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Amino acids in healthy aging skeletal muscle

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TABLE OF CONTENTS

1. ABSTRACT

Life expectancy in the U.S. and globally continues to increase. Despite increased life expectancy quality of life is not enhanced, and older adults often experience chronic age-related disease and functional disability, including frailty. Additionally, changes in body composition such as the involuntary loss of skeletal muscle mass (i.e. sarcopenia) and subsequent increases in adipose tissue can augment disease and disability in this population. Furthermore, increased oxidative stress and decreased antioxidant concentrations may also lead to metabolic dysfunction in older adults. Specific amino acids, including leucine, cysteine and its derivative taurine, and arginine can play various roles in healthy aging, especially in regards to skeletal muscle health. Leucine and arginine play important roles in muscle protein synthesis and cell growth while cysteine and arginine play important roles in quenching oxidative stress. Evidence suggests that supplemental doses of each of these amino acids may improve the aging phenotype. However, additional research is required to establish the doses required to achieve positive outcomes in humans.

2. INTRODUCTION

2.1. Aging and chronic disease

Life expectancy from birth and from the age of 60 years continues to increase with the average life expectancy in the United States being 78.8. years and 83.2. years for men and women, respectively, an approximate 16 year increase since 1940 (1). The percentage of the total population over the age of 65 years is now ~13% (40,229,000 older adults) and is expected to reach ~20% by 2030 (2).

Although human life expectancy is increasing, older individuals are faced with increased risk for chronic disease, reduced ability to perform activities of daily living, and general loss of independence, suggesting a larger window of diminished quality of life for aging adults. Many chronic diseases and physical impairments are associated with changes in body composition such as increases in fat mass and decreases in skeletal and bone masses. Age-related changes in skeletal muscle such as the involuntary loss of muscle mass (sarcopenia) and the associated decrease in strength increase the risk for falls and fractures. These changes have also been linked to loss of mobility and independence and to physical

Table 1. Classification of amino acids in humans

Essential amino acids	Conditionally essential amino acids	Nonessential amino acids
Histidine	Arginine	Alanine
Isoleucine	Cysteine	Aspartate
Leucine	Glutamine	Asparagine
Lysine	Glycine	Glutamate
Methionine	Proline	Serine
Phenylalanine	Tyrosine	
Threonine		
Tryptophan		
Valine		

disabilities in older adults (3-5). Such changes in body composition can lead to debilitating physical conditions that impact the ability of older adults to complete instrumental activities of daily living. In 2010, over 25% of those aged ≥ 65 years reported difficulty walking and climbing stairs, while 18.5.% reported difficulty doing errands independently (6). Dietary modifications may improve adverse age-related changes in body composition, and therefore, may impact the development of many chronic conditions (e.g. sarcopenia, obesity). Current recommendations for improving body composition in all populations, but most importantly for older adults, include exercise and consumption of a healthy diet with adequate dietary protein (7, 8).

Approximately 35-45% of the adult human body mass is comprised of skeletal muscle; however, between the ages of 30-80 y, skeletal muscle mass decreases by approximately 30% (i.e. sarcopenia), declining at a rate of 3-8% per decade after the age of 30 y (9, 10). The aging skeletal muscle phenotype is characterized by visible adverse changes in composition and function that begin as early as the 4th decade of life (10). Interestingly, the relationship between age-related loss of skeletal muscle mass and strength is not linear (11). Goodpaster *et al.* reported a 3-4% decrease in leg lean mass but a 5-9% decrease in specific torque among older adults over a 3 y period (12). Changes in amino acid and protein metabolism in response to stimuli may contribute to these changes in skeletal muscle mass and strength in older adults. Evidence suggests that older adults have a blunted anabolic response to amino acid ingestion, although basal muscle protein degradation and turnover remains unchanged (13, 14). Additionally, age-related changes in the skeletal muscle transcriptome and methylome partially underlie and likely precede the observed phenotypic changes in aging muscle (15-18).

Age-related changes in skeletal muscle morphology and physiology accelerate with advancing age, and are accentuated by intrinsic and extrinsic factors (19-26). For example, sarcopenia is characterized by chronic low-grade inflammation and is associated with increased reactive oxygen species production, altered mitochondrial function, and elevated apoptotic signaling (27, 28). Accumulation of reactive oxygen species damages lipid membranes, nuclear DNA, and mitochondrial DNA. Subsequently, these cellular damages decrease protein synthesis and induce apoptosis and myofiber death. Factors influencing muscular changes have been identified at all levels: the single myofiber, the skeletal muscle organ, and the whole organism. However, skeletal muscle aging is complex and multifactorial, and the molecular mechanisms instituting adverse changes in skeletal muscle with age are yet unclear.

2.2. Endogenous protein and amino acids

Endogenous proteins play many structural and functional roles in the mammalian body. All enzymes and many cellular structures including actin and myosin filaments, membrane carriers, and hormone receptors are proteins. Amino acids are the basic building blocks of all proteins and are often classified as essential, conditionally essential, or non-essential (Table 1), although essentiality is species and age dependent. In humans, nine amino acids are classified as essential. These amino acids have carbon skeletons that cannot be synthesized *de novo* by the body and are therefore required from dietary sources (29). Additionally, in humans, six amino acids are classified as conditionally essential. Conditionally essential amino acids can be synthesized by the human body, but their synthesis is limited by a variety of factors including amino acid precursor availability. When synthesis is limited for any reason, the amino acid becomes an essential part of the diet. Alternatively, five amino acids are known to be nonessential in humans. These amino acids are assumed to be synthesized in adequate amounts as long as the total protein requirement is met. However, whether or not these quantities are sufficient for optimal health and growth remains unknown. Furthermore, although the importance of conditionally essential and nonessential amino acid intake has been explored in other mammals, its research is limited in humans (30). Future research is needed to explore the role and dietary requirements of conditionally essential and nonessential amino acids in human health.

