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

Branched-Chain Amino Acids Activate Key Enzymes in Protein Synthesis after Physical Exercise[1–3]

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ABSTRACT BCAAs (leucine, isoleucine, and valine), particularly leucine, have anabolic effects on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation in resting human muscle. Also, during recovery from endurance exercise, BCAAs were found to have anabolic effects in human muscle. These effects are likely to be mediated through changes in signaling pathways controlling protein synthesis. This involves phosphorylation of the mammalian target of rapamycin (mTOR) and sequential activation of 70-kD S6 protein kinase (p70 S6 kinase) and the eukaryotic initiation factor 4E-binding protein 1. Activation of p70 S6 kinase, and subsequent phopsphorylation of the ribosomal protein S6, is associated with enhanced translation of specific mRNAs. When BCAAs were supplied to subjects during and after one session of quadriceps muscle resistance exercise, an increase in mTOR, p70 S6 kinase, and S6 phosphorylation was found in the recovery period after the exercise with no effect of BCAAs on Akt or glycogen synthase kinase 3 (GSK-3) phosphorylation. Exercise without BCAA intake led to a partial phosphorylation of p70 S6 kinase without activating the enzyme, a decrease in Akt phosphorylation, and no change in GSK-3. It has previously been shown that leucine infusion increases p70 S6 kinase phosphorylation in an Akt-independent manner in resting subjects; however, a relation between mTOR and p70 S6 kinase has not been reported previously. The results suggest that BCAAs activate mTOR and p70 S6 kinase in human muscle in the recovery period after exercise and that GSK-3 is not involved in the anabolic action of BCAAs on human muscle. J. Nutr. 136: 269S–273S, 2006.

KEY WORDS: • *BCAAs* • *exercise* • *protein synthesis* • *skeletal muscle*

Regular resistance exercise increases muscle mass due to a higher rate of protein synthesis in relation to protein breakdown. The synthesis of the myofibrillar protein increases and, because it constitutes ~80% of the muscle protein, the effect of resistance exercise can be measured as an increase in muscle volume or fiber cross-sectional area after about 2 mo of training (1). When protein metabolism was studied in human subjects using the stable isotope technique, it was found that protein synthesis was increased for 24 h and even up to 48 h after one training session (2–4). Protein degradation was also increased and only in combination with nutritional intake was a net gain in protein achieved (5,6).

Endurance training increases mitochondrial protein content, including the concentration and maximal activity of the oxidative enzymes, thereby leading to improved endurance and greater utilization of fat (7,8). The effect of a single bout of endurance exercise on protein synthesis and degradation is less clear. It is probably influenced by such factors as preexercise muscle glycogen content, duration and intensity of exercise, and exogenous nutritional intake during exercise (9–11). Data from animal studies suggest that protein degradation is unchanged or increased following endurance exercise and that protein synthesis remains unchanged or decreases during the actual exercise, but increases again after exercise to a level higher than before exercise (12–14). This is partly supported by results from a study in humans, which shows that muscle protein synthesis is elevated during a 4-h recovery period after 4 h of treadmill walking (15). In contrast, no change in protein synthesis was found in the deltoid muscle of female swimmers after 4,600 m of intense interval swimming (16). Different intensity and duration of exercise or the training status of the subjects may explain the discrepancies.

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Intramuscular signaling

The anabolic effect of exercise and nutrition is likely to be mediated through changes in signal transduction. The signaling networks controlling protein synthesis through translation initiation involve phosphorylation of the mammalian target of rapamycin (mTOR)[5] and sequential activation of 70-kD S6 protein kinase (p70 S6 kinase), the eukaryotic initiation factor 4E-binding protein (4E-BP1), and the eukaryotic initiation factor (eIF) 2B (17–19). p70 S6 kinase is activated by phosphorylation on several Ser/Thr residues, including Ser^{424}/Thr^{421}, which facilitates phosphorylation on Thr^{389} that is required to fully activate the kinase (20). Activation of p70 S6 kinase increases the phosphorylation of the ribosomal protein S6 and thereby enhances the translation of specific mRNAs (i.e., mRNAs that encode for ribosomal proteins). A stimulatory effect of oral administration of BCAAs, particularly leucine, on mTOR, p70 S6 kinase, and the eukaryotic initiation factors has been demonstrated in studies in rats (17). However, leucine did not stimulate the insulin pathway, including phosphoinositide 3-kinase and protein kinase B (or Akt) in rat muscle (17).

