Nutraceutical Effects of Branched-Chain Amino Acids on Skeletal Muscle[1,2,3]

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ABSTRACT BCAA catabolism in skeletal muscle is regulated by the branched-chain α-keto acid dehydrogenase (BCKDH) complex, located at the second step in the BCAA catabolic pathway. The activity of the BCKDH complex is regulated by a phosphorylation/dephosphorylation cycle. Almost all of BCKDH complex in skeletal muscle under normal and resting conditions is in an inactive/phosphorylated state, which may contribute to muscle protein synthesis and muscle growth. Exercise activates the muscle BCKDH complex, resulting in enhanced BCAA catabolism. Therefore, exercise may increase the BCAA requirement. It has been reported that BCAA supplementation before exercise attenuates the breakdown of muscle proteins during exercise in humans and that leucine strongly promotes protein synthesis in skeletal muscle in humans and rats, suggesting that a BCAA supplement may attenuate muscle damage induced by exercise and promote recovery from the damage. We have examined the effects of BCAA supplementation on delayed-onset muscle soreness (DOMS) and muscle fatigue induced by squat exercise in humans. The results obtained showed that BCAA supplementation prior to squat exercise decreased DOMS and muscle fatigue occurring for a few days after exercise. These findings suggest that BCAAs may be useful for muscle recovery following exercise. J. Nutr. 136: 529S–532S, 2006.

KEY WORDS: • *BCKDH complex* • *exercise* • *branched-chain amino acids* • *muscle fatigue* • *muscle soreness*

Leucine, isoleucine, and valine possess a similar structure with a branched-chain residue and therefore are referred to as BCAAs. All are essential amino acids for animals and share a common membrane transport system and enzymes for their transamination and oxidative decarboxylation (see below) (1,2), indicating that they are closely related in their metabolic fate.

BCAAs account for 35–40% of the dietary essential amino acids in body protein and 14–18% of the total amino acids in muscle proteins (3,4). The muscle mass of humans is ~40% of the body weight; the muscle protein pool therefore represents a very large reservoir of BCAA in the body. On the other hand, animals have a free amino acid pool, which appears to be constant, and the content of free BCAAs in the human skeletal muscle is only ~0.1 g (0.6–1.2 mmol)/kg muscle (2). This pool of free BCAAs is extremely small compared with the BCAA content of muscle proteins. The total concentration of BCAA in human blood (0.3–0.4 mM) is relatively high compared with that of the other amino acids (except glutamine) (5,6). However, the amount of BCAAs in human blood is also very small compared with that in muscle proteins. Recent studies have demonstrated that free BCAAs, especially leucine, play a very important role in protein metabolism; leucine promotes protein synthesis and inhibits protein degradation via mechanisms involving the mammalian target of rapamycin (7,8). These findings suggest that leucine is not only a building block of proteins but also a modulator of protein metabolism. From this background, it is interesting to consider the efficacy of BCAAs when these amino acids are ingested as a supplement. Here, we describe regulation of the BCAA catabolism during exercise and a nutraceutical effect of these amino acids on skeletal muscle in relation to exercise.

Regulation of BCAA catabolism

All of the steps of the BCAA catabolic pathway are located in mitochondria (1). The first two steps in the pathway are common to the three BCAAs (**Fig. 1**). The first reaction,

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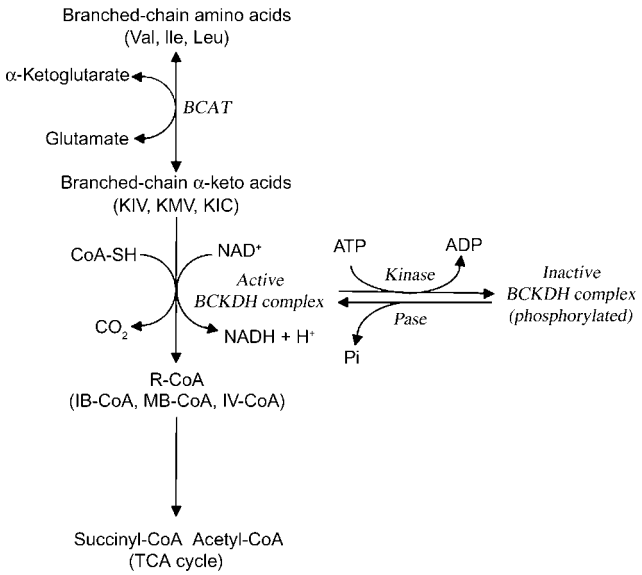