The skeletal muscle is a key organ system for the degradation and synthesis of amino acids. Degradation and synthesis of amino acids depends on both the distribution of macronutrients and the availability of macronutrients for energy production (i.e., fed vs. fasted). For example, skeletal muscle is the major organ for initiating the transamination of branched-chain amino acids (leucine, isoleucine, and valine). Branched chain ketoacids can then be released from muscle for oxidation in the liver and

Table 2. Amino acid dietary reference intakes

Amino acid	DRI Recommendation (mg · g protein^{-1} · day^{-1})	Grams amino acid per day based on 77.4. kg[1]	Grams amino acid per day based on 90 kg[2]	References
Histidine	18	1.11	1.30	42, 62
Isoleucine	25	1.55	1.8	42, 62
Leucine	55	3.4	4.0	42, 62
Lysine	51	3.16	3.67	42, 62
Methionine & cysteine	25	1.5	1.8	42, 62
Phenylalanine & tyrosine	47	2.9	3.38	42, 62
Threonine	27	1.67	1.97	42, 62
Tryptophan	7	0.43	0.50	42, 62
Valine	32	1.98	2.3	42, 62

[1]: Based on average weight of a U.S. woman 60-69 years old. [2]: Based on average weight of a U.S. man 60-69 years old

other organs. The degradation of branched-chain amino acids provides products for the synthesis of amino acids such as glutamine in the muscle. Additionally, the release of amino acids (e.g., alanine and glutamine) from the skeletal muscle provides necessary products for synthesis of other amino acids and molecules, such as glucose, by other organs. While this is only a brief overview, the importance of skeletal muscle for amino acid metabolism is highlighted.

2.3. Exogenous protein and amino acids

Adequate dietary protein is essential for overall human health with recommendations differing throughout the human lifespan. As there are no true body stores for protein, insufficient protein intake to satisfy body requirements leads to a negative protein balance (i.e., protein synthesis less than breakdown). Imbalanced protein metabolism during inadequate protein intake generally occurs in the skeletal muscle, resulting in clinical manifestations such as skeletal muscle atrophy, impaired muscle growth or regrowth, and functional decline. Some populations, such as older adults, are particularly vulnerable to insufficient protein.

Animals and plants are both sources of exogenous protein and amino acids. Meat, poultry, seafood, dairy, and eggs are excellent sources of high biological value proteins, meaning the protein mixture in the individual food source contains each of the essential and conditionally essential amino acids at a level that equals or exceeds the human requirement for the amino acid as a percentage of the total protein requirement. Whey and casein are two common protein supplements derived from dairy products. When milk is curdled for cheese, it separates into two phases: the watery phase and the curds. Whey protein is derived from the watery phase. It represents about 20% of the proteins in cow's milk, and it is rich in leucine. Alternatively, casein is insoluble at acid pH and is found in the curds. It represents about 80% of the proteins in cow's milk, but it has less leucine than whey. Legumes, nuts, seeds, vegetables, and whole grains are lower biological protein sources; they provide limited amounts of one or more of the essential amino acids.

The current protein Recommended Dietary Allowance (RDA) is 0.8 g · kg body weight^{-1} · day^{-1} for all adults over the age of 19 years. Infants, children, pregnant women, and lactating women have increased protein needs (31). A committee within the Institute of Medicine (the health arm of the United States National Academy of Science) set the adult protein RDA after analyzing several nitrogen balance studies. Furthermore, they also set requirements for many of the essential and conditionally essential amino acids after analyzing results of isotopic tracer studies (see Table 2 for Dietary Reference Intakes).

Adequate protein intake is essential for muscle mass maintenance and growth, especially in older adults. Several studies have shown that when compared to younger adults, older adults may have greater total protein needs and may benefit from higher protein intakes (32-39). In relatively healthy, older adults, acute studies demonstrated that old (vs. young) adult skeletal muscle has an impaired protein synthesis response to protein intakes of 20 g or less, but a comparable protein synthesis response to protein intakes of 25-30 g in a single meal (40). These varying responses could more specifically reflect the availability of specific amino acids (14, 41).

Additionally, two recent studies using the indicator amino acid oxidation method found that the mean protein requirement for elderly women was is 0.96 g · kg^{-1} · day^{-1} or 0.85 g · kg^{-1} · day^{-1} (34, 35). Both of these values are above the current estimated average requirement of 0.66 g · kg^{-1} · day^{-1} and even

Table 3. Summary of the effects of single-dose or long-term protein or amino supplementation in older adults

Supplement	Dose and duration	Physiological effect	Conclusion
[1]EAA (52)	6.7 g EAAs (26% (1.7 g) or 41% (2.7 g) leucine); single dose	Following EAA ingestion containing 2.7 g leucine only: ↑ FSR, comparable to the younger adults consuming 2.7 g leucine ↑ Phenylalanine net balance (reflects muscle balance)	Increasing the proportion of leucine in a mixture of EAA can overcome the attenuated MPS response in elderly
Whey protein isolate (53)	0, 10, 20, or 40 g of whey protein isolate; single dose	Following the 20 g dose: ↑ MPS by 65% above basal level ↑ MPS by 90% over basal level No effects observed after ingestion of lower doses	Twenty grams of whey protein is sufficient to increase myofibrillar MPS in non-frail older adults
EAA+arginine (56)	11 g EAA+arginine; 2x per day, between meals for 16 weeks	↑ LBM after 12 weeks ↑ Lower extremity strength measure score ↑ Gait speed ↓ 5-step test time ↓ Floor-transfer test time	Supplementing the diet with EAA+arginine improves lean body mass, strength, and physical function compared to baseline values in glucose intolerant elderly individuals
EAA (57)	8 grams EAA (2.5 g leucine) given at 10 am and 5 pm for 12 months	↑ Whole-body lean mass ↑ Circulating IGF-1 ↓ Circulating TNF-alpha	Nutritional supplements with oral EAA mixture increased whole-body lean mass in elderly subjects with sarcopenia
EAA (58)	7.5 g EAA (1.39 g leucine); 2x per day for 3 months between meals	↑ Basal FSR ↑ Lean body mass ↑ Basal IGF-1 protein expression	EAA improved LBM and basal muscle protein synthesis in older individuals
Leucine (63)	4 g/meal; 3 meals/day; 2 weeks	↑ Postabsorptive FSR ↑ Phosphorylation of mTOR ↑ Phosphorylation of 4E-BP1 ↑ Phosphorylation of p70S6K1	Leucine supplementation during meals may improve MPS and mechanisms underlying anabolic responses in older skeletal muscle
Leucine (64)	2.5 g leucine consumed with meals for 3 months	No change in skeletal muscle mass or strength No change in whole-body insulin No change in glycated hemoglobin content No change in plasma lipids	Long-term leucine supplementation does not augment skeletal muscle mass or strength and does not improve glycemic control or blood lipid profile in healthy elderly men
Leucine (65)	2.5 g leucine consumed with meals for 6 months	No change in lean tissue mass (not different b/w groups) No change in body fat percentage No change in muscle strength No change in muscle fiber type No change in insulin sensitivity or plasma lipid concentration (did not change b/w groups)	Prolonged leucine supplementation does not modulate body composition, muscle strength, muscle mass, glycemic control, or lipidemia in elderly, type 2 diabetic patients who habitually consume adequate dietary protein

