Modification by Exercise Training of Activity and Enzyme Expression of Hepatic Branched-Chain α-Ketoacid Dehydrogenase Complex in Streptozotocin-Induced Diabetic Rats

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Summary The branched-chain α-ketoacid dehydrogenase (BCKDH) complex is the rate-limiting enzyme in the catabolism of branched-chain amino acids. In the present study, we examined the effects of exercise training on the activity and enzyme expression of the hepatic BCKDH complex in diabetic rats. The rats were prepared by intravenous injections of streptozotocin (50 mg/kg BW), and exercise training was accomplished by treadmill running for 45 min/d for 4 wk. The total and actual activities of hepatic BCKDH complex were significantly increased to ~160% by 4 wk of diabetes. On the other hand, diabetic rats in the trained group had the same level of activities as those in the normal rats, indicating that exercise training inhibited the diabetes-induced increase in the enzyme activities. The activity state (% active form) of the enzyme complex was about 100% in all groups and was not affected by diabetes or training. The protein amounts of the enzyme subunits (E1α and E2) and the abundance of mRNA for the E2 subunit, but not for the other subunits, in the liver had the same trend as the activities. These results suggest that the capacity for branched-chain amino acid catabolism in streptozotocin-induced diabetic rats is reduced by exercise training and that this modification is associated with the suppression of diabetes-induced BCKDH complex expression in the liver.

Key Words treadmill running, insulin-deficient diabetes mellitus, branched-chain amino acids

In the catabolism of branched-chain amino acids (BCAA), the initial step is reversible transamination catalyzed by branched-chain aminotransferase, and the second is irreversible oxidative decarboxylation of the branched-chain α-ketoacids (BCKA) catalyzed by the branched-chain α-ketoacid dehydrogenase (BCKDH) complex. The latter is the committed step in the oxidation of the BCAA (1, 2).

The BCKDH complex is an intramitochondrial multienzyme complex composed of E1 (3-methyl-2-oxobutanate dehydrogenase (lipoamide): EC 1.2.4.4), E2 (dihydrolipoamide acyltransferase: no EC number), and E3 (dihydrolipoamide dehydrogenase: EC 1.8.1.4) components. This enzyme complex is subject to covalent modification (3); BCKDH kinase (EC 2.7.1.115) (4) inactivates the complex by phosphorylation of the E1 component, and BCKDH phosphatase (EC 3.1.3.52) reactivates it by dephosphorylation.

The regulation of BCAA catabolism has been intensively studied in rats. Skeletal muscles have a markedly higher activity of the aminotransferase and a lower activity of BCKDH complex, and rat liver has the reverse situation with respect to enzyme activities (3, 5–7). From these findings, the concept of an interorgan relationship in BCAA and BCKA metabolism has been proposed (8). It has been suggested that hepatic BCKDH complex functions as a sink for the disposal of extra circulating BCKA derived from the transamination of BCAA in peripheral tissues (9).

The diabetic state is characterized by an elevation of plasma levels of glucose as well as BCAA in rats. The elevation of BCAA is attributed to the increased proteolytic state of body tissues and hyperphagia in the diabetic animals (10, 11). It has been reported that diabetes increased the activity of BCKDH complex in rat liver (12–14).

Exercise training has dramatic effects on energy metabolism (15, 16). Although such effects on BCAA catabolism in normal rats have been studied in some detail (5, 7, 17), BCAA catabolism in diabetic rats has been largely ignored. In the present study, we hypothesized that exercise training would improve the BCAA metabolism when training ameliorates diabetic conditions. Since the rate-limiting step of BCAA catabolism is catalyzed by BCKDH complex as described above, we ex-
amined in the present study whether exercise training would have effects on the activities and protein contents of BCKDH complex and mRNA levels for the enzyme components in streptozotocin (STZ)-induced diabetic rat liver.

**MATERIALS AND METHODS**

**Animal care and experimental design.** Male Sprague-Dawley rats aged 7 wk (body weight 220–240 g) were obtained from CLEA Japan (Tokyo, Japan). The rats were fed a laboratory chow (CE-2, CLEA Japan) and tap water ad libitum and housed in a room with controlled temperature (24±1°C) and light (12-h cycles: lighting from 05:00 to 17:00) conditions. All procedures involving animals were approved by the experimental animal care committee of Nagoya Institute of Technology.

