The 7th Workshop on the Assessment of Adequate Intake of Dietary Amino Acids: Summary of General Discussion

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Abstract
Extensive discussion sessions were held at the end of each of the 2 d of the workshop. Through the course of the workshop, it became clear that there were different opinions on how to use uncertainty factors to obtain upper levels of intake from no observed adverse effect levels of a particular nutrient and that the selection of an appropriate uncertainty factor would be rather arbitrary. Much of the discussion centered around the potential for using metabolic limits, expressed as the level of intake at which the major pathway of metabolism may approach saturation and at which the amino acid is metabolized by alternative pathways, as a measurable early or surrogate marker for amino acid excess and possible toxicity. After extensive discussion on various conditions that would need to be satisfied for metabolic limits to be used as markers of excessive intake of amino acids, there was a general consensus that methods such as measuring oxidation limits are an attractive approach that merit future investigation. It was noted that there are many data on the clinical use of glutamine, whereas data for proline are very scarce. There was recognition that regardless of the available data, there is regulatory pressure for setting upper levels of intake for amino acids and that much more data are required. J. Nutr. 138: 2050S–2052S, 2008.

Introduction
The first day took the form of an open symposium on upper levels of intake for nutrients organized jointly by the International Council on Amino Acid Science and the International Life Sciences Institute Japan, and the second day was a closed conference centered on the available data for glutamine and proline. The discussions at the end of each day focused mainly on the possibilities and difficulties in setting upper levels of intake for individual amino acids, because many do not have well-established toxicity and fewer clinical data are available compared with vitamins or minerals. Furthermore, it was recognized that even for vitamins and minerals for which there are enough clinical data to obtain no observed adverse effect levels (NOAEL) in humans, there is no international consensus on what uncertainty factors to apply in setting upper levels of intake. Consequently, there was extensive discussion of the potential for using measurable parameters as a way to obtain upper levels of intake. The discussions from both days are summarized in this report.

Issues in determining upper levels of nutrient intake
Information was presented on the movement in various countries and international bodies toward requiring the identification of upper levels of intake for most nutrients, generally coupled with the setting of dietary reference intakes. The current methodology for setting upper levels of intake for nutrients is based on a risk assessment model with hazard identification, hazard characterization, dietary intake assessment, and risk characterization as components. Examples of setting upper levels of intake for vitamins and minerals were presented through the course of the workshop and it became clear that although the same data had been used for risk assessment by various groups, there were differences in approaches to selecting uncertainty factors to convert the NOAEL into upper levels of intake. With respect to amino acids, previous workshops have shown that an extensive database was needed to undertake risk assessments for higher intakes and, consequently, there was interest in whether there are ways in which data could be...
obtained that have direct relevance to setting upper levels of intake.

**Metabolic levels of intake and measurable markers**

The issue of whether metabolic limits, such as a dose-dependent inflection point in the generation of CO2 from a stable-isotope-labeled amino acid (2,3) or in the urinary excretion of vitamins (4), could serve as biological markers for upper levels of intake for these nutrients was discussed at length. It was proposed that a metabolic limit could be expressed as the level of intake at which the major pathway(s) of metabolism approach saturation and at which the amino acid is metabolized by alternative pathways. The basic concept is that in general, the nutrients themselves are not toxic per se, as observed in a number of cases of inborn errors of amino acid metabolism where extremely high amino acid plasma concentrations were not associated with adverse effects (6), but that certain metabolites in the main pathways or metabolites originating from alternate pathways may have had higher toxicity. There was agreement that for each amino acid, its major routes of metabolism and also any metabolic pathways thought to be linked to adverse effects need to be considered in defining a metabolic upper level of intake. It was pointed out that it would not be possible to develop a common metabolic limit for all amino acids, because their catabolic pathways are very different. For example, the toxicities seen for methionine excess stem from the specific pathways associated with methionine catabolism and therefore would not apply to other amino acids. It was noted that although toxicities can be shown at very high doses in animals, it is unethical to perform experiments in humans that result in adverse effects; consequently, many of the data regarding amino acid toxicity in humans have come from inadvertent dosing. It was noted that a metabolic limit would be a surrogate or early marker for possible toxicity at higher intakes and could be investigated ethically in humans. It was suggested that precautionary upper levels of intake could be defined if a metabolic limit were viewed as a predictor of adverse metabolic effects and treated as if it were the lowest observed adverse effect level. To use metabolic limits as surrogates for upper levels of intake, it was noted that it will be necessary to show that the metabolic limits are reached before any toxicological signs are manifested; it was pointed out that such examples exist in the pharmaceutical area (7).

There was extensive discussion on how metabolic limits could be measured in humans. It was pointed out that it would be difficult to undertake experiments with methionine in humans because of the likelihood of well-known toxicity being expressed at high doses. It was suggested that oxidation may not be the only marker for metabolic limits and that in the case of methionine, metabolites such as S-adenosyl methionine could be a marker. It was noted that animal data on the correlations between toxicity and any postulated metabolic limits are necessary before proceeding to human studies. Questions were raised regarding the similarities of amino acid metabolism between species and it was noted that, apart from the metabolism of certain amino acid isomers, metabolism across species is generally similar, although only primates and humans use glutamine for the conjugation of organic acids for detoxification and excretion (8). However, an important difference between humans and other animals in amino acid utilization is the fact that humans grow at much slower rates than most other animals.

