Mechanisms of activation of muscle branched-chain α-keto acid dehydrogenase during exercise in man

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1. Exercise leads to activation (dephosphorylation) of the branched-chain α-keto acid dehydrogenase (BCKADH). Here we investigate the effect of low pre-exercise muscle glycogen content and of branched-chain amino acid (BCAA) ingestion on the activity of BCKADH at rest and after 90 min of one-leg knee-extensor exercise at 65% maximal one-leg power output in five subjects.

2. Pre-exercise BCAA ingestion (308 mg BCAAs (kg body wt)$^{-1}$) caused an increased muscle BCAA uptake, a higher intramuscular BCAA concentration and activation of BCKADH both at rest (9 ± 1 *versus* 25 ± 5% for the control and BCAA test, respectively) and after exercise (27 ± 4 *versus* 54 ± 7%).

3. At rest the percentage active BCKADH was not different, 6 ± 2% *versus* 5 ± 1%, in the normal and low glycogen content leg (392 ± 21 and 147 ± 34 μmol glycosyl units (g dry muscle)$^{-1}$, respectively). The post-exercise BCKADH activity was higher in the low (46 ± 2%) than in the normal glycogen content leg (26 ± 2%).

4. It is concluded that: (1) the mechanism of activation by BCAA ingestion probably involves an increase of the muscle BCAA concentration; (2) BCKADH activation caused by exercise and BCAA ingestion are additive; (3) low pre-exercise muscle glycogen content augments the exercise-induced BCKADH activation without an increase in muscle BCAA concentration; and (4) the mechanism of BCKADH activation via BCAA ingestion and low muscle glycogen content are different.

The branched-chain amino acids (BCAAs) – leucine, valine and isoleucine – are three of the nine essential amino acids in mammals. After ingestion of a protein-containing meal most of the essential amino acids are degraded by the liver. The BCAAs, however, largely escape from hepatic uptake and are primarily degraded in peripheral tissues. Skeletal muscle is able to degrade BCAAs and it has been suggested that the BCAAs are the third fuel for skeletal muscle (Shinnick & Harper, 1976; Goldberg & Chang, 1978). The first step in BCAA degradation is a reversible BCAA aminotransferase reaction in which the BCAAs are converted to their respective branched-chain α-keto acids (BCKAs) and the amino group acceptor α-ketoglutarate is converted to glutamate. BCAAs have also been suggested to play a role in fatigue mechanisms in muscle, supposedly by a carbon-draining effect of this BCAA aminotransferase reaction on the tricarboxylic acid cycle (Wagenmakers, Coakley & Edwards, 1990). The BCKAs are further degraded in an irreversible oxidative-decarboxylation reaction by the branched-chain α-keto acid dehydrogenase complex (BCKADH). This reaction is the rate-limiting step in BCAA degradation in most tissues and the activity of the BCKADH decides whether the carbon skeletons of the BCAAs remain available for protein synthesis or are broken down in the oxidative pathway.

The BCKADH is regulated by a phosphorylation–dephosphorylation cycle with dephosphorylation causing activation. At rest 2–10% of the enzyme is active in rat (Wagenmakers, Schepens & Veerkamp, 1984; Aftring, Miller & Buse, 1988) and human skeletal muscle (Wagenmakers, Brookes, Coakley, Reilly & Edwards, 1989). Prolonged exercise at 50–70% maximal power output (W_{max}) in the overnight fasted state is known to lead to a 2- to 5-fold increase of the percentage of active BCKADH in rat (Kasperek & Snider, 1987; Fujii, Shimomora, Tokuyama & Suzuki, 1994) and human skeletal muscle (Wagenmakers *et al.* 1989; Wagenmakers *et al.* 1991). The nutritional status appears to influence the activation of the BCKADH by exercise. Carbohydrate loading and carbohydrate ingestion during exercise prevented the exercise-induced activation, whereas low resting muscle glycogen without carbohydrate supplementation caused a 3·6-fold activation (Wagenmakers *et al.* 1991). Furthermore, an increased circulating leucine concentration achieved by ingestion of BCAAs activated the

BCKADH in rat muscle (Aftring, Block & Buse, 1986). BCKADH activity in rat muscle was also increased 3 h after feeding of a high amount of protein that increased BCAA concentration in both plasma and muscle (Block, Aftring, Mehard & Buse, 1987).

