Knowledge Gained from Studies of Leucine Consumption in Animals and Humans\textsuperscript{1,2,3}

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Abstract

Leucine's wide-ranging metabolic influences have made it subject to special interest. It is abundant in the diet, especially in some milk and cereal proteins, in part due to its allocation of 6 codons in the genetic code, and individual dietary intakes range up to >250 mg \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1}\). It influences many cell functions by various mechanisms, which include allosteric activation of enzymes, enabling ATP generation and insulin secretion from the pancreatic islet cell, and activation of signaling pathways. It is a mediator of the anabolic drive of dietary amino acids, stimulating anabolic hormone secretion and directly signaling protein deposition and growth through the stimulation of protein synthesis and restraint of proteolysis. Its signaling may involve the mammalian target of rapamycin complex and rapamycin-insensitive pathways responding to a leucine "transceptor," which combines leucine cellular transport, fueled by the intracellular-extracellular glutamine gradient, and a signaling response to changes in ionic and water balance and cell volume. In animal studies, dietary leucine supplementation has reversed many of the adverse influences of a high-fat diet, consistent with a benefit for healthy weight maintenance in humans for which evidence is accumulating. The implications for safety of leucine-supplemented diets are discussed in terms of adversely lowering valine and isoleucine concentrations and inducing hyperammonemia through overloading peripheral glutamine synthetic pathways. Finally, the apparently high human leucine requirement is explained in terms of an adaptive metabolic demand model of requirements and the design and analysis of human studies, which may overestimate values. J. Nutr. 142: 2212S–2219S, 2012.

Introduction

Supplements of leucine and the BCAA are advocated and widely used in relation to performance and in various clinical situations \( \textsuperscript{1,2} \) to maintain muscle mass during weight loss \( \textsuperscript{3} \) and in the elderly, although its efficacy in some of these circumstances remains to be firmly established \( \textsuperscript{4} \). It is quite clear that in animals and especially humans, consumption of leucine and the BCAA seems to be without identifiable hazards and the task of identifying the upper limits of human tolerance of leucine remains extremely difficult because of a lack of firm data. Here, the intention is to examine some selective aspects of the biology of leucine, including its regulatory role and those features of its metabolism that might relate to its safety and toxicity at high intakes and that may be useful in understanding its dietary requirement in human nutrition.

Dietary Content of Leucine and the Genetic Code

The genetic code is degenerate; 64 codons identify 22 coded amino acids. Thus, statistically, the number of codons assigned to each amino acid in a protein will to some extent influence its frequency in protein. The highest number is 6, assigned to leucine and in the >0.5-m protein sequences held in the UniProtKB/Swiss-Prot protein knowledgebase \( \textsuperscript{5} \), the frequency of leucine is 9.7% compared with 2.4 and 1.1% for methionine and tryptophan (1 codon each), respectively. However, serine (6.6%) and arginine (5.5%) also have 6 codons, with 4 and 3

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codons for valine (6.9%) and isoleucine (6.0%). This indicates that codon number is by no means the only determinant of frequency of occurrence. Leucine’s hydrophobic side chain conveys a specific structural character to protein and its high frequency of occurrence involves sequences of leucine-rich repeats, which characterize the structure and function of proteins and which is responsible for many protein-protein interactions (6). The richest sources of leucine (Table 1) are some cereals such as maize (and sorghum), and milk proteins, especially β-casein (10.5%), and the whey proteins, α-lactoglobulin (10.6%), and β-lactoglobulin (13.3%) (7).