A role for Akt/mTOR/p70 S6 kinase activation in controlling muscle growth was first reported by Bodine et al. (21). The authors showed that activation of this pathway was necessary for adaptive hypertrophy and recovery after atrophy in rat muscle. In two recent studies, resistance exercise in rats or high-frequency stimulation of rat muscle in vitro were also reported to activate this pathway, supporting the view that the Akt/mTOR/p70 S6 kinase pathway is involved in contraction-induced muscle growth (22,23). Another enzyme that is regulated by Akt is glycogen synthase kinase-3 (GSK-3). Phosphorylation and deactivation of GSK-3 and subsequent activation of eIF2B can also enhance protein translation (24).

Administration of amino acids/BCAAs in humans

At rest. Studies on resting human muscle indicate that administration of BCAAs, particularly leucine, has an anabolic effect on protein metabolism either by increasing the rate of protein synthesis or by decreasing the rate of protein degradation, or both (25–27). The time course for the stimulatory effect of amino acids on protein metabolism is largely unknown; however, a relatively rapid response to increased availability of amino acids was recently reported (28). Infusion of an amino acid mixture into human subjects gave a stimulatory effect on protein synthesis 30 min after the start of the infusion and the rate of synthesis remained elevated for another 90 min (28).

Infusion of BCAAs or leucine alone in human subjects for 2 or 6 hr increased the phosphorylation of p70 S6 kinase and 4E-BP1 in skeletal muscle (29,30) without activating the Akt signaling pathway (29). Furthermore, after a 6-h infusion of an amino acid mixture, the increase in p70 S6 kinase phosphorylation was confirmed and, in addition, the authors reported that amino acid infusion did not alter GSK-3 phosphorylation in skeletal muscle (31). In contrast, Carroll et al. (32) did not detect any increase in p70 S6 kinase and 4E-BP1 phosphorylation after infusing amino acids for 3 h despite an increase in the rate of protein synthesis in two different muscle groups, the vastus lateralis and the soleus, in human subjects.

In relation to resistance exercise. Intake of an amino acid mixture or a protein hydrolysate after a bout of resistance exercise stimulates the rate of protein synthesis in human muscle and leads to a positive protein balance (i.e., the rate of protein synthesis is greater than the rate of protein breakdown) (5,6,33–35). Different theories to explain this effect have been presented. Increased availability of amino acids increases the transport of these into muscle and it was suggested that this stimulates the rate of protein synthesis in muscle (36). Another possibility is that the effect is due to a stimulatory effect of a single amino acid or a group of amino acids, for example, the BCAAs, and particularly leucine, rather than the increased availability of amino acids. It was recently reported that addition of leucine to a protein hydrolysate led to a greater stimulation of protein synthesis than the intake of the hydrolysate without leucine after a session of resistance exercise (37). Whether this effect on protein synthesis is mediated by the extracellular concentration of amino acids, for example, leucine (via a specific receptor), or to intramuscular changes in amino acid levels still remains to be answered (18,38).

