

An Approach to Defining the Upper Safe Limits of Amino Acid Intake¹⁻³

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Abstract

The existing data on the safe upper limits of amino acid intake in humans is essentially observational; how much do individuals ingest and what side effects do they have? There are numerous studies in humans comparing the effects of high doses of amino acids given as protein bound vs. as free amino acids. These studies have shown that protein-bound amino acids have much less effect on plasma levels of the test amino acid, because protein intake stimulates protein synthesis as another sink for the increased amino acid intake. In practice, the highest amino acid intakes occur with free amino acid supplements that may be ingested by athletes who believe that the amino acids will benefit them in training and/or performance. Previously, in a piglet study, we were able to define the point at which maximal phenylalanine oxidation occurred, above which plasma phenylalanine concentration and body balance rose exponentially. We regard this value of maximal disposal (oxidation) of an amino acid as one metabolic marker of the upper limit of intake. Recently, others have demonstrated a similar maximal oxidation rate for leucine in rats. Based on these experimental data and the paucity of published human data in controlled experiments, we think that a systematic approach needs to be undertaken to define the maximal oxidation rate for all dietary indispensable amino acids and other amino acids that may be ingested in excess by humans. We believe that this will provide a rational basis to begin to define the upper limits of tolerance for dietary amino acids. However, some amino acids, such as threonine and methionine, will be more difficult to study, because they have more than 1 route of disposal or very complex metabolic regulation, in which case defining their upper limits will be more multifaceted. J. Nutr. 138: 1996S–2002S, 2008.

Introduction

The advent of stable isotope-based carbon oxidation studies has allowed a systematic reevaluation of the mean dietary requirement for most indispensable amino acids (IDAA)⁸ in humans (1,2). The mean requirement, defined as the estimated average

requirement (EAR), is the experimentally defined value and satisfies the requirement in 50% of the individuals in a population (3). Using repeated measures within the same subject, progress has been made toward defining the population variance and hence toward defining the recommended dietary allowance, which meets the requirement for ~97.5% of the population (1,3). The recommendations for dietary reference intakes (DRI) also include the definition of a tolerable upper intake level (UL) for amino acids, the highest average daily intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As amino acid intake increases above the UL, the potential risk of adverse effects may increase (3). Existing data on the upper limits of amino acid intake is observational in humans and therefore no UL could be established during the DRI process for any of the amino acids. This is despite the fact that some amino acids, including methionine and phenylalanine, are known to result in toxic effects at high doses (3–5).

There are numerous studies in humans comparing the effects of high doses of amino acid given as protein-bound vs. as free amino acids (6–9). These studies have shown that protein-bound amino acids have much less effect on plasma levels of the test amino acid, because protein intake stimulates protein synthesis as another sink for the increased amino acid intake. The

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⁸ Abbreviations used: ALAT, alanine aminotransferase; BCAA, branched chain amino acid; DRI, dietary recommended intakes; EAR, estimated average requirement; IDAA, indispensable amino acid; SAA, sulfur amino acid; TDG, threonine dehydrogenase; UL, tolerable upper intake level; VA, amino acid solutions high in phenylalanine.

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consumption of a mixture of amino acids, either as protein or amino acid mix, also has less negative impact than the excess consumption of individual amino acids. In practice, the highest amino acid intakes are likely to occur with free amino acid supplements, which may be ingested by athletes who believe the amino acids will give them a benefit in training and/or performance; an example is glutamine (10). Therefore, potential approaches to define the UL for amino acids need to be identified to make recommendations to prevent adverse events from occurring due to excess amino acid intakes. In this review, we will briefly outline one such approach based on stable isotope carbon oxidation to identify the UL for amino acids.