[1]EAA: Essential amino acids; FSR: Fractional synthesis rate; MPS: Muscle protein synthesis; LBM: Lean body mass

above the current RDA of 0.8 g · kg^{-1} · day^{-1} for adults. Of functional importance, Campbell *et al.* showed that older adults lost muscle mass when placed on a controlled diet with 0.8 g · kg^{-1} · day^{-1} for 14 weeks (32). This study was tightly controlled, and each subject resided at the General Clinical Research Center at The Pennsylvania State University for the 14-week duration. The results of these studies collectively suggest that older adults have protein needs greater than the current RDA.

Consumption of other macronutrients is also important for muscular health. Protein and amino acid requirements are determined under conditions of sufficient dietary energy intake. Amino acids will be increasingly oxidized for energy under conditions of inadequate energy intake from carbohydrate and fat. Although glucose is the primary energy source for both the working muscle and the brain under postprandial conditions, amino acids can be used as a fuel, both directly and after

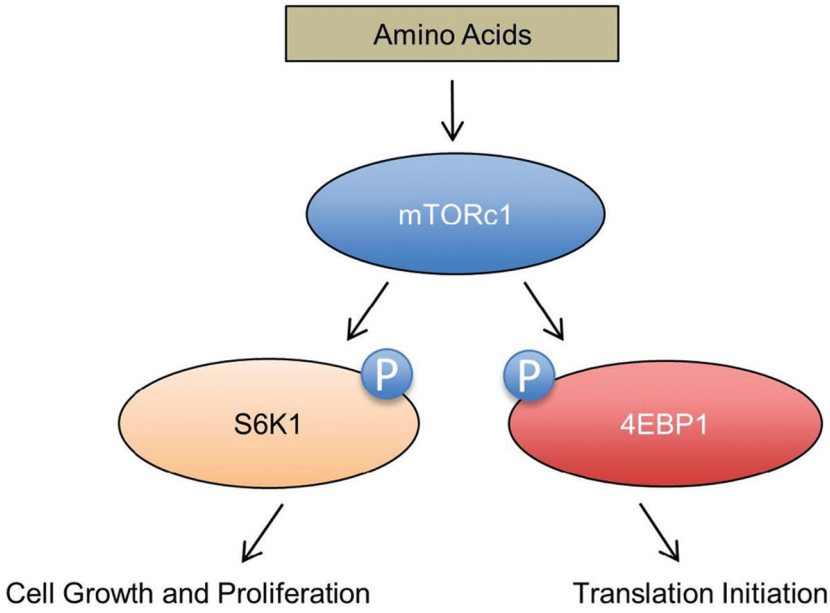

Figure 1. Biochemical mechanisms for the beneficial effects of BCAA supplementation on aging skeletal muscle.

gluconeogenesis from amino acid carbon chains, when energy intake is inadequate. Therefore, adequate energy intake, including some carbohydrate intake, is essential for sparing amino acids for anabolic purposes. With adequate energy and carbohydrate intake, protein can be utilized for its many structural and functional roles.

Specific amino acids are known to play integral roles in muscular health throughout the lifespan; these include leucine, cysteine and its derivative taurine, and arginine. Furthermore, aging affects the availability and physiologic effects of several of these amino acids. The purpose of this review is to summarize the roles of each of these amino acids in healthy aging, with a particular focus on skeletal muscle health.

3. LEUCINE

Leucine is a hydrophobic, branched-chain amino acid metabolized predominately in the liver, adipose tissue, and skeletal tissue (42). The leucine dietary reference intake is 55 mg · g dietary protein $^{-1}$ · day^{-1} (~3 grams leucine for a 70 kg individual consuming the RDA for dietary protein (0.8 g · kg^{-1} · day^{-1}) (43). Food sources include soybeans, beef, chicken, eggs, oats, nuts, and milk. Leucine is predominantly known for its role in the stimulation of muscle protein synthesis (44). However, leucine has several additional functions in the body. Unlike other amino acids, leucine cannot be converted into glucose. Rather, leucine is a significant precursor for lipid biosynthesis in adipose tissue and, to a lesser extent, in muscle and liver (42). Leucine, like other amino acids, is essential for the synthesis of proteins. In the brain, leucine is a component of leucine-enkephalin, an endogenous opioid peptide neurotransmitter (45). Additionally, on the molecular level, leucine residues are critical components of the leucine zipper structural motif commonly found in DNA binding proteins. Each leucine zipper motif has four leucine residues, each separated by six amino acids (46).

3.1. Leucine-stimulated protein synthesis

The importance of essential amino acids, particularly leucine, in protein synthesis has been well established (47). Ingestion of essential amino acids, leucine in particular, increases mammalian target of rapamycin complex 1 (mTORC1) activity (47). mTORC1 is a serine/threonine protein kinase that increases muscle protein synthesis through translational control. Upon activation, mTORC1 phosphorylates many downstream targets including S6K1 and 4EBP1 (Figure 1). Phosphorylated S6K1 promotes cell growth and cell proliferation through phosphorylation of further downstream targets (48). Phosphorylation of 4EBP1, a translational repressor, results in its dissociation from the inactive eIF4E-4EBP1 complex which frees eIF4E to bind with eIF4G to form the active eIF4G-eIF4E complex (49). The eIF4G-eIF4E complex plays an integral role in eukaryotic translation initiation (50).

Even when isolated from other essential amino acids, leucine can independently activate mTORC1 (51) and enhance muscle protein synthesis (52). Multiple studies have shown that essential amino acid bolus feedings that include 3 grams of leucine significantly increase muscle protein fractional synthetic rate in older adults (53-55). In young adults, 10 grams of essential amino acids (~2 grams of leucine) mixed in a

noncaloric, noncaffeinated carbonated beverage was sufficient to induce maximal postprandial muscle protein synthesis (56). Other human studies have shown that essential amino acid supplements with 1.39 to 3.95 grams of leucine given between meals increase lean body mass, strength, and physical function independently of exercise (57-59).

