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Efficacy and Safety of Leucine Supplementation in the Elderly[1–3]

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Abstract

Leucine supplementation has grown in popularity due to the discovery of its anabolic effects on cell signaling and protein synthesis in muscle. The current recommendation is a minimum intake of 55 mg · kg^{-1} · d^{-1}. Leucine acutely stimulates skeletal muscle anabolism and can overcome the anabolic resistance of aging. The value of chronic leucine ingestion for muscle growth is still unclear. Most of the research into leucine consumption has focused on efficacy. To our knowledge, very few studies have sought to determine the maximum safe level of intake. Limited evidence suggests that intakes of ≤1250 mg · kg^{-1} · d^{-1} do not appear to have any health consequences other than short-term elevated plasma ammonia concentrations. Similarly, no adverse events have been reported for the leucine metabolite β-hydroxy-β-methylbutyrate (HMB), although no studies have tested HMB toxicity in humans. Therefore, future research is needed to evaluate leucine and HMB toxicity in the elderly and in specific health conditions. *J Nutr* 2016;146(Suppl):2625S–9S.

Keywords: amino acids, hydroxyl-beta-methylbutyrate (HMB), muscle, protein, sarcopenia

Introduction

The BCAA leucine directly affects skeletal muscle anabolism through activation of mechanistic target of rapamycin complex 1 (mTORC1)[8] signaling (1). Activation of this signaling pathway results in an increase in protein synthesis (1). Typically, individuals are catabolic when in the postabsorptive state and thus are in negative net protein balance (i.e., protein breakdown exceeds protein synthesis) (2). An anabolic stimulus such as BCAAs can shift protein net balance from catabolism to anabolism, which is mainly the result of protein synthesis exceeding protein breakdown (3).

After the ingestion of protein, BCAAs, or isolated leucine, the amino acid leucine reaches the cell and moves into the lysosome. Leucine entry in the lysosome triggers the amino acid–sensing mechanism believed to be responsible for initiating protein synthesis (4). However, the mechanism of leucine transport from the membrane into the lysosome has yet to be determined. The incorporation of leucine into the lysosome initiates the colocalization of the lysosome with mTORC1. After lysosomal activation, the lysosomal membrane protein vacuolar ATPase (v-ATPase) transduces the amino acid–sensing signal to the Rag GTPases. v-ATPase elicits the binding of the Ragulator proteins to mTORC1 (4). The Ragulator proteins form a scaffold on the lysosome for Rag proteins and mTORC1 (5). Amino acids such as leucine must be present within the cell for colocalization of the lysosome with mTORC1. When amino acids are not present, the GTPase Activating Protein (GAP) Activity Toward Rags (GATOR) 1 protein acts as a negative regulator of amino acid sensing. High amino acid availability allows GATOR2 to serve as a positive regulator of this pathway by inhibiting GATOR1 (6, 7). After lysosome-mTORC1 interaction, the Ras homolog enriched in brain (Rheb) protein stimulates mTORC1 kinase activity. Rheb binds to the catalytic domain of mTORC1 to commence mechanistic target of rapamycin (mTOR) signaling and phosphorylation of downstream effectors (8).

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[8] Abbreviations used: CaHMB, calcium β-hydroxy-β-methylbutyrate; EAA, essential amino acid; eIF, eukaryotic initiation factor; GATOR, GTPase Activating Protein (GAP) Activity Toward Rags; HMB, β-hydroxy-β-methylbutyrate; HMBFA, β-hydroxy-β-methylbutyric free acid; KIC, α-ketoisocaproate; MPS, muscle protein synthesis; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; MyoPS, myofibrillar protein synthesis; S6K1, S6 kinase β1; 4E-BP1, eukaryotic translation initiation factor 4E–binding protein 1.

