5*, *6*).

FIGURE 1.1
Risk function of deficiency and excess for individuals in a population related to food intake, assuming a Gaussian distribution of requirements to prevent deficit and avoid excess



The shaded ranges correspond to different approaches to defining requirements to prevent deficit and excess, respectively. The estimated average requirement (EAR) is the average daily intake required to prevent deficit in half of the population. The recommended nutrient intake (RNI) is the amount necessary to meet the needs of most (97.5%) of the population, set as the EAR plus 2 standard deviations. The tolerable upper intake level (UL) is the level at which no evidence of toxicity is demonstrable.

1.2.3 Apparently healthy

The term, "apparently healthy" refers to the absence of disease based on clinical signs and symptoms of micronutrient deficiency or excess, and normal function as assessed by laboratory methods and physical evaluation.

1.2.4 Protective nutrient intake

The concept of protective nutrient intake has been introduced for some micronutrients to refer to an amount greater than the RNI which may be protective against a specified health or nutritional risk of public health relevance (e.g. vitamin C intake of 25 mg with each meal to enhance iron absorption and prevent anaemia) (*7*). When existing data provide justifiable differences between RNI values and protective intake levels comment to that effect is made in the appropriate chapter of this document. Protective intake levels are expressed either as a daily value or as an amount to be consumed with a meal.

1.2.5 Upper tolerable nutrient intake level

Upper limits (ULs) of nutrient intake have been set for some micronutrients and are defined as the maximum intake from food, water and supplements that is unlikely to pose risk of adverse health effects from excess in almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group (see Figure 1.1). ULs should be based on long-term exposure to all foods, including fortified food products. For most nutrients no adverse effects are anticipated when they are consumed as foods because their absorption and/or excretion are regulated. The special situation of consumption of nutritional supplements which, when added to the nutrient intake from food, may result in a total intake in excess of the UL is addressed for specific micronutrients in subsequent chapters, as appropriate. The ULs as presented here do not meet the strict definition of the "no observed effect level" (NOEL) used in health risk assessment by toxicologists because in most cases, a dose–response curve for risk from exposure to a nutrient will not be available (*8*). For additional details on derivation of ULs, please refer to standard texts on this subject (*9*, *10*).

The range of intakes between the RNI and UL should be considered sufficient to prevent deficiency while avoiding toxicity. If no UL can be derived from experimental or observational data in humans, the UL can be defined from available data on the range of observed dietary intake of apparently healthy populations. In the absence of known adverse effects a default value for the UL of 10 times the RNI is frequently used (*5*, *10*, *11*).

1.2.6 Nutrient excess

Traditional toxicology-based approaches to assessing adverse health effects from nutrient excess start by defining either the highest intake level at which no observed adverse effects of biological significance are found (i.e. the no observed adverse effect level (NOAEL)), or the lowest intake level at which adverse effects are observed (i.e. the lowest observed adverse effect level that are (LOAEL)). Uncertainty or modifying factors are then used to adjust a known NOAEL or LOAEL to define reference doses which represent chronic intake levels that are considered safe, or of no significant health risk, for humans. The nature of the adjustment used to modify the acceptable intake indicated by the NOAEL or LOAEL is based on the type and quality of the available data and its applicability to human populations (*5*, *9*, *11*).

Uncertainty factors are used in several circumstances: when the experimental data on toxicity is obtained from animal studies; when the data from humans are insufficient to fully account for variability of populations or special sensitivity subgroups of the general population; when the NOAEL has been obtained in studies of insufficient duration to assure chronic safety; when the database which supports the NOAEL is incomplete; or when the experimental data provide a LOAEL instead of a true NOAEL. The usual value for each uncertainty factor is 10, leading to a 10-fold reduction in the acceptable intake level for each of the considerations listed above. The reductions may be used in isolation or in combination depending on the specific micronutrient being assessed.