3.2. Anabolic resistance to protein and leucine ingestion in aging

Aging is associated with a blunted skeletal muscle anabolic response to ingestion of 20 grams or less of high quality protein (14, 53). Additionally, when normalized to body weight, older men require a greater relative protein intake than younger men to reach equivalent muscle protein synthesis rates suggesting the existence of an anabolic threshold (60). However, the anabolic response to the ingestion of 30 grams of protein is similar in old and young skeletal muscle (61). This bolus-dependent anabolic response may be clinically relevant; merely supplementing the diet of older adults with 20 grams of protein twice per day was not sufficient to attenuate skeletal muscle loss with disuse (62). Rather, current evidence suggests that older adults may benefit more from an even meal distribution of protein with 25-30 grams of protein at each of the three daily meals, ~90 grams per day (40). Importantly, 30 grams of high quality protein contains ~3 grams of leucine. Therefore, the amount of leucine recommended to overcome the impaired anabolic response in older adults is ~3 grams per meal, or ~9 grams per day, a level that is 3 times the current leucine RDA (Table 3) (43, 63).

Supplementing older adults whose protein intake was controlled at 0.8 g · kg^{-1} · day^{-1} with additional leucine at meals increased fractional synthetic rate and mTORC1 signaling (64). However, when older adults who were already consuming ~1.0 g protein · kg^{-1} · day^{-1} were supplemented with leucine at meals, no effects on changes to fractional synthetic rate or lean body mass were observed (65, 66). Collectively, this evidence suggests that essential amino acid supplements given between meals or leucine supplements given with meals may only be beneficial for muscle mass maintenance in those consuming less than or equal to the current protein RDA.

Despite the increasing evidence that older adults may have increased dietary protein and/or leucine needs, protein intake, and therefore leucine intake, generally decreases with age (67, 68). This decrease in protein intake may be attributed to changes in appetite, especially in those consuming liquid supplements (68, 69). Essential amino acid or leucine supplements may be an effective strategy for increasing protein intake, muscle protein synthesis, and lean body mass in older adults without adversely affecting satiety or the normal metabolic response to a later meal (70, 71).

3.3. Inactivity and bedrest

Inactivity and bedrest further attenuate the anabolic response to protein/leucine intake (72). In healthy, older adults seven days of bedrest significantly reduces leg lean mass and muscle protein synthesis in response to 12 grams of essential amino acids. Seven days of bedrest is comparable to the average length of a hospital stay for older adults with acute illness (73). Older adults also lose significantly more leg mass after just 10 days of bedrest compared to the amount younger adults lose after 28 days of bedrest (74, 75). These large reductions in muscle mass with inactivity in older adults may result from blunted muscle protein synthesis in response to essential amino acid stimulation. Drummond *et al*. reported that the reduction in leg lean mass and muscle protein synthesis after bed rest is accompanied by a reduction in mTORC1 signaling and a reduction in amino acid transporter protein content (72). Augmentation of anabolic resistance following muscle disuse could explain why protein supplementation alone does not attenuate disuse atrophy. Interestingly, exercise training alone is inadequate to fully regain the muscle mass lost during two weeks of bedrest (76, 77). Therefore, nutritional interventions that stimulate mTORC1 activity, a positive regulator of muscle protein synthesis, may have important implications for skeletal health in older adults, especially when combined with exercise training. Research investigating the therapeutic effects of combined physical activity and protein/leucine supplementation post bedrest or immobilization is needed.

3.4. Leucine and weight loss in aging

Leucine may also play an important role in muscle mass maintenance during weight loss, especially in older adults (78). Along with the age-related decrease of skeletal muscle mass there is an accumulation of fat within and around the muscle (sarcopenic obesity) which, combined with skeletal muscle loss, likely increases one's risk for functional disability and metabolic dysregulation (79, 80). Additionally, there is an age-associated redistribution of fat mass to the abdominal region which is closely linked to morbidity and mortality as well as increased risk of chronic diseases (e.g., cardiovascular disease, insulin resistance, metabolic syndrome, etc.) (81-83). Further, the global rise in obesity is affecting all generations and is a health concern for the older population (84). A major problem that older adults face when trying to combat obesity with caloric restriction is the further loss of skeletal muscle mass; in some older adults, the risks associated with muscle atrophy may outweigh the benefits of weight loss. Sarcopenia itself is independently associated with insulin resistance (79). Therefore, utilizing nutritional strategies that promote muscle mass maintenance is imperative during weight loss, especially in older adults.

Although the ideal macronutrient ratios during weight loss remain controversial, higher protein diets

are likely to promote better body composition during hypocaloric conditions (8, 85-88). Furthermore, because of leucine's unique role in muscle protein synthesis, leucine has the potential to independently stimulate muscle protein synthesis and protect lean body mass during weight loss (89-91). Several studies have reported benefits of whey protein or dairy consumption on adipose tissue loss, lean muscle mass maintenance, and muscle protein synthesis rates during weight loss (78, 92, 93). Preserving muscle mass during weight loss is important for the prevention of sarcopenia as well as for improvements in metabolic function and insulin sensitivity.

3.5. Leucine and insulin resistance

Amino acids, especially the essential amino acids, increase circulating insulin levels, and high protein intakes have been associated with the development of type II diabetes (94-96). Alternatively, higher protein diets have also been associated with improved body composition through greater loss of fat mass and reduced loss of lean muscle mass (85, 97). Improvements in glycemic control often accompany these improvements in body composition (85, 97).

Skeletal muscle plays an important role in insulin sensitivity; it accounts for ~75% of the body's insulin-stimulated glucose uptake (98, 99). Despite the positive data surrounding leucine's beneficial effect on muscle mass maintenance and sarcopenia prevention in older adults, recent epidemiologic data suggests an association between elevated circulating branched-chain amino acids and the development of type II diabetes (100-102). However, evidence from animal feeding studies suggests that the association of branched-chain amino acids with type II diabetes development may only occur in the background of a high-fat diet or in humans who preferentially use fat for energy production (100, 103, 104). Similarly, elevated leucine levels observed in obese individuals decline rapidly following bariatric surgery (105). Interestingly, while excess leucine from dietary protein intake may increase the risk of metabolic disease in adults 50-65 y, higher protein intake may be beneficial at reducing overall and cancer-related mortality in adults over the age of 66 y (106). Whether leucine is the cause or effect of insulin resistance is unclear, but further investigation is warranted, particularly if leucine is protective against mortality in older adults.

