Nutraceutical Effects of Branched-Chain Amino Acids on Skeletal Muscle

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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

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• muscle soreness

Leucine, isoleucine, and valine possess a similar structure with
a branched-chain residue and therefore are referred to as
BCAAs. All of the three BCAAs when these amino acids are ingested as a supplement.

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
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 mecha-
nisms 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,
Branched-chain amino acids

α-Ketoglutarate

Glutamate

BCAT

CoA-SH

CO₂

NAD⁺

ATP

ADP

Pase

Inactive BCKDH complex (phosphorylated)

Active BCKDH complex

Succinyl-CoA, Acetyl-CoA

(TCA cycle)

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

The first two steps in the BCAA catabolic pathway.

- **α-Ketoglutarate** is converted to **Glutamate** by the enzyme **BCAT**.
- **CoA-SH** is converted to **CO₂**.
- **NAD⁺** and **ATP** are used in the reaction.
- **ADP** and **Pase** (phosphatase) are involved in the inactivation of the BCKDH complex.
- **Succinyl-CoA** and **Acetyl-CoA** are the final products of the TCA cycle.

**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 ± 0.8 kg/m² for females and 22.2 ± 0.5 kg/m² for males). Squat exercise was used to induce delayed-onset muscle soreness (DOMS) and muscle fatigue. The composition of the test solutions used in 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 μg tissue for skeletal muscle and ~1500 μg 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.

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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.
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 Comstarh) 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 first and third days in the placebo trial, indicating that DOMS occurred following the squat exercise trials (Fig. 2A). 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. 2A). DOMS on days 3–5 in females was also significantly lower in the BCAA trial than in the placebo (Fig. 2A). 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. 2B). 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 may 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.

LITERATURE CITED


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).