To examine the acute (short period) effects of STZ-induced diabetes on hepatic BCKDH complex activities and serum BCAA and BCKA concentrations, rats at the early stage of STZ-induced diabetes and normal (control) rats were prepared. The rats were randomly divided into control (n=6) and diabetic (n=6) groups. Diabetes was induced by an intravenous injection of STZ (50 mg/kg body weight) to rats under the fed condition. STZ was dissolved in a 10 mM citrate buffer (pH 4.5) just prior to use. The control rats were injected with the citrate buffer without STZ in the same manner. Four days after treatment with or without STZ, livers were collected under pentobarbital anesthesia from 17:00 to 18:00 in a day, immediately freeze-clamped in liquid nitrogen, and stored at −80°C until use. Blood was collected from the heart by using a syringe for the preparation of serum, which was stored at −80°C until analyses.

To investigate the effects of exercise training on the enzyme activities and expression and the concentrations of serum components in long-term diabetic rats, the animals were randomly divided into control (normal) (n=12) and diabetic (n=12) groups. Each group of rats was subdivided into sedentary and exercise-trained groups. Diabetic rats were prepared, and normal rats were treated as described above. Blood was obtained from the tail vein 4 d after injection to analyze the glucose concentration. When the induction of diabetes was confirmed, the rats were trained on a motor-driven treadmill (KN-73, Natsume-Seisakusho, Tokyo, Japan) for 45 min/d, 6 d/wk for 4 wk. The intensity of exercise was gradually increased during the first week of the program from a speed of 15 to 25 m/min (up a 6° incline). The rats were run 25 m/min for the following 3 wk. The trained rats were sacrificed 48 h after the final exercise bout to minimize the effect of acute exercise. On the final day of the experiment, livers and blood were collected and treated as described above.

**Analytical methods.** The concentration of serum glucose was determined by the colorimetric method, using a commercial kit (Wako Chemical Industries, Osaka, Japan). The concentration of serum insulin was determined by radioimmunoassay (18). The concentration of the sum of the serum BCAA (leucine, isoleucine, and valine) was determined by the enzymatic fluorimetric method of Gleson and Maughan (19). The concentration of the sum of the serum BCKA (α-ketoisocaproate, α-keto-β-methylvalerate, and α-ketosovalerate) was determined by the method of Goodwin et al. (20) with minor modifications: 1/20 volume of serum samples described previously (20) was used and NADH production was measured by the fluorimetric method (19). The activity of liver citrate synthase was measured by the method of Srere (21).

**Extraction and assay of BCKDH complex and BCKDH kinase.** An extraction of the BCKDH complex from rat liver was performed as described previously (7). The activities of the BCKDH complex in the active form (actual activity) and in the totally dephosphorylated form (total activity) were determined spectrophotometrically by measuring the rate of NADH production as described previously (22). The total enzyme activity was obtained by measuring the enzyme activity after complete dephosphorylation of the enzyme complex, using a broad specificity protein phosphatase (7). The activity state of the complex was calculated as a percentage by multiplying the ratio of the actual activity to the total activity by 100.

The assay of the hepatic BCKDH kinase activity was performed by measuring ATP-dependent inactivation of BCKDH as described previously (4). Kinase activity is expressed as the first order rate constant of BCKDH inactivation.

**Western blotting analysis.** Western blotting was performed as described previously (23, 24). 125I-labeled protein A was used as the secondary antibody. Radioactivities on blotted membranes were determined by using a BAS1000 Bio Imaging Analyzer (Fuji Film, Tokyo, Japan).

**Northern blotting analysis.** The total cellular RNA was isolated from liver by using a reagent of ISOGEN (Wako Chemical Industries) as described in the manufacturer’s instructions. The concentrations of RNA were spectrophotometrically determined (23). Northern blotting was carried out by the use of a standard protocol (25, 26), using 10 μg of total RNA for the E1α and E2 subunits and 30 μg for the E1β subunit. cDNAs for E1α, E1β, and E2 subunits of rat BCKDH complex and β-actin were used as probes for the hybridization (27). Radioactivity associated with the bands was determined quantitatively with the BAS1000 Bio Imaging Analyzer.

**Statistics.** Data are expressed as means±SE. For the acute (short period) effects of diabetes, unpaired Student’s t-tests were used to determine significant difference (28). All other results were analyzed by a two-way analysis of variance (ANOVA) (diabetes×exercise). Significant differences between groups were further established by the Fisher’s PLSD test (28). Differences with p<0.05 were considered significant.
RESULTS

Acute (short period) effects of diabetes on serum glucose, BCAA, and BCKA concentrations and hepatic BCKDH complex activity

Four days of STZ-induced diabetes markedly increased serum glucose and BCAA concentrations and had only a minor effect on serum BCKA concentrations (Table 1). The total and actual activities of hepatic enzyme complex were slightly increased by the short period of diabetes, though the significant difference was observed only in the actual activity. On the other hand, the activity state was little affected by diabetes because the complex was almost fully dephosphorylated/activated under normal conditions (Table 1).