Inflammation is known to play a key role in obesity-linked insulin resistance (107). Burrill *et al.* recently reported that proinflammatory cytokine treatments down-regulated the expression of branched-chain amino acid transport and oxidation genes in 3T3-L1 cells (108). Furthermore, in rat liver epithelial cells, insulin inhibits branched-chain α-ketoacid dehydrogenase, the rate-limiting enzyme in branched-chain amino acid catabolism (109). The cumulative effects of chronic inflammation and hyperinsulinemia may induce gene expression changes that result in decreased branched-chain amino acid uptake in critical tissues. Future research needs to continue elucidating the mechanistic links between branched-chain amino acids and insulin resistance; no causal relationships have been established to date.

4. CYSTEINE

Cysteine is a semi-essential amino acid that may be consumed as such in the diet, or it can be synthesized in the body from the sulfur atom from methionine and the amino acid serine. Because of this, the requirements for methionine and cysteine must be considered collectively. The dietary reference intake for cysteine and methionine is 25 mg · g protein $^{-1}$ · day^{-1} (43). Food sources of cysteine include soy, eggs, dairy, whole grains, meats, and poultry.

Cysteine is a precursor to glutathione, the most abundant intracellular thiol, as well as iron-sulfur proteins, such as NADH dehydrogenase (110). Cysteine residues are often covalently bound to other cysteine residues in disulfide bonds. These disulfide bonds play important roles in crosslinking proteins and supporting a protein's tertiary structure. Although clinically diagnosed cysteine deficiency is rare, evidence suggests that supplemental cysteine may be beneficial, especially in older adults (111).

4.1. Cysteine and glutathione synthesis

Cysteine is required for both synthesis of proteins and synthesis of the tripeptide gamma-glutamylcysteinylglycine, or glutathione. Glutathione is the major intracellular thiol and an important antioxidant molecule, and the availability of cysteine limits the rate of glutathione synthesis. The normal turnover of glutathione in adults has been estimated to be < 40 mmol per day, which is slightly greater than estimates of the magnitude of cysteine turnover in the body protein pool (112-115). Because tissue glutathione levels become depleted at sulfur amino acid intakes that are marginal but adequate for protein synthesis, marginal protein intakes are likely to be associated with low tissue glutathione levels (116-119).

Sulfur amino acid nutrition is of significant interest in the context of aging because it is known that oxidative stress increases as organisms age, and oxidative stress may contribute to the sarcopenia associated with aging (120, 121). Furthermore, human plasma and erythrocyte glutathione levels decrease in aging, and animal studies have shown that muscle, blood cell, liver, and lung glutathione concentrations decrease with age (122-127). Because cysteine is the limiting substrate for glutathione synthesis, decreased cysteine availability may contribute to lower glutathione levels in older adults.

Several studies have shown associations of glutathione depletion with increased oxidative stress and increased muscle cell apoptosis. Dam *et al.* reported that depletion of glutathione increased reactive oxygen species production, DNA fragmentation, calpain activity, and caspase-independent apoptotic signaling in rat skeletal muscle (128). Sekhar *et al.* reported that, compared to younger adults, older adults had lower cysteine and glutathione levels in red blood cells, lower fractional rates of glutathione synthesis, and higher levels of three markers of oxidative stress (i.e., hydroperoxides, F_2-isoprostanes, and lipid peroxides) (127). Sinha-Hikim *et al.* observed elevated markers of oxidative stress, inflammation, and muscle cell apoptosis, as well as decreased muscle weight, in aged mice compared with young mice (129).

Short-term cysteine supplementation studies in humans have shown positive effects on glutathione pools (127, 130, 131). Sekhar *et al.* reported that supplementing older subjects with *N*-acetylcysteine equivalent to 0.81 mmol cysteine·kg^{-1}·d^{-1} plus 1.33 mmol·kg^{-1}·d^{-1} glycine for 14 days increased the rate of intracellular glutathione synthesis and nearly doubled red blood cell glutathione concentrations, resulting in glutathione concentrations in supplemented older adults that were comparable to the levels observed in young subjects (127). Supplementation also led to a significant reduction in concentrations of plasma markers of oxidative stress (reactive oxygen metabolites, F_2-isoprostane, and lipid peroxides) and in the ratio of oxidized glutathione to reduced glutathione in red blood cells. Sekhar *et al.* also reported similar positive outcomes of the *N*-acetylcysteine + glycine supplementation of patients with uncontrolled type 2 diabetes (131). In addition, Nguyen *et al* showed that supplementation of older HIV-infected men with this same *N*-acetylcysteine + glycine supplement for 14 days increased red blood cell glutathione level, reduced markers of oxidative stress, increased muscle strength, and improved mitochondrial function and insulin sensitivity (132).

Sinha-Hikim *et al.* and Vidal *et al.* conducted studies with longer term cysteine supplementation in rodents (129, 133). Sinha-Hikim *et al.* found that supplementation of aged male mice with a formulation containing cysteine, glycine, selenomethionine and glutamine for 6 months prevented age-related increases in muscle cell apoptosis (129). Supplementation also prevented increases in oxidative stress and inflammation marker levels; tissue glutathione levels were not reported for this study but presumably were increased by the sulfur amino acid-containing supplement. Vidal *et al.* reported the physiologic effects of long-term cysteine supplementation in rats (133). Twenty-one month old rats were clustered into one of two groups, non-inflamed or low-grade inflamed, according to their natural α_2- macroglobulin plasma level. Rats with an α_2- macroglobulin level greater than two standard deviations above the mean for adult rats were categorized as low-grade inflamed. None of the rats showed any observable signs of disease at baseline. After rats were categorized, they were fed either a nonpurifed rodent diet supplemented with 4.0 g·kg^{-1} of cysteine or the same diet supplemented with an isonitrogenous amount of alanine (control) for 14 weeks. Cysteine-supplemented rats had higher free cysteine and free glutathione concentrations in the liver than did the alanine-supplemented groups, regardless of inflammation grade. There was no difference in acute-phase protein levels in plasma of cysteine-supplemented and control rats. Interestingly, however, cysteine-supplemented rats did not demonstrate the age-related decline in food intake that was observed in the control rats supplemented with alanine. This association of cysteine and glutathione status with food intake is consistent with previous findings; Hernadfalvi *et al.* reported that the attenuation of anorexia in lipopolysaccharide-pretreated mice was associated with increased glutathione levels in brain and liver (134). No causation between brain glutathione levels and food intake has been proven. However, the effect of tissue glutathione levels on food intake should be further investigated given that decreased food intake has been negatively associated with survival in elderly patients (135).