Modifying factors are additional uncertainty factors which have a value of 1 or more but less than 10, and are based on expert judgement of the overall quality of the data available. Given the paucity of human data, the limitations of animal models and uncertainties of interpretation, the traditional toxicological approach to determining limits for intake, as summarized here, may in fact lead to the definition of intakes which promote or even induce deficiency if followed by a population. This has recently been recognized by the WHO International Programme on Chemical Safety, and a special risk assessment model has been derived for elements that are both essential and have potential toxicity (*5*, *9*).

1.2.7 Use of nutrient intake recommendations in population assessment

Recommendations given in this report are generally presented as population RNIs with a corresponding UL where appropriate. They are not intended to define the daily requirements of an individual. However "healthy" individuals consuming within the range of the RNI and the UL can expect to minimize their risk of micronutrient deficit and excess. Health professionals caring for special population groups that do not meet the defined characterization of "healthy" should, where possible, adjust these nutrient-based recommendations to the special needs imposed by disease conditions and/or environmental situations.

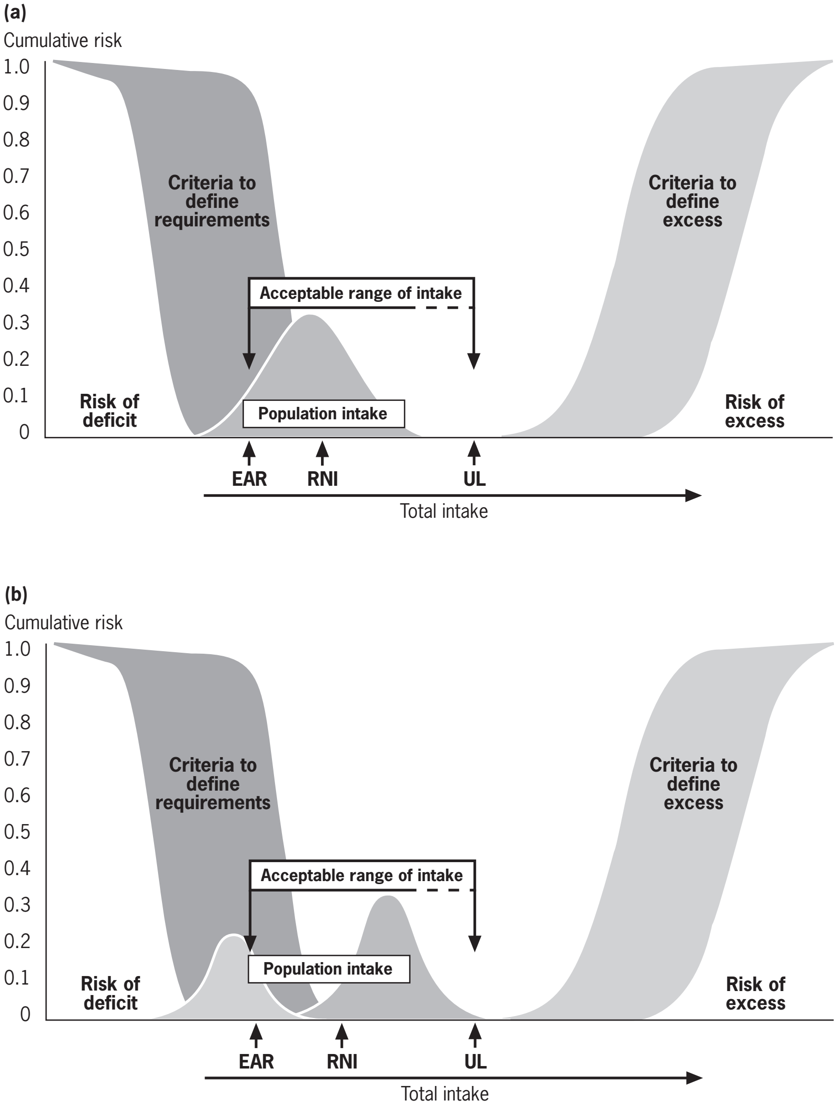
The use of dietary recommendations in assessing the adequacy of nutrient intakes of populations requires good quantitative information about the distribution of usual nutrient intakes as well as knowledge of the distribution of requirements. The assessment of intake should include all sources of intake, that is, food, water and supplements; appropriate dietary and food composition data are thus essential to achieve a valid estimate of intakes. The day-to-day variation in individual intake can be minimized by collecting intake data over several days. There are several statistical approaches that can be used to estimate the prevalence of inadequate intakes from the distribution of intakes and requirements. One such approach the EAR cut-point method which defines the fraction of a population that consumes less than the EAR for a given nutrient. It assumes that the variability of individual intakes is at least as large as the variability in requirements and that the distribution of intakes and requirements are independent of each other. The latter is most likely to be true in the case of vitamins and minerals, but clearly not for energy. The EAR cut-point method requires a single population with a symmetrical distribution around the mean. If these conditions are met, the prevalence of inadequate intakes corresponds to the proportion of intakes that fall below the EAR. It is clearly inappropriate to examine mean values of population intake and RNI to define the population at risk of inadequacy. The relevant information is the proportion of intakes in a population group that is below the EAR, not below the RNI (*4*, *5*).

