

Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application

Amino Acids and Muscle Loss with Aging¹⁻³

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ABSTRACT Aging is associated with a progressive loss of muscle mass (sarcopenia), which increases the risks of injury and disability. Although the mechanisms of sarcopenia are not clearly elucidated, age-associated alterations in the muscle anabolic response to nutritional stimuli and a decline in protein intake may be significant contributing factors. The most recent findings regarding the role of nutritional intake on protein metabolism in the elderly will be reviewed. Specifically, aging is associated with changes in the muscle protein metabolism response to a meal, likely due to alterations in the response to endogenous hormones. Nonetheless, the older muscle is still able to respond to amino acids, mainly the essential and BCAAs, which have been shown to acutely stimulate muscle protein synthesis in older individuals. It is likely that this stimulatory effect of essential and BCAA is due to the direct effect of leucine on the initiation of mRNA translation, which is still present in older age, although it appears to be attenuated in aged animals. Recent data suggest that excess leucine may be able to overcome this age-related resistance of muscle proteins to leucine. For this reason, long-term essential amino acid supplementation may be a useful tool for the prevention and treatment of sarcopenia, particularly if excess leucine is provided in the supplement. *J. Nutr.* 136: 277S-280S, 2006.

KEY WORDS: • aging • amino acids • muscle • sarcopenia • protein metabolism

Sarcopenia is an age-related progressive loss of muscle mass that leads to muscle weakness, limited mobility, and increased susceptibility to injury. The mechanisms involved in sarcopenia have not been clearly understood. Indeed, an imbalance between protein synthesis and protein breakdown rate can lead to the loss of muscle proteins.

Previous studies suggest that sarcopenia may be due to a reduced basal rate of muscle protein synthesis (1-5). However, these age-related differences in muscle protein synthesis might be due to dietary manipulations and limitation of physical activity before the experiments. In fact, we have recently reported in the largest cohort of healthy community indwelling men that, despite a decline in muscle mass, basal muscle protein synthesis and net protein balance are not reduced with healthy aging when usual diet and normal physical activity levels are not manipulated before the experiments (6). Because

most protein catabolism in daily life occurs in the basal, post-absorptive state, the results of this study suggest that sarcopenia is more likely caused by reduced stimulation or altered sensitivity to anabolic factors. Additionally, taken together, these data suggest that older muscle may be more sensitive to short-term inactivity or may require different dietary intakes of nutrients to maintain a normal synthesis rate. In this article we focus on the role of nutritional intake, an anabolic stimulus, and its effects, on protein metabolism in the elderly.

Muscle anabolic response to protein and amino acid intake in aging

Several studies indicate that protein intake decreases in frail elders (7), and that even healthy older adults may need more protein than what was recommended by the most recent protein Dietary Recommended Intakes (DRI) for persons 55 and older (8,9). Yet, studies in which nutritional intervention with commercial nutritional supplements or high-protein diets were tested in elderly subjects to determine whether they could increase muscle mass and strength mostly reported negative results (10-12).

There are 2 possible mechanisms that can explain these negative results. First, it has been reported that if physical activity is not increased to enhance the energy requirements, the addition of nutritional supplements to the diet of elderly subjects results in a calorie-for-calorie reduction in dietary intake so that the total daily energy intake did not change (11). This suggests that a nutritional supplement for the elderly should be better

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considered as a meal substitute rather than a true nutritional supplement. It also suggests that if the nutrient pattern and nutritional content of the supplement given to the elderly is similar to a regular meal, such a nutritional supplement will likely be ineffective. Therefore, to be effective, nutritional supplements for the elderly should be more efficient than regular food in terms of anabolic effect per energy unit. Second, it is also possible that the ability to utilize dietary protein/amino acids for muscle anabolism is reduced with aging so that the older population requires a larger amount of amino-nitrogen to maintain muscle protein balance and muscle mass. Previous studies on muscle protein metabolism in response to nutritional intake in the elderly seem to support both of these mechanisms.

