Determination of the Tolerable Upper Intake Level of Leucine in Adult Men1–3

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Abstract
Leucine is purported to improve athletic performance. Therefore, the BCAA, especially leucine, are popular as dietary supplements among strength-training athletes. There are, however, concerns regarding possible adverse effects of excessive leucine intake. The objective of the current study was to determine the metabolic and adverse effects of the acute ingestion of very high intakes of leucine supplements. Five healthy men (20–35 y) each received graded stepwise increases in leucine intakes of 50, 150, 250, 500, 750, 1000, and 1250 mg · kg−1 · d−1 corresponding to the Estimated Average Requirement, and Estimated Average Requirement ×3, ×5, ×10, ×15, ×20, and ×25 to a total of 29 studies. The graded stepwise approach was used rather than a randomization of leucine intake to minimize the possibility of severe adverse effects. Participants were given a maintenance diet for 2 d prior to each leucine level containing 1 g · kg−1 · d−1 of protein and 1.7 × measured the resting metabolic rate. Leucine oxidation was determined using L-[1–13C]leucine and the appearance of 13CO2 (calculated as F13CO2) in breath. A range of markers was used to monitor for adverse effects, including glucose, insulin, alanine aminotransferase, and ammonia. Plasma leucine concentrations significantly increased beyond an intake of 500 mg · kg−1 · d−1. The metabolic limit to oxidize leucine was between 550 and 700 mg · kg−1 · d−1. An increase in blood ammonia concentrations was observed at leucine intakes >500 mg · kg−1 · d−1. There were no changes in liver alanine aminotransferase. Glucose concentrations fell (P < 0.004) but remained within the normal range and without any change in insulin. This study is the first to our knowledge to directly estimate the safe upper limit of leucine intake in humans and raises concerns that intakes >550 mg · kg−1 · d−1 or ~39 g/d may be a risk to health. It is important to note that these are acute studies, where each participant was exposed to graded increases in leucine intake. Longer term adaptation was not studied. J. Nutr. 142: 2220S–2224S, 2012.

Introduction
The use of dietary supplements is on the rise, especially among athletes. Reports suggest that almost 85% of professional athletes consume some form of nutritional supplements, with the most popular ones being vitamins, antioxidants, and protein, including creatine and amino acids (1). In the United States, ~3.4% of the general population uses amino acid supplements, 62% on a daily basis (2), and thus we must be concerned that these individuals may consume excessive amounts of amino acids. 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., stimulation of protein synthesis by leucine, synthesis of catecholamines from aromatic amino acids, methyl and sulfur donation from sulfur amino acids, NO from arginine, etc. Therefore, dietary supplementation with specific amino acids in excess of the requirement for protein synthesis may have adverse effects, or it may be beneficial in some situations. For these reasons, additional knowledge is necessary regarding the highest possible intake of each amino acid at which no adverse effect occurs (3,4).

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The BCAA include leucine, valine, and isoleucine and are popular as dietary supplements among strength-training athletes (5). Leucine in particular has been shown to stimulate protein synthesis and promote anabolism (6) and is widely used by athletes who think it helps improve performance. Most of the initial studies to test for leucine supplementation and effect of performance were negative (7,8), although recent studies seem to suggest benefits with leucine supplementation (9,10), particularly when combined with whey protein, which has a high leucine concentration (11,12). Furthermore, the most recent focus has been on leucine supplementation on decreasing exercise-induced muscle damage (13–16), which shows promising results and has potential for numerous clinical applications. Thus, there is a critical and urgent need to identify the safe upper limit of leucine that can be consumed.

A review of the literature can be conducted based on the numerous leucine supplementation studies conducted to identify a safe intake. Fernstrom (17) earlier summarized studies in athletes, normal adults, and patients with clinical disorders and reported that intakes of 15–60 g · kg\(^{-1}\) · d\(^{-1}\) (2200–8500 mg · kg\(^{-1}\) · d\(^{-1}\) for a 70-kg man) of total BCAA did not result in adverse event outcomes for the variables monitored. A considerable problem with interpreting the reported human studies is the great variability in approaches; these experiments included supplements of all 3 BCAA at different doses, different ratios among the BCAA, different routes of infusion/feeding (i.v. vs. oral), and different athletic training regimes. Thus, there is a need to identify direct approaches to determine a safe upper limit of leucine intake. Recently, we proposed one such approach (18) and the following article describes our approach with the experimental evidence (19).

