Hepatic Branched-Chain α -Keto Acid Dehydrogenase Complex in Female Rats: Activation by Exercise and Starvation

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(Received January 14, 1999)

Summary The effects of acute exercise and starvation on hepatic branched-chain α -keto acid dehydrogenase (BCKDH) complex activity were examined in female rats fed high (30%)- or low (8%)-protein diets. The total activity of the complex was significantly higher in the high protein-fed rats than in the low protein-fed rats but was not affected by acute exercise and starvation in either diet group. The proportion of the active form of BCKDH complex was less than 10% in both diet groups. Acute exercise and starvation markedly increased the active form of the complex in both diet groups. The activity of BCKDH kinase, which is responsible for inactivation of the BCKDH complex by phosphorylation, tended to be decreased by acute exercise and starvation in both diet groups. These results suggest that the activity of the BCKDH kinase is an important factor determining the proportion of the active form of BCKDH complex in exercise and starvation, and that the female rat is a useful model for studying the regulation of hepatic BCKDH complex activity.

Key Words branched-chain α -keto acid dehydrogenase (BCKDH) complex, BCKDH kinase, exercise, starvation, female rat liver

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The branched-chain α -keto acid dehydrogenase (BCKDH) complex is the most important regulatory enzyme of branched-chain amino acid catabolism. This enzyme is subject to regulation by covalent modification, being inactivated by a specific kinase (BCKDH kinase) and activated by a specific phosphatase (1, 2). The hepatic BCKDH complex serves as a means for disposing extra circulating branched-chain α -keto acids derived from the transamination of leucine, isoleucine and valine in rat peripheral tissues (3). Shutting off its activity by phosphorylation serves to conserve the branched-chain α -keto acids and therefore the branched-chain amino acids during times under which dietary protein is absent or limited in amount.

Investigations of the mechanisms responsible for regulation of branched-chain amino acid catabolism have focused on the role played by the BCKDH complex. A number of factors have been reported to affect BCKDH complex activity, including dietary protein, exercise and starvation (4). Since many papers have reported that the hepatic BCKDH complex is almost 100% active in male rats fed high-protein diets (4), it has been necessary to feed male rats with a low-protein diet to decrease the active form of the complex in studies designed to identify physiological factors that activate the complex (3). However, we recently found that a diurnal rhythm in the activity state of the hepatic BCKDH complex occurs in the female rat (5). At the end of the light period, the proportion of the active form is less than 10% in female rats fed a chow diet, whereas it is approximately 80% in male rats at the same time point (5). In the present study, we examined the effects of exercise and starvation on the activities of the hepatic BCKDH complex and its kinase using female rats fed high- and low-protein diets. The results of the present study clearly showed that the female rat is a very useful model for studies designed to determine factors involved in the regulation of BCKDH complex activity.

MATERIALS AND METHODS

Materials. Reagents were obtained as previously described (5) or from Sigma Chemical Company. Female Sprague-Dawley rats (7 wk old) were obtained from CLEA Japan, Tokyo. Semipurified diets containing 8 or 30% protein were purchased from CLEA Japan and consisted of the following ingredients in percentage by weight: AIN 76 mineral mix, 3.5; AIN 76 vitamin mix, 1; soybean oil, 5; choline bitartrate, 0.2; D,L-methionine, 0.3; inositol, 0.01; cellulose, 5; milk casein, 8 or 30; corn starch, 61.99 or 39.99; and sucrose, 15.

Animal care. All procedures involving animals were approved by the Experimental Animal Care Committee of Nagoya Institute of Technology. Rats were individually caged in an animal room (temperature at $24 \pm 2^\circ\text{C}$; light from 05:00 to 17:00). Rats were maintained on a commercial chow diet (CE-2; CLEA Japan; approximately 25% protein content) for 1 wk and randomly divided into two dietary groups, high (30%)-protein diet and low (8%)-protein diet groups, and were fed ad libitum with appropriate experimental diets for 3 wk.