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Phosphorylation of these mTORC1 downstream effectors directly increases protein synthesis due to changes in translation initiation and protein elongation (9). Briefly, mTORC1 phosphorylates its downstream target, ribosomal protein S6 kinase β1 (S6K1). Phosphorylated S6K1 increases phosphorylation of its downstream target rpS6, which in turn increases translation initiation. Phosphorylation of S6K1 also prevents the binding of eukaryotic elongation factor 2 (eEF2) to the ribosome. Inhibition of eEF2 binding to the ribosome permits protein translation. mTORC1 phosphorylation also phosphorylates the eukaryotic translation initiation factor 4E–binding protein 1 (4E-BP1). When not phosphorylated, 4E-BP1 binds the eukaryotic initiation factor (eIF) 4E, preventing its assembly into the eIF-4F complex, consequently inhibiting cap-dependent translation. Phosphorylation of 4E-BP1 by mTORC1 releases eIF-4E and permits its assembly into the eIF-4F complex, activating translation initiation.

Because BCAAs have been shown to increase skeletal muscle protein synthesis and net balance, the amino acids that comprise the BCAAs—leucine, isoleucine, and valine—have become a popular nutritional supplement for those seeking to improve athletic performance as well as for those hoping to attenuate muscle loss due to illness, bed rest, or aging (10–13). Leucine, in particular, has garnered extra attention due to its effects on muscle anabolism (14). Leucine is an essential amino acid (EAA) and cannot be synthesized within the body. Potent dietary sources of leucine include meat, fish, dairy, soy, and nuts (15).

According to data collected by the NHANES III (16) for 1994–1998, the population, on average, consumed 6.08 g leucine/d. The highest intake was observed in men 19–30 y of age, with 8.64 g/d. NHANES data also indicated that ~10% of those surveyed reported the use of a leucine supplement. For those individuals, the average intake of leucine from supplementation was 1.86 g/d. The heaviest users, classified as being in the top 90th percentile for supplement usage, ingested an additional 5.92 g leucine/d, independent from meals. The NHANES results revealed that the highest total daily leucine intake was ~14.5 g/d (16).

Although leucine intake can vary widely across populations, 2 small studies examined the minimum daily leucine requirement in order to maximize protein synthesis and to minimize leucine oxidation. el-Khoury et al. (17) tested leucine oxidation over a 24-h period in 5 young adults. They found that the recommendation of 38.3 mg · kg^{-1} · d^{-1} established in 1994 was insufficient to maintain positive leucine balance. Riazi et al. (18) tested higher leucine doses in 7 young men. The results confirmed that a higher leucine dose is necessary to maximize the benefits of leucine intake. They postulated that 55 mg leucine · kg^{-1} · d^{-1} is optimal in adults and thus should be the recommended dosage level. This recommendation was derived in studies in younger individuals alone. Optimal leucine intake for older individuals is still undetermined.

Leucine Supplementation Efficacy in the Elderly

Reduced muscle mass and strength (sarcopenia) are well-recognized predictors of frailty and increase the risk of falls in older adults. Loss of muscle can result from inactivity, illness, or the natural course of aging. One likely mechanism of sarcopenia is the anabolic resistance of older muscle to nutritional stimulation (19, 20). Aging reduces the sensitivity of skeletal muscle to EAA stimulation at low doses, whereas the maximal response at higher doses is preserved (21). One proposed method for attenuating sarcopenia is nutritional supplementation by utilizing BCAAs or protein (10, 13). These interventions provide an anabolic stimulus to the muscle, resulting in increased muscle protein synthesis (MPS) (22–24) and decreased muscle protein breakdown (12, 23, 25). These benefits of leucine supplementation have been shown over the short term in younger and older adults. Younger men showed equivalent increases in skeletal muscle myofibrillar protein synthesis (MyoPS) after ingesting either 25 g whey protein containing 3 g leucine or 6.25 g whey protein supplemented with additional leucine for a total of 5 g leucine (12). Similarly, older men who ingested 3.5 g leucine as part of a 10-g EAA cocktail showed elevated MyoPS 24 h postsupplement. Control subjects who received 1.8 g leucine did not show this sustained elevation in MyoPS (26). The results of these 2 acute studies recognize the short-term benefits of leucine ingestion, which activates MPS in both younger and older individuals.