Figure 1.2 serves to illustrate the use of nutrient intake recommendations in risk assessment considering the model presented in Figure 1.1; the distributions of nutrient intakes for a population have been added to explore risk of excess or deficit (*2*, *4*, *5*). Figure 1.2a presents the case of a single population with intakes ranging from below the EAR to the UL with a mean intake close to the RNI. The fraction of the population that is below the EAR represents the prevalence of deficit; as depicted in the figure this is a sizeable group despite the fact that the mean intake for the population is close to the RNI. Figure 1.2b presents the case of a bimodal distribution of population intakes where the conditions to use the EAR cut-point method are not met. In this case it is clear that a targeted intervention to increase the intake of one group but not the other is needed. For example, if we examine the iron intake of a population we may find that vegetarians may be well below the recommended intake while those who consume meat may be getting sufficient iron. To achieve adequacy in this case we need to increase iron intake in the former but not the latter group (*2*, *12*).

1.3 Approaches used in estimating nutrient intakes for optimal health

The methods used to estimate nutritional requirements have changed over time. Four currently used approaches are briefly outlined below: the clinical approach, nutrient balance, functional indicators of nutritional sufficiency (biochemical, physiological, molecular), and optimal nutrient intake. A detailed analysis of the relative merits of these approaches is beyond the scope of this chapter, but additional information on each can be found in subsequent chapters of this report. When no information is available the default approach to define a recommended intake based on the range of observed intakes of "healthy" populations is used.

FIGURE 1.2
Distribution of population intakes and risk of deficit and excess



a) Examines the risk of inadequacy for a given distribution of intakes as shown by the shaded bell-shaped area. In this example, the proportion of individuals that have intakes below the EAR are at risk of deficiency (see text for details).
b) Illustrates the need to examine whether there is more than one group within the population distribution of intakes. In this case, the overall mean intake is above the RNI, suggesting a low risk of deficit. However, while a large proportion of the population (represented by the right-hand bell-shaped area) is over the RNI, there is in fact a significant proportion of the population (represented by the left-hand bell-shaped area) below the EAR, and thus at risk of deficiency. The intervention here should be targeted to increase the intake for the group on the left but not for the one on the right; the right-hand group may exceed the UL and be at risk for excess if their intake is increased.