Sulfur amino acids are present in adequate amounts in most diets that include high-protein foods. However, when dietary sulfur amino acid intake is inadequate, cysteine supplements may be beneficial. Cysteine-rich, un-denatured whey protein can be used as a cysteine supplement. Daily whey protein consumption (2-3 servings of 10-15 grams) has been shown to increase plasma glutathione levels in patients with nonalcoholic steatohepatitis, HIV, and cystic fibrosis (136-138). Because whey protein is also a good source of leucine, whey protein supplements may confer multiple health benefits in older adults. In clinical and research situations, precursors of cysteine are frequently used because cysteine and its precursor methionine are both easily oxidized. As a supplement to research diets, the disulfide form of cysteine, cystine, can be used; much of the cysteine present in dietary proteins is present in disulfide linked form and is released as cystine during proteolytic digestion (129, 139). Clinically, cysteine derivatives, especially *N*-acetylcysteine and 2-oxothiazolidine-4-carboxylate have been used to provide cysteine, especially when intravenous administration is required, because these derivatives are much more water-soluble than the disulfide cysteine (140, 141). Supplementation with cysteine or its various derivatives are not entirely equal because of differences in the uptake and metabolism of cysteine and its derivatives, but cysteine and glutathione depletion or inadequacy favor the utilization of all forms of cysteine for glutathione synthesis as opposed to their catabolism to taurine and inorganic sulfur (142-145).

Overall, these results suggest that increased cysteine intake may be beneficial for older adults in that they may prevent or minimize decreases in tissue glutathione levels and increases in oxidative stress that occur with aging. However, the etiology of decreased cysteine availability in older adults, the effects of restored glutathione levels on inflammatory disease progression in humans, and the long-term effects of cysteine supplementation on clinical outcomes or disease attenuation have received little investigation. The potential therapeutic effects of restored glutathione levels on sarcopenia, chronic disease progression, and mortality remain largely unknown.

4.2. Taurine

Taurine, a derivative of cysteine, has antioxidant, anti-inflammatory, and metabolic actions that may be therapeutic for aging individuals. Muscle cannot synthesize taurine from cysteine; therefore, it is dependent upon taurine uptake. Taurine is found naturally in fish and meat but is absent in plants outside of algae. Although taurine is an acid with an amino group, taurine has a sulfonic acid group instead of a carboxylic acid group like the proteogenic amino acids; taurine is not used as a building block for proteins in the body. However, it is the most abundant free amino acid in skeletal and cardiac tissue, and it plays a role in many cellular functions including muscle excitability, oxidant attenuation, and inflammatory control (146, 147). Over the past decade, taurine's direct and indirect properties have been recognized as important attenuators of skeletal muscle health, cardiovascular disease, and diabetes.

Taurine plays an integral role in chloride ion conductance and calcium homeostasis – two important regulators of muscle excitability. Several studies have shown that changing the taurine concentration within muscle leads to changes in muscle function including reduced force production, action potential speed, and exercise capacity (148, 149). Ito *et al.* recently showed that taurine depletion, via a taurine transporter knockout, accelerates aging-associated skeletal muscle tissue damage in mice (150). Knockout mice showed significant skeletal muscle defects, elevated aging biomarker mRNA levels, and increased activation of the unfolded protein response (150). These mice also had shorter lifespans, decreased mitochondrial complex 1 activity, and centralized nuclei distribution in muscle cells, a histological change indicative of muscle injury (150). Together these defects indicate severe muscle injury in taurine-deficient mice.

Aged muscle develops a phenotype with many of the same functional and histological characteristics as younger taurine-depleted muscles. An age-associated reduction in taurine content has been reported in serum and tissues, including skeletal muscle (151-153). Because many of the defects observed in muscles of aged animals are also observed in young taurine-depleted animals, several studies have evaluated the effect of taurine supplementation on taurine restoration and muscle function in aged animals. Treating aged rats with 2% taurine in their drinking water (water was administered until rats drunk a daily dose of 1g taurine $\cdot$ kg^{-1} bodyweight) for 3 months significantly increased muscle taurine content and resting chloride conductance (152). Similarly, feeding aged rats normal chow plus water supplemented with 0.12 mol$\cdot$ L^{-1} of taurine for 8 months increased serum and tissue taurine concentrations to the levels found in younger rats and reduced age-related markers of oxidative damage (154).

As previously noted, bedrest further exacerbates the aging muscle phenotype. Several studies suggest that taurine supplementation may help alleviate the harmful physiologic effects of bedrest. Supplementing rats with 5 g taurine$\cdot$ kg^{-1} bodyweight after 14 days of hindlimb unloading (which induces muscle atrophy in rats) restored cytosolic calcium concentration and resting chloride conductance (155). However, although taurine supplementation helped restore muscle function, it did not prevent muscle atrophy in this model (155). Alternatively, pretreatment of C2C12 mouse myotubes with taurine counteracted muscle atrophy and restored mitochondrial function when the myotubes were exposed to cisplatin, a pharmacological inducer of muscle weakness and cachexia (156).

Understanding the effect of taurine on muscle atrophy is also important for cardiovascular health interventions. Human cross-sectional and case control studies suggest that increased taurine is negatively associated with cardiovascular disease (157, 158). Taurine transporter knockout mice show that taurine deficiency alters the structure of ventricular cardiomyocytes, induces cardiomyopathy, and increases expression of heart failure genes (159, 160). Although the role of taurine in cardiac and skeletal muscle atrophy remains controversial, evidence suggests that taurine plays a role in preventing age related muscle deterioration in animal and cell models. Furthermore, supplementing with taurine may alleviate the age-associated decline in taurine content and restore several muscle functions.

Taurine's antioxidant and anti-inflammatory properties may also have beneficial roles in the treatment of type II diabetes. Taurine supplementation may improve diabetic outcomes through alleviating the negative effects of reactive oxygen species on insulin release and resistance. In animal models, taurine supplements counteract oxidative stress, improve insulin sensitivity by inhibiting c-Jun N-terminal kinase activation, and improve beta-cell stimulus-secretion coupling (161, 162). Similarly, in overweight, non-diabetic male patients with heparin and intravenous intralipid induced insulin resistance,

taurine supplementation (3 g · day^{-1} for 2 weeks) improved insulin sensitivity and beta cell function (163).