FIGURE 1 The first two steps in the BCAA catabolic pathway. KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; KIC, α-ketoisocaproate; CoA-SH, coenzyme A, reduced form; IB-CoA, isovaleryl-CoA; MB-CoA, α-methylbutyryl-CoA; IV-CoA, isovaleryl-CoA; R-CoA, acyl-CoA; Pase, phosphatase. Adapted from Shimomura et al. (9).

catalyzed by branched-chain aminotransferase (BCAT),[5] is a reversible transamination to form branched-chain α-keto acids (BCKAs). The second reaction, catalyzed by the branched-chain α-keto acid dehydrogenase (BCKDH) complex, is an irreversible oxidative decarboxylation of BCKAs. It has been suggested that the second reaction is the rate-limiting step in the overall BCAA catabolic pathway (1,10).

The BCKDH complex is regulated by covalent modification; BCKDH kinase is responsible for inactivation of the complex by phosphorylation of the E1α subunit (11,12), while BCKDH phosphatase is responsible for activation of the complex by dephosphorylation (13). Many reports indicate that the activity of the BCKDH complex is inversely correlated with kinase activity, suggesting that the kinase plays an important role in the regulation of the complex activity (14). Although BCKDH phosphatase has been purified from bovine kidney (13), little information about BCKDH phosphatase is available.

Tissue specificity of BCAA catabolism in animals

It is known that BCAAs are oxidized primarily in the skeletal muscle, while other essential amino acids are catabolized mainly in liver. Although the liver cannot directly catabolize BCAAs, it degrades BCKAs derived from the corresponding amino acids (1,10). This organ specificity for BCAA catabolism is attributed to the unique distribution of the first two enzymes (BCAT and the BCKDH complex) of the catabolic pathway in the body: extremely high and low activities of BCAT and the BCKDH complex, respectively, in skeletal muscle and the reverse situation in liver (15). This organ specificity is typical in rats (15,16).

The importance of low activity of the BCKDH complex in skeletal muscle for protein synthesis

Because leucine is a potent stimulator of protein synthesis in muscle cells (7), it is interesting to consider the effects of BCAA catabolism on muscle protein synthesis. In rat skeletal muscle, the degradation of BCAAs is tightly regulated by the BCKDH complex: the total enzyme activity in skeletal muscle of rats fed a chow diet is only ~2% that in the liver (~30 mU/g tissue for skeletal muscle and ~1500 mU/g tissue for liver) (10,16), and the activity state (percentage of active form of the enzyme complex) in skeletal muscle is only 4–6% under normal, resting conditions in rats (16), whereas the activity state of the hepatic enzyme (especially in male rats) is close to 100% (10). The relative state of inactivity of rat skeletal muscle BCKDH complex, compared to liver, may reflect the relatively higher amount of BCKDH kinase in muscle (17).

Clofibric acid, a well-known antihyperlipidemic drug, is reported to be a kinase inhibitor (18). It has been demonstrated that administration of clofibric acid to rats greatly activates the BCKDH complex in skeletal muscle and liver (19). It is known that long-term treatment of rats with the drug causes myopathy and decreased skeletal muscle protein concentration (20,21). These findings suggest that muscle protein synthesis may be inhibited by chronic activation of the BCKDH complex, thereby promoting BCAA oxidation (19). Therefore, the low activity of the BCKDH complex in the skeletal muscle under resting conditions may be important for normal growth of skeletal muscle.

