Effects of protein and amino acid supplementation on muscle protein metabolism in relation to exercise

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Abstract Exercise promotes protein and amino acid breakdown in skeletal muscle. Proper nutrient intake in relation to exercise is thus important to maintain or build up skeletal muscle. It has been demonstrated that supplementation of branched-chain amino acids (BCAAs) before exercise has beneficial effects on skeletal muscle, such as decreasing exercise-induced muscle damage and soreness. Protein synthesis in skeletal muscle is enhanced after exercise, and proper timing of supplementation of protein and amino acids for effectively stimulating muscle protein synthesis is herein discussed. Treatment of elderly individuals with exercise and nutrient supplementation is also demonstrated to have efficacy against age-related sarcopenia.

Keywords: resistance exercise, protein synthesis, supplement, essential amino acids, branched-chain amino acids

Introduction

Skeletal muscles with high power and flexibility are very important for participants in athletic competitions. Many athletes incorporate resistance training in their training programs to build up suitable skeletal muscle, and during such training, good nutrition in vitally important. As resistance training is also common in the general public for the purpose of health promotion, good nutrition is also important for public health.

In many developed countries, age-related sarcopenia characterized by a gradual, but progressive, loss in skeletal muscle mass and strength is a problem1,2). In the USA, medical expenses related to sarcopenia reached 18.5 billion dollars in 20003). It is therefore important to maintain skeletal muscles in the elderly in order to avoid sarcopenia.

In this paper, the authors summarize the effects of exercise on protein and amino acid metabolism and discuss the most effective timing of nutrient intake in relation to exercise in order to increase the mass and strength of skeletal muscles.

Protein and amino acid metabolism in skeletal muscles

As muscle accounts for approximately 40% of body weight of humans and contains more or less 20% protein, muscle tissue acts as a reservoir of amino acids in the human body. Furthermore, muscle contains 3-5 g of free amino acids, which play an active role in protein metabolism4,5). Therefore, proteins and amino acids are very important nutrients to sustain or increase skeletal muscle mass in humans. However, both nutritional treatment and exercise training is necessary for development of skeletal muscles.

1. Increased protein breakdown by exercise

There are 3 major protease systems in muscles: 1) the lysosome system, 2) the ATP-dependent ubiquitin-proteasome system, and 3) the Ca\(^{2+}\)-dependent protease system6). Among these systems, the lysosome system is known to be activated by acute exercise. A very recent study using gene-manipulated mice demonstrated that autophagy, which is involved in the lysosome protease system, is important for exercise performance6). However, there is little information on the roles of protease systems in exercise.

It is known that intensive acute exercise causes muscle damage, resulting in increased serum creatine kinase release from skeletal muscles7). Therefore, enzyme activity in serum is commonly used as an index of muscle damage, which is caused by non-routine exercise, particularly in untrained individuals8). Muscle damage is accompanied by increased muscle protein breakdown. This protein breakdown is detected by monitoring blood urea concentrations. Urea is formed in the liver from ammonia derived from protein/amino acid degradation. It has been reported that the blood urea concentration increases proportionally with duration of exercise9), thus suggesting that protein degradation is promoted by prolonged exercise.

On the other hand, leucine oxidation during exercise,
which can be intensively examined using stable isotope-labeled leucine, was elevated in the early stages of exercise without an increase in urea concentration. These findings suggest that protein breakdown during exercise is underestimated in analyses based on measurement of urea production, and that some amino acids (e.g., leucine) are used as substrates for energy production, even in the early stages of acute exercise.

2. Increased amino acid breakdown by exercise

In the amino acid catabolic system, the carbon skeletons of the amino acids ultimately yield precursors and intermediates of the citric acid cycle (Fig. 1). Numerous types of amino acids, in excess, are catabolized in the liver, whereas some amino acids (Leu, Ile, Val, Ala, Asp and Glu) can be directly oxidized for energy production in muscles. Branched-chain amino acids (BCAAs: Leu, Ile and Val) are typical amino acids that are catabolized in muscles, but not the liver, and Ala and Glu (particularly Glu/Gln) act as transporters of amino groups from the muscles to liver.

The BCAA catabolic pathway (Fig. 2) exclusively exists in mitochondria. The first 2 steps of the pathway are common to the 3 BCAAs, and the second step catalyzed by branched-chain α-keto acid dehydrogenase complex (BCKDC) is important for regulation of catabolism. BCKDC is regulated by a phosphorylation/dephosphorylation cycle; the E1α subunit of BCKDC is inactivated by phosphorylation and reactivated by dephosphorylation. The inactivation of BCKDC is catalyzed by branched-chain α-keto acid dehydrogenase kinase (BDK), and the activation by branched-chain α-keto acid dehydrogenase phosphatase (BDP).

BDK appears to be the main regulator of BCKDC activity, and it has been reported that almost all BCKDC in the skeletal muscles of rested rats exists in an inactive/phosphorylated state, suggesting that BCAA oxidation is very low in resting skeletal muscles. This was also confirmed in human skeletal muscles. These findings suggest that the low BCKDC activity in resting skeletal muscle contributes to providing BCAAs for protein synthesis. It has been reported that chronic activation of BCKDC, by administration of a BDK inhibitor (clofibrate) into rats, induces a decrease in muscle proteins.