Effects of exercise training and diabetes on rat body weights

The body weights of the normal rats was 426±9 g for the sedentary group and 393±4 g for the trained group, and those of the diabetic rats were 350±14 g for the sedentary group and 350±7 g for the trained group. Exercise training had no significant effect on body weight gain in normal and diabetic rats, but diabetes suppressed the body weight gain in sedentary and trained groups, resulting in body weights in the sedentary and trained groups being significantly less in diabetic rats than in normal rats.

Effects of exercise training on serum glucose, BCAA, and BCKA concentrations in long-period diabetic rats

After 4 wk of the experimental period, the concentration of serum glucose in sedentary diabetic rats was as high as 37.0±1.1 mm (Table 2). This value appeared to be higher than that of short-period diabetic rats, suggesting a progression of the diabetic condition over the 4 wk period. On the other hand, the concentration of serum glucose in trained diabetic rats was significantly lower than that of sedentary diabetic rats (Table 2), indicating that exercise training suppressed to some extent the progression of diabetic conditions. Training had no effect on the serum glucose concentration in control rats (Table 2). The serum insulin concentration was markedly lowered by diabetes, but it was not significantly affected by training in either control or diabetic rats (Table 2). The serum free fatty acid (FFA) concentrations were significantly increased by diabetes and tended to be decreased by training in diabetic rats (p=0.06) (Table 2). The serum BCAA concentrations in sedentary-diabetic rats were up to twofold higher than that of control rats and were at the same level as those of the short-period diabetic rats. Exercise training significantly decreased the BCAA concentrations of diabetic rats and had a minor effect on the concentration in control rats (Table 2). The concentrations of serum BCKA in sedentary groups of rats were at the same level between control and diabetic rats and were significantly decreased by training in both control and diabetic rats (Table 2).

Effects of exercise training on the activities of hepatic BCKDH complex and citrate synthase in diabetic rats

The actual and total activities of hepatic BCKDH complex in sedentary rats were significantly increased to ~160% by the long period of diabetes (Table 3). On the other hand, the activities of trained diabetic rats were at the same level as those of control rats (Table 3), indicating suppression of the diabetes-induced increase in BCKDH activities by exercise training. The enzyme activities in control rats were not affected by training (Table 3). The activity state was not affected by either diabetes or exercise training, though the BCKDH kinase activity was decreased by diabetes (Table 3).

The activity of citrate synthase, the marker enzyme for mitochondria, was affected neither by diabetes nor by training (Table 3).

Effects of exercise training on the protein contents of hepatic BCKDH complex subunits in diabetic rats

E1α and E2 subunits of BCKDH complex in liver extracts were detected in the Western blot analyses, using
Table 3. Effects of exercise training on the activities of hepatic BCKDH complex, BCKDH kinase, and citrate synthase in diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Diabetic</th>
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<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Trained</td>
<td>Sedentary</td>
<td>Trained</td>
</tr>
<tr>
<td>BCKDH complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total activity, mU/g wet tissue</td>
<td>1.240±9.6a</td>
<td>1.228±42a</td>
<td>1.934±107b</td>
<td>1.301±184a</td>
</tr>
<tr>
<td>Actual activity, mU/g wet tissue</td>
<td>1.230±72a</td>
<td>1.190±43b</td>
<td>1.958±117b</td>
<td>1.330±218b</td>
</tr>
<tr>
<td>Activity state, %</td>
<td>100±3</td>
<td>97±1</td>
<td>102±2</td>
<td>101±5</td>
</tr>
<tr>
<td>BCKDH kinase, min⁻¹</td>
<td>0.11±0.01a</td>
<td>0.10±0.01a</td>
<td>0.08±0.01b</td>
<td>0.07±0.01b</td>
</tr>
<tr>
<td>Citrate synthase, U/g wet tissue</td>
<td>10.6±0.6</td>
<td>10.5±0.4</td>
<td>11.8±0.4</td>
<td>10.6±0.7</td>
</tr>
</tbody>
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Values are means±SE for 6 rats. Values designated by different superscript letters are significantly different (p<0.05).