Here we investigated the effect of low pre-exercise muscle glycogen content on the activation of the BCKADH and whether BCAA ingestion, causing an increase in muscle BCAA uptake (MacLean, Graham & Saltin, 1996), is related to activation of BCKADH during 90 min of one-leg knee-extensor exercise in man.

METHODS

Subjects

Ten healthy male volunteers participated in the study. Five subjects were studied during one-leg knee-extensor exercise with a normal and low muscle glycogen content leg. The other five subjects were studied during exercise with and without pre-exercise BCAA ingestion. Mean age, weight, height, and one-leg W_{max} were 27 ± 3 years, 72 ± 3 kg, 1·79 ± 0·05 cm and 63 ± 4 W, respectively. All subjects were healthy and physically active and participated regularly in leisure sport. The subjects were informed about possible risks and discomfort involved in this experiment before giving their consent to participate. The study was performed according to the Declaration of Helsinki, and the study was approved by the Ethical Committee of the Karolinska Institute, Stockholm and of the Copenhagen-Fredrikberg committee.

Protocol

Subjects performed one-legged exercise in the upright position on an ergometer that permits the exercise to be confined to the quadriceps femoralis muscle group (Andersen & Saltin, 1985). Three days before the actual experiment subjects performed a graded exercise test to determine their W_{max}. The exercise protocol consisted of 90 min one-leg knee-extensor exercise at a workload of 60–65% W_{max}. After the 90 min of exercise the subjects were not completely exhausted, but experienced moderate to quite intense sensations of exertion localized in the exercised leg. The subjects that participated in the study with a normal and low glycogen content quadriceps femoralis muscle performed the two tests on one day. In the morning subjects started at random with the normal or low glycogen content leg and in the afternoon with the other leg after a resting period of 1 h (Fig. 1). In order to obtain one normal and one low glycogen content leg the subjects underwent a glycogen-depletion protocol on the evening before the actual test (van Hall, Saltin, Van der Vusse, Söderlund & Wagenmakers, 1995). The five subjects that were studied with or without BCAA ingestion performed the control test during the morning and after 1 h of rest the other leg was exercised after BCAA ingestion. The BCAAs were provided as three boluses; 45 and 20 min before and 5 min after the start of the exercise bout. The first bolus that was provided contained 154 mg (kg body wt)$^{-1}$ BCAAs and the second and third bolus contained 77 mg (kg body wt)$^{-1}$ BCAAs with a composition of 1·7 : 1·15 : 1 of L-leucine, L-valine and L-isoleucine, respectively (Fig. 1).

On the experimental day subjects reported to the laboratory in the morning after an overnight fast. The subjects were fasted throughout the day. Catheters for blood sampling were placed in the femoral artery and vein in the inguinal region of the leg to be exercised and advanced with the tip placed 5–6 cm proximal to the inguinal ligament. A thermistor was inserted through the venous catheter for blood-flow measurements by the constant-infusion thermodilution technique (Andersen & Saltin, 1985). After placement of the catheters the subjects rested for 1 h in the supine position. During the resting period between the two tests a femoral venous catheter was placed in the leg to be exercised in the afternoon. Arterial and venous blood were sampled simultaneously before exercise and after 30, 60 and 90 min of exercise. Femoral venous blood flow was measured by the thermodilution technique (Andersen & Saltin, 1985) just before the blood samples were taken. Local anaesthesia was applied to the skin (Xylocaine 0·5%) and percutaneous incisions were made before exercise for subsequent muscle biopsies at rest, just before the exercise started and exactly after 90 min of exercise for analysis of the BCKADH, amino acids and glycogen.