Population Intakes of Leucine

Food intake surveys underestimate nutrient intakes because of underreporting and my analysis of the UK adult National Diet and Nutrition Survey (8), trimmed of under-reporters (i.e., energy intakes <1.35 × predicted basal metabolic rate), indicates median and 90th percentile intake values for protein of 1.25 and 1.61 g · kg⁻¹ · d⁻¹ (14.2 and 17.3% energy) and 108 and 138 mg/kg leucine at 8.3% (9) of the protein intake. Modeling the more recent UK National Diet and Nutrition Survey protein intakes of 17.6% food energy (10) for a physically active 70-kg young man (physical activity level = 2.2) indicates a protein intake of 2.41 g · kg⁻¹ · d⁻¹ or 200 mg · kg⁻¹ · d⁻¹ of leucine. Our studies of 90-kg body builders (11) indicated protein intakes up to 3.05 g · kg⁻¹ · d⁻¹ (28% protein calories) of a mainly animal protein diet [8.6% leucine (9)]; i.e., a food leucine intake of 262 mg · kg⁻¹ · d⁻¹ (~7 × the recommended dietary allowance). Although little is known about the long-term health effects of these high-protein intakes, it can be assumed that such intakes are widespread.

Regulatory Influences of Leucine

Leucine mediates insulin secretion, regulatory anabolic and anti-catabolic signaling to regulate protein deposition and growth. It also influences food intake and body weight maintenance, macronutrient disposal and metabolism, insulin resistance, and the symptoms of type II diabetes. Finally, leucine can influence behavior in terms of mental and physical function, although these actions are probably indirect and mediated through metabolic interactions of leucine with neurotransmitters in the nervous system (1,2). Here, selected aspects of leucine’s regulatory role will be highlighted.

Leucine and Protein Metabolism

Food provides energy and amino acid substrates, which together exert a regulatory influence, an anabolic drive (12,13), on tissue protein balance through the stimulation of protein synthesis (S)⁴ and inhibition of degradation (D) (14,15). Much of this effect is explained by the action of insulin and amino acids (15,16), with leucine being the most effective amino acid mediator of the response in all circumstances examined to date (17–19). In the whole body, effective postprandial protein utilization (PPU) is achieved through an insulin-mediated, protein-conserving influence of dietary energy, inhibiting amino acid oxidation and D [an influence also observed in human muscle (20)] and an amino acid-mediated augmentation of the inhibition of D and stimulation of S (15). The overall efficiency of PPU [Δleucine deposition/Δleucine intake] is highest when postprandial increases in tissue and blood amino acid concentrations and associated oxidation rates are minimized by a maximal inhibition of D (16). Thus, the key physiological feeding response at the whole body level, determining maximal PPU, is the sensitivity of the insulin inhibition of D to further inhibition by amino acids, i.e., the ability to inhibit D with only minor increases in plasma leucine.

Protein Degradation

How leucine and insulin interact to inhibit proteolysis remains poorly understood. Studies of regulation of autophagy in yeast and mammalian cells have identified a key signaling role for mammalian target of rapamycin (mTOR), a highly conserved Ser/Thr kinase, with evidence that both the complex containing the rapamycin-dependent binding partner, raptor (mTORC1), and containing the rapamycin-insensitive binding partner, rictor (mTORC2) are involved (21). In myotubes, most of the insulin/IGF-1, Akt/Protein Kinase B (PKB)-mediated inhibition of lysosomal autophagy is mediated through an mTORC2 pathway involving the FOXO transcription factor FOXO3 (22). In human muscle in vivo, the inhibitory influence of insulin on D at moderate physiological concentrations, which increased Akt/PKB kinase activity, had no effect on either mTORC2 signaling

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<td>187</td>
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¹ Protein composition values are from Robinson (7).
² Mean of plant or animal values shown in this table.
via the FOXP1α-atroge (proteosome) axis or on mTORC1 signaling to eEF2, or p70S6k, or eIF4BP1 (20). In the isolated perfused rat liver, insulin inhibits autophagic proteolysis by inducing hepatocyte swelling through osmotic water influx (23). This is sensed by the integrin system (cell surface receptors that interact with the extracellular matrix and mediate intracellular signaling), mainly activating the p38 MAP-kinase pathway (24,25), and is insensitive to rapamycin, so it does not involve mTORC1 (26). Whether mTORC1 is involved in leucine’s inhibitory action on proteolysis in muscle is not clear. In a rat skeletal muscle cell line, the leucine inhibition of proteolysis is abolished by inhibition of mTOR by rapamycin (27), suggesting the mTORC1 pathway is involved. However, in perfused rat liver, although leucine interacts strongly with the mTORC1 complex, activating rapamycin-sensitive downstream signaling, leucine’s marked inhibition of proteolysis is insensitive to rapamycin, suggesting that either mTORC2 or some other leucine-mediated signal transduction is involved in this action (26).