An approach to define the upper limits for amino acid intake

The ideal marker for identifying excess intake of an amino acid should have very specific dose-response characteristics. In particular, its response to increasing intake should display an inflection point that could be used to identify the onset of the excess situation. Previously, in neonatal piglets, we observed an upper inflection point in the dose-response curve for phenylalanine retention and $^{14}\text{CO}_2$ production from phenylalanine oxidation with graded phenylalanine intake (Fig. 1) (11). Piglets received graded phenylalanine intakes ranging from 0.2 to 1.2 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Apparent phenylalanine balance, which was calculated as the difference between phenylalanine intake and oxidation, increased linearly between 0.2 and 0.5 g phenylalanine $\text{kg}^{-1} \cdot \text{d}^{-1}$. Apparent phenylalanine balances did not differ for intakes between 0.5 and 0.8 g phenylalanine $\text{kg}^{-1} \cdot \text{d}^{-1}$. At the highest phenylalanine intakes, the apparent phenylalanine balance was significantly higher than the plateau values (Fig. 1). Phenylalanine balance reflects the net rate of accretion or retention of phenylalanine in body tissues. The increase in phenylalanine balance above the intake of 0.8 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ indicated that oxidative processes were unable to keep pace with the increasing intake of phenylalanine. The inflection point at 0.8 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was the “metabolic limit” to oxidize or catabolize phenylalanine in neonatal piglets and was supported by the observation of significantly higher plasma concentrations of phenylalanine and tyrosine in the piglets receiving 1.2 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (11). Once the maximum level of phenylalanine oxidation was reached, plasma phenylalanine concentrations and reten-

tion rose rapidly. Hence, we reasoned that a suitable marker to define the tolerable UL for a dietary amino acid would be the level at which the maximum oxidation level was reached. This approach was described and proposed by our group initially during the 3rd Amino Acid Assessment Workshop (12) and reinforced subsequently during the 5th Amino Acid Assessment Workshop (13).

The above-described response pattern for increasing phenylalanine intake should be observable for most amino acids and can be applied to define the UL. When the amino acid intake is low, protein synthesis, oxidation, and excretion of the amino acid and related metabolites will be low (Fig. 2). With increasing intake of the limiting amino, retention of the amino acid will increase as a result of increasing utilization of the limiting amino acid for protein synthesis and other required metabolic functions. Therefore, a positive slope in amino acid retention will be observed (Fig. 3). Once the requirement for the amino acid is met for protein synthesis and related functions, additional increments of the test amino acid will be primarily catabolized in proportion to the extra intake. This increasing catabolism in proportion to intake will occur because each additional increment in intake will be in excess of the requirements for metabolism and will be oxidized. In this zone, the catabolic pathways are sufficient to deal with the excess intake and the excess amino acid is broken down and used for energy (Fig. 2). For the range of amino acid intakes in this zone, a minimum slope or no slope in the retention of the amino acid will probably be observed, depending on the amino acid being tested (Fig. 3). Further increases in amino acid intake will lead to a positive slope in the amino acid retention curve due to increased retention of the amino acid in body pools. This increase in retention is a result of dietary intake exceeding the metabolic capacity to catabolize the amino acid in direct proportion to intake. This point, at which the metabolic capacity to catabolize or oxidize the excess amino acid is exceeded, can be regarded as one estimate of the UL, because it represents the intake where the normal regulatory mechanisms are no longer sufficient to dispose of the excess. The amino acid intake corresponding to this inflection point does not represent a toxic intake level but rather suggests that with increasing dietary intakes above this level, the potential or risk for adverse events will increase. Also, amino acid intakes above this point are usually characterized by