Previous studies have demonstrated that amino acid availability is critical in the regulation of muscle protein metabolism (13,14). Specifically, hyperaminoacidemia acutely stimulates muscle protein synthesis by increasing the amino acid transport into muscle cells (13). An age-related decline in the ability to utilize exogenous amino acids could be due to specific problems of muscle fibers, including decreased transmembrane amino acid transport for protein synthesis, reduced availability of substrate for protein synthesis due to the alterations in the whole body amino acid turnover, or alterations in the endogenous hormonal response and/or in the response of muscle to the hormonal stimuli after meal intake.

We investigated whether an age-associated decline in the ability of skeletal muscle to efficiently transport exogenous amino acids was responsible for a reduction in the response of muscle protein synthesis to an amino acid load by measuring amino acid transport and muscle protein turnover in healthy older subjects in the postabsorptive state and during the intravenous infusion of an amino acid mixture (15). However, we found that under these circumstances amino acid delivery to the leg and transport into the muscle, and muscle protein synthesis were normally stimulated in older subjects (15). Because protein breakdown did not change during amino acid infusion, a positive net balance of amino acids across the muscle was achieved, indicating acute muscle protein accretion. Thus, we concluded that although muscle mass decreases with aging, muscle protein anabolism can nonetheless be directly stimulated by increased amino acid availability.

Nonetheless, in the physiological condition, that is, during meal absorption, in order for dietary amino acids to reach the systemic circulation and be made available for the muscle tissue, they have to pass through the splanchnic tissues. It is well known that the splanchnic tissues in young individuals use a significant portion of the dietary amino acids for their own metabolism (16). It has been also demonstrated that the first-pass splanchnic extraction of leucine increases with age (17). Thus, if the splanchnic tissues utilized more amino acids in the elderly, the flow and availability of dietary amino acids for the peripheral tissues, including muscle, would be reduced; hence the response of muscle protein anabolism to a given dose of amino acids would be blunted. To test this hypothesis, we compared the response of muscle protein metabolism to the oral administration of a large dose of mixed amino acids in young and elderly subjects using stable isotope methodologies to measure the parameters of muscle protein metabolism (18). Interestingly, despite a higher phenylalanine first-pass splanchnic extraction in the elderly, we found that the delivery of amino acid to the leg increased to the same extent in both the young and elderly. Additionally, amino acid transport into the muscle, its utilization for protein synthesis, the mixed muscle protein fractional synthetic rate, and the net protein balance increased significantly and similarly in both age groups (18). Thus, from these data we concluded

that although the splanchnic first-pass extraction of oral amino acids increases with age, it does not prevent the dietary amino acids from reaching the systemic circulation and stimulating net muscle protein anabolism in the elderly as it does in the young. It is likely that the higher amino acid first-pass splanchnic extraction in older subjects is more a reflection of a higher splanchnic turnover rate (i.e., higher synthesis and breakdown) rather than of an isolated higher splanchnic utilization rate for synthesis and oxidation such that splanchnic net balance of amino acid is similar between young and old subjects during meal absorption.

These data, supporting the notion that amino acids acutely stimulate muscle protein anabolism in older as well as in younger individuals, were apparently at odds with the previous studies in which nutritional supplementation failed to improve muscle mass (10,11). However, this was not necessarily the case, because different combinations of nutrients were used. We used a balanced mixture of amino acids alone (15,18), whereas all the other studies used a mixture of protein, fat, and carbohydrate (10–12). In a subsequent study, we found that whereas the addition of carbohydrate to an amino acid meal enhanced the amino acid stimulation of muscle protein synthesis in young subjects (19,20), such a combination did not have any additive effect on muscle protein synthesis in older subjects (20). On the contrary, the carbohydrate blunted the amino acid-induced increase in muscle protein synthesis, thereby blunting the anabolic effect of the meal on muscle proteins (20). Guillet et al. (21) recently confirmed these data by simulating the prandial state using the systemic hyperinsulinemic-euglycemic clamp in the presence of increased amino acid concentrations. These results are intriguing, particularly because in both studies the older subjects were healthy and nondiabetic, and in our experiment we documented that their whole-body glucose turnover, muscle glucose uptake, and insulin concentrations were not different from those of the younger control group (20).