Concept behind UL of amino acid oxidation

Markers for identifying excess intake of an amino acid should have very specific dose-response characteristics. In particular, variation with intake should display an inflection point that would identify the onset of the amino acid excess situation (18). Previously, in neonatal piglets, we observed an upper inflection point in the dose-response curve for phenylalanine retention and \(^{14}\)CO\(_2\) production from phenylalanine oxidation with graded phenylalanine intake (20). Piglets received graded phenylalanine intakes ranging from 0.2 to 1.2 g · kg\(^{-1}\) · d\(^{-1}\). The apparent phenylalanine balance, which was calculated as the difference between phenylalanine intake and oxidation, increased linearly between 0.2 and 0.5 g phenylalanine · kg\(^{-1}\) · d\(^{-1}\). Apparent phenylalanine balances did not differ for intakes between 0.5 and 0.8 g phenylalanine · kg\(^{-1}\) · d\(^{-1}\). At the highest phenylalanine intakes, the apparent phenylalanine balance was significantly higher than the plateau values. Phenylalanine balance reflects the net rate of accretion or retention of phenylalanine in body tissues. The increase in phenylalanine balance beyond the intake of 0.8 g · kg\(^{-1}\) · d\(^{-1}\) indicated that oxidative processes were unable to keep pace with the increasing intake of phenylalanine. The inflection point at 0.8 g · kg\(^{-1}\) · 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 mg · kg\(^{-1}\) · d\(^{-1}\) (20). Once the maximum level of phenylalanine oxidation was reached, the plasma phenylalanine concentrations and retention rose rapidly. Hence, we reasoned that a suitable marker to define the tolerable upper intake level (UL) for a dietary amino acid would be the level at which the maximum oxidation level was reached (Fig. 1). This approach was initially described and proposed by our group during the 3rd Amino Acid Assessment Workshop (21) and subsequently reinforced during the 5th (22) and 7th Amino Acid Assessment Workshops (18).

Leucine UL in animal models

Sakai et al. (23) used the above-described approach to identify leucine excess in rats. They identified the metabolic limit by measuring \(^{13}\)CO\(_2\) production from U-\(^{13}\)C-leucine with increasing leucine intake in the excess range. The maximum limit to oxidize excess leucine was reached at 10% of dietary intake or 8900 mg · kg\(^{-1}\) · d\(^{-1}\); the oxidation achieved a plateau and this inflection point was identified as the UL of leucine intake in rats. The same authors, in an earlier study using a similar strain of rats that received a diet similar in composition, observed significant growth inhibition in rats fed 15% leucine or 12,400 mg leucine/kg (23). 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 more appropriately identify the UL.

Tsukuba et al. (24) in a controlled experiment studied oral supplements of leucine excess in 4-wk-old rats and estimated ~3500 mg · kg\(^{-1}\) · d\(^{-1}\) as the no observed adverse effect level based on body weight, food consumption, and hematological measurements. Similarly, Mawatari et al. (25) in 10-wk-old female rats estimated that oral leucine at 1000 mg · kg\(^{-1}\) · d\(^{-1}\) did not affect the outcome of pregnancy and did not cause fetal toxicity.

Leucine UL in adult humans

We recently reported a study of the effects of graded doses of leucine in healthy young men aimed at defining the UL of leucine in the acute phase (19). Participants received increased dietary leucine in a graded stepwise intake of 50, 150, 250, 500, 750, 1000, and 1250 mg · kg\(^{-1}\) · d\(^{-1}\) corresponding to the Estimated Average Requirement and Estimated Average Requirement × 3, × 5, × 10, × 15, × 20, and × 25 on separate study days (26). All study days were separated by a minimum of 2 wk to ensure a sufficient washout period between the leucine excess study day diets.