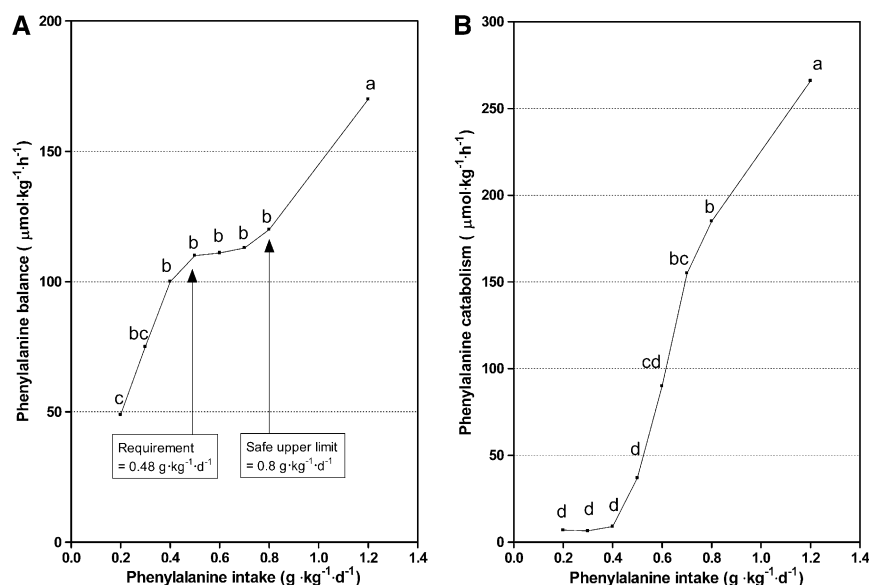


FIGURE 1 Phenylalanine balance (A) and catabolism (B) in neonatal pigs fed graded levels of phenylalanine. Phenylalanine balance was calculated as the difference between rates of intake and oxidation. Phenylalanine catabolism was calculated using the specific radioactivity of liver free phenylalanine. Values are means, $n = 3$; pooled SE = 18 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and 36 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for phenylalanine balance and catabolism, respectively. Means for a variable without a common letter differ, $P < 0.05$. Adapted with permission from House et al. (10).

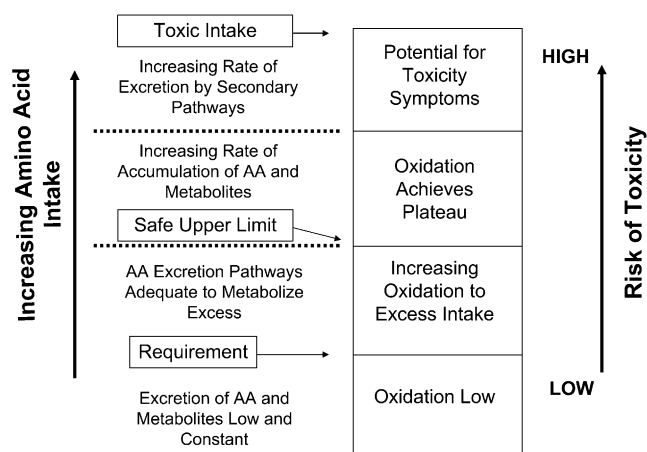


FIGURE 2 Schematic representation of body responses to excess amino acid (AA) intake.

an increasing rate of accumulation in blood and excretion of the amino acid and its secondary catabolites in urine.

Recently, Sakai et al. (14) used this approach to identify an excess intake of leucine in rats. They identified the “metabolic limit” to catabolize leucine by measuring $^{13}\text{CO}_2$ production arising from graded leucine intakes ranging from 0 to 30% of the diet. The maximum limit to oxidize excess leucine was reached at 10% of dietary intake; the oxidation achieved a plateau above the intakes of 10% dietary leucine or $8.9 \text{ g leucine} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. This inflection point was identified as the UL for leucine intake in rats (14). Sakai et al. (14) also reported that plasma leucine and other plasma amino acid concentrations did not differ among all the leucine intakes. Therefore, they were not able to identify accumulation of excess leucine and other metabolites as a potential biomarker or surrogate marker. In an earlier study (14) using a similar strain of rats and receiving a diet similar in composition, they observed significant growth inhibition in rats

fed 15% leucine or $12.4 \text{ g leucine} \cdot \text{kg}^{-1}$. This suggests that the inflection point at which the maximum limit to oxidize excess leucine is reached is an early marker to identify the potential for an adverse event (in this case, growth inhibition) and may identify the UL more appropriately.