Protein Synthesis

For human adults, the in vivo stimulation of muscle protein synthesis (MPS) by feeding (28) appears to be mediated mainly by amino acids (29) rather than insulin (30,31), with leucine as the main stimulus (32,33). Thus, moderate insulin levels that inhibit D do not stimulate MPS (20). Human and animal studies have unraveled some of the signaling involved, but it remains only very partially understood, especially in relation to the interplay between insulin and leucine on signal transduction and the extent to which amino acids act through more than one pathway. It is generally agreed that the stimulatory effect of leucine on protein S is mediated through upregulation of both initiation and elongation phases of mRNA translation, promoting both global translation and discrimination in the selection of mRNA for translation (34). mTORC1 appears to play a central role in mediating this aspect of leucine’s influence (35), phosphorylating ribosomal protein kinase S6 (S6K1) and 4E-binding protein 1 (4EBP1), among many other substrates (36), although an mTORC1-independent amino acid signaling pathway to protein S may also be involved in human adults (M. J. Rennie, University of Nottingham, personal communication). Insulin’s action is mediated by the Akt/PKB kinase pathway (37). In human muscle in vivo, an oral protein bolus increased phosphorylation of both S6K1 and 4EBP1, which mirrored the increases in muscle leucine concentrations and MPS, whereas increases in plasma insulin concentrations, Akt/PKB activity, and eIF4G phosphorylation preceded the increase of MPS (38).

The molecular mechanisms by which leucine interacts with mTORC1 and any other signaling pathway is beyond the scope of this article (35,39,40). However, one interesting development relates to the concept of dual-function amino acid transporter/receptor (“transceptor”) proteins as the initiating mechanism (41,42). The transporter System L operates as an obligatory 1:1 heteroexchanger facilitating uptake of leucine in exchange for glutamine, which is accumulated via secondary (Na+ linked) active transporters (e.g., SNAT2). The uptake of leucine by such a system requires glutamine reuptake to maintain the high glutamine gradient. This will establish an osmotic gradient for the passive influx of water that promotes cell swelling and activates anabolism (26), events observed in skeletal muscle (43,44). SNAT2 appears to be capable of sensing and directly signaling amino acid availability in the extracellular pool to intracellular signaling pathways, including mTOR, possibly through phosphoinositide 3-kinase-dependent mechanisms. The inhibition of hepatic autophagy by leucine was reported some years ago to involve receptor-mediated signaling (45), although, as indicated above (26), mTORC1 may not be involved. Human MPS does appear to respond to extracellular rather than intramuscular amino acid availability (46). Thus, Hundal and Taylor (41) argue that leucine’s influence on cell signaling critically depends on maintaining both cellular hydration and the glutamine transmembrane concentration gradient (and other SNAT2 amino acid substrates), identifying glutamine as physiologically indispensable from the perspective of preserving the primacy of leucine in terms of mTORC1 signaling (42). This is consistent with the direct relationship between muscle glutamine concentration and ribosomal capacity, activity, and protein S rate in rat muscle we reported some years ago (47).

After a protein meal, the leucine-stimulated increase in MPS in humans is only transient, ≤3 h, even though leucine concentrations and mTORC1 signaling remain increased (38). This is consistent with the cessation of muscle growth in early adulthood regardless of the dietary intake. Rennie et al. (38) call this a “muscle full” effect, i.e., muscle gauging its capacity to synthesize new proteins once postabsorptive losses have been replaced by feeding. Some years ago, we suggested that muscle fiber growth, i.e., an increase in fiber cross-sectional area, was volume limited by the surrounding connective tissue matrix (endomysium) (13). If muscle fiber swelling is an essential part of insulin and leucine activation of muscle anabolism, as already suggested, then it might be predicted that once postprandial replacement of postabsorptive muscle protein losses is complete, the structural volume limitation may terminate the anabolic response through inhibiting MPS and/or increasing D. Such a response could involve integrin signaling, which plays an important regulatory role in insulin action on muscle (48), possibly involving mTORC2 signaling to the actin cytoskeleton (49). If increases in D were part of the muscle-full response, then this could account for the apparent return to baseline of MPS while leucine concentrations and mTORC1 signaling remained elevated, i.e., newly synthesized polypeptides could be degraded, possibly through endoplasmic reticulum-associated D (50) preventing their incorporation into muscle protein.