1.3.1 The clinical approach

The traditional criteria to define essentiality of nutrients for human health require that a) a disease state, or functional or structural abnormality is present if the nutrient is absent or deficient in the diet and, b) that the abnormalities are related to, or a consequence of, specific biochemical or functional changes that can be reversed by the presence of the essential dietary component. Endpoints considered in recent investigations of essentiality of nutrients in experimental animals and humans include: reductions in ponderal or linear growth rates, altered body composition, compromised host defense systems, impairment of gastrointestinal or immune function, abnormal cognitive performance, increased susceptibility to disease, increased morbidity and changes in biochemical measures of nutrient status. To establish such criteria for particular vitamins and minerals requires a solid understanding of the biological effects of specific nutrients, as well as sensitive instrumentation to measure the effects, and a full and precise knowledge of the amount and chemical form of nutrients supplied by various foods and their interactions (*2*, *12*).

1.3.2 Nutrient balance

Nutrient balance calculations typically involve assessing input and output and establishing requirement at the point of equilibrium (except in the case of childhood, pregnancy and lactation where the additional needs for growth, tissue deposition and milk secretion are considered). However, in most cases, balance based on input–output measurements is greatly influenced by prior level of intake, that is, subjects adjust to high intakes by increasing output and, conversely, they lower output when intake is low. Thus, if sufficient time is provided to accommodate to a given level of intake, balance can be achieved, and for this reason, the exclusive use of nutrient balance to define requirements should be avoided whenever possible (*1*, *5*, *13*).

In the absence of alternative sources of data, a starting point in defining nutritional requirements using the balance methodology is the use of factorial estimates of nutritional need. The "factorial model" is based on measuring the components that must be replaced when the intake of a specific nutrient is minimal or nil. This is the minimum possible requirement value and encompasses a) replacement of losses from excretion and utilization at low or no intake, b) the need to maintain body stores and, c) an intake that is usually sufficient to prevent clinical deficiency (*6*). Factorial methods should be used only as a first approximation for the assessment of individual requirements, or when functional clinical or biochemical criteria of adequacy have not been established. Furthermore, although nutrient balance studies may be of help in defining mineral needs, they are of little use for defining vitamin requirements (*14*, *15*). This is because the carbon dioxide formed on the oxidation of vitamins is lost in expired air or hard to quantify, since it becomes part of the body pool and cannot be traced to its origin unless the vitamin is provided in an isotopically labelled form (*15*).

1.3.3 Functional responses

Various biomarkers are presently being evaluated for their specificity and sensitivity to assess nutrient-related organ function and thus predict deficiency or toxicity.

In terms of defining nutrient needs for optimal function, recent efforts have focused on the assessment of:

- *Neurodevelopment*: monitoring electro-physiologic responses to defined sensory stimuli; sleep–wake cycle organization; and neurobehavioural tests (*16*, *17*, *18*).
- *Bone health*: measuring bone mineral density by X-ray absorptiometry; markers of collagen synthesis and turnover; and hormonal responses associated with bone anabolism and catabolism (*19*, *20*).
- *Biochemical normalcy*: measuring plasma and tissue concentrations of substrates or nutrient responsive enzymes, hormones or other indices of anabolic and catabolic activity; and plasma concentrations and tissue retention in response to a fixed nutrient load (*21*, *22*).
- *Immune function*: measuring humoral and cellular response to antigens and mitogens in vitro or in vivo; antibody response to weak antigens such as immunizations; T-cell populations; cytokine responses; and mediators of inflammation related to tissue protection and damage (*23*, *24*).
- *Body composition and tissue metabolic status*: using stable isotope assessment of body compartments (e.g. body water, lean and fat mass); radiation-determined body compartments measured by dual energy X-ray absorptiometry (DEXA) and computerized tomography; electrical impedance and conductivity to determine body compartments; and finally, magnetic resonance imaging and spectroscopy of body and organ compartments (i.e. brain and muscle high energy phosphate content) (*25*, *26*).
- *Bioavailability*: evaluating stable and radioactive isotopes of mineral and vitamin absorption and utilization (*7*, *27*).
- *Gene expression*: assessing the expression of multiple human mRNA with specific fluorescent cDNAs probes (which currently evaluate from 10 000–15 000 genes at a time and will soon be able to assess the expression of the full genome); and laser detection of hybridized genes to reveal mRNA abundance in relation to a given nutrient intake level. These novel