**Upper levels of intake for health and disease**

The issue of the differences in metabolism between health and disease and the impact on upper levels of intake was discussed. It was noted that there are examples of disease states where there are clear differences in the metabolism of amino acids; for example, it was reported that the requirement for branched-chain amino acids increases by 80% in children with liver disease. Based on the influence of glutamine deficiency on stimulation of AMP-activated protein kinase activity (9) heat-shock proteins (10), and glutathione metabolism (11), it has been hypothesized that only patients having lower plasma glutamine levels may benefit from glutamine supplementation (12,13). This is in accordance with a study showing that glutamine depletion is an independent factor in addition to acute physiology and chronic health evaluation II score for the prediction of mortality in intensive care unit patients (14). There are also differences in the effects of short-term and long-term supplementation. Although there were questions on the relevance of the application of upper levels of intake for healthy individuals to diseased patients, there was much support for the view that differences in susceptibility should be treated as a continuum with values for the healthy individual serving as reference points. It was also noted that the concept of measuring metabolic limits could be applied to diseased patients as well. On the other hand, upper levels of intake are usually set for the healthy general population and the inclusion of subjects with disease in the same data set as healthy subjects would increase the variability too much, so that any uncertainty factor used to set the upper level of intake would be unnecessarily excessive for the general population. The need to consider whether the amino acid was taken with food or without food was also pointed out. People taking tryptophan supplements because they think it helps them sleep through serotonin synthesis and people taking lysine for herpes-induced cold sores were given as examples of when amino acid supplements were taken on an empty stomach. The issue of whether metabolic limits may need to be exceeded for information on certain clinical effects to be gained, such as the evaluation of pituitary function by acute infusion of a high dose of arginine, was also discussed. It was noted that it would be possible to measure the various metabolic pathways for arginine to see if any proposed metabolic limit would need to be exceeded to gain beneficial effects; for example, there was work on the balance between nitric oxide synthesis and arginase in lung tissues of mice with cystic fibrosis or other lung diseases that could provide useful insights (15,16).

**Relevance of animal data**

The issue of extrapolation of animal data to humans was discussed. It was noted that in the current process of upper level of intake evaluations, animal data are considered only when there is difficulty in hazard identification or dose-response assessment using the available human data (17). There were differences of opinion on the relevance of animal data to human upper level of intake determination. Whereas some had the opinion that animal experiments do not predict the efficacy of amino acids and that the extrapolation from animals to humans may be limited to adverse effects, others expressed that amino acid requirements for human neonates could be estimated from amino acid requirements in pigs by using the total daily protein intake data as a reference. It was reported that this has been successfully applied to methionine and threonine requirements and that the requirements for these amino acids are proportional to the protein intake. It was suggested that the same principle might also apply to setting upper metabolic limits. However, it was pointed out that for glutamine, there are vast species differences in muscle glutamine concentrations (18) and doubts were raised as to the validity of a simple
model based on percentages of protein intake being applicable to all amino acids. On the other hand, others pointed out that although the basal levels may be different, various species show similar percentage reductions in glutamine levels after burn injuries, so that differences in basal levels do not necessarily reflect different metabolic requirements (19).

Glutamine and proline

There was much discussion regarding glutamine and it was noted that in the clinical setting, at least 40 g/d has been given to patients whose glutamine clearances are slower than those of healthy individuals (20,21). These patients showed no adverse effects after glutamine dosing and so it was suggested that these data could be used for setting upper levels of intake for healthy individuals. Others noted that it was difficult to see any specific toxicities associated with excess glutamine in animal experiments. This made it difficult to undertake hazard identification, a problem for risk analysis, and it was suggested that where toxicity could not be observed at any dose, the highest observed intake with appropriate data could be set as the upper level on intake. There was some discussion on the differences between glutamine and glutamate. It was noted that there are reports that feeding 147 g/d monosodium glutamate was without any adverse effects (22). It was noted that data on proline are sparse compared with most amino acids. There was a discussion on the difference in metabolism between neonates and adults with regard to arginine synthesis, with proline being the only precursor for arginine in the neonate, but glutamate and glutamine being used as substrates in adults (23). It was noted that in neonates, only one-half the required arginine could be produced from proline (24–26).

The discussion ended with the general consensus that regard- less of the available data, there is regulatory pressure for setting upper levels of intake for amino acids and that much more data are required. The following points were generally recognized: 1) there are differences in opinions on what uncertainty factors to apply to NOAEL to obtain upper levels of intake; 2) the use of metabolic limits as an early marker or surrogate marker for upper levels of intake merits investigation; 3) measuring oxidation limits is an attractive approach for obtaining metabolic limits for amino acids; and 4) there is a considerable deficiency of data for certain amino acids, such as proline, and much more data are needed in general for considering upper levels of intake.

Other articles in this supplement include references (27–32).

Literature Cited