Leucine, Insulin Secretion, Pancreatic β-Cell Function, Insulin Resistance, and Body Weight Maintenance

Layman et al. (3) have championed the potential importance of leucine for treatment of obesity and metabolic syndrome and there is now detailed animal experimental evidence for leucine’s influence on insulin secretion and sensitivity, food intake and dietary macronutrient disposal, and body composition. Leucine and α-ketoisocaproate (KIC) act as potent insulin secretagogues. A leucine-rich protein such as whey appears to mediate insulin secretion because of its BCAA content (51) rather than through its effect on the incretins, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (52). Leucine was thought to act as a metabolic fuel within the islet β-cell through provision of KIC for oxidation after transamination with α-keto glutarate (αKG) by the mitochondrial branched-chain aminotransferase (BCATm) (53). However, in BCATm knockout mice, the stimulation of insulin secretion by KIC is abolished, whereas leucine stimulation is maintained, as is that by all other secretagogues (54). This suggests that leucine acts to stimulate fuel production from other
substrates rather than serving as a fuel itself and KIC action requires its transamination to leucine. Leucine allosterically activates glutamate dehydrogenase, which aids glutamine uptake, glutaminolysis, and oxidation, fueling β-cell K<sub>ATP</sub> channel activity, exporting K<sup>+</sup>, and enabling an increase in free cytosolic Ca<sup>2+</sup> through the calcium channel. This triggers insulin secretory granule exocytosis via various mechanisms. Leucine has also been shown to regulate gene transcription and protein S in islet β-cells by both mTOR-dependent and -independent pathways (53,55).

These new findings suggest that leucine may exert a long-term impact on β-cell function by regulating gene expression. Upregulation of β-cell ATP synthase β-subunit has been reported (55) and this is of interest, because inhibition of mitochondrial ATP synthesis has been suggested as a key event in the progression of β-cell dysfunction, itself a decisive cause of type 2 diabetes. Long-term treatment with leucine improves insulin secretory dysfunction of human diabetic islets (55). This provides one mechanism for a regulatory role of dietary leucine and high-protein diets on glycemic control in overweight and obese populations and those with type 2 diabetes (3,56). Thus, better glycemic control and body weight maintenance would be linked through a limitation of hunger and snacking. Also, better maintenance of lean tissue would result from leucine’s influence on MPS during energy restriction and the modulation of insulin signaling and glucose use by skeletal muscle (56). Such actions of leucine have been identified in a mouse high-fat feeding model of obesity and insulin resistance (57). Doubling dietary leucine by its provision within their drinking water reversed many of the metabolite abnormalities and caused a marked improvement in insulin sensitivity and signaling and glucose tolerance, with a decrease in hepatic steatosis and inflammation in adipose tissue.