Collectively, an abundance of experimental evidence suggests that taurine plays a role in the prevention and/or treatment of many chronic conditions. These conditions include, but are not limited to, age-related skeletal muscle decline, cardiovascular disease, and type II diabetes. However, before recommendations for nutritional therapy can be established, large-scale clinical trials are needed to confirm the findings from animal models and cross-sectional and case control observations.

5. ARGININE

Arginine is a conditionally essential amino acid that plays a role in cell signaling and insulin and growth hormone release (164, 165). An arginine dietary reference intake value has not been established. However, food sources include soy products, seeds, crustaceans, meats, and poultry. Within the body, arginine's biosynthesis depends on the availability of its carbon and nitrogen precursors: glutamate, glutamine, and proline (166). Available arginine can be converted to L-ornithine, an important intermediate in the urea cycle, or to phosphocreatine, an important source of readily available and quickly used ATP within skeletal muscle cells (167, 168). Additionally, arginine is a precursor for nitric oxide, a neurotransmitter, mediator of the immune response, and potent vasodilator. Functionally, arginine plays important roles in cardiovascular function, blood pressure control, and wound healing (169, 170).

5.1. Arginine, growth hormone, and insulin-like growth factor 1

Insulin and growth hormone both play important roles in cell growth and other anabolic processes within the body. Growth hormone also stimulates the production of insulin like growth factor-1 (IGF-1), a hormone involved in muscle mass maintenance and accretion (171). Aging is associated with decreased circulating levels of growth hormone and IGF-1 (22). Growth hormone therapy has shown positive effects on body composition, strength, and circulating IGF-1 levels in adults with confirmed growth hormone deficiencies (172, 173). However, growth hormone interventions have a high incidence of adverse effects including fluid retention, joint pain, and muscle pain (174). These side effects may further limit mobility in older adults. Additionally, growth hormone interventions are expensive – another important limitation for their use in the elderly (175). Therefore, alternative therapies, such as arginine supplementation, that induce growth hormone release and promote muscle mass accretion are being investigated.

Recent evidence suggests that arginine positively affects skeletal muscle growth through actions dependent and independent of the growth hormone axis in animal and cell culture models (176-180). He *et al.* found that 120-day-old pigs supplemented for 46 days with 1.0 % arginine in their corn and soybean meal-based diet had significantly greater skeletal muscle mass and lower fat mass (176). Metabolomic analysis of serum from treated pigs showed that dietary arginine enhanced skeletal muscle protein synthesis (176). Creatinine, lysine, tyrosine, and tricarboxylic acid cycle metabolites were all higher in arginine-supplemented pigs. Although growth hormone and insulin levels were not evaluated in this study, they were measured in a similar study conducted by the same group (177). In this latter study, 60 days of arginine supplementation did not significantly alter growth hormone or insulin-like growth factor-1 levels measured 12 hours after feeding. Similarly, mice supplemented with 1.51% L-arginine-HCl in their drinking water increased muscle weight by 12% although no increase in growth hormone was observed 5 hours after feeding (178).

In 2005, Collier *et al.* first reported the oral doses of arginine required to elicit the growth hormone response (181). Compared to placebo, both 5 and 9 grams of ingested arginine induced a transient increase in circulating growth hormone concentrations that were apparent by 30 minutes post-ingestion with peak levels around 60 minutes post-ingestion. Intriguingly, when compared to placebo, 13 grams of arginine did not result in a significant growth hormone response. Most subjects reported gastrointestinal distress after the 13 gram dose; therefore, much of the arginine may have been excreted before it was absorbed. This work produced a new understanding of the time dependency of the growth hormone response following arginine ingestion. Previously, this time-dependent response was not always considered when evaluating arginine's effect on the growth hormone response. Therefore, the effect of arginine on the growth hormone response may have been missed by sampling at an inappropriate time.

Collectively, these results suggest that arginine or dietary protein sources rich in arginine can improve skeletal muscle health in animals. However, whether similar effects occur in humans is unknown but is an important area for future investigation.

5.2. Arginine and muscle protein synthesis

Aside from the effect of arginine on the growth hormone axis, arginine supplementation may also affect other signaling cascades, including the mTORC1 pathway. In 2008, Yao *et al.* first analyzed the effect of dietary arginine supplementation on the mTORC1 pathway in neonatal pigs (179). Treated pigs were supplemented with 0.6 % L-arginine in a milk-based diet for 7 days. Control pigs were fed the same diet supplemented with an isonitrogenous amount of alanine. After their last meal, the abundance and

phosphorylation state of mTORC1, S6K1, and several eukaryotic initiation factors were assessed in the skeletal muscle. Although arginine did not alter phosphorylated levels of S6K1, it did increase levels of phosphorylated mTORC1 and 4EBP1. Protein fractional synthetic rate and formation of the active eIF4G-eIF4E complex also increased in the skeletal muscle. It is important to note, however, that arginine supplementation also increased levels of circulating insulin, an independent stimulator of mTORC1. Other growth factors, such as growth hormone, were not measured. Collectively, these results suggest that arginine supplementation potentiates mTORC1 signaling compared to controls fed a milk-based diet only. However, it was not clear whether these results occurred independently of growth factor release.

Ham *et al.* investigated the direct role of arginine on skeletal muscle protein synthesis in growth factor and nutrient deprived C2C12 cells (180). C2C12 myotubes were incubated in HEPES buffered saline with arginine or equimolar concentrations of alanine for up to 4 hours. Compared to alanine, arginine supplementation increased protein synthesis, myotube diameter, and phosphorylation of mTORC1 and its downstream targets rS6 (a phosphorylation target of S6K1) and 4EBP1 during the first hour of treatment (180). Furthermore, experiments using identical methodology with the inclusion of rapamycin, an inhibitor of mTORC1, did not show any effect of arginine on protein synthesis or myotube diameter (180). This was the first study to show the direct effect of arginine in protection against muscle wasting during catabolic conditions *in vitro*. Because cells were deprived of growth factors and nutrients, the results of this study support a direct role for arginine in the regulation of mTORC1 in skeletal muscle. Collectively, these results suggest that arginine positively influences cell growth and muscle protein synthesis through both growth factor-independent and growth factor-dependent stimulation.