The two major variables introduced with the addition of carbohydrate to the oral amino acid meal (20) or with the hyperinsulinemic-hyperaminoacidemic clamp (21) were increased energy (carbohydrate) and increased insulin (endogenous secretion). Muscle protein synthesis is an energy-consuming process, requiring ~ 0.7 kcal/g of protein synthesized (~ 240 kcal/d for an average person) (22). Consequently, if additional energy had any influence on muscle protein synthesis, such an effect should have been positive rather than negative. Insulin is a potent anabolic stimulus for muscle proteins (23–33), and a number of studies have reported that hyperinsulinemia can increase muscle protein synthesis, particularly when muscle amino acid availability is increased (21,23,24,28,31–33).

Therefore, it is possible that muscle protein synthesis in the elderly is resistant to the anabolic action of insulin, and that this effect of aging is independent of a normal glucose tolerance. Preliminary data suggest that when skeletal muscle is exposed to hyperinsulinemia, insulin can significantly increase muscle protein synthesis when amino acid availability to the muscle is maintained. On the contrary, such an anabolic effect of insulin is absent in older subjects, suggesting that muscle protein synthesis is resistant to the anabolic action of insulin in healthy, nondiabetic older humans. The data also suggest that age-associated insulin resistance of muscle proteins is the primary reason for the reduced muscle anabolic response to feeding, and this may play an important role in the development of sarcopenia. Indeed, accumulating evidence indicates that the anabolic response of skeletal muscle proteins to mixed feeding decreases with age in both humans (20) and animals (34,35), despite the fact that amino acids alone can normally stimulate protein synthesis in older muscle (15,18,36,37).

Essential amino acids and muscle protein anabolism in aging

Although the anabolic efficacy of a mixed meal is reduced with aging, the observation that pure amino acids can still stimulate muscle protein anabolism in elders can be exploited to design nutritional approaches for the treatment of sarcopenia. From the previous unsuccessful nutritional intervention studies (10–12) it emerges that nutritional interventions to increase muscle mass in older people should be performed with nutrients or supplements more efficient than the food normally consumed. Considering that carbohydrate appears to have a negative rather than a positive effect on muscle protein turnover in older muscle, it seems logical that pure protein or amino acid mixtures should be evaluated. Among the different proteins available for nutritional supplementation, rapidly absorbed proteins, such as whey, have been shown to exert a better anabolic effect as compared with slowly absorbed proteins like casein (38).

However, even high-quality proteins may not be adequately efficient, because studies indicate that the anabolic action of amino acids on muscle proteins is due mainly to the essential amino acid (39,40). These findings have been confirmed in older subjects by administering nutritional supplements containing only essential amino acids or a balanced essential and nonessential amino acid mixture containing the same amount of essential amino acids but double the amount of amino acids and amino-nitrogen (37). Under these circumstances, muscle protein synthesis and net balance, an index of muscle protein accretion, were stimulated to the same extent by either supplement. Because the essential amino acid supplement contained half the amino-nitrogen and the amino acids of the balanced supplement, its anabolic efficiency (anabolic effect/energy or nitrogen delivered) was double compared with that of the balanced supplement (37). These data were obtained by feeding fairly large doses of amino acids as small boluses over a 3-h period to perform steady-state measurements of muscle protein turnover. Thus, in a subsequent study a more physiological approach was taken by administering 15 g of essential amino acids in a single bolus to determine if age-related differences in acute amino acid absorption might be responsible for a differential anabolic effect on muscle proteins. In this case, some kinetic differences were found with regard to the profile of blood amino acid concentration over time, with older subjects displaying a slower but more prolonged amino acid peak. Similarly, the net amino acid balance, the index of net muscle protein accretion or degradation, peaked later in the elderly, but remained positive for a longer time, so that when the area under the net balance curve was calculated, no differences between older and younger subjects were found in the net anabolic effect of the amino acid bolus (41).