In this mouse model, the metabolic improvements in insulin resistance were achieved without changes in food intake. However, leucine has a direct influence on satiety and food intake, which is another mechanism by which dietary leucine in protein can enable weight control. In our randomized controlled trial of commercial weight loss regimes showing similar rates of weight loss on an unrestricted high-protein diet as with low-fat, energy-restricted diets (58), we showed that with the high-protein diet there was a similar voluntary reduction in food intake as the target reductions with the low-fat diets, which is consistent with the well-known satiety effects of protein shown in test meal studies (59). mTORC1 signaling in the arcuate nucleus of the hypothalamus has been implicated in satiety responses, and intracerebroventricular administration of leucine but not valine increased hypothalamic mTORC1 signaling and decreased food intake and body weight, an effect also observed with leptin treatment (60). Also, in rats and ob/ob mice, a leucine-supplemented diet decreased food intake and attenuated weight gain and expansion of fat mass with no changes in lean body mass (61). In these studies, intracerebroventricular administration of leucine reduced food intake in a dose dependent manner, enhancing pro-opiomelanocortin (anorexigenic peptides) and diminishing neuropeptide Y (orexigenic peptide) expression in the hypothalamus with increased ATP, reduced AMP, and a decrease in the activity of the energy-sensing AMP-activated protein kinase and its phosphorylated substrate, acetyl-CoA carboxylase. Increased mTORC1 signaling to p70S6K and eIF4E was also observed and abolished by rapamycin. How leucine activates mTORC1 was not explained, but we might speculate that the transceptor concept discussed above in the context of leucine signaling in skeletal muscle could also be involved, especially given the evidence of the increased energy status of the neurons involved.

Within populations, the evidence base relating higher protein and especially leucine intakes to improved glycemic and body weight control is not extensive, but there is some recent epidemiological support for the idea. Thus, risk of overweight/obesity fell significantly as BCAA intakes (percent of protein intake) increased (Q4 compared with Q1) within an international cohort of healthy adults from China, Japan, the UK, and the US (62). Although the overall increase in total protein intakes (as a percentage of the energy intake) was small (13%), animal protein intake was >40% higher (Q4 compared with Q1) and because dairy proteins contain higher BCAA levels than meat, the body weight effects may reflect the beneficial influence of calcium intake, for which there is considerable evidence (63).

**Peripheral Leucine Nitrogen Metabolism**

This is important in the context of disposal of high leucine intakes and potential toxic metabolic effects, and there are probably 2 important issues. First, leucine disposal interacts with the disposal of valine and isoleucine, because the first 2 enzymes in their catabolism, i.e., BCAT and the mitochondrial branched-chain α-keto acid dehydrogenase enzyme complex (BCKDC) are common to all 3 BCAA (64,65). Thus, their oxidation occurs in lockstep (i.e., correlated changes in their concentrations after a meal), suggesting to some authors that the plasma concentrations of the individual BCAA are not tightly defended and therefore not particularly important (66). We showed some years ago that leucine transamination is freely reversible and much faster than the rate-limiting decarboxylation step (67). The activity of the BCKDC is regulated by covalent (inhibition by phosphorylation) and allosteric mechanisms, which include both end-product inhibition (NADH and branched-chain acyl-CoA esters) and activation, mainly by KIC (acting to inhibit the BCKD kinase, and by adaptive changes in enzyme expression in response to varying protein intakes (64). The dominance of KIC for BCKD kinase activation is consistent with leucine’s greater dietary supply and higher tissue concentrations compared with valine and isoleucine and the joint pathway appears designed to managing BCAA disposal within a limited range of relative concentrations of the 3 BCAA. However, it is poorly designed for selective conservation of individual BCAA in unbalanced mixtures, which might occur with leucine supplementation. In this case, increased leucine concentration and oxidation will promote the oxidation of valine and isoleucine, potentially lowering their concentration. This occurs in growing animals when the ratio of leucine:valine and isoleucine is higher than usual and can inhibit growth (68). Whether high levels of leucine intake unbalanced by valine and isoleucine would eventually inhibit protein S through lowering isoleucine and valine concentrations to limitingly low levels has not been investigated.

The second issue relates to the fate of leucine nitrogen from excessive intakes of leucine. BCAT and BCKDC are widespread in peripheral tissues (65,69), with dietary BCAA largely escaping first-pass hepatic catabolism. The preferred substrate for BCAT is α-KG, so glutamate will be the primary product, although according to relative tissue concentrations of other transaminases and the tissues’ need for glutamate, BCAA amino acid nitrogen can be transferred to other dispensable amino acids. Measurements of BCAT activities in various human tissues (65,69) indicate that BCAA transamination occurs mainly in muscle (~65%), brain, and adipose tissue (together ~25%), with the rest in liver, kidney, intestine, and heart. This peripheral location of BCAA metabolism means that not only can they serve as a source α-amino nitrogen to the periphery but that they
require nitrogen transport back to the splanchnic bed for ureagenesis and nitrogen excretion. This normally occurs as alanine and glutamine (69), but any limitation on the synthesis of these nitrogen carriers would result in increased ammonia levels, which would be problematic.