tools provide a powerful means of assessing the amount of nutrient required to trigger a specific mRNA response in a given tissue. These are in fact the best criteria for defining selenium needs without having to measure the key selenium dependent enzymes (i.e. liver or red blood cell glutathione peroxidase [GSHPx]) (*28*). In this case the measurement of sufficiency is based on the GSHPx–mRNA response to selenium supply rather than measuring the enzymatic activity of the corresponding protein. Micro-array systems tailored to evaluate nutrient modulated expression of key genes may become the most effective way of assessing human nutritional requirements in the future (*29*).

1.3.4 Optimal intake

Optimal intake is a relatively new approach to deriving nutrient requirements. The question "Optimal intake for what?" is usually answered with the suggestion that a balanced diet or specific nutrients can improve physical and mental performance, enhance immunity, prevent cancer, or add healthy years to our life. This response is unfortunately often used too generally, and is usually unsupported by appropriate population-based controlled randomized studies (*15*). The preferred approach to define optimal intake is to clearly establish the function of interest and the level of desired function (*30*). The selected function should be related in a plausible manner to the specific nutrient or food and serve to promote health or prevent disease.

If there is insufficient information from which to derive recommendations based on actual data using any of the approaches described above, the customary intake (based on an appropriate knowledge of food composition and food consumption) of healthy populations becomes a reasonable default approach. Indeed, the presently recommended nutrient intakes for term infants of several vitamins and minerals are based on this paradigm. Thus, the nutrient intake of the breast-fed infant becomes the relevant criteria since it is assumed that human milk is the optimal food for human growth and development. In this case, all other criteria are subservient to the estimate obtained from assessment of the range of documented intake observed in the full term breast-fed infant. Precise knowledge of human milk composition and volume of intake for postnatal age allows for the definition of the range of intakes typical for breast-fed infants. A notable exception, however, is the requirement for vitamin K at birth, since breast milk contains little vitamin K, and the sterile colon does not provide the vitamin K formed by colonic microorganisms.

Planners using RNIs are often faced with different, sometimes conflicting numbers, recommended by respectable national scientific bodies that have used varying approaches to define them (*31*, *32*). In order to select the most appropriate for a given population, national planners should consider the information base and the criteria that led to the numerical derivation before determining which correspond more closely with the setting for which the food-based dietary guidelines are intended. The quantified RNI estimates derived from these various approaches may differ for one or more specific nutrients, but the effect of these numeric differences in establishing food-based dietary guidelines for the general population is often of a lesser significance (*2*, *12*, *33*). Selected examples of how various criteria are used to define numerical estimates of nutritional requirements are given below. More detail is provided in the respective chapters on individual micronutrients that follow.

Calcium

Adequate calcium intake levels suggested for the United States of America are higher than those accepted internationally, and extend the increased needs of adolescents to young adults (i.e. those aged < 24 years) on the basis of evidence that peak bone mass continues to increase until that age is reached (see Chapter 4). Results of bone density measurements support the need for calcium intake beyond that required for calcium balance and retention for growth. However, the situation in most Asian countries suggests that their populations may have sufficient calcium retention and bone mass despite lower levels of intake. This report acknowledges these differences and suggests that calcium intake may need to be adjusted for dietary factors (e.g. observed animal protein, sodium intake, vitamin D intake) and for sun exposure (which is related to geographic location/latitude, air pollution and other environmental conditions), since both affect calcium retention.

Iron

In the case of iron, the differences in quantification of obligatory losses made by various expert groups is possibly explained by differences in environmental sanitation and the prevalence of diarrhoea (*34*). In addition, the concern about iron excess may be greater in places where anaemia is no longer an issue, such as in northern Europe, while in other areas iron deficiency is of paramount significance. The use of different bioavailability adjustment factors in the definition of iron RNIs is a useful concept because the presence of dietary components that affect bioavailability differs between and within a given ecological setting. The present Expert Consultation established a recommendation based on absorbed iron; the RNI thus varies according to the bioavailability of iron in the diet. Recommended RNIs are provided for four bioavailability factors, 5%, 10%, 12% and 15%, depending on the composition of the typical local diet (see Chapter 13).

