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Regulation of branched-chain amino acid catabolism in rat models for spontaneous type 2 diabetes mellitus

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ABSTRACT

The branched-chain α-keto acid dehydrogenase (BCKDH) complex is the most important regulatory enzyme in branched-chain amino acid (BCAA) catabolism. We examined the regulation of hepatic BCKDH complex activity in spontaneous type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Zucker diabetic fatty rats. Hepatic BCKDH complex activity in these rats was significantly lower than in corresponding control rats. The amount of BCKDH complex in OLETF rats corresponded to the total activity of the complex. Activity and abundance of the bound form of BCKDH kinase, which is responsible for inactivation of the complex, showed an inverse correlation to BCKDH complex activity in OLETF rats. Dietary supplementation of 5% BCAAs for 10 weeks markedly increased BCKDH complex activity, and decreased the activity and bound form of BCKDH kinase in the rats. These results suggest that BCAA catabolism in type 2 diabetes is downregulated and enhanced by BCAA supplementation.

Branched-chain amino acid (BCAA) catabolism is regulated by the mitochondrial branched-chain α-keto acid dehydrogenase (BCKDH) complex, which catalyzes the irreversible oxidative decarboxylation of the three branched-chain α-keto acids (BCKAs) generated by reversible transamination of BCAAs in the BCAA catabolic pathway [1]. The BCKDH complex is a multienzyme complex composed of E1 (consisting of α and β components), E2 and E3 [2], and its activity is subject to regulation through reversible phosphorylation (inactivation) and dephosphorylation (activation) of the E1α component by a specific kinase (BCKDH kinase) and a specific phosphatase, respectively [1]. The BCKDH kinase bound to the complex (bound form) plays a particularly important role in the regulation of BCKDH complex activity, as the amount of bound kinase is inversely correlated with complex activity [2].

The activity of the BCKDH complex is responsive to alterations in various nutritional and metabolic conditions [1]. Several hormones that regulate energy metabolism, such as insulin, are known to be regulators of BCKDH complex activity [3]. It has been reported that diabetes is characterized by elevation of plasma BCAA concentrations and promotion of BCAA catabolism [4–7]. Most investigators have reported that diabetes increases hepatic BCKDH complex activity in rats. However, almost all reports regarding to the BCAA catabolism are based on data for type 1 diabetes, which is characterized by severe insulin deficiency [4–7]. In a very recent study, hepatic BCKDH activity in ob/ob obese mouse was reported (total activity: 592 ± 32 nmol/min/g tissue vs. 1103 ± 39 for lean mouse) [8], but the mechanisms responsible for the regulation of BCKDH complex activity in type 2 diabetes remain uncertain.

In the present study, with the objective of elucidating the regulation of BCAA catabolism in type 2 diabetes, we examined enzyme activity and protein levels for the hepatic BCKDH complex and its kinase in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of spontaneous type 2 diabetes, and also examined the effects of dietary supplementation of BCAAs on the activity of this enzyme in rats. In addition, in order to confirm the effects of type 2 diabetes on hepatic BCKDH complex activity, we measured the enzyme activities in another rat model for spontaneous type 2 diabetes, Zucker diabetic fatty (ZDF) rats.

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Materials and methods

Animals and experimental design. All procedures were approved by the Animal Care Committees of Nagoya University School of Medicine and Nagoya Institute of Technology. Male OLETF rats aged 4 weeks obtained from Tokushima Research Institute of Otsuka Pharmaceutical Co. (Tokushima) and male ZDF/CrlCrlj-Lepr^{fa} rats aged 8 weeks from Japan Charles River Laboratories, Inc. (Yokohama) were used as rat models for spontaneous type 2 diabetes, and age-matched male non-diabetic rats (Long-Evans Tokushima Otsuka (LETO) rats and ZDF/CrlCrlj-?/+ rats, respectively) were used as normal controls. All animals were housed in a conventional animal room with controlled temperature (22 ± 2 °C) and a 12-h light/dark cycle. Rats had free access to tap water and diet during the experimental period unless otherwise stated.

OLETF and LETO rats were fed the pellet chow diet CE-2 (CLEA Japan, Inc., Tokyo, Japan) from 4 to 8 weeks of age. At 9 weeks of age, they were randomly divided into four groups as follows: LETO/control diet, LETO/BCAA diet, OLETF/control diet, and OLETF/BCAA diet groups (n = 9 in each group), and were fed the corresponding diets through 19 weeks of age. The AIN93G rodent diet was used as the control diet, and the BCAA diet was prepared by replacing the 5% cornstarch in the control diet with 5% BCAA (2.50% Leu, 1.25% Ile, and 1.25% Val). Pellet-type diets were manufactured by CLEA Japan. At 16 weeks of age, rats underwent an intraperitoneal glucose tolerance test (IPGTT), as reported previously [9], in order to confirm the development of diabetes in OLETF rats. At 19 weeks of age, body weights of OLETF rats were 629 ± 19 g for the control diet group and 611 ± 15 g for the BCAA diet group, and those of LETO rats were 454 ± 8 and 445 ± 6 g, respectively. On the final day of the experiment, food was withdrawn from cages at the end of the dark phase (08:00 h), and 6 h later, rats were injected intraperitoneally with sodium pentobarbital (60 mg/kg body weight) and sacrificed by blood sampling from the inferior vena cava to prepare serum and plasma. Livers were rapidly removed, freeze-clamped at liquid nitrogen temperature, and stored at −80 °C until analyses.