**Blood and urine biochemical variables.** Baseline and hourly vital signs (blood pressure, heart rate, body temperature) and blood glucose (One Touch Ultra) were measured during the study day to ensure patient safety and did not reveal any significant changes. Alanine aminotransferase, as a marker of liver function, blood urea, creatinine, electrolytes, CBC, urinary creatinine, or urea, did not show any significant changes due to increased leucine intakes. Blood ammonia concentrations at...
the end of all study days were higher (P < 0.0001) than fasting blood ammonia concentrations, except at a leucine intake of 50 mg · kg⁻¹ · d⁻¹. With increasing intakes of leucine, blood ammonia concentrations rose (P = 0.0001) by the end of the study day and were above the normal range of 35 μmol/L after leucine intakes of 500 mg · kg⁻¹ · d⁻¹. When blood ammonia concentrations at the end of the study were beyond the upper limit of the normal range, participants were contacted and requested to come back to the clinical investigational unit the next day to provide a blood sample to check ammonia concentrations. In all cases, the blood ammonia concentrations were within the normal range (<35 μmol/L) when tested on the following day, indicating that the adverse effect due to increased leucine intake was not observed when the participants returned to their normal diet. Plasma glucose was decreased at all intakes of leucine compared with 50 mg · kg⁻¹ · d⁻¹ (P = 0.0004), from 6.2 ± 0.3 μmol/L (mean ± SEM) to a low of 4.7 ± 0.2 μmol/L, although all values stayed within the normal range of 3.3–6.1 μmol/L. Leucine has been suggested to act as an insulin secretagogue (6,27,28), but we did not observe significant changes in plasma insulin due to increasing leucine intakes.

Plasma BCAA concentrations were measured in all participants, because previous studies have documented that increased intakes of leucine decrease the plasma concentrations of valine and isoleucine (29–32). The plasma leucine concentration significantly increased and plasma valine and isoleucine concentrations significantly decreased with increasing leucine intakes, as previously reported (31). The BCAA share a common catabolic pathway with the branched-chain ketodehydrogenase controlling the irreversible catabolic step, which commits the carbon skeleton of the BCAA to the TCA cycle. Leucine concentrations have been shown to stimulate branched-chain ketodehydrogenase as well as to compete with the other 2 BCAA for metabolism in vivo (33); this phenomenon, referred to as BCAA antagonism, is well documented (31).

\[ \text{L-}[1^{-13}C]\text{Leucine oxidation and estimation of the UL of leucine} \]

Three baseline and 4 plateau breath samples were collected for measurement of leucine oxidation during each study day. The oxidation of L-[1⁻¹³C]leucine to ¹³C₂O₂, calculated as F¹³C₂O₂, responded to increasing leucine intakes (P < 0.0001). F¹³C₂O₂ increased with increasing leucine intakes up to 500 mg · kg⁻¹ · d⁻¹, after which oxidation remained at a plateau up to intakes of 1250 mg · kg⁻¹ · d⁻¹. Using 2-phase linear regression analysis (34,35), a breakpoint in F¹³C₂O₂ was identified at 350 mg · kg⁻¹ · d⁻¹ (r² = 0.83) (19). This breakpoint identifies the maximum oxidative potential in adult humans to dispose of excess dietary leucine intake. The lower and upper 95%CI was calculated as 454 and 647 mg · kg⁻¹ · d⁻¹, respectively.

Analysis of results from leucine UL study in adult humans

The primary objective of our recent study (19) was to define the metabolic capacity to dispose of excess leucine intake in vivo in adult men. Oxidation of L-[1⁻¹³C]leucine to ¹³C₂O₂ in breath (F¹³C₂O₂) was the primary endpoint measured with increasing intakes of dietary leucine. F¹³C₂O₂ increased with increasing intakes of leucine until 500 mg · kg⁻¹ · d⁻¹, after which oxidation remained at a plateau. Two-phase linear regression analysis identified a breakpoint at a leucine intake of 550 mg · kg⁻¹ · d⁻¹ and this represents the maximum leucine oxidative potential in vivo in adult men (19). There was a concomitant increase in blood ammonia concentrations beyond the normal range (<35 μmol/L) in all participants with intakes of leucine >500 mg · kg⁻¹ · d⁻¹. There is an increasing risk of adverse effects, as observed with the increasing ammonia levels, with leucine intakes beyond the metabolic capacity to oxidize leucine. Our study results are acute in nature and it is unknown whether chronic consumption of leucine in adult humans will result in a similar upper limit of leucine oxidation.