Human brain avidly extracts leucine and releases glutamate (69), with the entry of leucine exceeding that of any other amino acid. Although not neuroactive, because leucine (and valine and isoleucine) shares the same large neutral amino acid transporter it can influence the uptake of the aromatic amino acids and the synthesis of neurotransmitters deriving from them (although I am not aware of any information of the impact of very high leucine intakes in humans on this aspect of brain amino acid metabolism). Leucine is especially important for brain glutamate metabolism being “trafficked” between cellular compartments, providing -NH₂ groups for glutamate synthesis in compartments of high glutamate concentration and supplying KIC to act as a sink for -NH₂ groups from glutamate in compartments of low glutamate concentration (69). Given the ample anaplerotic capacity of the brain to synthesize αKG, it might be predicted that increased leucine uptake into the brain when plasma leucine levels are very high would result in increased glutamine release without excessive ammonia accumulation.

In skeletal muscle, the uptake of BCAA represents ~50% of all muscle amino acid uptake, with their nitrogen released as alanine and glutamine (69). Alanine synthesis can occur through the mitochondrial form of alanine-amino-transferase, which is expressed in muscle, allowing glutamate produced by BCATm to be recycled back to α-KG through transamination with pyruvate (69). Because leucine uptake into muscle is likely to involve a 1:1 exchange with glutamine via the system L transporter, maintenance of the high intracellular glutamine concentration by both an Na⁺-dependent SNAT transporter mediating glutamine reuptake and by de novo synthesis in muscle is essential to allow leucine uptake. In the rat hemisephorus perfused with leucine alone, glutamine accounted for 80% of the increased nitrogen output as alanine and glutamine, and intracellular concentrations of alanine, glutamine, and glutamate were increased (70). In human muscle, glutamine concentrations are much higher (~20 mmol/L (71)) than in rat muscle (<10 mmol/L (47)), suggesting that glutamine synthesis is particularly important for leucine disposal in human muscle. The source of the substrates for glutamine synthetase, however, has not been clarified (69). Cytosolic ammonia for glutamine synthesis could come from mitochondrial ammonia via glutamate dehydrogenase 1 if this is sufficiently active in human muscle, because the purine nucleotide cycle may be a significant source of ammonia only in exercise (71). Leucine is entirely ketogenic and cannot provide carbon for cytosolic glutamate, and a suitable anaplerotic enzyme such as pyruvate carboxylase (allowing α-KG synthesis from glucose) is not abundantly expressed in muscle (69). In some circumstances such as during exercise in the postabsorptive state, muscle extracts circulating glutamate in substantial quantities, releasing glutamine and ammonia (72), and this occurs in the postprandial state after a balanced AA supply from a meal. However, with high leucine intakes, there may be insufficient circulating glutamate to enable the required muscle glutamine synthesis. In this case, ammonia would eflux out of muscle, with the potential for hyperammonemia, which would provide an end point indicating leucine toxicity. In rats fed leucine at 30% of the diet, increases in blood ammonia were not reported (at least as measured during amino acid analysis) even though blood urea nitrogen increased 3-fold (73). However, in the recent human toxicity trials of leucine by Pencharz and Ball (74), leucine intakes >500 mg · kg⁻¹ · d⁻¹ were associated with hyperammonemia. Clearly, more work is required in this area.