Exercise enhances BCAA catabolism

Skeletal muscle is the major tissue for oxidation of BCAA, as described above. It is known that BCAA oxidation in skeletal muscle is enhanced by exercise (2). Exercise activates the BCKDH complex in human and rat skeletal muscles (16,22) and in rat liver (23) by dephosphorylation of the enzyme complex. Especially in rat skeletal muscle, almost all of BCKDH complex is in an inactive/phosphorylated state under resting conditions (16), and it appears that tight control of the complex activity by the kinase may be downregulated by exercise. We examined the mechanism responsible for activation of the complex in rat skeletal muscle by exercise using an electrically stimulated muscle contraction model (24) and found that increases in leucine and α-ketoisocaproate concentrations in the muscle may be one of the factors responsible for BCKDH activation in skeletal muscle because α-ketoisocaproate is a potent inhibitor of the kinase (18).

Effects of BCAA supplementation on muscle soreness and muscle fatigue induced by squat exercise in humans: a preliminary study

An oral BCAA supplement (77 mg/kg body weight) before exercise has been reported to increase intracellular and arterial BCAA levels during exercise, resulting in the suppression of endogenous muscle protein breakdown (25). Oral BCAA administration (12 g/d for 2 weeks and additionally 20 g each before and after the exercise test) also reportedly suppresses the rise in serum creatine kinase activity for several days after exercise (26). These findings suggest that BCAA supplementation might reduce muscle damage induced by exercise. Therefore, we conducted a preliminary human study to evaluate whether BCAA supplements might attenuate muscle soreness and muscle fatigue induced by exercise.

Young healthy female and male adults, who did not take regular exercise, were recruited, and 16 female and 14 male subjects aged 21–24 y old participated (BMI 21.6 ± 0.5 kg/m^2 for females and 22.2 ± 0.8 kg/m^2 for males). Squat exercise was used to induce delayed-onset muscle soreness (DOMS) and muscle fatigue. The composition of the test solutions used in

[5] Abbreviations used: BCAT, branched-chain aminotransferase; BCKA, branched-chain α-keto acid; BCKDH, branched-chain α-keto acid dehydrogenase; DOMS, delayed-onset muscle soreness.

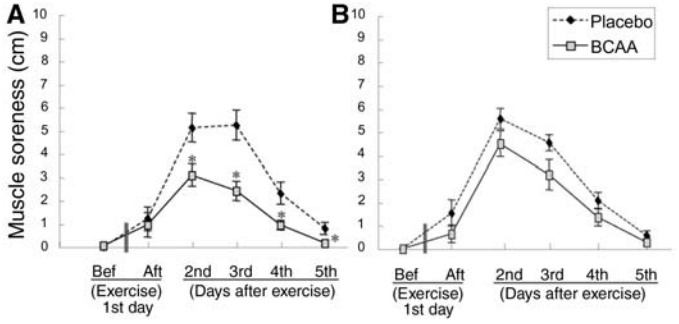

FIGURE 2 Effect of the BCAA supplement on DOMS induced by squat exercise. (*A*) females; (*B*) males. Values are means ± SEM for 16 females and 14 males. $^*P < 0.05$ to the corresponding placebo trial (Wilcoxon signed-rank test).

this study were as follows: a BCAA solution (150 mL) containing 5 g of a BCAA mixture (Ile:Leu:Val = 1:2.3:1.2), 1 g green tea powder (Instant Green Tea, Ajinomoto General Foods), and 1.2 g non-nutritive sweetener (Pal Sweet, Ajinomoto); and a placebo solution (150 mL) containing the same ingredients as the BCAA solution, but substituting 5 g dextrin (Sanwa Cornstarch) for the BCAAs. The BCAA mixture was based on an amino acid composition reported by the Food and Agricultural Organization of the World Health Organization (27). The two solutions were designed to look and taste similar; the green tea powder was used to mask the bitter taste of the BCAAs. The BCAA intake per body weight was 92 ± 2 mg/kg for females and 77 ± 3 mg/kg for males. The exercise test consisted of 7 sets of 20 squats/set (total 140 squats), with 3-min intervals between each set. During each set, squats were performed every 2 s. The experiment was conducted with a crossover design, so that each subject was tested with placebo and BCAA solutions, separated by a 12-week interval. The subjects were randomly divided into two groups, with half taking BCAA and half placebo during each trial. Subjects were blind to the test solution.