Experimental design and statistical considerations

Genetics and other factors may influence the responses to excess amino acid intakes in different species. The large variability in response to various amino acid intakes in humans makes the identification of a clear UL for amino acids especially challenging. It has been our experience with defining the EAR for amino acids that studying a range of test intakes within each individual allows the definition of a statistically stronger estimate of the EAR (1,2). This is primarily because a repeated measures model accounts for some of the large interindividual variability in humans and multiple data points per individual on the dose-response curve increases the statistical confidence associated with the regression lines. A dose-response curve to identify a breakpoint requires multiple data points on both the sloping line and the nonsloping line. A minimum of 3 data points is required to define a line (1). Unlike the stable isotope-based carbon oxidation studies conducted to determine the EAR for amino acids, where the test intakes were chosen to bracket an expected breakpoint estimate that was based upon previous research, it will be more difficult to choose excess intakes more appropriately, because there are no studies available from which to estimate the upper metabolic oxidative capacity. Therefore, it will be prudent to choose a wide range of excess intakes initially to ensure that there will be a minimum of 3 intakes on each line.

Practical reasons for defining an upper limit for amino acid intake

In the United States, $\sim 3.4\%$ of the population uses amino acid supplements, 62% on a daily basis (15), and thus we must be concerned that these individuals may consume excessive amounts of amino acids. The most common group of people who consume nutritional supplements tends to be athletes (10,16). The branched-chain amino acids (BCAA; leucine, valine, and isoleucine) are especially popular among athletes who either consume them as individual supplements or in combination with protein drinks (17).

Excessive intakes of free amino acid may have adverse effects; however, there are very few data to either confirm or deny this position. Some amino acids have specific metabolic functions in addition to the requirements for protein synthesis; e.g. control of protein synthesis by leucine, synthesis of catecholamines from aromatic amino acids (phenylalanine, tyrosine, and tryptophan), methyl and sulfur donation from sulfur amino acids (SAA; methionine and cysteine), nitric oxide synthesis from arginine, etc. Supplemental lysine doses have been reported to suppress chronic cold sores caused by herpes simplex (18) and lysine pills are available as dietary supplements in many health food stores. Furthermore, lysine fortification in people consuming a predominantly cereal-based diet and living in a low socioeconomic situation has been recently shown to be beneficial in reducing stress and anxiety (19). Also, a combination of oral lysine and arginine supplementation has been shown to reduce anxiety and basal levels of stress hormones in healthy humans with high subjective levels of mental stress and anxiety (20). Therefore, dietary supplementation with specific amino acids in excess of the requirement for protein synthesis may be beneficial in some situations. The questions remains, however, how much extra amino acid can be consumed safely.

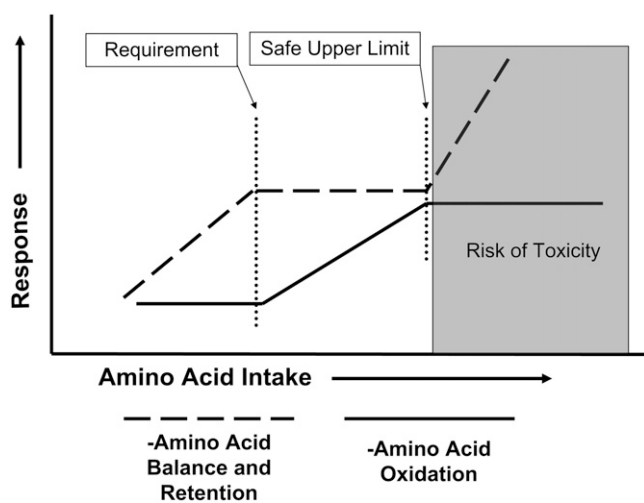


FIGURE 3 Patterns of response expected due to increasing intakes of amino acids. With increasing intakes of the amino acid, oxidation of the amino acid will increase. Once the “metabolic limit” to oxidize the excess amino acid is reached, amino acid oxidation will plateau and this inflection point will identify the UL for the test amino acid. As amino acid intake increases above the UL, the potential risk of adverse effects and toxicity may increase.