The Metabolic Demand for Leucine

Defining the metabolic demand for leucine is problematic. Although its demand for net protein S is obvious, it is not clear to what extent other aspects of its metabolism generate an additional, irreducible metabolic demand (Fig. 1). No specific essential metabolite or unique biologically active molecules are known to be synthesized from its carbon skeleton. Although its regulatory actions are clear, to what extent these various influences determine the leucine intake required to achieve overall nitrogen, protein, or leucine balance and maintain the lean body mass and general well-being is difficult to judge. It can only be assumed that the short-term balance studies that are used to determine human amino acid and protein requirements do identify intakes that optimize tissue protein S and balance. The adaptive nature of the oxidative disposal of leucine and the other BCAA can be assumed to be an important part of the explanation of the adaptive nature of overall nitrogen losses. However, this adds a further difficulty to the identification of the minimum leucine requirement, i.e., allowing sufficient time for complete adaptation, especially in studies in which valine and isoleucine intakes are maintained at usual intakes while leucine intakes are reduced. If the minimum maintenance demand is quite low, as observed in pigs, for example, the high leucine concentration in most food proteins and the use of amino acid mixtures with normal levels of valine and isoleucine may mean that it is extremely difficult to identify the true minimum leucine intake that allows leucine and protein balance with minimum rates of leucine oxidative disposal. Most importantly, the metabolic demand for leucine needs to be considered in the context of the human diet and human feeding characteristics.

Estimates of the Leucine Requirement

Currently, leucine requirements are defined for infants, children, adolescents, and adults (75). For infants, the requirement is assumed to be that indicated by the leucine content of human

![FIGURE 1](https://academic.oup.com/jn/article-abstract/142/12/2212/4630823) Simple amino acid metabolic demands model for growth and maintenance. The demand is the flow of amino acids to protein S and other metabolic pathways, with any excess amino acids flowing through oxidative pathways and with the demand met by amino acids deriving from protein synthesis, de novo synthesis, or diet. Because amino acids deriving from protein synthesis will largely match the amount and pattern going to protein synthesis, the demand that needs to be met from the diet will be dominated by the flow to net protein synthesis when this occurs as growth and to other pathways, which irreversibly remove amino acids, i.e., maintenance. S, synthesis.
breast milk (96 mg/g protein). For older children and adolescents, no studies of the leucine requirement (or that of any other amino acid) were identified of sufficient quality, so the expert committee devised a factorial model based on growth and maintenance. The growth component is a dietary intake sufficient to provide for average rates of protein deposition (observed in longitudinal studies of body composition) after adjusting for a 58% efficiency of dietary protein utilization and assuming a leucine content of human tissue protein of 75 mg/g protein. The maintenance component is assumed to be the same as the adult requirement of 39 mg·kg\(^{-1} \cdot \text{d}^{-1}\). On this basis, the total leucine requirement at 6 mo, 1–2 y, 3–10 y, and ≥18 y is 73, 54, 44, and 39 mg·kg\(^{-1} \cdot \text{d}^{-1}\), respectively.

The adult leucine requirement of 39 mg·kg\(^{-1} \cdot \text{d}^{-1}\) is the highest requirement of any indispensable amino acid (75). The reanalysis by Kurpad et al. (76) of all the leucine oxidation data indicates either 44 or 48 mg·kg\(^{-1} \cdot \text{d}^{-1}\) for leucine according to his analytical approach. The maintenance requirement for leucine for pigs is only 13 mg·kg\(^{-1} \cdot \text{d}^{-1}\) and has been shown to be one of the least limiting amino acids for maintenance (77).

One reason for this apparent species difference is the requirements model. In growing animals, maintenance and growth requirements are separately evaluated, with net protein S not assumed to be part of maintenance. Thus, there is no a priori reason why the amino acid pattern of the maintenance demand should be similar to that for growth. In human nutrition, growth is only a significant part of the requirement in the first few years of life, after which nutritional demands are dominated by maintenance. This is conceptually complex and practically difficult to evaluate because of 2 features of human behavior in relation to dietary protein that influence protein and amino acid requirements. First, human diets contain a wide range of protein intakes up to quite high levels, resulting in adaptive changes in the capacity for amino acid oxidation to match habitual protein intakes and the adapted level appears relatively resistant to changes in intake in the short term (78). This increases the metabolic demand above the obligatory minimum level (79). Second, the sustained periods of postabsorptive state that characterize human feeding patterns result in tissue protein losses that must be replaced during feeding. As a result, dietary amino acids must provide for not only the obligatory metabolic demands, conceptually the same as the maintenance requirement in growing pigs, but also for the adaptive metabolic demand and net postprandial protein S (Fig. 2) (79). Within this model, the overall amino acid composition of the demand is not predictable because of variation in the length of the postabsorptive state, consequent postabsorptive losses and required postprandial gains, and the unknown composition of both the obligatory and adaptive metabolic demand (78).