5.3. Arginine in aging

Both cell culture and animal model experiments report positive effects of arginine on muscle protein synthesis. However, limited evidence exists on the role of arginine alone on muscle mass maintenance in aging adults. Studies have shown that a mixture of β-hydroxy-β-methylbutyrate (HMB, a metabolite from leucine catabolism), arginine, and glutamine increases fat free mass in adults with various wasting diseases, including AIDS and cancer-related cachexia (182, 183). Additionally, supplementing elderly women with a combination of 2 g HBM, 5 g arginine, and 1.5 g lysine for 12 weeks improved protein synthesis, strength, and functionality (184). Although these studies support the beneficial effects of arginine mixed with HMB, lysine, and/or glutamine, there is limited evidence for the long-term effects of arginine alone on the age-related decline in skeletal muscle mass and/or function.

Arginine has also been investigated for its use in the treatment of coronary endothelial dysfunction. Arginine is the precursor of nitric oxide, the endothelium-derived vasodilator. Experimental studies have shown that oral L-arginine supplementation improves coronary endothelial function in patients with coronary artery disease, hypercholesterolemia, and hypertension (185-187). Bode-Boger *et al.* also reported the positive effects of L-arginine supplementation in healthy older adults with age-related progressive endothelial dysfunction (188). Although several reports and initial clinical trials show the beneficial effects of arginine on endothelial function, the direct mechanism remains unclear. In the normal physiologic state, intracellular concentrations of arginine far exceed those needed by endothelial nitric oxide synthase (NOS), the enzyme that converts arginine into nitric oxide. The effect of supplemental arginine on nitric oxide production may be explained, in part, by the enzyme arginase. Like NOS, arginase also requires arginine as a substrate, and it is upregulated in several disease conditions including hypercholesterolemia and hypertension (189, 190). Increased arginase activity and expression may decrease the amount of arginine available for nitric oxide synthesis (191). When additional arginine is supplemented to those with upregulated arginase, arginine's availability for nitric oxide production may be restored.

6. EFFECTS OF EXCESS AMINO ACID INTAKE

There is no evidence that amino acids derived from usual or even high intakes of protein from food present any risk. For individuals consuming typical foods, intake of protein is unlikely to exceed 25% of energy, and these levels are well within safe intakes of protein. However, it does not follow that a high intake of a purified protein supplement or of an individual L-amino acid is safe. At this time, no tolerable upper intake levels have been established by the Food and Nutrition Board of the Institute of Medicine; a detailed summary of the available data related to risk of excess intakes of individual L-amino acids is included in their publication "Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (31)."

The majority of the research on adverse effects of excess intakes of individual L-amino acids has been conducted in animals, with reductions in feed intake and growth rate being the most apparent outcomes. Excess amino acids are most toxic when they are given in amounts that are disproportionate to the normal amino acid composition of the diet (192-194). Consistent with this association of adverse effects with the degree of imbalance of the overall amino acid pattern, Imamura *et al.* found adverse effects of 3% (w/w) L-leucine supplementation on growth of rats fed a low protein diet (6% casein), whereas there was no effect of up

to 8% L-leucine supplementation on growth of rats fed a moderate (12% casein) or high (40% casein) diet (195). This relationship of adversity to degree of imbalance can be seen as related to the degree to which protein synthesis can serve as a sink for the excess amino acid and the degree of competition among amino acids for shared transporters or shared metabolic enzymes. In addition to effects on feed intake and growth rate, excess intake of individual amino acids by animals have been reported to have a variety of effects, including alterations in hormone levels, one-carbon metabolism, mineral bioavailability and blood lipid profiles.

There is little dose-response data for the effects of excess amino acid intake, either acute or chronic, by humans. Pencharz *et al.* studied the effect of acute ingestion of excess L-leucine by young men on leucine catabolism and a panel of markers for adverse effects (196). They estimated that the metabolic limit for leucine oxidation was between 550 and 700 mg. $kg^{-1} \cdot d^{-1}$ (~39 g/d), a level that was associated with increased plasma leucine and ammonia concentrations. Hiratsuka *et al.* studied the effect of intake of various doses of L-tryptophan (1 to 5 g/d) for 3-week periods in young women and found no adverse effects (197). The urinary excretion of nicotinamide and other tryptophan catabolites increased in proportion to the ingested amounts of tryptophan, demonstrating that the metabolic capacity was not saturated. In rare cases, excess intake of tryptophan combined with use of serotonin drugs has resulted in “serotonin syndrome” due to toxic effects of excess serotonin on the nervous system.

Although almost all studies suggest that supplementation with an L-amino acid at several times its requirement level is not associated with adverse effects in humans, little data is available that allows evaluation of possible adverse effects associated with long-term intake of excess L-amino acid. Those using or promoting the use of supplemental amino acids should also be aware of potential hazards related to use of any supplement, in particular errors in dosage calculation and the possible presence of contaminants. Two examples serve to underscore the importance of these potential, though rare, risks of supplement use. Although oral doses of L-methionine (100 mg/kg body wt) are widely used to test for a tendency to develop hyperhomocysteinemia and have been regarded as very safe, one subject died after a methionine loading test in which the subject is believed to have been accidentally given 10-times the specified amount (198). A condition called eosinophilia myalgia syndrome appeared in 1989 that was linked to supplemental L-tryptophan use and subsequently shown to be due to a contaminant in certain production batches (199). Future research and/or clinical case studies of observed effects are necessary to determine adverse effects of amino acid supplementation.

7. CONCLUSION AND FUTURE DIRECTION

As evidenced in this review, amino acids play a role in healthy aging. Knowledge of the interactions between specific amino acids and age-related physiological and metabolic impairment has advanced through discovery research. Research using animal and cell culture models has led to a better understanding of amino acid signaling cascades and potential impacts of amino acids on health outcomes. However, there are several important differences between animal and cell culture models and humans. Of the amino acids presented in this paper, only leucine has been well-studied in humans. Human data support increased protein intakes in older adults and bolus doses of leucine and/or essential amino acids between meals for skeletal muscle health. Although discovery research has shown positive effects for cysteine, taurine, and arginine in mammalian physiology, the effects of supplemental doses of each of these amino acids in humans remains unclear. Future research to characterize the role of these and other amino acids (not discussed) in healthy human aging is also necessary.

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