Plasma analysis

Blood was sampled with heparinized syringes and analysed for haemoglobin and haematocrit; the remainder was immediately centrifuged to obtain plasma. Part of the plasma was deproteinized with sulphosalicylic acid (6 mg (100 μl plasma)$^{-1}$), vortex-mixed and frozen in liquid nitrogen and analysed for amino acids by HPLC (Van Eijk, Rooyakkers & Deutz, 1993).

Muscle analysis

Muscle biopsies were obtained within 10 s of termination of the exercise bout. Fresh muscle tissue (10–30 mg) was directly weighed and transferred to 700 μl ice-cold buffer (composition (mM): 250 sucrose, 2 EDTA, 10 Tris–HCl; pH 7·4) for homogenate preparation. The remainder of the muscle tissue was immediately frozen in liquid nitrogen for amino acid and glycogen analysis. Homogenates for BCKADH measurements were prepared with a Teflon–glass Potter-Elvehjem homogenizer. The actual activity and total activity were measured as described previously (Wagenmakers *et al.* 1984) from the $^{14}CO_2$ production from [1-^{14}C]-α-ketoisocaproic acid. The incubation time in this study was 20 min and the specific activity of the α-ketoisocaproic acid was 10 000 d.p.m. nmol^{-1}.

Muscle tissue for amino acid and glycogen analysis was freeze dried and blood and connective tissue were removed. For amino acid analysis 2–5 mg dried muscle were extracted with 5% sulphosalicylic acid while being vortex-mixed vigorously and analysed by HPLC (Van Eijk *et al.* 1993). The remainder was powdered and extracted with PCA for determination of muscle glycogen determined by a fluorometric enzymatic method (Van der Vusse, Janssen, Coumans, Kuipers, Does & Ten Hoor, 1989).

Statistical analysis

All data are means ± S.E.M. Statistical analysis of the data was done using the non-parametric Wilcoxon ranked-sign test to determine differences between data obtained in the low and normal glycogen leg test and between data obtained in the test with or without BCAA supplementation. Statistical significance was set at $P < 0·05$.

RESULTS

Muscle glycogen

The glycogen-depletion protocol the evening before the normal/low muscle glycogen content test caused a considerable lowering of muscle glycogen concentration in the depleted leg; 147 ± 34 μmol glycosyl units (g dry muscle)$^{-1}$ for the low glycogen leg and 392 ± 21 μmol glycosyl units (g dry muscle)$^{-1}$ for the normal glycogen leg.

