Exercise Promotes BCAA Catabolism: Effects of BCAA Supplementation on Skeletal Muscle during Exercise[1]

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ABSTRACT Branched-chain amino acids (BCAAs) are essential amino acids that can be oxidized in skeletal muscle. It is known that BCAA oxidation is promoted by exercise. The mechanism responsible for this phenomenon is attributed to activation of the branched-chain α-keto acid dehydrogenase (BCKDH) complex, which catalyzes the second-step reaction of the BCAA catabolic pathway and is the rate-limiting enzyme in the pathway. This enzyme complex is regulated by a phosphorylation-dephosphorylation cycle. The BCKDH kinase is responsible for inactivation of the complex by phosphorylation, and the activity of the kinase is inversely correlated with the activity state of the BCKDH complex, which suggests that the kinase is the primary regulator of the complex. We found recently that administration of ligands for peroxisome proliferator-activated receptor-α (PPARα) in rats caused activation of the hepatic BCKDH complex in association with a decrease in the kinase activity, which suggests that promotion of fatty acid oxidation upregulates the BCAA catabolism. Long-chain fatty acids are ligands for PPARα, and the fatty acid oxidation is promoted by several physiological conditions including exercise. These findings suggest that fatty acids may be one of the regulators of BCAA catabolism and that the BCAA requirement is increased by exercise. Furthermore, BCAA supplementation before and after exercise has beneficial effects for decreasing exercise-induced muscle damage and promoting muscle-protein synthesis; this suggests the possibility that BCAAs are a useful supplement in relation to exercise and sports. J. Nutr. 134: 1583S–1587S, 2004.

KEY WORDS: • *exercise* • *branched-chain amino acids* • *branched-chain α-keto acid dehydrogenase complex* • *peroxisome proliferator-activated receptor-α* • *rat*

The branched-chain amino acids (BCAAs)[3] leucine, isoleucine, and valine are among the nine essential amino acids for humans and account for ~35% of the essential amino acids in muscle proteins and ~40% of the preformed amino acids required by mammals (1). Because animal and human cells have a tightly controlled enzymatic system for BCAA degradation, BCAAs that are ingested in excess are quickly disposed of (2). Although BCAAs are absolutely required for protein synthesis, some intermediates formed in their catabolism [e.g., branched-chain α-keto acids (BCKA)] can be toxic at high concentrations (1). Therefore, the disposal of excess BCAAs is critically important for maintaining normal body conditions.

It is known that BCAAs can be oxidized in skeletal muscle, whereas other essential amino acids are catabolized mainly in liver (3). Exercise greatly increases energy expenditure and promotes oxidation of BCAAs (3). It is believed that BCAAs contribute to energy metabolism during exercise as energy sources and substrates to expand the pool of citric acid–cycle intermediates (anaplerosis) and for gluconeogenesis. In contrast, leucine is special among the BCAAs, because it promotes muscle-protein synthesis in vivo when orally administered to animals (4). As a consequence of these findings, BCAAs are receiving considerable attention as potentially helpful dietary supplements for individuals who enjoy exercise and participate in sports. We describe here what is known about the mechanism responsible for the promotion of BCAA oxidation by exercise and summarize the effects of BCAA supplementation (as a dietary supplement) in relation to exercise.

Regulation of BCAA catabolism

Enzymes regulating the BCAA catabolism. The entire catabolic pathway for BCAAs is located in mitochondria. The

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[3] Abbreviations used: BCAA, branched-chain amino acid; BCKA, branched-chain α-keto acid; BCKDH, branched-chain α-keto acid dehydrogenase; CoA, coenzyme A; HIByl-CoA, β-hydroxyisobutyryl-coenzyme A; KIC, α-ketoisocaproate; PPARα, peroxisome proliferator-activated receptor-α.

first two steps are common to all three BCAAs and have characteristic features of catabolism. The first reaction in the pathway is the reversible transamination of BCAA to produce BCKA, which is catalyzed by branched-chain aminotransferase. The second reaction is the irreversible oxidative decarboxylation of BCKA to form coenzyme A (CoA) compounds, which is catalyzed by the branched-chain α-keto acid dehydrogenase (BCKDH) complex (**Fig. 1**). The latter reaction is the rate-limiting step of BCAA catabolism and is therefore understandably subject to tight regulation (2,5).

