Bioavailability of Nutrients and Other Bioactive Components from Dietary Supplements

Methodological Issues in Assessing Bioavailability of Nutrients and Other Bioactive Substances in Dietary Supplements: Summary of Workshop Discussion

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Overview

The discussion leaders presented a brief overview, summarized below, of some current in vitro and in vivo methods for estimating the bioavailability of iron and folate, which were used as illustrative case studies that may be relevant to other nutrients and bioactive substances found in dietary supplements. The remainder of the discussion session was devoted to the identification of gaps in our knowledge and the development of research priorities.

In vitro methods: iron case study

The group agreed that the bioavailability of substances from supplements cannot be assumed to be adequate based simply on tablet dissolution and disintegration criteria alone, for example, as established by the U.S. Pharmacopoeia for the purpose of establishing good manufacturing practices. Furthermore, using the case of iron as illustrative of this general principle, the Working Group noted that estimates of relative bioavailability from food based on measurements of iron solubility alone have proven disappointing (1). Thus, there is a need to develop simple and reliable in vitro methods to predict bioavailability of a variety of nutrients and bioactive substances found in dietary supplements.

The group cited in vitro iron bioavailability studies using a current state-of-the-art technique involving cultured human Caco-2 cells as an illustrative case study. Caco-2 cells are a human intestinal cell line originally derived from a colon adenocarcinoma and have various properties that make them suitable for studying cellular aspects of iron metabolism and iron transport (2). Iron-dependent systems that can be studied with this model include iron-dependent regulation of the hereditary hemochromatosis gene, HFE, and of DMT1, a proton-coupled iron transporter gene (3), and iron-dependent ferritin synthesis (4). Investigators have developed in vitro approaches, based either on radioactive iron uptake (5) or cellular ferritin synthesis (6) to estimate iron bioavailability from food and recently, dietary supplements (7).

Using these techniques, the bioavailability of iron can be determined from an enzyme digest after dialysis of the digest iron across a low-molecular-weight cutoff membrane. Relative iron bioavailability is estimated by measuring either Caco-2 cell iron uptake from radioisotope-labeled digests (5,8) or cellular ferritin synthesis (6) using nonradioactive starting materials usually in comparison to ferrous ascorbate.

The group acknowledged that one potential shortcoming of the Caco-2 cell approach is that there has been no published work to directly compare estimates of relative iron bioavailability determined in the Caco-2 cell system with human iron absorption studies. However, based on the results of a new study, Au and Reddy (9) report a significant correlation ($r = 0.92$) between iron uptake in Caco-2 cells from various food digests and human iron absorption. Thus, although more validation studies are necessary, especially with iron supplements, it seems that an in vitro approach using Caco-2 cells is promising in predicting human iron bioavailability.

Despite the obvious screening value of an in vitro system for predicting relative iron absorption, the Working Group concluded that human absorption studies will remain the gold standard for estimating human iron bioavailability.

In vivo methods: folate case study

Although the ultimate goal is the in vivo estimation of human bioavailability of nutrients and other bioactive substances in dietary supplements, researchers often encounter difficulties in performing human studies because of numerous methodological or ethical limitations. This discussion group...
reviewed various methods and limitation of human studies using the B vitamin folate as an illustrative example.

In brief, both short-term and long-term studies have been used to estimate folate bioavailability in humans. These in vivo studies were based on the determination of folate content in blood or urine, and the studies often combined the use of radioactive or stable isotope-labeled oral folates. The Working Group delineated several limitations and caveats associated with in vivo human folate bioavailability studies including:

- Studies of relative bioavailability should be performed by measuring the relative response using a linear portion of the response curve irrespective of the endpoint that is used to assess bioavailability.
- Often in an attempt to keep the experimental test conditions physiological, a small oral folate dose is used. This experimental approach only gives a minimal rise in serum or urinary folate that is often difficult to detect. Further, because there are large intersubject variations in the postprandial folate response, the estimation of folate bioavailability using these physiological doses of folate is difficult (10). Moreover, information on the absolute bioavailability (fractional absorption) of various forms of folate is extremely scarce.