The effects of acute exercise and starvation were examined in individual experiments. However, rats in both experiments were prepared by the same procedure, as follows. During the experimental period (3 wk), all of the rats were accustomed to running on a motor-driven treadmill (KN-73, Natsume-Seisakusho, Tokyo) at 35 m/min for 30 min/d, 5 d/week. Exercise training was performed between 15:00 and 17:00 each day. On the final day of the experiment, rats were deprived of food for approximately 7 h before being sacrificed, with the exception of the starvation group which was starved for 24 h. In the preliminary experiment, we confirmed that exercise training for 3 wk had no effect on the total activity and proportion of the active form of hepatic BCKDH complex in rested female rats fed either a high or low-protein diet. Rats of the acutely exercised group were run for 85 min at 30 m/min just before being sacrificed. All of the rats were sacrificed between 16:00 and 17:00 by cervical dislocation; livers were rapidly removed, freeze-clamped at liquid nitrogen temperature and stored at -80°C until analyzed. Serum prepared from blood collected from the heart was stored at -80°C until use.

Extraction and assay of BCKDH complex and BCKDH kinase. Extraction of the BCKDH complex from rat liver was performed as described previously (6). The activities of the BCKDH complex in the active form (actual activity) and totally dephosphorylated form (total activity) were determined spectrophotometrically by the rate of NADH production as described previously (7). One unit of the complex catalyzed the formation of $1\text{ }\mu\text{mol}$ of NADH/min. The proportion of the active form of the complex was calculated as a percentage by multiplying the ratio of the actual activity to the total activity by 100.

The activity of BCKDH kinase was measured by ATP-mediated inactivation of the BCKDH complex as reported previously (7). Apparent first-order rate constants for BCKDH inactivation were calculated using the least-squares linear regression analysis.

Determination of branched-chain α -keto acids in serum. The concentrations of branched-chain α -keto acids in the serum were measured using high-performance liquid chromatography in the procedure described by Koike and Koike (8).

Statistics. Data are expressed as means $\pm$ SE. Statistical analysis was performed using a 2×2 factorial ANOVA and Scheffé's test (9). Differences with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

In reasonable agreement with previous findings (10), the total activity of hepatic BCKDH complex was considerably higher in the high-protein diet group than in the low-protein diet group (Table 1). Nevertheless, at the time these rats were sacrificed, there was no significant difference in the proportion of the active form of the BCKDH complex in the sedentary rats fed high- and low-protein diets (Table 1). Likewise, the total activities of each diet group were not affected by exercise (Table 1). Acute exercise dramatically increased the proportion of the active complex

Table 1. Effects of acute exercise on activities of hepatic BCKDH complex and BCKDH kinase.

Group	<i>n</i>	BCKDH complex activity		Ratio of active form (%)	BCKDH kinase (min ⁻¹)
		Total (mU/g wet wt)	Active form		
High-protein diet					
Rested	8	1,186 ± 85	114 ± 19	10 ± 1	1.66 ± 0.18
Exercised	8	1,319 ± 117	1,217 ± 118 [‡]	92 ± 3 [‡]	1.07 ± 0.07
Low-protein diet					
Rested	9	450 ± 35*	22 ± 2	5 ± 1	2.77 ± 0.14*
Exercised	9	437 ± 34*	191 ± 53*	40 ± 10* [‡]	1.97 ± 0.20* [‡]

Values are means ± SE. n, number of rats.

* Significantly different from high protein diet group ($p < 0.05$).

[‡] Significantly different from rested rats fed the same diet ($p < 0.05$).

in both high and low protein-fed rats, although the effect was more than twice as great in the high protein-fed rats (Table 1). The activity of hepatic BCKDH kinase was significantly lower in the high-protein diet group than in the low-protein diet group, regardless of whether the rats were acutely exercised or not (Table 1). Acute exercise tended to decrease the kinase activity in both dietary groups. In fact, the decrease caused by exercise was statistically significant in the low-protein diet group (Table 1). It was not clear that acute exercise activates the hepatic BCKDH complex in male rats fed a chow diet because the hepatic BCKDH complex in the rats is almost 100% active (4). In the present study, we clearly show a dramatic effect of exercise on the activity of the hepatic BCKDH complex in high protein-fed female rats sacrificed at the end of the light period in a day. It was also found that BCKDH kinase activity tended to be decreased by acute exercise, which may account, in part, for the increase in the active form of the BCKDH complex.

It has been reported that acute exercise significantly increases the concentrations of branched-chain amino acids in rat serum, muscle and liver (6), suggesting that the concentrations of branched-chain α -keto acids are also increased by acute exercise. To examine this possibility, serum branched-chain α -keto acids were measured in acutely exercised animals. Only α -ketoisocaproic acid, which is known to be a potent inhibitor of BCKDH kinase (11), was increased significantly by acute exercise (Table 2). These results suggest that the inhibition of BCKDH kinase activity by this α -keto acid may also be partially responsible for the increase in BCKDH complex activity by acute exercise. It is of interest why exercise activated the complex nearly completely in the high-protein diet group but only partially in the low-protein diet group. This is presumably because the relatively high BCKDH kinase present in rats fed the low-protein diet obstructed activation of the complex.