The catabolic pathway of BCAAs has been most intensively studied in rats. Many studies have focused on regulation of the activity state of the BCKDH complex in rat liver. The BCKDH complex is regulated by a phosphorylation-dephosphorylation cycle. BCKDH kinase is responsible for inactivation of the complex by phosphorylation of the E1 component of the complex (6,7), and BCKDH phosphatase is responsible for reactivation of the complex by dephosphorylation (8). Much evidence suggests that the BCKDH kinase primarily regulates the activity state of the complex (7). The BCKDH phosphatase may also be important, but very limited information about the phosphatase is available, although purification of the phosphatase from bovine kidney has been reported (8).

Regulation of the activity state of BCKDH complex by α-ketoisocaproate derived from leucine. Many reports show that the activity of BCKDH kinase is inversely correlated with the activity state of the BCKDH complex in vivo, which suggests that the kinase may regulate BCAA catabolism (7). It was demonstrated (9) that α-ketoisocaproate (KIC; formed by transamination of leucine) is a potent inhibitor of the kinase. α-Keto-β-methylvalerate and α-ketoisovalerate, which are derived from isoleucine and valine, respectively, have an effect similar to that of KIC but with greatly reduced potency (9). Therefore, when KIC has accumulated in tissues under some physiological conditions, BCAA catabolism is promoted by activation of the BCKDH complex.

It was reported that feeding rats a leucine-rich diet decreases plasma concentrations of isoleucine and valine (10) and activates the hepatic BCKDH complex (11). This indicates that administration of leucine alone induces BCAA imbalance presumably because of inhibition of BCKDH kinase by KIC.

Effect of exercise on BCAA catabolism and its regulation

Activation of BCKDH complex by exercise. Endurance exercise increases energy expenditure and promotes protein and amino acid catabolism (3). BCAAs can be oxidized in skeletal muscles, and their oxidation is enhanced by exercise (3). It was reported that endurance exercise activates the BCKDH complex in human and rat skeletal muscles (12,13) and rat liver (14). We showed that BCKDH kinase activity in rat liver is decreased significantly by 85 min of running exercise (14), which may be a main factor contributing to activation of the hepatic BCKDH complex. Although the detailed mechanism is not known, it is unlikely that altered gene expression of the kinase can be responsible for the decrease in kinase activity caused by such a short period of exercise. In our study using an electrically stimulated muscle-contraction model, increases in leucine and KIC concentrations in the muscle are suggested to be one of the factors responsible for the muscle BCKDH activation (15). We also demonstrated that the amount of the kinase bound to the complex is reduced by exercise (16).

In addition to the acute effects of exercise as described, it was reported that exercise training (repeated exercise bouts) decreases the kinase protein in rat skeletal muscle and thereby results in greater activation of the BCKDH complex in skeletal muscle of trained rats by acute exercise (17).

It is well known that feeding a low-protein diet (or protein starvation) inactivates the BCKDH complex by phosphorylation of the enzyme in rat liver (1,2). The activity of BCKDH kinase and the amount of the kinase bound to the complex are inversely correlated with the activity state of the complex (18). Inactivation of the BCKDH complex provides a mechanism for conservation of BCAAs for protein synthesis under BCAA-deficient conditions. When the rats fed the low-protein diet performed running exercise, hepatic BCKDH complexes were significantly increased (14), which suggests that BCAA