It has also been demonstrated that acute exercise significantly activates the muscle BCKDC in rats; after 2 hours of running on the treadmill (30 m/min), ~80% of muscle BCKDC was in an active state. This enzyme activation is associated with increased BCAA oxidation in rats, and these phenomena were also confirmed in humans. Hepatic BCKDC activity is low in rats fed a low-protein diet (BCAA-deficient condition), and this low activity was significantly elevated by acute exercise, thus suggesting that BCAA oxidation was promoted in the animals, even under BCAA-deficient conditions. Therefore, the BCAA requirement may be increased by exercise.

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**Fig. 1** The pathways for amino acid oxidation
Physiological effects of BCAA supplementation on skeletal muscles

1. Regulation of protein metabolism by leucine.

It has been demonstrated that leucine stimulates protein synthesis by promoting mRNA translation\textsuperscript{20-22}. This mechanism involves activation (phosphorylation) of the mammalian target of rapamycin (mTOR, particularly mTOR complex 1), which phosphorylates ribosome protein S6 kinase 1 (S6K1), that subsequently phosphorylates ribosome protein S6 and eukaryotic initiation factor 4E-binding protein 1 (eIF4E-BP1). The latter allows eIF4E to form an eIF4F complex with eIF4A and eIF4G, and this complex is associated with mRNA in the translation processes. Furthermore, it has been reported that activated mTOR inhibits the autophagy system\textsuperscript{23} and the ubiquitin-proteasomal system\textsuperscript{24}. From these findings, it is evident that leucine stimulates protein accretion in skeletal muscles.

2. Inhibition of exercise-induced muscle protein breakdown by BCAA supplementation.

MacLean et al.\textsuperscript{25} reported evidence suggesting that skeletal muscle protein breakdown is suppressed by BCAA supplementation before exercise in humans, although muscle NH$_3$ production is increased during exercise. In this study, the subjects ingested a total of 77 mg BCAAs/kg BW (two doses of 38.5 mg/kg BW) 20-45 min before the onset of exercise comprising 70-75% of one-legged maximal knee extension for 60 min. These findings suggest that supplemented BCAAs are degraded in the muscle during exercise, resulting in suppression of BCAA mobilization from muscle proteins. This phenomenon is probably related to increased BCAA oxidation during exercise, and the relatively low concentrations of free BCAAs in the muscle (~0.65 mM) (4) and plasma (0.45 mM)\textsuperscript{26}.

3. Effects of BCAA supplementation on exercise-induced muscle damage and soreness.

Muscle damage is commonly assessed based on increases in serum creatine kinase activity, which is released from damaged muscle cells. BCAA supplementation has been reported to partially suppress the exercise-induced increase in blood creatine kinase activity\textsuperscript{27,28}.

Muscle damage induced by exercise is commonly associated with muscle soreness. Delayed-onset muscle soreness (DOMS), which is most strongly felt at 24 to 72 h after exercise, is caused by non-routine or strenuous exercise. The effects of BCAA supplementation on squat exercise-induced DOMS has been examined using untrained young women\textsuperscript{29-31}, and these studies demonstrated that BCAA supplementation before exercise significantly reduces the level of DOMS. This effect has also been demonstrated in male subjects\textsuperscript{32}. Furthermore, it has been reported that BCAA supplementation reduces exercise-induced muscle damage and improves exercise performance in the successive exercise program of athletes under fatigue conditions\textsuperscript{33}. These findings support the notion that BCAA supplementation is useful for sports, although the
detailed mechanism responsible for the action of BCAA remains to be clarified. However, it may be related to the regulatory functions of amino acids on muscle protein metabolism.

The other physiological functions of BCAA supplementation in relation to exercise are as follows. BCAA supplementation:
1) decreases central fatigue during exercise in humans34,35),
2) spares muscle glycogen during exercise in rats36), and
3) prolongs endurance exercise during heat stress in humans37) and under normal conditions in rats38).

An interesting study demonstrated a protective effect of BCAA supplementation against muscle cramps in patients with liver cirrhosis39). In Japan, a BCAA formulation is used in liver cirrhosis patients to improve decreases in plasma albumin levels. It is commonly observed that plasma BCAA levels are significantly lower in such patients, corresponding to decreased plasma albumin levels. Therefore, BCAA administration to patients is effective in improving albumin levels. Although the mechanisms responsible for the protective effects of BCAAs against muscle cramp are uncertain, it is thought that BCAA supplementation could suppress muscle protein breakdown by increasing the free BCAA concentrations in the tissue. This hypothesis may also be applied to muscle cramps during exercise; BCAA supplementation may be useful in suppressing muscle cramps during sporting events.