Table 2. Effects of acute exercise on serum branched-chain α -keto acid concentrations.

Group	<i>n</i>	KIC	KMV (nmol/mL)	KIV
High-protein diet				
Rested	8	8.1 $\pm$ 0.8	6.6 $\pm$ 0.6	6.3 $\pm$ 0.5
Exercised	8	13.4 $\pm$ 0.8 [‡]	10.2 $\pm$ 0.7	7.9 $\pm$ 0.6
Low-protein diet				
Rested	9	10.1 $\pm$ 0.8	7.3 $\pm$ 1.1	7.1 $\pm$ 0.7
Exercised	9	15.0 $\pm$ 1.9 [‡]	11.0 $\pm$ 0.8 [‡]	10.0 $\pm$ 1.4 [‡]

Values are means $\pm$ SE. *n*, number of rats.

KIC, KMV, and KIV, α -ketoisocaproate, α -keto- β -methylvalerate, and α -ketoisovalerate, respectively (derived by transamination from leucine, isoleucine, and valine, respectively).

[‡]Significantly different from rested rats fed the same diet ($p < 0.05$).

Table 3. Effects of 24 h-starvation on activities of hepatic BCKDH complex and BCKDH kinase.

Group	<i>n</i>	BCKDH complex activity		Ratio of active form (%)	BCKDH kinase (min ⁻¹)
		Total (mU/g wet wt)	Active form		
High-protein diet					
Control	4	1,154 $\pm$ 42	104 $\pm$ 56	9 $\pm$ 5	1.50 $\pm$ 0.17
24 h-Starved	6	1,197 $\pm$ 84	802 $\pm$ 117 [‡]	67 $\pm$ 8 [‡]	0.88 $\pm$ 0.12
Low-protein diet					
Control	4	527 $\pm$ 38*	23 $\pm$ 6	4 $\pm$ 1	2.85 $\pm$ 0.13*
24 h-Starved	6	704 $\pm$ 46*	369 $\pm$ 27* [‡]	54 $\pm$ 6 [‡]	1.46 $\pm$ 0.17 [‡]

Values are means $\pm$ SE. *n*, number of rats.

*Significantly different from high protein diet group ($p < 0.05$).

[‡]Significantly different from control rats fed the same diet ($p < 0.05$).

The effects of starvation on the activity of the BCKDH complex was also investigated. Total activities of the BCKDH complex in each diet group were not affected by 24 h starvation (Table 3). In contrast, the proportion of the active complex in both high- and low-protein diet groups was significantly increased after starvation (Table 3). Like acute exercise, starvation tended to decrease kinase activity in both dietary groups. In fact, the decrease was statistically significant in the low-protein diet group (Table 3). It has been reported that 48 h starvation increased the proportion of active BCKDH complex (3). These experiments were done with male rats that had been fed a low-protein diet. In the present study, we found, by using female rats, that an effect of starvation on the complex can be readily

demonstrated in rats fed a high-protein diet.

The concentration of serum α -ketoisocaproate in the starved rats was 11.6 ± 0.7 nmol/mL for the high-protein diet group and 13.4 ± 1.1 for the low-protein diet group. Since we failed to collect enough blood from the control rats for determination of branched-chain α -keto acids, we could not conclude whether the concentration of the α -keto acid was increased by starvation. However, the concentrations in starved rats seemed to be lower compared to those in acutely exercised rats (Table 2), suggesting that adaptive changes in kinase activity caused by starvation may be more important than direct inhibition by the α -keto acid.

We demonstrate in this study that the female rat is a good experimental model for investigation of factors regulating the activity of the liver BCKDH complex. The results of the present study suggest that the level of BCKDH kinase activity is an important factor in determining the activity state of the liver BCKDH complex. The mechanisms responsible for the decrease in kinase activity after only 85 min of exercise and 24 h of starvation require further investigation.

This work was supported in part by a grant from the U.S. Public Health Service (DK 19259 to R. A. H.) and a Grant-in-Aid for Scientific Research (08680110 to Y. S.) from the Ministry of Education, Science, Sports and Culture, Japan.

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