Mechanism to explain hyperammonemia observed with increasing leucine intakes

Leucine is an activator of glutamate dehydrogenase, which results in α-ketoglutarate and ammonia production. It has been reported that leucine at concentrations >800 μmol/L activates glutamate dehydrogenase to dispose of the surplus amino acids (36,37). Intracellular glutamate concentrations are very high (5–10 mmol/L) compared with extracellular concentrations (30–50 μmol/L). Although amino acids can be converted to glutamate via transamination reactions, glutamine serves as the major precursor for glutamate via the phosphate-dependent glutaminase, which is also regulated by the energy potential (36). Thus, flux into glutamate comes from glutamine, especially when leucine concentrations increase (as it did in the current study, up to 2000 μmol/L), leading to increased glutamine synthesis.

An additional explanation is also possible. Due to the increased accumulation of isovaleryl CoA, which is formed from leucine catabolism, inhibition of N-acetylglutamate synthase (NAGS) has been shown to occur in isovaleric aciduria (38). NAGS is an activator of carbamoyl-phosphate synthetase-1, which regulates the urea cycle (38). With a decrease in NAGS, carbamoyl-phosphate synthetase-1 activation would not have occurred and this is also shown by the lack of increase in urea concentrations in both blood and urine in our current study.

Summary

The DRI proposed by the Institute of Medicine defines the UL as the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases beyond the UL, the potential risk of adverse effects may increase. Based on the above definition and the maximum oxidative potential observed in the present study, the measured breakpoint (350 mg · kg⁻¹ · d⁻¹) may or may not be the UL for leucine intake in all individuals, because blood ammonia concentrations were greater than the normal range at 500 mg · kg⁻¹ · d⁻¹ of leucine intake. Furthermore, the current study was conducted with an acute supplement of dietary leucine in normal adult men, so whether chronic leucine supplementation in normal/trained athletes will provide similar results is unknown.

An analysis of the current habitual leucine intake was conducted in strength-training athletes who are chronic amino acid supplement users. The average protein intake in these athletes was ~2 g · kg⁻¹ · d⁻¹ (39). The mean leucine content in food is ~15% (40). Therefore, for an athlete weighing 80 kg, the dietary leucine intake is 300 mg · kg⁻¹ · d⁻¹. Available BCAA supplements contain a maximum 1800 mg/serving (41), with a recommended regimen of 3 doses/d, which equals ~67.5 mg · kg⁻¹ · d⁻¹. Thus, the habitual total exposure to leucine in adults consuming 2 g · kg⁻¹ · d⁻¹ and the amino acid supplement is ~367 mg · kg⁻¹ · d⁻¹. These calculations reveal that most people, including athletes, consume less than the UL for leucine oxidation determined in the current study (550 mg · kg⁻¹ · d⁻¹). However, there is probably a range in protein and leucine intake among athletes, with some consuming more than the recommended dose and thus at potential risk of adverse effects. The impact of chronic consumption by humans of excess leucine less than, at, or greater than the maximum oxidation limit for nonadapted
individuals remains unknown. A high chronic intake may either reduce the risk of adverse effects by increasing the basal leucine oxidation rate or increase the risk of adverse effects by gradual accumulation of metabolic events associated with excess intake.

In conclusion, with increasing intakes of leucine, a clear dose response in leucine oxidative capacity, measured as E13CO2 from the oxidation of L-[1-13C]leucine, was observed. The maximum oxidative potential for leucine in vivo under acute feeding conditions was identified using 2-phase linear regression analysis with a breakpoint of 550 mg·kg^-1·d^-1 or 39 g·d^-1. Significant increases in blood ammonia concentrations were observed at leucine intakes >500 mg·kg^-1·d^-1. At a leucine intake of 250 mg·kg^-1·d^-1, the mean fed-state ammonia concentrations are within normal limits, but at a leucine intake of 500 mg·kg^-1·d^-1, the mean ammonia concentration is beyond the normal limits. We did try nonlinear regression analysis on the fed-state ammonia results and did not obtain a significant model. It could therefore be argued that when chronic ingestion studies are designed, leucine intake levels should obtain a significant model. It could therefore be argued that when chronic high-dose leucine ingestion studies need to be performed. Further, such studies need to be considered in young women as well as in elderly women and men.

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Literature Cited

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