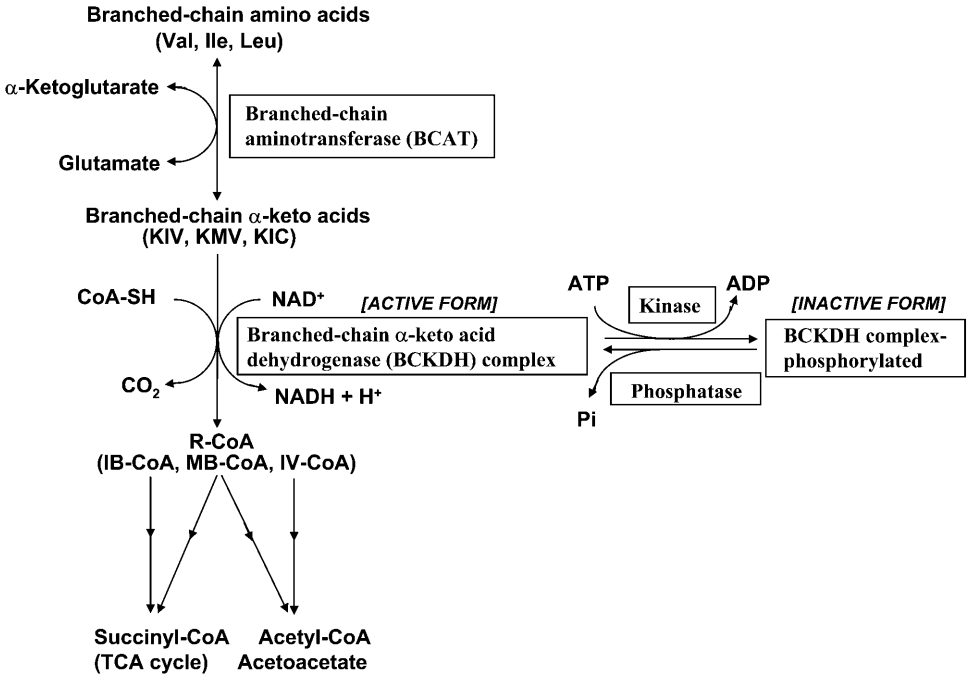

FIGURE 1 The BCAA catabolic pathway, which occurs within mitochondria, includes two primary reactions: the reversible transamination of BCAAs and the irreversible decarboxylation of BCKA to form CoA compounds. The latter reaction is the rate-limiting step of BCKA catabolism. TCA, tricarboxylic acid cycle; KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; CoA-SH, reduced form of CoA; IB-CoA, isobutyryl-CoA; MB-CoA, α-methylbutyryl-CoA; IV-CoA, isovaleryl-CoA; R-CoA, acyl-CoA.

catabolism is promoted by exercise even in rats that are deficient in BCAA.

Effects of stimulation of the fatty acid oxidation on the BCKDH complex activity. A number of physiological conditions including exercise stimulate fatty acid oxidation as well as BCAA oxidation (1,14). It is interesting to consider the relationship between the two catabolic systems. Clofibric acid is well known as a hyperlipidemic drug that stimulates fatty acid oxidation. It was reported that this compound also stimulates BCAA catabolism and causes muscle wasting upon long-term treatment of animals with the drug (19,20). Studies examining the long-term effects of clofibric acid on BCKDH kinase expression in rat liver revealed decreases in kinase activity, protein, and message in response to the treatment (21). It is believed that clofibric acid exerts many of the long-term effects by activation of peroxisome proliferator-activated receptor-α (PPARα). Starvation also stimulates fatty acid oxidation. Because starvation increases free fatty acid levels in the circulation and fatty acids are naturally occurring ligands for PPARα, the increase in free fatty acid levels caused by starvation may downregulate kinase expression in rat liver (22). Because fatty acid oxidation is also promoted by exercise, the hypothesis of activation of the BCKDH complex in association with increased fatty acid oxidation might be applicable to the mechanisms for promotion of BCAA oxidation by exercise training.