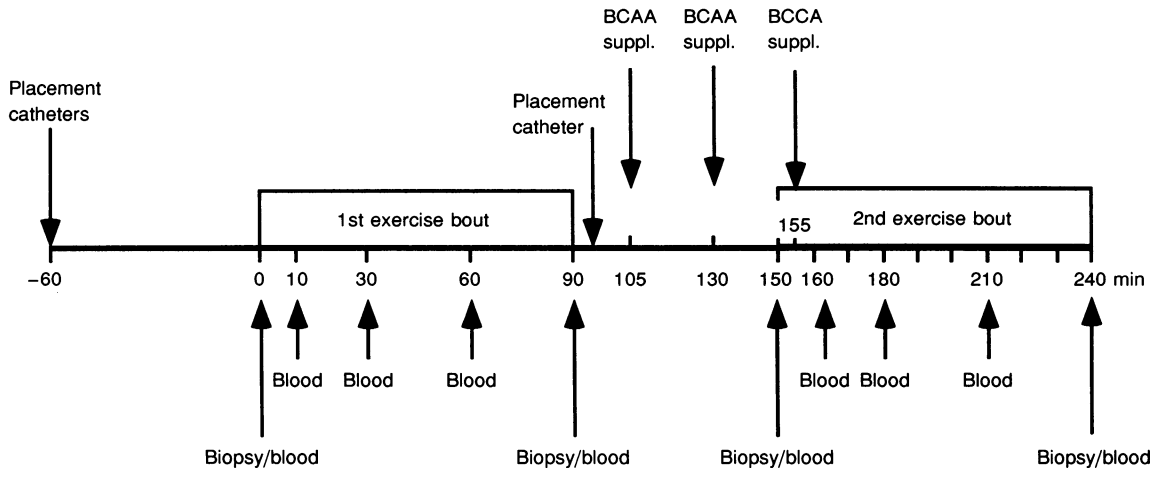

Figure 1. Flow diagram of the exercise protocols

Five subjects were studied during one-leg knee-extensor exercise with a normal and low muscle glycogen content leg. The first exercise bout was performed at random with the normal or low glycogen content leg and the second exercise bout with the other leg. Five other subjects were studied without (first exercise bout) and with (second exercise bout) BCAA ingestion.

During the 90 min of exercise muscle glycogen concentration decreased considerably in both legs. Glycogen usage during the 90 min of exercise was higher when exercise was performed with normal muscle glycogen stores. Muscle glycogen content after exercise was 160 ± 45 and 91 ± 19 μmol glycosyl units (g dry muscle)$^{-1}$ for the normal and low glycogen content leg, respectively. The difference between legs was not significant.

BCKADH activity

Pre-exercise muscle glycogen content did not affect the percentage active BCKADH at rest (Fig. 2). However, the exercise-induced activation of BCKADH was considerably higher in the glycogen-depleted leg after pre-exercise muscle glycogen lowering. The exercise-induced increase in the percentage active BCKADH was 4-fold in the normal glycogen leg, whereas exercise caused a 9-fold increase of the percentage active BCKADH in the glycogen-depleted leg (Fig. 2).

BCAA ingestion caused a nearly 3-fold increase in the resting BCKADH activity (Fig. 3). The exercise-induced increase in BCKADH activity after BCAA supplementation had nearly the same magnitude as the exercise-induced increase without BCAA supplementation implying that the effects of BCAA ingestion and exercise are additive.

The total BCKADH activity was similar in both experiments and the resting actual activity was the same except when BCAAs were ingested. In that case the actual activity increased approximately 3-fold (Table 1).

BCAA fluxes and muscle concentration

At rest and during the first hour of exercise there was no significant exchange of BCAAs across the exercising leg whether normal or glycogen depleted (Fig. 4). However, after 90 min of exercise an uptake of BCAAs was found. Significance ($P < 0{\cdot}04$) was only reached in the leg with normal muscle glycogen content. The BCAA concentration

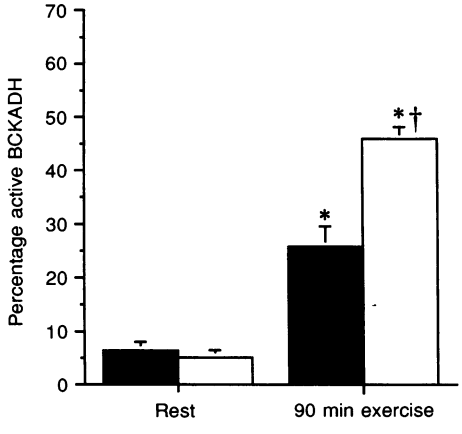

Figure 2. Effect of muscle glycogen content on the exercise-induced activation of the branched-chain α-keto acid dehydrogenase

Percentage active branched-chain α-keto acid dehydrogenase in skeletal muscle at rest and after 90 min of exercise with a normal (black columns) or low muscle glycogen content leg (open columns). * Significant differences from rest and † significant difference from normal glycogen content muscle ($P < 0{\cdot}05$).

