Population reference intakes and micronutrient bioavailability: a European perspective

Peter J Aggett

ABSTRACT
The processes of setting population reference intakes in the European Union and elsewhere have highlighted the paucity of data for informing the assessments and the need to take the opportunity to establish transparent, physiologically based approaches to setting reference values for safe and adequate intakes, including considerations of excess exposures. The confusion arising from the European exercise contributed to a number of initiatives to rationalize approaches to setting reference levels and safe upper levels of intake. A biologically based approach to nutrient risk assessment, which has many features that could be extended advantageously to the creation of a similar approach to setting nutrient reference values, has been proposed. This approach has yet to be explored, but an additional product of the earlier confusion has been the development of proposals for the international harmonization of approaches to setting nutrient-based dietary standards that could lead to internationally agreed-upon standards for nutrient risk assessment and for setting key intake values. Am J Clin Nutr 2010;91(suppl):1433S–7S.

INTRODUCTION
This article reflects on the experience of setting reference values within the European Union (1) and illustrates the confusion and concerns experienced by many of those involved. It discusses how attitudes toward establishing reference values have evolved to reach an appreciation of the need to adopt a harmonized international approach to setting reference values in which the data are used critically and in which the limitations of the assessments are explicit. An important element of the interpretation and translation of core data into reference values is the intelligent use of the many factors that are the key components that are collectively incorporated into the concept of bioavailability and then used in the derivation of the particular value of the bioavailability factor, which is used in translating a physiologic requirement into a dietary requirement. These aspects are not always presented fully and transparently, even though they are a major source of uncertainty in the assessment of nutrient requirements, particularly micronutrient requirements and reference values. However, understanding the uncertainties and variabilities of the components of bioavailability, rather than the overall bioavailability, is integral to the process of assessing reference values and to developing a biological or physiologic approach to the identification of safe upper limits of exposure to micronutrients in nutrient risk assessment. It is possible to draw on this experience to envisage a systematic approach for determining reference values and limits across the continuum of assessing inadequate, adequate, and excessive intakes of micronutrients.

The report of the Scientific Committee for Food on Nutrient and Energy Intakes for the European Community (1) followed a number of similar exercises that had been conducted in the United States, Canada, The Netherlands, Germany and the German-speaking communities, France, the United Kingdom, Australia, New Zealand, and the Nordic countries and with collaboration between the World Health Organization (WHO), the International Atomic Energy Agency, and the Food and Agriculture Organization (FAO) (2). Each committee produced its own terminology, and although their definitions differed only marginally, the multiplicity of terms was a source of confusion among regulators, policy makers, planners, and health and dietary practitioners alike (3, 4). In addition, the intended application of the reference values varied, as did the uses to which they had been put. These uses include guidance values for food labeling, assessing dietary adequacy, assessing the adequacy of intakes by individuals and populations, and determining “nutritional status.”

The core data used by the various panels were essentially the same for all of these exercises (2, 4, 5). From these data the committees estimated a physiologic requirement that was then adjusted by a factor (the bioavailability factor) to provide a value that served as an average requirement. From this value, in turn, a lower level of intake at which there was considered to be a significant risk of deficiency and a higher level of intake that would be reasonably expected to meet the needs of a defined majority of the population was set; usually this was done on the assumption of a normal Gaussian distribution of dietary requirements. Although this process was acknowledged to be rather arbitrary, it may be the best available approach because of the lack of quantitative information to enable assessors to consider systemic and intestinal adaptation in determining the distribution of dietary requirements. These adaptive features are in

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3 Supported by the International Life Sciences Institute Europe Addition of Nutrients to Food Task Force.
4 Address correspondence to PJ Aggett, Littlefield, Parbold Hill, Parbold WN8 7TG, United Kingdom. E-mail: profpjaaggett@aol.com.
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fact components of bioavailability (see below), and therefore, in addition to using a “bioavailability factor” in determining a mean or average requirement, the adjustment for allowing for the distribution of population requirements may actually involve a further “bioavailability factor.” As well as setting reference values for requirements, some committees advised on a level of intake above which there was a significant risk of adverse effects or toxicity, and the diversity of this advice has prompted separate exercises to derive upper levels. These exercises used toxicologic risk assessment processes that produced values that were not necessarily consonant with the nutritional reference values (6–8).

THE USE OF BIOAVAILABILITY

Bioavailability is defined as “the efficiency with which a dietary component is used systemically through normal metabolic pathways.” It is expressed as a percentage of intakes and is known to be influenced by dietary and host factors. Thus the bioavailability factor represents the complete systemic fate of the nutrient, which includes the following: the release of the nutrient from the physiocochemical dietary matrix; the effects of luminal and mucosal digestion; binding and uptake by the intestinal mucosa; transfer across the gut mucosa (transcellular or paracellular pathways or both) to the portal, systemic, or lymphatic circulation; systemic distribution; systemic deposition (stores or sequestration); metabolic and functional use; and excretion (renal, hepatobiliary, or intestinal).