Another reason for the apparent species difference is the conduct and interpretation of amino acid requirements studies. All recent stable isotope studies of the leucine requirement have used purified amino acid mixtures modeled on egg and this will overestimate minimum requirements. This is because egg protein is limited by nonessential nitrogen (NEN). It has long been known that utilization of the indispensable amino acids is maximized with a diet containing generous amounts of NEN (75). Also, purified amino acid diets rather than whole proteins will reduce the efficiency of utilization and overestimate the requirement (80). Finally, unlike classical balance studies that identified a “zone of equilibrium,” current stable isotope approaches adopt regression approaches of one sort or another that invariably identify an intake at the upper range of the range of equilibrium. Figure 3 shows 24-h 13C-1 leucine and nitrogen balance studies of healthy Indian adults (81), probably the best leucine requirement data available. This indicates zero balance statistically from intakes of 30 mg·kg\(^{-1} \cdot \text{d}^{-1}\), the start of the zone of equilibrium. The regression analysis indicated leucine requirement values of 37 and 38 mg·kg\(^{-1} \cdot \text{d}^{-1}\) for the 13C and N balance studies, respectively, which the authors argued confirmed their “tentative requirement” of 40 mg·kg\(^{-1} \cdot \text{d}^{-1}\) (81). This was much higher than the value obtained by N balance with an optimized diet (added NEN) (82), which after recalculation (75) indicated 26 mg·kg\(^{-1} \cdot \text{d}^{-1}\). All these factors account for why the currently accepted requirement

![FIGURE 2](https://academic.oup.com/jn/article-abstract/142/12/2212S/4630823)

**FIGURE 2** Obligatory and adaptive metabolic demands during the diurnal cycle. As discussed in the text, the diurnal pattern of feeding and fasting in human nutrition complicates the protein and amino acid requirement because of the need to provide for a variable amount of net protein deposition to replace fasting losses, the extent of which varies with the adaptive metabolic demand, which is in turn a function of the habitual protein intake.

![FIGURE 3](https://academic.oup.com/jn/article-abstract/142/12/2212S/4630823)

**FIGURE 3** Estimates of the human adult leucine requirement. Twenty-four-hour 13C-1 leucine and nitrogen balance studies of healthy Indian adults studied after 7 d of leucine intakes of 14, 22, 30, and 40 mg·kg\(^{-1} \cdot \text{d}^{-1}\) (81). Values, redrawn from (81), are mean ± SD, n = 10 (leucine) and 9 (nitrogen). The nitrogen balance is adjusted on the basis of 5 mg N·kg\(^{-1} \cdot \text{d}^{-1}\) unmeasured losses rather than the value of 8 mg N·kg\(^{-1} \cdot \text{d}^{-1}\) assumed by the authors. The regression analysis as shown indicates leucine requirement values (13C-1 leucine or N equilibrium) of 37 and 38 mg·kg\(^{-1} \cdot \text{d}^{-1}\). An alternative interpretation is a zone of equilibrium from intake of 30 mg·kg\(^{-1} \cdot \text{d}^{-1}\), which is closer to the recalculated N balance value of 26 mg·kg\(^{-1} \cdot \text{d}^{-1}\) (75) obtained with an optimized diet of added nonessential nitrogen (82).
values for leucine and the BCAA appear high. Why those using stable isotope studies design and analyze their studies in a way that will indicate higher than minimum values is a question that only the study authors can answer, but in my view, the current values are overestimated. Fortunately, because no human diet is likely to be limited by leucine, the public health implications of an overestimation of the leucine requirement is probably of minor importance.

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Literature Cited