The use of isotopically labeled folates greatly increases the sensitivity of measuring folate absorption and can provide an estimate of fractional folate absorption when combined with an intravenous dose. For example, Pfeiffer et al. (11) reported that the bioavailability of stable isotope-labeled folic acid (pteroylglutamic acid) cooked into wheat flour-based foods was 80–85% compared with that of an orally administered dose of stable isotope-labeled folic acid alone in healthy adult subjects. Likewise, Buchholz et al. (12) reported that a small dose of 14C-labeled folic acid was ~90% absorbed in healthy adult males. In the latter study, it was necessary to use accelerator mass spectrometry to measure extremely small amounts of radioactivity in blood, urine and feces. The estimation of folate bioavailability using these isotope methods is promising.

The Working Group concluded that additional studies using these methods are likely to provide us with solid quantitative data of the fractional absorption of extrinsically labeled folates. Similar novel approaches to estimate bioavailability might be developed for other bioactive substances as well.

The Working Group also noted that a specific limitation confronting those attempting to evaluate folate bioavailability is the lack of readily available sensitive means to accurately assess folate status, which is often used in experiments as an endpoint for determining folate bioavailability.

Research recommendations

The following research needs were identified for determining the bioavailability of nutrients and bioactive substances in dietary supplements.

In vitro methods

- There is a continuing need to develop simple and reliable in vitro methods to screen bioavailability of dietary supplements. However, it will be necessary to validate the information obtained from these in vitro methods on a case-by-case basis against human clinical trials.
- Based on currently available information, a Caco-2 cell-based technique is a promising in vitro method for estimating iron bioavailability. However, additional validation under a variety of conditions and additional comparisons with human iron absorption data are needed. This or similar approaches may be useful in principle for estimating bioavailability of other nutrients or bioactive substances in dietary supplements. Further development of such methods is warranted.

In vivo methods

- A need exists for the development of readily available certified reference material standards for many nutrients and bioactive substances commonly found in dietary supplements. These standards then may be used in human studies as a standard to compare experimental data among various studies and laboratories.
- There is a need to provide quantitative estimates of bioavailability based on measurements of fractional absorption. Appropriate methods should be developed for nutrients and other bioactive substances found in dietary supplements.
- To provide practical information about bioavailability from dietary supplements, human studies are needed using doses, levels and delivery forms comparable to actual commercial formulations. Furthermore, these studies should be conducted in the target population of intended use of the supplements by taking race, gender and age into consideration.
- Critical evaluation should be given to evaluate whether analyses of epidemiological data, such as the intake of supplements and/or tissue contents of the bioactive component or metabolite, provide a useful approximation of the bioavailability of certain nutrients or bioactive substances.
- Investigation is needed on the interaction between the target nutrients or bioactive substances and other nutrients or components in supplements because these factors may influence bioavailability.
- The methodological approaches to estimate the bioavailability of an active ingredient in a botanical preparation are not necessarily similar to those of nutrients. Bioavailability tests based on classical drug bioavailability criteria, such as determining the area under the curve after oral and intravenous loading, may be useful in determining bioavailability of bioactive compounds found in botanical preparations and additional studies are warranted.
- Because the exact nature of the bioactive components of various botanicals and their physiological roles in the maintenance of health and disease prevention are mostly unknown, it is often difficult to design appropriate bioavailability studies for these bioactive substances. Thus, there is a critical need for careful identification of these bioactive components and additional understanding of their physiological functions.

In vivo estimations of bioavailability in humans are the holy grail but can be quite expensive and may be seriously hampered by numerous technical or ethical considerations. Newer stable isotope and low enrichment 14C-based absorption methods are promising but may have limited potential to the measurement of bioavailability by the community at-large because of demanding analytical procedures or limitations in access to equipment. Thus, there will continue to be a need to develop reliable in vitro methods to screen the bioavailability of dietary supplements and rank the more promising formulations for additional direct studies in humans. However, the Working Group cautioned about the uncritical application of these surrogate techniques because these in vitro methods will need to be evaluated for efficacy on a case-by-case basis and it is highly unlikely that any particular experimental approach will suffice for all bioactive substances of interest.
LITERATURE CITED