On trial days, the subjects in the fasting state reported to the laboratory at 0830 h and then ingested a jelly-type food (200 g in weight containing 100 kcal from sugar) (Otsuka Pharmaceutical) at 0900 h and, 30 min later, the BCAA or placebo solution. The squat exercise session commenced ~15 min after ingestion of the test solution. BCAA (or placebo) ingestion occurred prior to the exercise trial because it has been reported that (1) BCAA supplementation before exercise attenuated muscle protein breakdown (25), (2) postexercise muscle protein synthesis was greater when essential amino acids were consumed before exercise, rather than after (28), (3) in a separate preliminary study, we found that plasma BCAA concentrations were elevated within 15 min and peaked 30 min after ingestion when the 5 g of BCAA mixture were ingested, and (4) dietary BCAAs may affect energy metabolism during exercise (29). Muscle soreness before and after exercise and for the following 4 d (from the second through the fifth day) was evaluated while sitting using a visual-analogue scale consisting of a 10-cm line with "no pain" printed at one end and "extremely sore" at the other (30). Muscle fatigue was evaluated at the same time using a visual-analogue scale consisting of a 10-cm line with "no fatigue" printed at one end and "extreme fatigue" at the other. The subject was instructed to make a mark on the line indicating the degree of muscle soreness and muscle fatigue he/she felt. Informed, written consent was obtained from all subjects before participating in the study. The study protocol was approved by the human research review committee of the Nagoya University School of Medicine.

Muscle soreness in females was highest on the second and third days in the placebo trial, indicating that DOMS occurred following the squat exercise trials (**Fig. 2***A*). However, although DOMS also occurred after the BCAA trial, peak soreness occurred only on the second day and was significantly lower than that which occurred following the placebo trial (Fig. 2*A*). DOMS on days 3–5 in females was also significantly lower in the BCAA trial than in the placebo (Fig. 2*A*). In male subjects, DOMS peaked on the second day and tended to be lower in the BCAA than in the placebo trial throughout the test period, although the differences did not attain statistical significance (Fig. 2*B*). However, the calculated area under the curve for muscle soreness over the 5-d period was lower in the BCAA trial than in the placebo trial in both sexes (data not shown). The suppression of DOMS by BCAA supplementation appeared to be slightly less in male subjects than in female subjects. The reason for the sex difference is not clear, though it may be related to the smaller BCAA dose ingested by males because of their greater body mass: male subjects ingested 77 ± 3 mg/kg body weight, whereas females consumed 92 ± 2 mg/kg body weight. Further study is required to clarify this point.

Muscle fatigue in female and male subjects was highest right after exercise and gradually decreased during the following 4 d in both the BCAA and placebo trials (data not shown). The fatigue reported during the 4 d after the exercise trial (from the second through fifth days) in both sexes tended to be lower in the BCAA trial than in the placebo trial.

The results obtained in this preliminary study indicate that the ingestion of 5 g of BCAAs before exercise can reduce DOMS and muscle fatigue for several days after exercise. The mechanisms that underlie these BCAA effects have not yet been examined. However, one possibility is that BCAA may attenuate exercise-induced protein breakdown, while leucine may stimulate muscle protein synthesis. If the finding is substantiated, the results could support the usefulness of BCAA in muscle recovery from exercise. Further studies are required to elucidate the mechanisms responsible for the effects of BCAA supplementation.

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