One session of resistance exercise did not change the phosphorylation of mTOR and GSK-3, but a decrease in Akt phosphorylation in human muscle was noted after exercise (**Fig. 1**). Ser^{424}/Thr^{421} phosphorylation of p70 S6 kinase in muscle was increased after exercise, but no change in Thr^{389} phosphorylation was found (**Fig. 2**). Only in combination with BCAAs was an increase in Thr^{389} phosphorylation of p70 S6 kinase seen 1 and 2 h after exercise (Fig. 2), indicating that p70 S6 kinase was activated, which is also supported by the increase in S6 phosphorylation (39). A stimulatory effect on p70 S6 kinase was also found in a recent study when skimmed milk protein powder and carbohydrates were supplied to human subjects after resistance exercise; a pronounced increase in p70 S6 kinase and 4E-BP1 phosphorylation was found in the muscle 3 h after exercise (40). Intake of BCAAs also led to an increase in mTOR phosphorylation 1 h after exercise, but had no significant effect on Akt, GSK-3α (data not shown), and GSK-3β phosphorylation (Fig. 1). Studies in experimental animals have provided indirect evidence that infusion of BCAAs or leucine alone phosphorylates and activates mTOR in skeletal muscle. For example, administration of rapamycin in food-deprived rats prevented the leucine-induced increase in p70 S6 kinase and 4E-BP1 phosphorylation in skeletal muscle (41). However, an effect of BCAAs on mTOR has not been reported previously in humans. The present results suggest that BCAAs increase mTOR phosphorylation on Ser^{2448} and activate p70 S6 kinase in human muscle via an Akt-independent pathway. However, preliminary results indicate that other phosphorylation sites on mTOR may be more important for activating the enzyme; changes in the Ser^{2481} phosphorylation of mTOR was found to correlate with changes in enzyme activity (L.S. Jefferson, personal communications). The lack of effect of BCAAs on Akt and GSK-3 phosphorylation is consistent with earlier findings in human subjects infused with BCAAs or a mixture of amino acids at rest (29,31), suggesting that the Akt/GSK-3 pathway is not involved in the anabolic action of BCAAs on human muscle.

Muscle hypertrophy through increases in protein synthesis may require activation of the mitogen-activated protein kinase (MAPK)-signaling cascades. For example, passive stretching of the mouse extensor digitorum longus muscle in vitro was recently reported to cause parallel increases in phosphorylation of p70 S6 kinase on Ser^{424}/Thr^{421} and p38 MAPK. Neither of these was blocked by rapamycin, suggesting no involvement of mTOR in the phosphorylation of p70 S6 kinase on these sites (42). In the study by Karlsson et al. (39), a profound increase in Ser^{424}/Thr^{421} phosphorylation in muscle was found when BCAAs were ingested (Fig. 2). Based on the results in mouse muscle, this may suggest that BCAAs phosphorylate p70 S6

[5] Abbreviations used: 4E-BP1, eukaryotic initiation factor 4E-binding protein; eIF, eukaryotic initiation factor; GSK-3, glycogen synthase kinase 3; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; p70 S6 kinase, 70-kD S6 protein kinase.