Folate

Food fortification or supplementation strategies will commonly be needed to satisfy the 400 μg/day folate recommended for adolescents and adults in this report (based on the intake required before conception and during early pregnancy to prevent neural tube defects) (*35*). Consumption from traditional food sources is not sufficient to meet this goal; however, food fortification and the advent of novel foods developed by traditional breeding or by genetic modification may eventually make it possible to meet the RNI with food-based approaches.

1.4 Conclusions

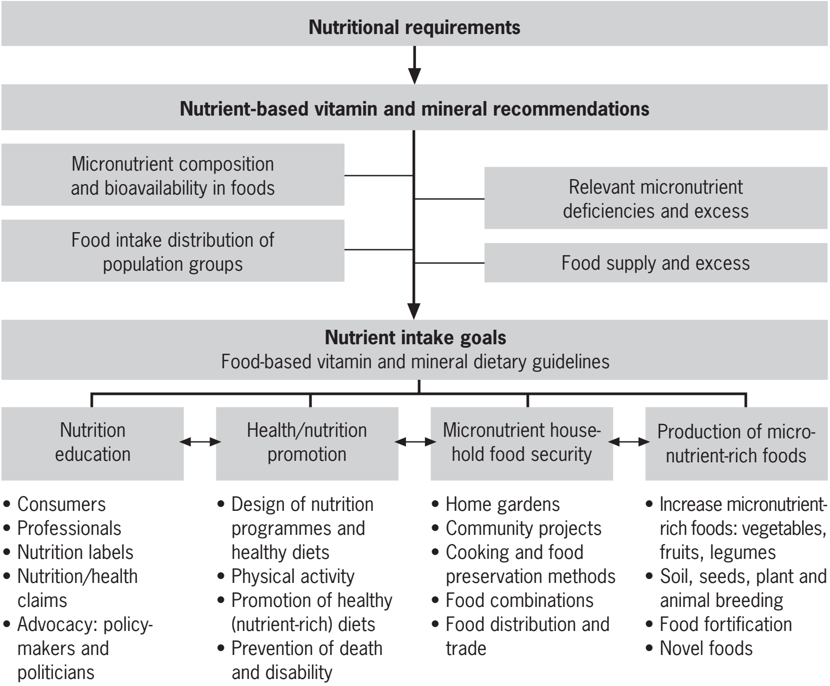
The quantitative definition of nutrient needs and their expression as recommended nutrient intakes have been important components of food and nutrition policy and programme implementation. RNIs provide the firm scientific basis necessary to satisfy the requirements of a group of healthy individuals and define adequacy of diets. Yet, by themselves, they are not sufficient as instruments of nutrition policy and programmes. In fact, single nutrient-based approaches have been of limited use in the establishment of nutritional and dietary priorities consistent with broad public health interests at the national and international levels (*36*).

In contrast to RNIs, food-based dietary guidelines (FBDGs) as instruments of policy are more closely linked to diet–health relationships of relevance to a particular country or region (*12*). FBDGs provide a broad perspective that examines the totality of the effects of a given dietary pattern in a given ecological setting, considering socioeconomic and cultural factors, and the biological and physical environment, all of which affect the health and nutrition of a given population or community (*2*, *5*). Defining the relevant public health problems related to diet is an essential first step in developing nutrient intake goals in order to promote overall health and reduce health risks in view of the multifactorial nature of disease. Thus, FBDGs take into account the customary dietary pattern, the foods available, and the factors that determine the consumption of foods and indicate what aspects should be modified.