Amino acids for which CO₂ can be used as an end point

The approach outlined earlier in this article can be applied for the identification of UL for amino acids where the carboxyl group is directly released into the bicarbonate pool and will therefore readily appear in breath. The amino acids in this category are phenylalanine, the 3 BCAA (leucine, valine, and isoleucine), and lysine. The 1-carbon in phenylalanine is catabolized to CO₂ in 3 steps. The initial step is catalyzed by phenylalanine-4-monooxygenase (EC 1.14.16.1) where tyrosine is formed (11). Tyrosine is converted to 4-hydroxy phenylpyruvate by tyrosine aminotransferase (EC 2.6.1.5), which is catalyzed by 4-hydroxy phenylpyruvate hydroxylase (E.C.1.13.11.27) to homogentisate and CO₂. Due to excess dietary phenylalanine intake, saturation of these catabolic pathways leading to a plateau in the appearance of breath ¹³CO₂ will identify the metabolic limit to dispose of the excess, as was previously observed in a piglet study (11). Also, simultaneous measurement of plasma and urinary concentrations of metabolites will provide insight as to which enzymes in the catabolic pathway are saturated. In the piglet study, we observed that with the increase in phenylalanine retention at the highest intake, there was a simultaneous increase in both plasma phenylalanine and tyrosine concentrations, suggesting that the first step in the catabolic pathway where phenylalanine is converted to tyrosine was not saturated. Therefore, the metabolic saturation must have been occurring later in the catabolic pathway. Other metabolites were not measured in that study, which would have permitted further understanding of the enzyme saturation process. In another piglet study, we examined administration of parenteral amino acid solutions high in phenylalanine (VA; Vamin; Kabi), low in phenylalanine (Vaminolact; Kabi), and Vaminolact + added phenylalanine on phenylalanine metabolism (21). We measured phenylalanine oxidation, plasma phenylalanine, and tyrosine concentrations, as well as excretion of alternate catabolites of phenylalanine (phenylpyruvate, phenylacetate, phenyl-lactate, and 2-hydroxyphenylacetate), and tyrosine (4-hydroxyphenylpyruvate, 4-hydroxyphenylacetate, and 4-hydroxyphenyl-lactate). Phenylalanine oxidation was increased in the piglets receiving VA, simultaneous with an increase in plasma phenylalanine concentration, and greater excretion of all measured catabolites of phenylalanine and tyrosine compared with piglets receiving amino acid solutions low in phenylalanine (21). In a similar study in human neonates, we observed that neonates receiving VA had significantly elevated urinary excretion of the phenylalanine catabolite, phenyl-lactate, and the tyrosine catabolite, 4-hydroxyphenylacetate (22). In the same group of infants, urinary excretion of phenylalanine and tyrosine was also significantly greater compared with neonates receiving the moderate phenylalanine parenteral solution. Therefore, to identify the metabolic limit of the body to dispose the excess phenylalanine, alternate catabolites of phenylalanine and tyrosine metabolism should be measured and correlated to the changes observed in phenylalanine oxidation resulting from excess phenylalanine intake.

The initial step for all 3 BCAA is transamination, a readily reversible reaction that yields the corresponding branched-chain ketoacid. This reaction is catalyzed by branched-chain aminotransferase (EC 2.6.1.42) (23). Branched-chain α -ketoacid dehydrogenase (EC 1.2.4.4) is the second and key enzyme that commits the carbon skeleton of the ketoacids to oxidative decarboxylation. The 1-carbon of all BCAA is readily released to CO₂ and therefore this concept was used in the direct amino acid oxidation method to determine the mean requirement for leucine (24) and valine (25) in humans. But in these earlier