Effect of BCAA supplementation on muscle performance in sport and exercise

Effects of BCAA supplementation on muscle protein metabolism in relation to exercise. The effects of BCAA supplementation before and after exercise on muscle-protein metabolism and exercise-induced muscle damage were examined in humans. It was reported (23) that an oral supplement of BCAAs (77 mg/kg body wt) before exercise increased intracellular and arterial BCAA levels during exercise and resulted in suppression of endogenous muscle-protein breakdown. It was also reported that oral BCAA administration (12 g/d for 2 wk and an additional 20 g each before and after the exercise test) suppressed the rise in serum creatine kinase activity for several days after exercise (24). Similar effects were also observed in a study in which subjects ingested an amino acid mixture (that contained 3.6 g of amino acids with 37% BCAAs) before and after the exercise test and 2 doses/d of the amino acid mixture for 4 d after the exercise test (25). The amino acid supplement also diminished muscle soreness that usually follows exercise. Although the mechanism responsible for the protective effects of BCAA supplementation against exercise-induced muscle damage and soreness have not been elucidated, it is presumed that stimulation of protein synthesis by leucine and suppression of exercise-induced protein breakdown by BCAAs may be involved. Furthermore, the most effective ratio of the three BCAAs for the beneficial effects is not known. Clearly these interesting observations should be followed up with studies designed to elucidate the mechanisms responsible for the phenomena and to clarify the most effective composition of BCAAs.

Specific features of valine catabolism. Valine catabolism is unique compared with the other BCAAs. The potentially toxic compound methacrylyl-CoA is formed in the middle of the valine catabolic pathway (**Fig. 2**). It was suggested that methacrylyl-CoA is a particularly reactive compound with considerable potential for cytogenic and mutagenic actions, because it is a thiol-reactive molecule through nonenzymatic Michael addition reactions (26). Crotonase and β-hydroxyisobutyryl-CoA (HIByl-CoA) hydrolase activities are critically important for rapid disposal of methacrylyl-CoA in cells. Furthermore, methacrylyl-CoA is generated during valine catabolism in the mitochondrial matrix space, where it can react with glutathione and thereby potentially interfere with the mechanism that protects mitochondria against damage by reactive oxygen species. Although methacrylyl-CoA has not been established to cause damage during exercise, stimulation of BCAA catabolism during exercise results in production of additional methacrylyl-CoA that must be rapidly destroyed by the combined actions of crotonase and HIByl-CoA hydrolase to prevent irreversible mitochondrial damage.