By utilizing the two approaches of FBDGs and RNIs, broad public health interests are supported by the use of empirically defined nutrient requirements. The role of RNIs in the development and formulation of FBDGs is summarized in Figure 1.3. The multiple final users and applications of these

FIGURE 1.3

Schematic representation of the process of applying nutritional requirements and recommendations in the definition of nutrient intake goals leading to the formulation of food-based dietary guidelines



The boxes at the bottom of the scheme exemplify the multiple final users of this knowledge and the implications for policy and programmes.

concepts are exemplified in the lower part of the scheme. Nutrition education, health and nutrition promotion, household food security and the production of micronutrient-rich foods all require nutritional requirements based on the best available scientific information. As the science base for nutrition evolves, so too will the estimates of nutritional requirements, which, when combined with FBDGs, will lead to greater accuracy with respect to applications and policy-making and will enhance the health of final users.

We have gone beyond the era of requirements to prevent deficiency and excess to the present goal of preserving micronutrient-related functions. The next step in this evolution will surely be the incorporation of the knowledge and necessary tools to assess genetic diversity in the redefinition of nutritional requirements for optimal health throughout the life course. The goal in this case will be to meet the nutritional needs of population groups, while accounting for genetic heterogeneity within populations (*37*). Though this may lead to the apparent contradiction of attempting to meet the requirements of populations based on the diverse and heterogeneous needs of individuals, it is in fact, a necessary step in providing optimal health—a long life, free of physical and mental disability—to all individuals.

References

1. Young VR. W.O. Atwater Memorial Lecture and the 2001 ASNS President's Lecture. Human nutrient requirements: the challenge of the post-genome era. *Journal of Nutrition*, 2002, 132:621–629.
2. Uauy R, Hertrampf E. Food-based dietary recommendations: possibilities and limitations. In: Bowman B, Russell R, eds. *Present knowledge in nutrition*, 8th ed. Washington, DC, International Life Sciences Institute Press, 2001:636–649.
3. Aggett PJ et al. Recommended dietary allowances (RDAs), recommended dietary intakes (RDIs), recommended nutrient intakes (RNIs), and population reference intakes (PRIs) are not "recommended intakes". *Journal of Pediatric and Gastroenterology Nutrition*, 1997, 25:236–241.
4. Food and Nutrition Board. *Dietary reference intakes: applications in dietary assessment*. Washington, DC, National Academy Press, 2001.
5. *Trace elements in human nutrition and health*. Geneva, World Health Organization, 1996.
6. *Energy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation.* Geneva, World Health Organization, 1985 (WHO Technical Report Series, No. 724; http://whqlibdoc.who.int/trs/WHO_TRS_724_(chp1--chp6).pdf, accessed 26 June 2004; http://whqlibdoc.who.int/trs/WHO_TRS_724_(chp7–chp13).pdf, accessed 26 June 2004).
7. Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *American Journal of Clinical Nutrition*, 2001, 73:93–98.
8. Olivares M, Araya M, Uauy R. Copper homeostasis in infant nutrition: deficit and excess. *Journal of Pediatric and Gastroenterology Nutrition*, 2000, 31: 102–111.
9. *Principles and methods for the assessment of risk from essential trace elements*. Geneva, World Health Organization, 2002 (Environmental Health Criteria, No. 228).
10. Food and Nutrition Board. *Dietary reference intakes. A risk assessment model for establishing upper intake levels for nutrients.* Washington, DC, National Academy Press, 1999.
11. *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits.* Geneva, World Health Organization, 1994 (Environmental Health Criteria, No. 170).
12. *Preparation and use of food-based dietary guidelines. Report of a Joint FAO/WHO Consultation.* Geneva, World Health Organization, 1996 (WHO Technical Report Series, No. 880).
13. Hegsted M, Linkswiler HM. Long-term effects of level of protein intake on calcium metabolism in young adult women. *Journal of Nutrition*, 1981, 111:244–251.
14. Food and Nutrition Board. *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington, DC, National Academy Press, 2002.