Fig. 1. Effects of exercise training on the protein contents of hepatic BCKDH complex subunits in diabetic rats. The expression of protein contents was measured by Western blot analysis, and typical autoradiograms are shown in the upper panel. Values are means±SE for 6 rats. Values designated by different superscript letters are significantly different (p<0.05).

Fig. 2. Effects of exercise training on the abundance of mRNAs for subunits of hepatic BCKDH complex in the diabetic rats. Total RNA (10 µg for E1α and E2 and 30 µg for E1β subunits) extracted from liver was analyzed by Northern blot analysis. Typical autoradiograms are shown in the upper panel. The mRNA level of each sample was normalized to the abundance of β-actin mRNA. Values are means±SE for 6 rats. Values designated by different superscript letters are significantly different (p<0.05). The abundance of β-actin mRNA was not significantly different among groups.

Effects of exercise training on the abundance of mRNAs for subunits of hepatic BCKDH complex in the diabetic rats

The abundance of mRNAs for E1α, E1β, and E2 subunits of hepatic BCKDH complex were determined by regular Northern blot analysis. Although an abundance of mRNA for E1α or E1β subunits was unaffected by diabetes or exercise training, diabetes significantly increased the abundance of mRNA for E2 subunit to ~135% in the sedentary group of rats (Fig. 2). The abundance of E2 mRNA in trained-diabetic rats, however, was at the same level as that of control rats (Fig. 2). The abundance of mRNA for β-actin was unaffected.
by diabetes or by training (data not shown).

**DISCUSSION**

STZ-induced diabetes markedly increased the concentrations of serum glucose and BCAA following only 4 d of STZ treatment, as reported previously (11). After 4 wk of diabetes, serum glucose concentration was further increased in the sedentary group of rats, suggesting a progression of the diabetic conditions during 4 wk. On the other hand, exercise training suppressed the further increase in glucose concentration that was observed in the sedentary group, indicating that exercise training to some extent ameliorated diabetic conditions. These results were also consistent with the findings reported previously (29, 30).

The serum BCAA concentrations in sedentary-diabetic rats were markedly increased by the short term of diabetes, but were not further increased by the long term of diabetes. Exercise training significantly decreased the BCAA concentrations in diabetic rats, resulting in the concentration not being significantly different between sedentary-control rats and trained diabetic rats. A similar trend was observed in the total and actual activities of hepatic BCKDH complex; the long term of diabetes markedly increased the enzyme activities in sedentary rats, and exercise training suppressed the diabetes-induced increase in the enzyme activities. The Western blot analyses showed that the alterations in protein amounts of the enzyme subunits were responsible for the changes in enzyme activities caused by diabetes and exercise training. However, the abundance of mRNAs for the subunits did not correspond to the protein amounts of subunits, suggesting that posttranscriptional regulatory mechanisms most likely determine the amounts of the BCKDH complex subunits. It has been reported that diabetes increases the activity of hepatic BCKDH complex (12, 13) and the content of the enzyme subunits (14) in rats. This is the first report that exercise training improves the serum BCAA concentrations and the activities of hepatic BCKDH complex and amounts of the enzyme subunits in diabetic rats.

BCKDH kinase is responsible for inactivation of the BCKDH complex by phosphorylation. In the present study, because the complex was almost fully activated in control and diabetic rats, the kinase played no significant role in the regulation of the BCKDH complex activity. Nevertheless, diabetes significantly decreased the hepatic kinase activity in sedentary rats, and this result is consistent with the previous report (14).

It has been reported that experimental diabetes significantly increases the serum BCKA concentrations in rats (31). In the present study, the rats were killed in the evening (postabsorptive period), whereas the animals were killed in the morning (postprandial period) in the other study (31). The concentrations of BCKA are considered to be an important factor for the regulation of BCKDH activity, because α-ketoisocaproate is a potent inhibitor of BCKDH kinase. However, BCKA concentrations were not very likely to have been the regulator of the enzyme complex activity in the present study, because BCKDH kinase can be ruled out from the regulators of BCKDH activities in this study. Furthermore, exercise training decreased the BCKA concentrations in all control and diabetic rats.

In conclusion, we clearly showed here that exercise training attenuated the diabetes-induced increases in serum BCAA concentrations and activities of hepatic BCKDH complex in rats, suggesting that exercise training ameliorates BCAA catabolism promoted by STZ-induced diabetes. Since skeletal muscles use BCAA as energy sources during exercise (5, 7), an exercise-induced increase in BCAA oxidation might be responsible for the amelioration of BCAA catabolism. Further study is required to elucidate the detailed mechanisms responsible for this training effect.

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