Estimating the Bioavailability Factors Needed for Setting Dietary Reference Values

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Abstract: Estimated average requirements for micronutrients are central to deriving Dietary Reference Values. These are used for nutrition policies and programs, and also for regulatory and labeling purposes, and are traditionally devised to cover the needs of virtually all individuals in any population group. In order to estimate the average requirement, an appropriate endpoint (biomarker) is selected which describes the relationship between dietary intake and health. However, for some micronutrients, such as zinc, there are no good biomarkers, and for others, such as iron, the intake-status relationship is confounded by variations in absorption. Average requirements for these nutrients are derived using a factorial approach in which physiological needs for tissue growth and maintenance and endogenous losses are estimated, and the total converted to a dietary requirement by taking into account the overall absorption from the diet; i.e. multiplying the requirement by a bioavailability factor. The latter can be determined using algorithms, or estimates from absorption studies, some of which are described in this short review paper.

Key words: bioavailability, absorption, micronutrient, iron, zinc, dietary reference values

Introduction

Recommended intakes for micronutrients are used for national and regional nutrition policies and programs, and for regulatory purposes, such as food labeling. They are traditionally devised to cover the needs of virtually all individuals in any population group by taking into account individual variation in physiological requirements. The estimated average requirement (EAR) is the critical step in deriving dietary reference values (DRVs), and its derivation should be made transparent by providing the evidence on which the calculations are based [1]. When the standard deviation (SD) of the endpoint used to set the average requirement is known, the quantity required to meet the needs of 97.5% of the population can be calculated. This derived quantity is known as the recommended dietary allowance (RDA) and is 2 SDs above the average. If the SD is not known, and this is the most usual situation, an estimate is made of the coefficient of variation (CV), usually between 10 and 20%.

Status biomarkers and health endpoints used for dietary reference values (DRVs)

In order to estimate nutrient requirements, the relationship between dietary intake and nutritional status/health is examined. Endpoints can range from the maintenance of a key biomarker of nutritional sta-
tus to the optimization of health through prevention of chronic, diet-related disease (Table I), with data generated from dietary interventions (randomized controlled trials, depletion-repletion, and balance studies). Although the most sensitive biomarker is generally selected, if more than one endpoint is taken into consideration, a weighting procedure may be required. Biomarker data that is currently being generated by "omics" techniques is especially pertinent to combinations of micronutrients, whole diets, and diet-related chronic diseases [2], and may enable the future development of re-constructions using a similar approach to that of the World Health Organization (WHO) in the calculation of disability-adjusted life years (DALYs) [3].

Currently, DRVs are set individually for each micronutrient as there are insufficient data to take into account known or, as yet unrecognized, nutrient interactions, let alone the effects of the diet as a whole. However, even this apparently simple task is fraught with problems, such as the lack of sensitive and robust biomarkers of status for some micronutrients, such as copper [4] and zinc [5]. Even when there are several useful biomarkers of status, there may be disagreement about the normal range and cutoff values for deficiency or excess, or for the protection against chronic disease, morbidity, and mortality. For example, in the absence of folate or vitamin B12 deficiency or anemia of chronic disease, hemoglobin concentration is a specific (although insensitive) functional iron status biomarker, but there is disparity in the normal values suggested by various bodies. In 1994, WHO [6] suggested a cutoff of 120 and 130 g/L for hemoglobin in women and men, respectively, whereas in NHANES III the 5th percentile is taken as the cut-off [7]. Mildly low hemoglobin concentration has been associated with increased mortality in adults, albeit without any evidence of cause and effect, and more importantly the thresholds for increased risk are affected by race/ethnicity [8]. Serum ferritin is an accurate measure of iron stores (provided there is no infection or inflammation) but there is uncertainty over the levels that are associated with risk of iron deficiency [9], and high concentrations indicative of overload are based on statistical rather than biological approaches [10]. Similar problems exist in using health endpoints for deriving DRVs. Chronic diseases are largely multifactorial and, therefore, be inappropriate for establishing single micronutrient recommendations. Consideration must also be given to different target populations and disease outcomes; e.g., primary versus secondary treatment and high-risk individuals.

In general, robust and responsive biomarkers of health that are specific to individual micronutrients have not yet been identified, even for well-studied diseases such as cardiovascular disease and cancer.

Bioavailability

In order to convert physiological requirements into dietary requirements, an adjustment is needed to take into account diet- and host-related variables that affect absorption and utilization (viz. bioavailability). These may include the chemical form of the nutrient and effects of cooking and processing (diet-related), presence of enhancers and inhibitors of absorption (diet-related), life-stage, and nutritional and health status (host-related), as reviewed by Gibson [11]. An estimate of average absorption from typical diets is required, and this will differ between and within population groups and countries. Furthermore, fractional absorption may be dose-dependent, so the mean habitual total intake of the nutrient in the population has to be taken into account. Additionally, for some nutrients, nutritional status may impact on retention (efficiency of absorption and/or endogenous losses) through homeostatic mechanisms. The use of different bioavailability factors is a tool used by risk managers to derive dietary recommendations that support public health policies, and is particularly relevant for personalized nutrition where a tailored factor can be calculated based on habitual diet, phenotype, and genotype.