15. Food and Nutrition Board. *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids.* Washington, DC, National Academy Press, 2000.
16. Fenstrom J, Uauy R, Arroyo P, eds. *Nutrition and brain*. Basel, Karger AG, 2001.
17. Lozoff B. Perinatal iron deficiency and the developing brain. *Pediatric Research*, 2000, 48:137–139.
18. Carlson SE, Neuringer M. Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. *Lipids*, 1999, 34:171–178.
19. Flohr F et al. Bone mineral density and quantitative ultrasound in adults with cystic fibrosis. *European Journal of Endocrinology*, 2002, 146:531–536.
20. Black AJ et al. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. *Journal of Bone and Mineral Research*, 2000, 15:557–563.
21. Prohaska JR, Brokate B. Lower copper, zinc-superoxide dismutase protein but not mRNA in organs of copper-deficient rats. *Archives of Biochemistry and Biophysics*, 2001, 393:170–176.
22. Mize CE et al. Effect of phosphorus supply on mineral balance at high calcium intakes in very low birth weight infants. *American Journal of Clinical Nutrition*, 1995, 62:385–391.
23. Chandra RK. Nutrition and the immune system from birth to old age. *European Journal of Clinical Nutrition*, 2002, 56(Suppl. 3):S73–S76.
24. Sandstrom B et al. Acrodermatitis enteropathica, zinc metabolism, copper status, and immune function. *Archives of Pediatrics and Adolescent Medicine*, 1994, 148:980–985.
25. Bertocci LA, Mize CE, Uauy R. Muscle phosphorus energy state in very-low-birth-weight infants: effect of exercise. *American Journal of Physiology,* 1992, 262:E289–E294.
26. Mayfield SR, Uauy R, Waidelich D. Body composition of low-birth-weight infants determined by using bioelectrical resistance and reactance. *American Journal of Clinical Nutrition,* 1991, 54:296–303.
27. Lonnerdal B. Bioavailability of copper. *American Journal of Clinical Nutrition,* 1996, 63(Suppl.):S821–S829.
28. Weiss Sachdev S, Sunde RA. Selenium regulation of transcript abundance and translational efficiency of glutathione peroxidase-1 and -4 in rat liver. *Biochemical Journal*, 2001, 357:851–858.
29. Endo Y et al. Dietary protein quantity and quality affect rat hepatic gene expression. *Journal of Nutrition*, 2002, 132:3632–3637.
30. Koletzko B et al. Growth, development and differentiation: a functional food science approach. *British Journal of Nutrition*, 1998, 80(Suppl. 1):S5–S45.
31. Howson CP, Kennedy ET, Horwitz A, eds. *Prevention of micronutrient deficiencies. Tools for policymakers and public health workers*. Washington, DC, National Academy Press, 1998.
32. *Preventing iron deficiency in women and children: technical consensus on key issues*. Boston, The International Nutrition Foundation, and Ottawa, The Micronutrient Initiative, 1999 (http://www.micronutrient.org/resources/publications/nvironbk.pdf, accessed 24 June 2004).
33. *Nutrition and your health: dietary guidelines for Americans*, 5th ed. Washington, DC, United States Department of Health and Human Services, and

United States Department of Agriculture, 2000 (http://www.health.gov/dietaryguidelines/dga2000/document/frontcover.htm, accessed 24 June 2004).
34. Albonico M et al. Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* or *Necator americanus*, on iron status of children. *International Journal of Epidemiology*, 1998, 27:530–537.
35. Oakley GP, Adams MJ, Dickinson CM. More folic acid for everyone, now. *Journal of Nutrition*, 1996, 126(Suppl.):S751–S755.
36. *International Conference on Nutrition. World declaration and plan of action for nutrition, 1992*. Rome, Food and Agriculture Organization of the United Nations, 1992.
37. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. *American Journal of Clinical Nutrition*, 2002, 75:616–658.