The benefit of chronic leucine supplementation is not as clear. Zeanandin et al. (27) tested a 6-mo leucine-supplemented diet in aged rats. Rats fed the additional leucine did not gain lean mass compared with controls. A recent meta-analysis by Xu et al. (28) in humans found that older subjects who consumed a leucine supplement showed a greater increase in MPS rates from baseline than did controls when the data were pooled across studies. This increase in MPS was not associated with concomitant increases in lean body mass. Essentially, although MPS increased postingestion, it did not result in improvements in muscle gains compared with the control. Therefore, the authors concluded that chronic leucine consumption may not provide a benefit to muscle accretion in older adults. In older adults, supplementation of 15 g EAAs twice daily during 10 d of bed rest improved MPS but did not prevent the loss of muscle mass with inactivity (29). In another study, Kim et al. (30) found that EAA supplementation in sarcopenic older women improved walking speed but not lean mass or strength. These paradoxical findings between acute and chronic investigations may be a result of dissimilar leucine doses between studies. Perhaps lower leucine doses within chronic studies are insufficient to induce muscle adaptations. Alternatively, it is possible that the chronic leucine and EAA supplementation studies were performed in subjects who were already consuming relatively high protein intakes or that the timing of the supplement relative to the meals might have reduced the supplement efficacy. Maximal MPS is achieved in older adults after the ingestion of 30 g of a high-quality protein (~2.8 g leucine), and higher doses taken within the same meal do not further improve MPS (31).

Leucine supplementation in older individuals with comorbidities such as obesity and diabetes has not been thoroughly studied. Binder et al. (32) showed no changes in body composition or glucose metabolism in obese mice fed a high-fat diet supplemented with leucine compared with controls. Newgard et al. (33) concluded that BCAA supplementation did not affect insulin resistance in rats fed a high-fat diet. Last, Leenders et al. (34) found no improvements in body composition, muscle strength, or insulin sensitivity in older men with type 2 diabetes after the ingestion of 2.5 g leucine with each meal over a 6-mo period compared with controls.

Leucine Supplementation Safety

Although the advantages of leucine supplementation over the long term remain to be shown, leucine supplementation is commonly used by the public (16). Because dietary supplement manufacturers are not required to obtain FDA approval before

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producing or selling dietary supplements (35), it is important to consider the safety of high leucine ingestion.

The previous research outlined above established that 55 mg · kg^{-1} · d^{-1} is the recommended daily intake for leucine ingestion in healthy young adults (18). This amount equates to ~4.4 g/d for an 80-kg person. Because the NHANES data suggested that leucine intake can reach 14 g/d in some individuals, Pencharz et al. (36) tested varying amounts of leucine ingestion for possible toxicity in human subjects. Five healthy young men took part in this trial. These men ingested 50, 150, 250, 500, 750, 1000, and 1250 mg leucine · kg^{-1} · d^{-1}. One subject experienced gastrointestinal distress after consuming 1000 mg/kg and did not complete the study. No changes outside of the normal range for glucose, insulin, urea, and creatinine were reported for the remaining subjects. The authors did report 2 potential issues with intakes above the control level of 50 mg. First, the subjects showed steep declines in both plasma valine and isoleucine values after increasing doses of leucine. Second, hyperammonemia occurred immediately after leucine intakes >500 mg · kg^{-1} · d^{-1}. The authors postulated that plasma ammonia concentrations increased in the blood beginning at this dose because it is the upper limit for leucine oxidation (maximal oxidative capacity). Thus, doses >500 mg · kg^{-1} · d^{-1} may be considered unsafe (36).

Elevated plasma ammonia concentrations can increase brain ammonia concentrations, which may lead to neurologic consequences and encephalopathies (37). However, in the study by Pencharz et al. (36), plasma ammonia concentrations had returned within normal ranges the day after the leucine intervention and thus there were no ill effects from elevated ammonia in the blood. To our knowledge, similar safety studies have not yet been conducted in the elderly.