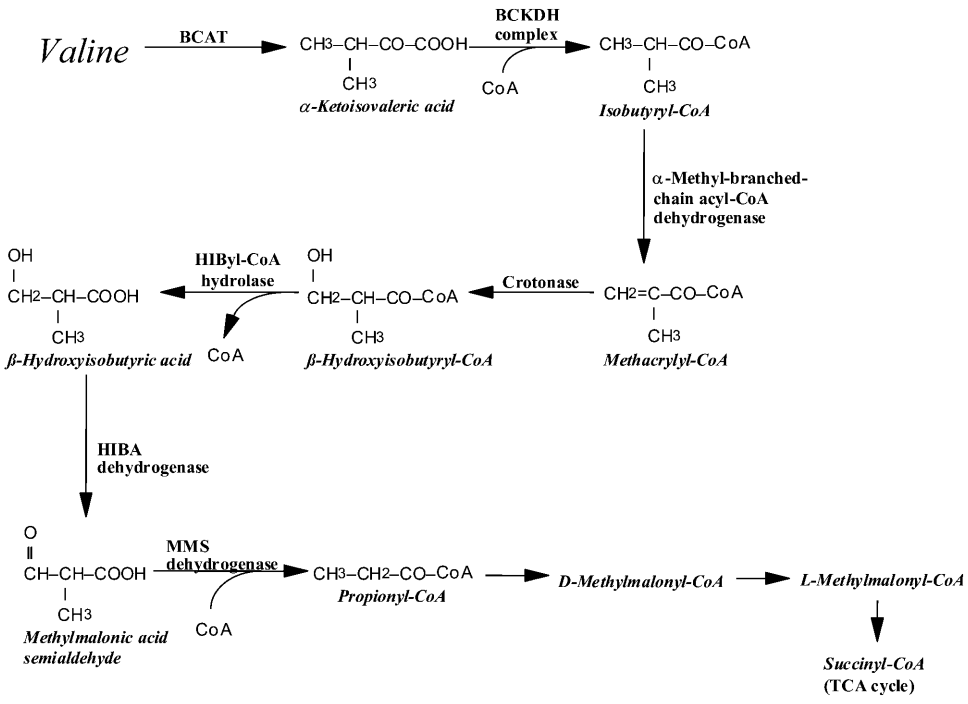

FIGURE 2 The valine catabolic pathway is unique when compared with other BCAAs in that the potentially toxic methacrylyl-CoA is formed in the middle of the pathway. Stimulation of BCAA catabolism during exercise results in production of additional methacrylyl-CoA that is rapidly destroyed by the combined actions of crotonase and HIByl-CoA hydrolase. BCAT, branched-chain aminotransferase; HIBA, β-hydroxyisobutyric acid; and MMS, methylmalonic acid semialdehyde.

We purified the HIByl-CoA hydrolase from rat livers and established a method for measurement of this enzyme activity in a coupled reaction with crotonase (26,27). The activities of both crotonase and HIByl-CoA hydrolase are extremely high compared to the activity of the BCKDH complex in the mammalian tissues examined [skeletal muscle of rat (26), dog (27), and human (28)]. Therefore, methacrylyl-CoA is rapidly degraded to the free acid and reduced form of CoA by the high activities of two enzymes. As a consequence, methacrylyl-CoA and HIByl-CoA are not detectable in liver cells even when incubated under conditions that should maximize the concentrations of valine pathway intermediates (29). These findings suggest that a supplement of valine as the BCAA mixture for sports should not be toxic for humans under normal conditions although exercise promotes valine catabolism.

Toxicity of BCAA. Acute and subacute toxicity studies of BCAAs using mice and rats (30) and a chronic toxicity study using rats (31) were reported. The BCAA composition used in these studies was a 2.1:1:1.2 leucine:isoleucine:valine ratio. No animals died from the single dose of 10 g of BCAA/kg body wt in the acute toxicity study, and the half-maximal lethal dose was estimated as >10 g/kg body wt. No toxic effects of BCAAs were observed at a dose of 2.5 $g \cdot kg^{-1} \cdot d^{-1}$ for 3 mo or 1.25 $g \cdot kg^{-1} \cdot d^{-1}$ for 1 y. There are no reports concerning BCAA toxicity in relation to exercise and sports.

Concluding remarks

It is clear that exercise promotes degradation of BCAAs. Promotion of fatty acid oxidation appears to be associated with greater rates of BCAA oxidation, which suggests that fatty acids may be regulators of BCAA oxidation. Furthermore, muscle-protein synthesis is enhanced after exercise. From these findings, it may be concluded that the BCAA requirement is increased by exercise. BCAA supplementation before and after exercise has beneficial effects for decreasing exercise-induced muscle damage and promoting muscle-protein synthesis; this suggests that BCAAs may be a useful supplement in relation to exercise and sports. Although in many human exercise studies, a dose of >5 g of BCAA was used as a supplement, the minimum dose to produce the beneficial effects of BCAA supplementation remains to be established. Furthermore, the most effective ratio of the three BCAAs is unclear. Toxicity studies of BCAAs using animals showed that BCAAs are quite safe amino acids when the three BCAAs are provided in a ratio similar to that of animal protein (e.g., a 2:1:1 leucine:isoleucine:valine ratio). Although leucine is the most potent amino acid among the BCAAs for stimulating protein synthesis, supplementation of leucine alone may cause BCAA imbalance via the activating effect of its keto acid on the BCKDH complex. A number of research groups examined whether BCAA supplementation might have a beneficial effect on endurance performance (32–36), but the results are inconsistent. Additional studies are required to clarify the appropriate amount of BCAA supplementation for beneficial effects and the responsible mechanisms.

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