Factorial Estimates

When the relationship between dietary intake and biomarkers of status/health or a functional endpoint is weak or non-existent and cannot be described mathematically, the requirement has to be derived from factorial estimates. These are the sum of obligatory losses, estimates of requirements for growth and body turnover, plus an allowance for storage if considered appropriate, expressed on a daily basis. The requirement figure has to be converted into a dietary reference intake value by multiplying by a factor that represents the mean efficiency of absorption of the nutrient for each population group (bioavailability factor).

A factorial approach is used for both iron and zinc to determine dietary reference intakes. The lack of a relationship between iron intake and status limits the use of status markers as suitable endpoints, and cut-off values for many of the health outcomes associated with iron deficiency are difficult to characterize (e.g., fatigue and physical performance). For zinc, poor quality status markers necessitate the use of a factorial model.

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**Table I: Examples of experimental approaches and endpoints that have been used to estimate micronutrient requirements**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarkers of status</td>
<td>Folate: red cell and serum folate, Vitamin B12: serum B, Vitamin D: serum 25-hydroxy Vitamin D</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>Selenium: plasma glutathione peroxidase</td>
</tr>
<tr>
<td>Function</td>
<td>Iodine: thyroid iodine accumulation and turnover</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>Vitamin A: night blindness</td>
</tr>
<tr>
<td>Factorial estimates</td>
<td>Iron, zinc</td>
</tr>
</tbody>
</table>

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**Table II: Use of algorithms to estimate iron and zinc bioavailability from different diets**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>WHO (1996) [14]</td>
</tr>
<tr>
<td>Low 15%</td>
<td>Moderate 30%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factorial estimates</th>
<th>Hotz &amp; Brown (2004) [16]</th>
</tr>
</thead>
<tbody>
<tr>
<td>phytate-Zn molar ratio &lt; 4</td>
<td>26% men</td>
</tr>
<tr>
<td>phytate-Zn molar ratio ≥ 18</td>
<td>18% men</td>
</tr>
</tbody>
</table>

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**Table III: Examples of algorithms to estimate iron and zinc bioavailability from different diets**

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Developing countries 5% and 10%</td>
<td>Western-type diets 12% and 15% (depending on meat content of diet)</td>
</tr>
</tbody>
</table>

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**Iron**

The main dietary factors that affect iron absorption are well characterized, with the notable exception of the "meat factor" [12], and data from single-meal studies have been used to develop algorithms to predict bioavailability (Table II). Body iron stores have a well-characterized role in modulating efficiency of iron absorption [18], and more recently hepcidin has been shown to be a regulator of iron homeostasis and may explain approximately a third of inter-individual variation in iron absorption [19]. There is new evidence from mother-child iron absorption studies indicating that genotype is largely responsible for determining the efficiency of iron absorption, once corrections for meal type and iron status have been made [20], which awaits further investigation. Despite major advances in our understanding of iron metabolism, the exact role of the effect of dietary enhancers and inhibitors in the context of the whole diet remains unclear and it has been acknowledged for some time that results of single-meal iron absorption measurements cannot predict absorption from the whole diet, presumably because the effects of inhibitors and enhancers are blunted due to the adaptive responses that maintain iron balance [21].

Studies measuring iron absorption from whole meals/diets generally "normalize" individual absorption data to take into account the effect of iron status (measured as serum ferritin concentration) in one of three ways:

1. Expression of results as relative bioavailability by comparing the test substance/food/meal with a reference dose of iron, often 3 mg heme iron, as well absorbed iron such as ferrous sulfate or ascorbate [22].
2. Correction of absorption to a mean reference value of 40% (corresponds to absorption by individuals with borderline iron stores) by multiplying by 40/R, where R is the reference dose absorption [23].
3. Correction of absorption to a value corresponding to low levels of iron stores: i.e. serum ferritin 40 µg/L [21] or 30 µg/L [24].

The variety of approaches used to measure iron absorption or bioavailability, and differences in the-
position of diets, make it impossible to compare and contrast results of different studies. This is illustrated in Table III, where the lack of agreement (primarily in relation to non-heme rather than heme iron), both between and within countries, is clearly shown.

Iron bioavailability is determined from a combination of dietary and host-related factors. The most important dietary constituents are phytate, polyphenols, and some animal proteins (all inhibitors) and ascorbic acid and animal tissue (both enhancers).