Branched-chain amino acids and muscle protein synthesis in aging

Based on the studies discussed above, essential amino acids appear to be the most efficient nutrient for the stimulation of muscle protein synthesis in older and in younger subjects; although the cellular mechanisms responsible for this effect are still under investigation. In addition to being the precursors for protein synthesis, it appears that essential amino acids may directly stimulate the synthetic process. Among the essential amino acids BCAAs have been shown to be the major carriers of amino nitrogen between the viscera and the peripheral tissues, including skeletal muscle (42), but more importantly they appear to be the ones most responsible for the direct

stimulation of muscle protein synthesis. Specifically, leucine is the most potent of the BCAAs for the stimulation of muscle protein synthesis. In general, acute increases in the rates of protein synthesis are determined by the number of ribosomes present in a cell as well as by the translational efficiency per ribosome. Studies on the mechanisms by which acute changes in nutritional status, such as those occurring after feeding, stimulate muscle protein synthesis have shown that feeding does not alter tissue ribosomal content but instead stimulates the initiation of mRNA translation (43–45). Leucine can activate several intracellular signals involved with initiating translation, the mammalian target of rapamycin (mTOR)⁵ signaling pathway, which includes 70-kDa ribosomal protein S6 kinase (S6K1), and eukaryotic initiation factor 4E binding protein-1 (4E-BP1) (46–48). Interestingly, it has been reported that in old rats, muscle protein synthesis becomes resistant to the stimulatory effect of leucine in the range of physiologic postprandial concentrations (49). This resistance has been correlated with a defect in the stimulation of S6K1 activity by leucine. More recently, Cuthbertson et al. (50) reported that in a small number of subjects, the sensitivity and responsiveness of myofibrillar and sarcoplasmic protein synthesis to various doses of essential amino acids were blunted in the elderly, which appears to be associated with a reduced activity of the mTOR signaling pathway (including mTOR and S6K1).

However, in old rats, the stimulation of muscle protein synthesis can be restored if leucine concentration is raised to supraphysiologic concentrations (49). In humans, Arnal et al. (51) demonstrated that the anabolic response of whole body protein turnover was normalized in the elderly if a protein-pulse feeding pattern (80% of daily protein in 1 meal) was used instead of spread-protein feeding (daily proteins equally distributed), suggesting that a large amount of amino acid may be needed in the elderly to obtain the same anabolic effect observed in the young. Furthermore, preliminary data from Katsanos et al. (52) indicate that muscle protein synthesis can significantly increase in older subjects after consuming a small amount of essential amino acids (6.7 g) if it contains a high proportion of leucine (2.8 g), whereas the same small dose of essential amino acids is ineffective if the proportion of leucine is lower (1.7 g). These results again suggest that aged muscle may be less sensitive to the stimulatory effect of amino acids, especially leucine, at low physiologic concentrations, but that this impairment can be overcome by the provision of a larger amount of leucine.

Conclusion

In conclusion, from the data available it appears that aging skeletal muscle may progressively lose its ability to respond to anabolic stimuli, including insulin and, to a lesser extent amino acids, particularly within the lower physiological range of concentrations. However, older muscle can still mount an anabolic response to the administering of protein/amino acid, and for this reason it is possible to devise nutritional interventions to maintain and restore muscle mass in the elderly. Among the most effective of possible strategies is the elimination of any inefficient sources of energy, including carbohydrate and even nonessential amino acids, if necessary, while increasing certain stimuli, particularly leucine, which can maximize the anabolic effect. In other words, to achieve the highest anabolic efficiency per energy unit it is important to deliver only nutrients that are absolutely necessary for the stimulation of muscle protein

⁵ Abbreviations used: mTOR, mammalian target of rapamycin; S6K1, 70 kDa ribosomal protein S6 kinase.

anabolism. Under these circumstances, if patients perform an isocaloric substitution of regular food with the nutritional supplements, such a change would still be advantageous for the muscle. However, because most of the data upon which we base our conclusions is obtained from acute experiments, long-term randomized clinical trials are necessary to ascertain the efficacy and cost-effectiveness of these potentially useful nutritional interventions for the reduction of muscle loss with aging.

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