Table 1. Total and actual activity of the skeletal muscle branched-chain α-keto acid dehydrogenase complex

	Normal glycogen		Low glycogen	
	Total activity	Actual activity	Total activity	Actual activity
Rest	4·3 ± 1·0	0·2 ± 0·03	5·6 ± 1·2	0·2 ± 0·02
90 min exercise	3·9 ± 0·8	1·0 ± 0·2*	5·2 ± 1·1	2·4 ± 0·5*†

	Control		BCAA supplementation	
	Total activity	Actual activity	Total activity	Actual activity
Rest	3·5 ± 0·9	0·4 ± 0·2	3·7 ± 1·0	1·0 ± 0·3†
90 min exercise	4·2 ± 1·2	1·2 ± 0·5*	4·1 ± 0·8	2·2 ± 0·5*†

Activities at rest and after 90 min of exercise with a normal and low muscle glycogen content and with or without pre-exercise BCAA supplementation. Values are expressed as means ± S.E.M. from 5 subjects in nmol min^{-1} (g muscle wet wt)$^{-1}$. * Significant differences ($P < 0·05$) from rest; † significant differences from normal glycogen in the glycogen effect test or from control in the BCAA supplementation test.

in muscle was not different between the legs at rest and after 90 min of exercise and did not change from the resting value during exercise (Fig. 4).

BCAA ingestion prior to exercise caused a substantial uptake of BCAAs in the muscle at rest and after 30 and 60 min of exercise (Fig. 5). No significant exchange of BCAAs was observed after 90 min of exercise and in the control test without BCAA ingestion. BCAA ingestion caused an increase in the BCAA concentration in the muscle both at rest and after 90 min of exercise in comparison to the control test (Fig. 5).

DISCUSSION

This study clearly demonstrates that the exercise-induced activation of the BCKADH is larger in a muscle with a low glycogen content than in a muscle with a normal glycogen content, regardless of whether the glycogen-depleted leg was exercised in the first or second exercise bout. Muscle glycogen was lower during exercise with the low glycogen content leg, albeit not significantly after 90 min. Previously, Wagenmakers *et al.* (1991) observed an inverse relationship between the post-exercise muscle glycogen content and the activity of the BCKADH during prolonged two-legged cycle exercise. Furthermore, a rapid activation of the BCKADH (from 17% at rest to 53% at exhaustion) was also observed in a patient with McArdle's disease which is a condition related to a glycogen breakdown defect in muscle (Wagenmakers *et al.* 1990). All these studies seem to indicate that the BCKADH is activated more during exercise when the glycogen availability or rate of glycolysis in muscle is lower.

ADP and ATP have been implicated in the activation of the BCKADH during exercise. ADP is an inhibitor of the kinase of the BCKADH (Lau, Fatania & Randle, 1982) and a rise in its concentration due to a decrease in the muscle energy state could, therefore, activate the BCKADH. A direct effect of ATP on BCKADH activity was proposed in rat muscle during exercise, as a negative correlation was found between BCKADH activity and the muscle ATP concentration (Kasperek, 1989). However, the exercise protocol as used in the present study does not lead to a change of the muscle ATP and ADP concentration compared with the resting value (van Hall *et al.* 1995) while the BCKADH is substantially activated. Furthermore, no difference was observed in the muscle ATP and ADP concentration of the normal and low glycogen leg, while more activation occurred in the low glycogen leg. Others have also observed activation of BCKADH during exercise without changes in muscle ADP and ATP concentration (Shimomura, Suzuki, Saitoh, Tasaki, Harris & Suzuki, 1990; Wagenmakers *et al.* 1991; Shimomura *et al.* 1993).

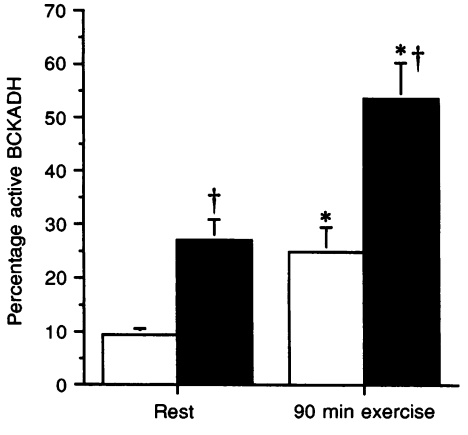

Figure 3. Effect of BCAA ingestion on the exercise-induced activation of muscle branched-chain α-keto acid dehydrogenase

Percentage active branched-chain α-keto acid dehydrogenase in skeletal muscle at rest and after 90 min of exercise without (control, open columns) and with oral pre-exercise BCAA ingestion (BCAA supplementation, black columns). * Significant differences from rest; † significant differences from normal glycogen content muscle ($P < 0·05$).