It is not easy to find many true measurements of bioavailability because it is difficult to measure. Few studies of bioavailability actually measure the totality of the components listed above, and a realization of this should warn us about the uncritical use of the concept. Therefore, in estimating reference values, mistaken assumptions about what “bioavailability” represents and the inappropriate use of poorly substantiated values are appreciable sources of uncertainty (3, 4).

More often than not the bioavailability factor that is being used to derive an average requirement from a physiologic requirement is actually a measure of bioefficacy, in that it expresses the efficiency with which ingested nutrients or their active components are absorbed, thereby reflecting only the dietary and intestinal components of bioavailability. This use of bioefficacy may or may not be a problem because it could be argued that this is really what is needed at this stage of assessment and that other components of bioavailability are more relevant to assessing the physiologic requirement or the confidence limits of the estimated values. Another issue is that application of a standard bioavailability factor may not allow for physiologic adaptations—eg, the expected increased uptake and transfer or systemic retention of nutrients when there is an increased demand for them during growth, pregnancy, and lactation and when systemic adaptations occur in response to low dietary intakes. The European population reference intakes uniquely tried to allow for this by assuming a higher bioavailability for some micronutrients during pregnancy and lactation and, in the case of zinc, by assuming a higher bioavailability at low intakes than was applied at what were presumed to be adequate intakes. On reflection, this was possibly a double application of a bioavailability factor, neither value of which was well substantiated: the first was used to derive an average requirement and the second was used on top of the first to derive the lower threshold intake.

Although there was an appreciation of the lack of appropriate data for the systematic derivation of reference values, panels did not necessarily have an effective way either of dealing with the lack of knowledge or of communicating the associated uncertainties and limitations to the intended users. There was a lack of a framework against which knowledge gaps could be identified, shown, and prioritized for filling. Not least, this applied to the components of bioavailability.

There are many other sources of uncertainty and variability in setting reference values. Sources of variability include interindividual differences arising from adaptation, polymorphisms, programming, age, sex, physiologic maturation, and sources of uncertainty reflect insecurity arising from imprecision in assessing diet, exposure, systemic depots, and bioavailability, as well as in the measurements and methodologies, and particularly in the extrapolations of values from one population group to another—eg, from adults to children or from men to women. Few, if any, of the assessments included clear descriptions of the uncertainties, variabilities, and assumptions involved in their considerations. Consequently, the subsequent recommendations give a false sense of security. Future assessments might be able to reduce any misplaced confidence in their derived values by developing a template based on the assessment process on which the sources of uncertainty and variability can be provided transparently.

SUMMARIZING CONFUSION

As has been said already, the many panels named their values differently, and these names have distinct connotations: “recommendation,” “reference,” “daily,” “dietary,” “allowances,” “amounts,” and “intakes,” for example, have different meanings and combinations that produce acronyms such as RNI (Reference Nutrient Intake), RDA (Recommended Dietary Allowance), or DRV (Dietary Reference Value) compound potential confusion (3, 4). It was a disappointment to those who drafted the commentary of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition Committee (4), from which much of this Introduction section is abstracted, that they were prevented from stating in their paper that “RDAs should not be confused with RDAs, nor with RDAs and vice versa,” although we did manage to slip in a similar comment about DRVs and DRVs (4).

The sentiment is obvious. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition Committee, of whom several members had been involved with setting the PRIs, and their own national recommendations observed (4) that:

...calculating dietary reference requirements is a difficult exercise. The estimated values are approximations and are often devised for safety rather than for preciseness. They reflect the often limited data available, and the need for an inductive extrapolation of data from small groups of individuals to large, heterogeneous populations. This is unreliable because there is no information on the population distribution of requirements, which is assumed to be Gaussian but is in reality probably skewed and influenced considerably by homeostatic mechanisms. The influence of such factors as systemic adaptation, polygenetic inheritance, and cultural culinary practices on the bioavailability and requirements and nutrients should be better characterized. It is hoped that better understanding of the cellular and
molecular basis of intestinal and systemic metabolic adaptation will help to clarify this area.

Reference intakes (eg, PRIs) better reflect their intended use than recommended intakes or allowances and remind us that these statistical terms require caution in their use. They are designed for populations rather than for individuals; and although they provide a means of assessing the probability that members of the population are vulnerable to nutrient deficiency, reference values cannot be used for the diagnosis of nutrient deficiency in individuals.