studies (24,25), the range of dietary BCAA intake was restricted to adequate levels and did not provide any information about the enzyme saturation process. In the rat study described earlier, Sakai et al. (14) used [U-¹³C]-leucine with excess leucine intakes to observe a saturation of the catabolic pathway and identified the inflection point where ¹³CO₂ appearance in breath plateaued due to the onset of the excess situation. The same approach can be applied for valine and isoleucine, although caution must be taken when interpreting the data obtained from individual BCAA excess studies due to antagonism among the BCAA (26). In young rats, supplementation of excess leucine led to growth inhibition (26). All 3 BCAA have been shown to compete for transport across membranes and high dietary intakes of leucine decrease plasma valine and isoleucine concentrations in animals (26). In humans, however, within the physiological range of dietary BCAA intake, there does not appear to be any significant interactions (27,28). With excess BCAA intake, the respective keto-acids should also accumulate in blood. Leucine also plays an important role in the stimulation of protein synthesis in animals along with a transient rise in insulin concentrations (29). Therefore, studies designed to identify BCAA excess must include measurement of other BCAA catabolites, especially the keto-acids, and hormone levels such as insulin and glucagon.

Lysine catabolism in mammalian tissues is regulated by the 2 initial reactions catalyzed by lysine ketoglutarate reductase (EC 1.5.1.8) and saccharopine dehydrogenase (EC 1.5.1.9) (30). This is the primary pathway for lysine catabolism, known as the saccharopine pathway, and 3 more separate enzymes of the lysine catabolic pathway must be present and receiving substrates before the release of the α -carboxyl group is possible. Although there are multiple steps prior to the release of the 1-carbon from lysine, lysine oxidation has been applied by us to examine lysine kinetics in a short-term fed-state model (31), as an indicator amino acid (32), and by others (33) in the direct amino acid oxidation method to determine amino acid requirements. Therefore, lysine oxidation should increase in response to excess lysine intakes, until the metabolic limit to catabolize lysine is reached. An upper inflection point in the oxidation dose-response curve may be observed, which will allow the definition of the UL for lysine intake. Studies designed for the identification of the UL for lysine should probably measure various catabolites in the pathway, such as saccharopine, α -amino adipic acid, and α -keto adipic acid. Inborn errors of lysine metabolism have been associated with increased concentrations of lysine and saccharopine in plasma and also with increased urinary excretion of lysine and saccharopine (34). In young pigs fed excess lysine, Edmonds and Baker (35) observed markedly higher concentrations of lysine and α -amino adipic acid in plasma, liver, kidney, and muscle. Urinary excretion of lysine and α -amino adipic acid was also increased in the pigs fed the excess lysine when compared with the pigs fed a basal diet (35). Lysine degradation in vivo also occurs by a secondary pathway, called the pipecolic acid pathway, which has been suggested to function as an overflow pathway for excess lysine intake (36). Pipecolic acid concentrations in plasma and urinary excretion in healthy subjects and patients with familial hyperlysinemia have been reported and therefore may act as a possible biomarker for excess lysine intake (36).

Amino acids for which sulfate excretion can be used as an end point

Methionine and cysteine, which constitute the SAA, have been extensively researched due to their involvement in multiple critical pathways, apart from being necessary for protein synthesis (13).

Homocysteine is the central product in the catabolism of methionine, which is formed by the transmethylation process and degraded to cysteine via the transsulfuration process. Hyperhomocysteinemia has been identified as an independent risk factor for cardiovascular disease in humans. Glutathione, the most prevalent intracellular thiol, is an important endogenous antioxidant synthesized de novo within all cells and intracellular availability of cysteine is believed to be the most important rate-limiting factor for glutathione synthesis (13). Methionine (37) and recently cysteine (38) have been suggested as the most toxic amino acids in animals. In humans, reports of toxicity are varied (5), although there has been a single report where an accidental overdose of methionine caused death in an apparently healthy subject (39). Catabolism of SAA predominantly leads to the production of sulfate and taurine, which are almost exclusively excreted in urine in both animals (40) and humans (41). Measurement of urinary sulfate has been validated as a simple and noninvasive way to measure SAA catabolism (40,41). Taurine as an end product in urine was more significant in pigs compared with humans (40). Therefore, the measurement of inorganic urinary sulfate in response to excess SAA intake should be an adequate marker to identify SAA excess. With increasing SAA intakes, sulfate excretion should increase until the catabolic pathways of transmethylation and transsulfuration are saturated. Excretion of sulfate will plateau once this point is reached. Also, we propose that simultaneous measurement of various thiols in various body fluids should be done to correlate with the sulfate excretion. Recently developed liquid chromatography-electrospray tandem mass spectrometry methods by us for measurement of homocysteine and cysteine concentrations in plasma and urine (42) and others for sulfate in biological fluids (43) provide a rapid and highly sensitive estimate of their concentrations and should be utilized.