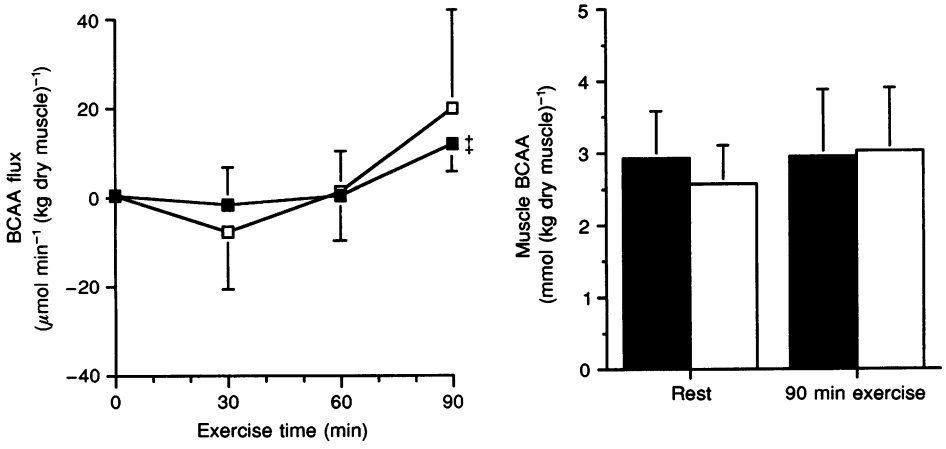

Figure 4. Effect of muscle glycogen content on leg exchange and muscle BCAA content

BCAA flux over the active leg and muscle BCAA content at rest and after 90 min of exercise with a normal (■ and black columns) and low glycogen content leg (□ and open columns). ‡ Significant uptake of the BCAAs by the muscle ($P < 0.05$).

In these studies only total tissue ATP and ADP were measured. However, it is possible that the free (not bound to myosin) cytosolic or more likely the mitochondrial ADP pool is involved in activation of the BCKADH kinase. In the present study, such changes may have occurred and may have been larger in the glycogen-depleted leg. An excessive rise in free-ADP concentration was also estimated to occur during exercise in patients with McArdle's disease, a condition in which glycogen cannot be broken down and which also leads to rapid activation of the BCKADH (Radda, 1986). Only small changes in free ADP and AMP are required for the control of mitochondrial respiration (Dudley, Tullson & Terjung, 1987), this could also apply for activation of the BCKADH. A major problem in investigating the role of the cytosolic or mitochondrial free-ADP concentration in the regulation of metabolism is that methods which directly estimate these are lacking at present.

Suggestions have also been made that an increase of the BCKA concentration in rat muscle during exercise leads to activation of BCKADH (Harris, Paxton, Powell, Goodwin, Kuntz & Han, 1986). The BCKA have an inhibitory effect on the BCKADH kinase (Lau, Fatania & Randle, 1982; Harris *et al.* 1986). Kasperek (1989) did not find increases in muscle BCAA and BCKA concentrations during exercise and suggested that the BCKAs are not the primary physiological regulators for activation of the BCKADH during exercise. Here no changes occur in the muscle BCAA concentration during exercise under control conditions. As a large (>60%) fall occurs in the muscle glutamate concentration during exercise (van Hall *et al.* 1995) the