The validity of reference values can be established only by long-term monitoring of the effects of outcomes of nutrient and energy intakes. This effort will require international collaboration and pooling the data and could be achieved by concomitant standardisation of nomenclature both for reference values and their use for food labelling. Initiatives to achieve such standardisation would be welcome. Despite these limitations, current reference values are an invaluable source of information for education programs and nutritional strategies for populations.

The final conclusion was to endorse Ruth Leverton’s observation that “RDAs are not for amateurs,” and the authors suggested that they may not be for professionals either (4).

The extracts above summarize a European perspective for setting core dietary reference values, but in parallel another problem has been emerging: the risk assessment of high exposure to micronutrients. Regulators needed to address potentially excessive exposure to micronutrient supplements, as well as set guidelines for the fortification of food products. It was accepted that such upper limits should be based on principles of safety rather than on arbitrary values based on further extrapolations from existing reference values. However, difficulties were then experienced because the safe upper levels or (toxicologic) reference doses derived by toxicologic risk assessment left limited margins of safety when balanced with the estimates of required dietary intakes recommended by nutritionists.

RATIONALIZATION AND HARMONIZATION

Biologically based model (acceptable range of intakes)

In essence, we needed a system that would enable a balanced risk assessment for intakes of essential nutrients across the spectrum of inadequacy through adequacy to potentially and frankly toxic exposures and that would address the accompanying spectrum of harm from inadequacy, benefit (or absence of harm) from adequate intake, and harm from excess intake. This spectrum was taken up by the International Program on Chemical Safety (IPCS) Environmental Health Criteria program, which established interdisciplinary groups to consider the “Principles and Methods for the Assessment of Risk from Essential Trace Elements” (6). Thus toxicologists and nutritionists were brought together to explore the concept of safe ranges of intakes. This Acceptable Range of Oral Intake (AROI) for essential trace elements is bracketed between, at lower intakes, the distribution of potential deficiencies, and, at higher intakes, the distribution of possible features of toxicity. This is the classic “U-shaped” curve of population and individual responses to low and high exposures; however, it was appreciated that in reality the shape of the curve would be influenced by a number of dietary and host factors and was unlikely to be truly U-shaped. The AROI lies at the base of this “U” and current reference values, and the safe upper levels of intakes would be expected to lie somewhere within that range. The factors influencing the shape of the curve at low, adequate, and high intakes are, of course, the components of bioavailability.

The Environmental Health Criteria group explored how existing data from high and low intakes could be applied to a biologically based template to assess the risk from essential trace elements: the principles involved, however, could be applied to any nutrient. They explored the spectrum of population or individual responses to a range of exposures to essential trace elements. This spectrum is summarized in Table 1, which shows the dose responses for reducing and increasing intakes passing through inadequate, adequate, and excess intakes, progressing through early adaptation to more ineffective adaptation marked by functional and structural disruption, to functional impairment, gross clinical effects, and death at both extremes. The physiologic and pathophysiologic stages and phenomena are balanced and provide a continuum; however, the shape of the dose–response spectrum is influenced by the host and dietary elements of bioavailability. However, homeostasis and bioavailability factors are inoperative at the extremes of inadequate and excessive intakes.

This pathophysiologic scenario prompts us to realize how far along the pathophysiologic pathways of low and high exposures the markers that are currently used to characterize deficiency or toxicity are placed. It also illustrates the potential of using adaptive stages of homeostasis as markers for establishing the limits of an AROI, including the setting of an upper level. In addition, it emphasizes the absence for nearly all micronutrients of dose–response data that would enable the systematic creation of an AROI. Copper is a possible exception to this generalization. However, even with copper, there is a lack of dose-response data to enable the identification of markers of adaptation that would enable a full “biologically based approach” to setting an AROI and dietary and toxicologic reference values (10).

Another point to consider is that the components of bioavailability used to inform the biologically based approach to setting reference values correspond to those of the absorption, distribution, metabolism, and excretion used by toxicologists for risk assessments of nonvolatiles. This commonality encouraged the IPCS group to support the notion of harmonized interdisciplinary risk assessments of high and low intakes. The

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1 Adapted from reference 9.
IPCS felt that the terminology used in determining and applying the AROI approach should be more extensively harmonized and that greater emphasis should be given to using homeostatic adaptive markers in risk characterization, emphasizing the strengths and weaknesses of hazard identification, dose–response, and exposure assessment components to increase the transparency of the process and to inform future developments and research investment (6).