Host-related factors include iron status, infection/inflammation, and genotype [12]. Not surprisingly, it has proved very difficult to reach a consensus on the factors that should be used to represent mean absorption of iron from whole diets when employing the factorial method to derive reference values [36]; different bodies use different values, as illustrated in Table IV. The factor used depends to some extent on the strategy of the policy makers. One option is to select a bioavailability factor using the absorption efficiency seen in individuals with virtually no iron stores (namely, serum ferritin 15 μg/L) and set the target outcome for the EAR to be normal, or to assume functional iron concentrations but no iron stores, as for example in the Australian and New Zealand reference values which assume 18% absorption [39]. Whether these levels are sufficient to meet the needs of individuals with iron stores and a lower efficiency of absorption is unclear. Conversely, a lower absorption value can be used with the aim of maintaining iron stores, especially important for women of childbearing age when they become pregnant [40]. The WHO considers the effect of iron status and total iron intake on efficiency of absorption and its consequent impact on iron stores, and concludes that borderline iron-deficient individuals absorb 15% of iron from a Western-type diet [15]. However, they also describe other diets with lower iron availability, represented by absorption values of 5, 10, and 12%, depending on the relative quantities of inhibitors and enhancers. Other methods use algorithms (Table II), the most comprehensive one to date having been developed from a large database of radio-isotope absorption studies [17]. This algorithm predicted iron absorption to be 14% in a Swedish diet, 16% in a French diet, and 16.6% in a US diet in women of child-bearing age with no iron stores. An algorithm that uses only serum ferritin to predict iron absorption was developed using isotope studies in a population of 31 men [18]. Although this approach benefits from requiring minimum population information, its development relied on a relatively small population and it may not be appropriate for non-Western diets.

**Table IV:** Examples of bioavailability factors used in factorial analysis to calculate dietary reference values

<table>
<thead>
<tr>
<th>Population Group</th>
<th>Iron</th>
<th>Zinc</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Diets with differing bioavailability</em></td>
<td>5%</td>
<td>10%</td>
<td>FAO/WHO (2004)</td>
</tr>
<tr>
<td>Men, women</td>
<td>18%</td>
<td>24%, 31%</td>
<td>Nutrient Reference Values for Australia and New Zealand (2005)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>25%</td>
<td>31%</td>
<td>Nutrient Reference Values for Australia and New Zealand (2005)</td>
</tr>
<tr>
<td>All population groups</td>
<td>15%</td>
<td>30%</td>
<td>UK (1991)</td>
</tr>
<tr>
<td><em>All population groups except children 6–12 m</em></td>
<td>15%</td>
<td>30%</td>
<td>EC (1995)</td>
</tr>
<tr>
<td>Men, women</td>
<td>10%</td>
<td>41%, 48%</td>
<td>IOM (2001)</td>
</tr>
<tr>
<td>Vegetarians</td>
<td>10%</td>
<td></td>
<td>IOM (2001)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>25%</td>
<td></td>
<td>IOM (2001)</td>
</tr>
<tr>
<td><em>All population groups</em></td>
<td>15%</td>
<td>40%</td>
<td>Nordic Recommendations (2004)</td>
</tr>
</tbody>
</table>

**No Recommended Nutrient Intake (RNI)/Population Reference Intake (PRI) is given for iron in pregnant women.**

**Zinc**

Plasma zinc has major limitations as a biomarker of status for individuals [5] and the relationship between dietary intake and plasma zinc concentration cannot be used for setting reference values. Requirements are therefore calculated using the factorial method. Fortunately, zinc is less complicated than iron in that there are fewer dietary modulators of absorption, and zinc status appears to have no significant impact on efficiency of absorption. There is, however, a dose-response relationship and absorption is also affected by age and the time over which the zinc is ingested [44]. Despite this, there are no universally agreed values for bioavailability factors used for factorial calculations, which is itself anything more diverse than those for iron, ranging from 15% in high-phytate diets with soy as the primary protein source [15], to 48% in men consuming Nordic diets (Nordic Nutrition Recommendations [43]). The principal modifier of zinc absorption is phytate (inositol hexa- and pentaphosphates), and results of a recent modeling exercise indicate that phytate is responsible for >80% of individual variation in zinc absorption [44]. This model, when finalized, can be used to make an ac-
curate estimate of zinc absorption in a population using information on the phytic and zinc content of the diet, and will undoubtedly replace the previously developed algorithms (Table II).

Conclusion

Bioavailability factors are a necessary additional consideration in setting DRVs for a number of micro-nutrients. Poor dietary bioavailability can, in some instances, be the predominant cause of micronutrient deficiency, rather than low intakes. However, absorption is difficult to predict and accurate measurements are costly and not always appropriate for whole diets consumed over a longer time span. Although several attempts have been made to predict absorption using algorithms, most have limitations. The development of new methods for modeling and predicting bioavailability was one of the key research priorities identified in a recent workshop of experts [45], which included discussions pertinent to both iron and zinc. Other important issues that require investigation include the influence of genotype, the quality of current food composition tables, and the need for long-term absorption studies to better characterize adaptive responses [45].

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References


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