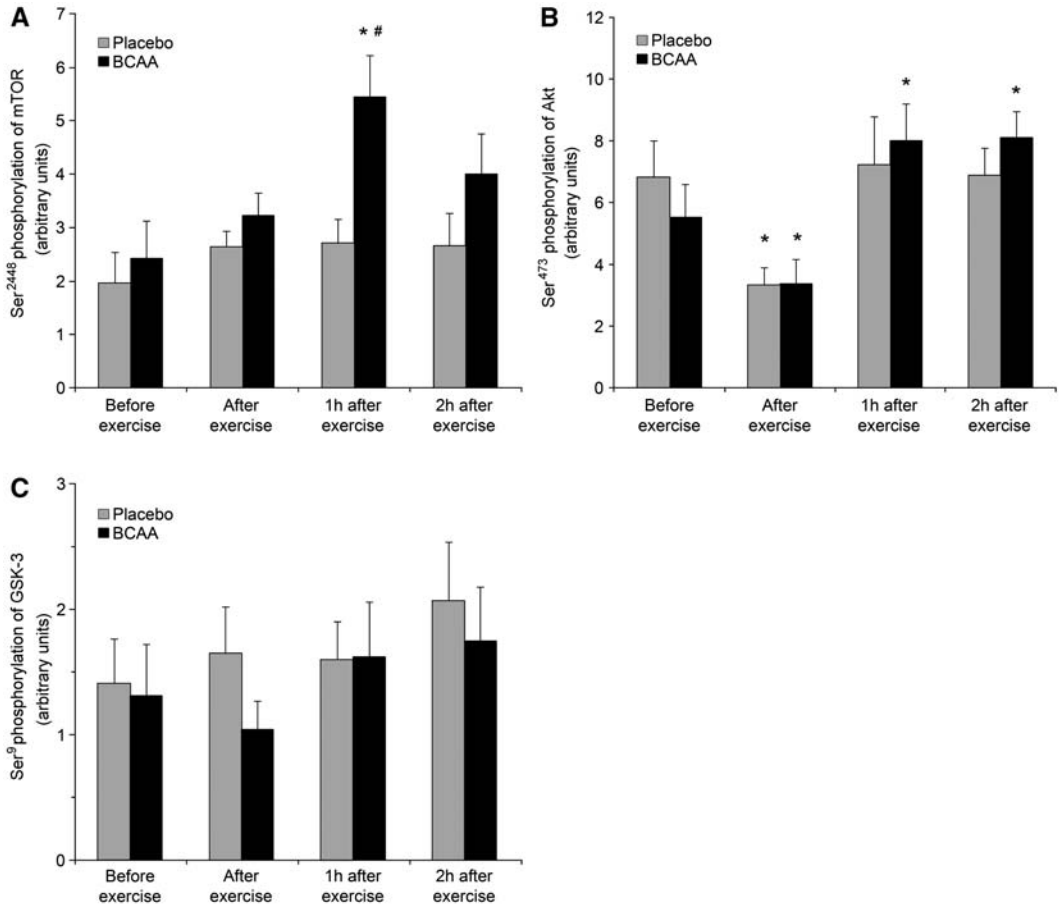

FIGURE 1 Phosphorylation of (*A*) mTOR on Ser^{2448}, (*B*) Akt on Ser^{473}, and (*C*) GSK-3β on Ser^{9} in muscle biopsy samples from vastus lateralis before, immediately after, and 1 and 2 h after resistance exercise. Phosphorylation of proteins was determined by western-blot analysis. The subjects ingested a solution of BCAAs or flavored water (placebo) during and after the exercise. A two-factorial (time, supplement) repeated-measures ANOVA was employed to analyze changes in the phosphorylation state of the kinases. When the ANOVA gave a significant main effect of time or supplement, Fisher's LSD post hoc test was used to verify where the difference occurred. Values are means ±SE of the mean for six subjects. $P < 0.05$ vs. before exercise (*); $P < 0.05$ BCAAs vs. placebo (#).

kinase on these sites without activating mTOR. There was a transient increase in p38 MAPK phosphorylation in muscle after resistance exercise, but the increase was unaffected by BCAAs, suggesting that p70 S6 kinase activation after BCAA intake is not dependent on MAPK pathway activation (39). **Figure 3** shows a proposed scheme for the activation of signaling pathways in protein synthesis by BCAAs in human muscle after resistance exercise.

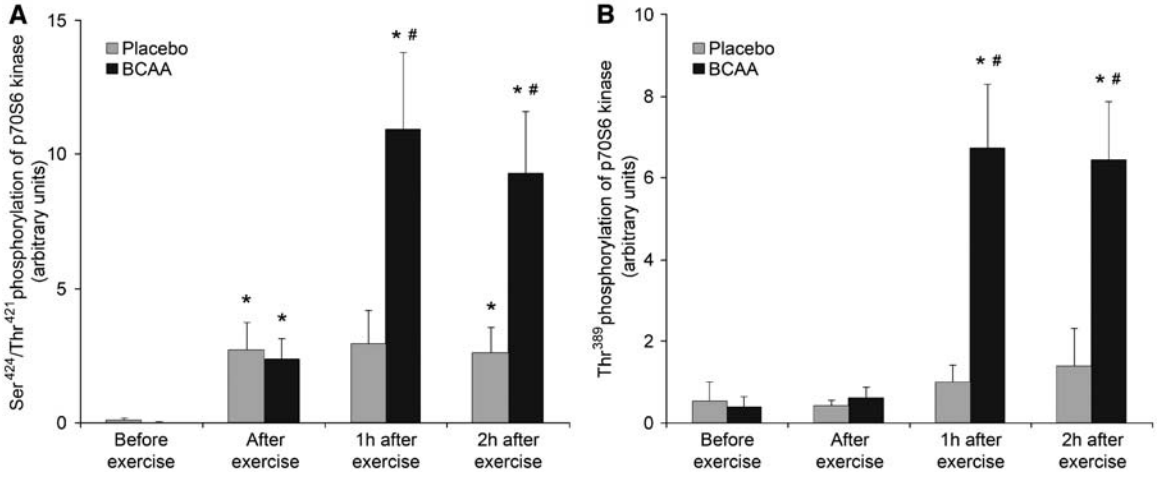

FIGURE 2 Phosphorylation of (*A*) p70 S6 kinase on Ser^{424}/Thr^{421} and (*B*) p70 S6 kinase on Thr^{389} in muscle biopsy samples from vastus lateralis before, immediately after, 1 and 2 h after resistance exercise. For explanation, see Figure 1. Values are means ±SE of the mean for seven subjects. $P < 0.05$ vs. before exercise (*); $P < 0.05$ BCAAs vs. placebo (#). Data from Ref. 39, with permission.