Amino acids that have complex metabolic pathways

For some IDAA, including threonine, histidine, and tryptophan, the catabolic pathways are more complex, and the 1-carbon is not released as readily into the bicarbonate pool to be excreted as CO₂. For these amino acids, identification of the UL might present problems. In the case of threonine, there are 2 major catabolic pathways: threonine is catabolized either by threonine dehydratase (EC.4.2.1.16) to ammonia and 2-ketobutyrate, which is rapidly converted to CO₂, or by threonine dehydrogenase (TDG; EC. 1.1.1.103) to form 2-amino-3 ketobutyrate, which eventually forms glycine and acetyl-CoA (8,44,45). The contribution of both of these pathways to threonine oxidation varies depending on age and species. For example, in human neonates, we observed that the TDG pathway accounted for 44% of total threonine oxidation (44), in contrast to adult humans, where the TDG pathway accounted for only 7–11% of total oxidation of threonine (8). In adults fed threonine-supplemented diets, either as free amino acids or as intact protein, we observed a positive relationship between plasma threonine concentrations and the rate of threonine catabolism to CO₂ up to a point, followed by a range of plasma threonine concentrations where no change in threonine catabolism was observed (Fig. 4) (8). Therefore, if the same relationship exists for excess threonine intakes well above what was tested previously and, assuming that the sequestration of the 1-carbon of threonine to glycine is still minimal, oxidation of threonine may be used to identify the UL using the oxidation approach outlined above.

Histidine is an amino acid that has large body pools in the form of hemoglobin and carnosine (46). Provision of histidine-free diets leads to extensive adaptation and accommodation in protein metabolism and there is controversy surrounding its

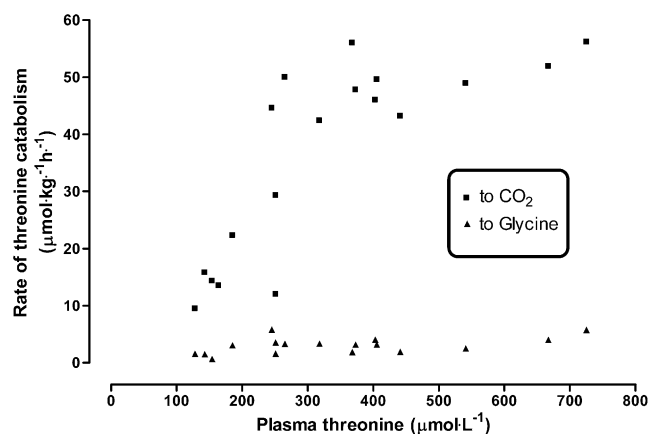


FIGURE 4 Plasma threonine concentration and rate of threonine catabolism to CO₂ via the threonine dehydratase pathway, or to glycine via the threonine dehydrogenase pathway in 6 adult humans. Subjects received 3 different dietary threonine treatments: Control diet with 65 mg threonine·kg⁻¹·d⁻¹, high-protein diet with 173 mg threonine·kg⁻¹·d⁻¹, and high free threonine diet with 173 mg threonine·kg⁻¹·d⁻¹. Adapted with permission from Darling et al (8).

essentiality in adults (46). Histidine may be degraded by 3 pathways. In the major catabolic pathway, histidine is degraded to urocanate and ammonia by histidase, which eventually leads to formation of products involved as part of the folate coenzyme system (47). Histidine is also degraded by histidine pyruvate amino transferase to form imidazole pyruvate and by histidine decarboxylase to form histamine. There are too few studies on histidine metabolism in humans to suggest the best approaches to measure potential excess intake in humans.