**Protein and amino acid supplementation in relation to exercise to promote muscle protein synthesis**

1. **Protein requirements**

   It is known that exercise training and sufficient levels of good quality proteins are required for both athletes and untrained individuals to build strong muscles. Resistance training, such as weight training, is known to be particularly effective in building skeletal muscle. Recent studies have demonstrated that relatively mild intensity exercise training with blood flow restriction is an effective means to increase muscle mass by elevating expression of muscle proteins40). Good quality proteins have amino acid compositions that are necessary as nutrients for humans, and many animal proteins (typical examples: milk and egg proteins) are considered to be good proteins. It is generally recognized that protein requirements increase with intensive exercise, although there is no consensus. However, as intensive exercise promotes protein and amino acid breakdown during exercise and protein synthesis after exercise, it is recommended that athletes increase protein intake by 1.5 to 2-fold during intensive exercise training periods41).

2. **Timing of protein and amino acid supplementation**

   Recent research in the exercise nutrition field has examined the appropriate timing of protein or amino acid intake to promote muscle protein synthesis in relation to exercise training. These studies are herein summarized in an effort to determine the best timing of nutrient intake in relation to exercise.

   As described above, protein and amino acid breakdown is promoted during exercise. It has been confirmed that muscle protein synthesis increases during the resting recovery period after exercise42,43), and that this increased protein synthesis lasts for at least 2 days after exercise41,42). This suggests that nutrient intake after exercise should effectively increase protein synthesis. Experimenting with dogs44), the muscle protein synthesis rate was found to be more effectively elevated by intravenous administration of amino acids and glucose at the end of (right after) exercise than at 2 h post-exercise. This finding was later confirmed in human studies.

   Levenhagen et al.45) measured the rates of muscle protein synthesis and breakdown during the post-exercise period in subjects aged 30-33 years, who were studied twice, with the same oral supplement (10 g of protein, 8 g of carbohydrate, and 3 g of fat) being administered either immediately after, or at 3 h after, 60 min of moderate-intensity exercise. The results clearly showed that the net protein synthesis rate was significantly higher in the former than in the latter cases. Esmarck et al.46) demonstrated, using elderly subjects (around 74 years of age), that protein supplementation immediately after - as compared to that at 2-h after - resistance exercise was more effective in increasing muscle strength and muscle mass. Subjects participated in a 12-wk resistance training program (3 times per week) receiving oral protein in liquid form (10 g of protein, 7 g of carbohydrate, and 3 g of fat) immediately after, or at 2 h after, each training session. Based on these findings, protein supplementation immediately after exercise is recommended to build up skeletal muscle.

   The effects of amino acid supplementation (6 g essential amino acid mixture and 35 g sugar) after resistance exercise on muscle protein synthesis has also been examined, and clearly shows that supplement ingestion at either 1 h or 3 h after resistance exercise equally increases muscle protein synthesis47). On the other hand, when this amino acid supplement was ingested immediately before and after resistance exercise, and comparisons made48), results showed that increased protein synthesis was significantly higher in the former than in the latter cases, thus suggesting that amino acid supplementation immediately before resistance exercise is more effective at building skeletal muscle. The efficacy of protein supplementation before or after exercise was also examined using whey protein, and the results showed no differences in protein synthesis49). Therefore, the beneficial effects immediately before exercise are greater with amino acid supplementation than with protein supplementation. This may be due to digestion not being necessary for amino acids, resulting in more rapid absorption from the gut. In contrast to the beneficial effects of amino acid supplementation immediately before exercise, it has been reported that ingestion...
EAA, essential amino acid; and BCAA, branched-chain amino acid.

It is recommended to ingest the EAA (or BCAA) supplement right before and during resistance training and the protein supplement (or meal with enough protein) after the training in order to enhance muscle protein anabolism during the post-exercise resting period. The BCAA supplement may be effective to decrease central fatigue during the endurance training (34).

The reason for this result is unclear, but it may be due to quick absorption of amino acids into circulation from the gut, and the short peak period for plasma concentrations.

In summary, the best time point for amino acid and protein supplementation, in relation to a training program, is shown in Fig. 3. Amino acid supplementation (particularly an essential amino acid mixture) should be ingested immediately before resistance exercise. When resistance exercise lasts more than 1 h, additional amino acid supplements should be ingested during training, because the peak of plasma amino acid concentration after ingestion of the supplement appears at approximately 30 min after ingestion and then decreases gradually. BCAAs in the essential amino acid mixture may be the main components responsible for physiological functions such as stimulation of muscle protein synthesis. After resistance training, protein supplements and/or a meal containing sufficient levels of proteins should be ingested without delay.

**Treatment of elderly individuals with amino acid supplements and resistance training to ameliorate age-related sarcopenia**

Aging induces insulin resistance, which promotes age-related sarcopenia. As noted above, sarcopenia is a serious problem in many developed countries, but nutritional treatment with amino acid supplements and exercise training have been demonstrated to be effective in the treatment of sarcopenia. Resistance exercise training with blood flow restriction is also effective for improving sarcopenia. However, these studies have mainly been conducted in the USA, and evidence for such treatments in elderly Japanese is lacking.

**References**


E1023-E1030.