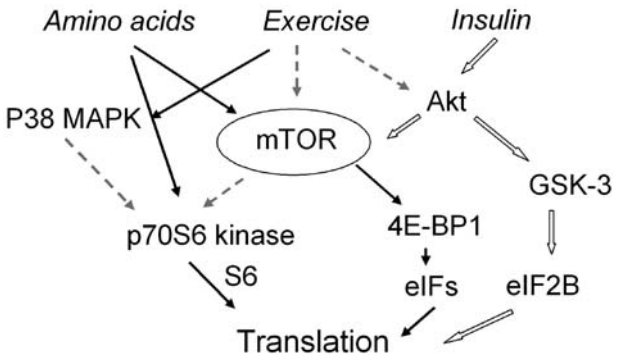

FIGURE 3 Proposed scheme for the activation of signaling pathways in protein synthesis by amino acids/BCAAs and resistance exercise in human muscle. Solid arrow, demonstrated effect; dashed arrow, possible effect; open arrow, effect of insulin.

In relation to endurance exercise. When BCAAs are ingested during endurance exercise, either running or standardized ergometer cycle exercise, the results indicate that the BCAAs have an effect in the recovery period after exercise rather than during the actual exercise (43,44). This conclusion is based on indirect measurements of protein metabolism (i.e., the release of tyrosine and phenylalanine from the muscle along with the change in the muscle concentration of these amino acids). Tyrosine and phenylalanine are neither synthesized nor degraded in skeletal muscle and changes in their release or concentration in muscle are therefore often used as markers for net protein degradation. It is a well-known fact that leucine is one factor that stimulates insulin release from the pancreas and it has been speculated that the anabolic effect of leucine is mediated through insulin. However, in the latter study, no significant increase was found in the arterial level of insulin when BCAAs were ingested, suggesting a direct effect of leucine on protein metabolism in muscle, in accordance with results in experimental animals (45). Only in one study has the effect of protein intake been investigated. Similar to the results from resistance exercise, ingestion of protein directly after endurance exercise stimulated protein synthesis in the recovery period and a positive leg protein balance was found, as measured by the stable isotope technique (46). Intake of the same protein mixture 3 h after the end of exercise did not stimulate protein synthesis (46).

There is little information in the literature on the effect of endurance exercise alone or with nutritional supply on the activation of signaling pathways controlling protein synthesis in human muscle. The results of a study conducted in our laboratory showed that standardized ergometer cycling at 75% of the maximal oxygen uptake for 1 h did not activate p70 S6 kinase (i.e., phosphorylation on Thr^{389} was unaltered up to 3 h after exercise, but phosphorylation on the positions Ser^{424}/Thr^{421} was markedly increased directly and 30 min after exercise) (47). However, the effect of nutritional supply was not investigated. Studies on rats have demonstrated that oral administration of leucine or a nutritionally complete meal after they had been running for 2 h on a treadmill restored the rate of muscle protein synthesis to the level before exercise and increased eIF4E availability for eIF4E:eIF4G complex formation (13,41,48).

In summary, acute resistance exercise increased Ser^{424}/Thr^{421} phosphorylation of p70 S6 kinase in skeletal muscle; however, phosphorylation on Thr^{389} was unaffected. Ingestion of BCAAs further enhanced the Ser^{424}/Thr^{421} phosphorylation and, in addition, increased phosphorylation of p70 S6 kinase on Thr^{389} 1 and 2 h after exercise. Phosphorylation of mTOR was also increased, but Akt and GSK-3 phosphorylation during recovery after exercise were not influenced by BCAA intake. This suggests that increased availability of BCAAs stimulates translation of specific mRNAs in muscle during recovery from resistance exercise. Corresponding data on BCAA supplementation in human subjects in relation to endurance exercise are lacking.

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