Tryptophan is another amino acid which, after the initial catabolic step catalyzed by tryptophan dioxygenase, branches into multiple pathways leading to the synthesis of various compounds from the ring structure of tryptophan, including kynurenate, anthranilate, xanthurenate, and NAD⁺ or amionmuconate semialdehyde (48). Tryptophan is the precursor for synthesis of many neurotransmitters, including serotonin and melatonin. Studies in animals (49,50) and humans (51) have shown that dietary tryptophan supplementation above the requirement level has considerable effects on behavior and brain neurotransmitters. In pigs, Chung et al. (52) did not observe toxic effects due to excess tryptophan intake and suggested that oral ingestion of tryptophan in pigs is safe. In humans, however, consumption of tryptophan in excess of the dietary requirement could be potentially dangerous. Due to the complex nature of tryptophan metabolism, other approaches need to be utilized to define the UL for tryptophan.

Also, other amino acids, including the conditionally IDAA, such as arginine (53,54), glutamine (10,55), and glutamate (56), should be considered for the definition of a UL. Because of their purported benefits, these amino acids are increasingly being used in clinical settings in excess levels with no knowledge of the safe level of dose. The potential for adverse events is increased in these patient populations and the UL of intake must be defined for these amino acids.

Identification of other potential biomarkers

The DRI (3) recommendations for identifying a UL included the definition of a lowest observed adverse effect level and a no adverse effect level. We hypothesize that the maximal level at which catabolism (or clearance) of an IDAA occurs will be an

early warning sign that excess accumulation of the IDAA and alternate (degradative) pathway products, which may be toxic, will begin to occur. Hence, identification of endpoints and early biomarkers of toxicity that could be measured in humans is necessary along with the definition of maximum oxidative potential of amino acids. The key questions of what those biomarkers might be and how they will allow us to identify the UL will depend on how accurately we can measure these potential biomarkers. All newly planned human studies to determine the UL should include basic biomarker measurements that correlate with the change in the oxidation potential. Collection of blood, urine, and saliva samples, and possibly tissue samples where applicable, should be conducted to identify other markers. Matsuzaki et al. (57) investigated changes in plasma metabolites, physiological measurements such as liver enzyme markers, including aspartate aminotransferase, alanine aminotransferase (ALAT), serum total cholesterol, glucose, total blood urea nitrogen, etc. in response to leucine intake ranging from 1.5 to 30% in rats. They used cluster analysis of multivariate correlations and DNA microarray analysis of liver samples to identify potential biomarkers for leucine excess. With an increase in leucine intake, ALAT levels increased and cluster analysis of multivariate correlations analysis revealed that α -keto isocaproic acid correlated with ALAT. DNA microarray analysis observed no changes in the expression of genes associated with leucine catabolism. Various approaches such as this must be continually defined and these will aid in developing robust methods to determine the UL, lowest observed adverse effect level, and no adverse effect level for various amino acids (58,59).

In conclusion, new methods need to be developed to identify the UL for safe intake of free amino acids. The approach described herein of using stable isotope-labeled carbon oxidation is applicable to many amino acids and may be systematically applied to most IDAA to define the UL. With increasing intake of the test amino acid, the oxidation of the amino acid will increase until the metabolic capacity to oxidize/catabolize the amino acid is reached. We propose that this maximal disposal rate of the amino acid be used as a metabolic marker of the UL, because this intake marks where homeostatic mechanisms have been exceeded and thus increasing intake in excess of this catabolic capacity increases the risk of toxicity. The catabolic mechanisms for most amino acids are upregulated with chronic intake in excess of requirement (60). In addition, there are overflow pathways for several amino acids that do not become evident until an excess intake is consumed for a period of time (60). The dietary intake at which the maximal disposal occurs in an experiment may therefore shift to the right (i.e. greater intake) following adaptation. The upregulated normal pathways or the new mechanisms that are recruited could either reduce or increase the toxic effects of the amino acid. Therefore, complete knowledge of the metabolism of the test amino acid is also required to interpret the results obtained from these studies.

Other articles in this supplement include references (61–70).

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