**Nutrient risk assessment**

Subsequently, a joint FAO/WHO Technical Workshop on Nutrient Risk Assessment (7, 8) readdressed the biologically or physiologically based model for establishing upper levels of intake for nutrients and related substances. The workshop evaluated the toxicologic risk assessment approach for nutrients and showed that the classic risk assessment methodology incorporating hazard identification and characterization offered opportunities for applying a template that enabled a clear presentation of assumptions, uncertainties, and variabilities; the use of a more extensive range of adverse events spanning the initial physiologic adaptations to high intakes or exposures; and the early pathophysiologic phenomena as markers well as the more customarily used gross effects. This dose-response spectrum of the adverse health effects used in hazard characterization is illustrated in Table 1 [adapted from Renwick et al (4)]. Here again it is evident how far along the pathologic chain the adverse events used in toxicologic risk assessment are. Most adverse health effects currently used in risk characterization lie at stages 5, 6, or 7. The workshop agreed that a biologically based model could be based on phenomena that occur either at lower exposures or after exposures of shorter duration, ie, at stages 3 or 4. These events could be identified by validated biomarkers that are predictive of the more serious sequelae that would occur at higher doses or with more prolonged exposures. It is arguable that this approach would enhance toxicologic risk assessment by enabling the use of events that are pathophysiologically closer and more specific to the exposure and for which it would be possible to perform research to identify and validate such markers and to characterize their variability, thereby reducing the need to apply large uncertainty factors in nutrient risk assessment. This was a valuable elaboration of the approach suggested by the IPCS Environmental Health Criteria Report (6), particularly for high intakes, but by analogy there is an appeal in seeing this extended to lower intakes and dietary reference values.

**Proposed harmonization of nutrient-based dietary standards**

The strands of assessing dietary reference values and of nutrient risk assessment, which, coincidentally, encompass the points raised by earlier reports, were brought together by a United Nations University initiative in collaboration with the FAO, WHO, and the United Nations Children’s Fund (UNICEF) (3), which convened a group to consider international harmonization on approaches for developing nutrient-based dietary standards. At this time an additional driver for the rationalization and harmonization of reference values and the transparency with which they are set emerged in the form of the need to avoid independent national or international reference values becoming a barrier to free trade.

This group agreed on 2 core reference values: Average Nutrient Requirement (ANR) and Upper Nutrient Level. An ANR would be based on the estimation of the distributions of requirements that are based on a specific criterion in healthy individuals; from this ANR, an Individual Nutrient Level x (INLx) based on a distribution centile x could be derived. An Upper Nutrient Level would be derived, usually but not necessarily so, by applying an uncertainty factor to a no or low observed adverse effect level in which the adverse effect may be an early pathophysiologic event as proposed by the FAO/WHO Technical Workshop on Nutrient Risk Assessment (2, 3).

This report on the international harmonization of dietary standards also provided a valuable description of the derivation of a physiologic requirement and average nutrient requirement (5). This derivation is of relevance to the EURECCA Network of Excellence and its aim of identifying the functional endpoints from which to build requirements because it considers the limitations of current approaches. It is an irony in the nutrition field that in the European Union the regulatory standard of evidential justification needed for establishing a nutritional health claim is arguably more stringent than that needed or used for setting dietary reference values. There is an appeal and logic in adopting the physiologic or biological approach for setting dietary reference values on the basis of a similar spectrum of responses to subphysiologic intakes. Reference values derived from such adaptive endpoints would be more defensible than would those based on a physiologic requirement, which in turn is based on a subjective functional outcome. Although there are insufficient data for applying a biological or physiologic approach to setting requirements for most nutrients, there probably is merit in structuring approaches to setting reference values against such a template. The aforementioned elements of bioavailability would be central to such an approach in that they should provide a continuum for addressing indicators of adequacy as well as inadequacy and excess.

In this article, bioavailability has been considered as a more integral aspect to deriving reference values than is commonly the case. The components involved individually and collectively represent critical considerations with regard to determining physiologically safe and adequate intakes of micronutrients. Their relative importance varies greatly according to the nutrient and the level of intake being considered. There is a tendency to base reference values on perceived desirable outcomes, which are often referred to as optimal nutritional outcomes. This tendency presents difficulties in that it involves a subjective concept and endpoint and has the potential to subvert the rationality apparent in establishing a physiologic requirement and average nutrient requirements for micronutrients on criteria based on the metabolism of that nutrient.

The biologically based approach also enables the exploitation of molecular biological approaches to developing critical markers of adaptation at both high and low doses, which could serve also as markers of status in that their adaptation would reflect the adequacy or otherwise of intake. As such, this approach would enable the development of a complementary research strategy to address both setting requirements and identifying the adequacy of supply in populations and individuals.
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