β-Hydroxy-β-Methylbutyrate Supplementation and Safety

β-Hydroxy-β-methylbutyrate (HMB) is a leucine metabolite that has emerged as a popular nutritional aid in the strength-training and muscle-performance communities. After leucine ingestion, the transamination of leucine results in α-ketoisocaproate (KIC). KIC is then metabolized into HMB in the cytosol within the muscle cell by KIC dioxygenase (38, 39). Approximately 5% of all leucine is converted into HMB. As such, a typical high-protein meal containing 3 g leucine would yield ~0.15 g HMB (40).

As with leucine supplementation, several studies have tested the efficacy of HMB supplementation on changes in muscle size, strength, and damage. Until recently, all studies tested calcium HMB (CaHMB) (41). van Someren et al. (42) observed reduced muscle damage and fatigue after 1-repetition-maximum biceps curl exercises with 3 g HMB supplementation in young, untrained adults; and Nissen et al. (43) found increased lean mass in subjects taking HMB compared with controls. Panton et al. (44) showed an increase in upper body strength with reduced fat mass in young men and women who ingested an HMB supplement compared with placebo controls. Last, Wilkinson et al. (45) observed increases in MPS in untrained young men after the ingestion of either 3.42 g leucine or 2.42 g HMB, with a concomitant decrease in MPB for the HMB group. Conversely, a meta-analysis by Rowlands and Thomson (46) found no benefit of HMB supplementation in young men, regardless of training status.

HMB supplementation has also been tested in older adults. The results for these trials have been encouraging (47). One study reported improved strength coupled with fat loss (48), whereas another found improvements in lean body mass, grip strength, and leg extensor strength compared with placebo controls (49).

Although HMB supplementation may increase lean mass and reduce fat mass in younger adults, the safety of HMB as a performance aid has only recently been investigated. One study tested the safety of HMB for long-term use in rats. Fuller et al. (50) conducted a 91-d study with the use of Sprague-Dawley rats that tested the safety of β-hydroxy-β-methylbutyric free acid (HMBFA). This new form of HMB results in higher HMB serum concentrations than CaHMB. In this study, rats were administered an HMBFA intervention of 0%, 0.8%, 1.6%, or 4% of the diet by body weight. The highest dose is the equivalent of ~400 mg · kg^{-1} · d^{-1} for humans. No adverse advents were observed for any treatment group. Similarly, blood and urine analyses were within the normal range in all groups, with no group differences. The authors concluded that HMBFA was safe for consumption in a rat model.

In humans, a daily 6-g HMB dose for 1 mo did not result in any adverse changes in blood metabolites or kidney or liver function according to Gallagher et al. (51). Similarly, a review by Nissen et al. (52) that included 9 chronic studies concluded that a daily 3-g HMB dose was safe for both young and old adults.

Baier et al. (53) used a cocktail containing CaHMB, arginine, and lysine in older adults for 1 y. In this study, they found no differences between the placebo and supplement groups for any blood or urine markers. None of the few studies that tested HMB in humans have reported negative health consequences of HMB supplementation.

Conclusions

Leucine, EAAs, protein, and HMB may be useful nutritional supplements to improve muscle protein anabolism, and possibly, muscle size and function in older adults, particularly in conditions of inactivity, malnutrition, and frailty. With the increasing popularity of nutritional supplementation, it has become important to test the safety of these performance aids, particularly in the elderly and in clinical populations.

No serious side effects have been reported with leucine, EAA, or HMB supplementation; and the health risks associated with these supplements are few and predictable. If the typical daily consumption matches the levels reported in the NHANES data, the average American consumes <15 g leucine/d. Although this dose far exceeds the recommended intake of 55 mg · kg^{-1} · d^{-1}, it is still far below the amounts needed to reach the body's maximal oxidation capacity (500 mg · kg^{-1} · d^{-1}). When leucine intake approximates the maximal oxidative capacity, plasma ammonia concentrations start to increase, but this effect is transient in healthy subjects. However, older adults and individuals with decreased renal or liver function may reach their leucine maximal oxidative capacity at much lower doses. Future safety studies are warranted.

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