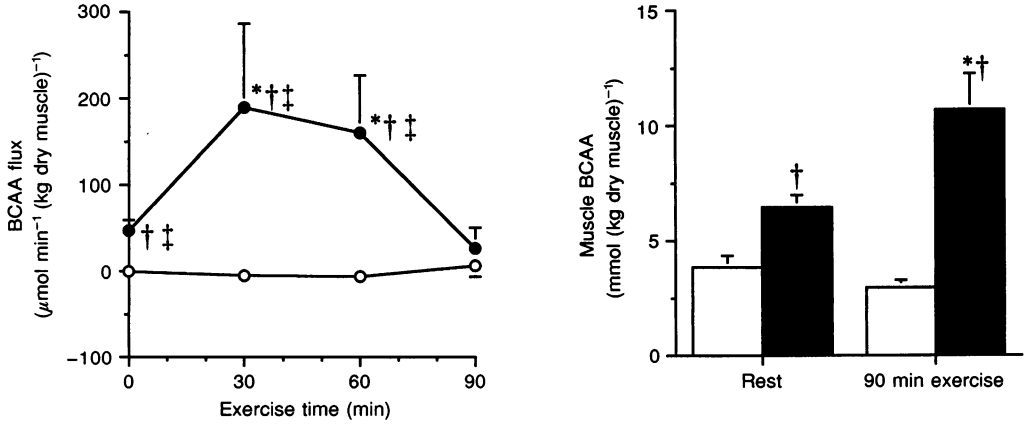

Figure 5. Effect of BCAA ingestion on leg exchange and muscle BCAA content

BCAA flux over the active leg and muscle BCAA content at rest and after 90 min of exercise without (control; ○ and open columns) or with pre-exercise BCAA ingestion (BCAA suppl.; ● and black columns). BCAA fluxes for 3 out of 5 subjects have been published before (MacLean *et al.* 1995). * Significant differences from rest; † significant differences from control experiment; ‡ significant uptake of BCAAs by the muscle ($P < 0.05$).

expectation is, however, that the BCAA aminotransferase reaction will shift in the direction of BCKA formation during exercise. This shift is potentially larger in the low glycogen leg (leading to more BCKADH activation) as the fall in muscle glutamate was larger in the low than in the normal glycogen muscle. Increases in the BCKA concentration have been reported during high intensity exercise in human muscle (Fielding, Evans, Hughes, Moldawer & Bistrain, 1986).

A new finding of this study is that BCAA ingestion leads to activation of the BCKADH under resting conditions and to a further increase of the BCKADH activity during exercise. The activation of BCKADH by BCAA supplementation and by exercise are additive effects; however, BCAA supplementation did not potentiate the exercise-induced stimulation of BCKADH activation. As the BCAA concentrations in muscle increase following BCAA ingestion, this will probably lead to higher BCKA concentrations due to a further shift of the BCAA aminotransferase reaction in the direction of BCKA formation. Activation of the BCKADH will then occur via inhibition of the BCKADH kinase. Aftring *et al.* (1986) observed that the intravenous administration of leucine to rats increased the circulating leucine concentration and rapidly increased the BCKADH activity at rest. Ingestion of a meal with a high protein content also led to a parallel increase of the plasma and muscle BCAA concentrations and of the muscle BCKADH activity for several hours following meal ingestion (Block *et al.* 1987). All these effects have been suggested to be mediated via increases of the muscle BCKA concentration and the inhibitor effect on the BCKADH kinase.

In conclusion this study clearly shows that the activation of the BCKADH is larger during exercise in muscle with a low, as opposed to a normal, glycogen content leg. It is also shown that oral ingestion of BCAAs leads to activation of the BCKADH and that the effects of exercise and BCAA ingestion are additive. The mechanism of activation by BCAA ingestion probably involves inhibition of the BCKADH kinase as a consequence of an increase of the BCAA and BCKA concentrations in muscle. The mechanism of the activation during exercise remains unclear. On the basis of the existing literature it is suggested that increases in the cytosolic or mitochondrial BCKAs and/or ADP concentration may both play a role.

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