ZDF and control rats were fed the AIN93G rodent diet for 2 weeks, and on the final day of the experiment, rats (body weight: 334 ± 10 g (n = 4) for diabetic and 274 ± 3 g (n = 5) for control rats) were treated by the same procedures as for OLETF and LETO rats.

Materials. Protein A-agarose was purchased from Upstate Biotechnology (Lake Placid, NY). Antisera against the BCKDH E1α, E1β, and E2 subunits, and monoclonal antibody against BCKDH kinase were prepared as described previously [10].

Blood component analyses. Concentrations of plasma glucose and serum insulin were measured as described previously [7]. Plasma BCAA concentrations were determined by the HPLC method. The concentration of total plasma BCKA was analyzed by a spectrophotometric endpoint assay [7].

Extraction and immunoprecipitation of enzymes from rat liver. Rat liver extracts were prepared as reported previously [2]. Immunoprecipitation of BCKDH complex associated with the kinase was performed using immunoadsorbents of protein A-agarose associated with polyclonal antibodies against the E2 component of the complex [2].

Immunoblotting and immunodetection. Immunoblotting analyses of the BCKDH E1α, E1β, and E2 subunits in the liver extracts, and the BCKDH kinase in the immunoprecipitates were performed as described elsewhere [10]. The kinase protein in the immunoprecipitates was measured as the bound form of the kinase. Analysis of immunoreactive bands on the films was performed using Scion Image software. The intensities of the bands are presented relative to the mean values of the LETO/control diet group.

Assays of enzyme activities. Total and actual activities of the BCKDH complex were measured by spectrophotometric assay [10]. One unit of BCKDH complex activity refers to the formation of 1 μmol of NADH/min. The activity state of the BCKDH complex is defined as the percentage of actual (active form) activity relative to total activity (activity of fully activated enzyme). Assay of BCKDH kinase activity was performed by measuring the ATP-dependent inactivation of the BCKDH complex [2]. Kinase activity is expressed as the first-order rate constant of BCKDH complex inactivation over time. The activity of citrate synthase, a marker enzyme of mitochondrial matrix, was measured as described previously [10].

Statistics. All values are expressed as means ± standard error (SE). Data for OLETF and LETO rats were analyzed by 2-way ANOVA and the Fisher's protected least significant difference test, and those for ZDF rats were analyzed by unpaired Student's t-test. Statistical analyses were performed using StatView (Version 5.0) software (SAS Institute, Cary, NC). Differences with $P < 0.05$ were considered significant.

Results and discussion

Concentrations of blood components in OLETF and LETO rats

Concentrations of blood components measured in OLETF and LETO rats are shown in Table 1. OLETF rats in both diet groups showed higher plasma glucose concentrations when compared to LETO rats. However, there were no significant differences in plasma glucose between the two diet groups in either LETO or OLETF rats. Serum insulin concentrations in both diet groups were markedly higher in OLETF than in LETO rats. However, serum insulin concentrations in OLETF rats were significantly lower in the BCAA diet group than in the control diet group, suggesting that insulin sensitivity is improved by BCAA supplementation. This effect of the BCAA diet in OLETF rats was also observed in the IPGTT conducted at 16 weeks of age (data not shown). The mechanisms underlying the effects of the BCAA diet on insulin sensitivity are unknown. However, it has been reported that chronically high levels of plasma BCAAs markedly improve the insulin sensitivity in branched-chain aminotransferase-deficient mice [11]. As plasma BCAA levels are relatively high during the postprandial period in rats fed the BCAA diet (data not shown), this observation may be related to the improved insulin sensitivity in OLETF rats.

The concentrations of individual plasma BCAAs in LETO rats were similar between the control and BCAA diet groups. On the other hand, those in OLETF rats were significantly higher in the BCAA diet than in the control diet group. When the concentrations of BCAAs were compared between LETO and OLETF rats, no differences were observed for the control diet, but the concentrations for

Table 1
Concentrations of blood components in LETO and OLETF rats

Blood component	LETO		OLETF	
	Control diet	BCAA diet	Control diet	BCAA diet
Glucose (mg/dl)	141.9 ± 2.5	135.7 ± 3.3	164.0 ± 4.7*	172.2 ± 3.6*
Insulin (ng/ml)	0.34 ± 0.07	0.38 ± 0.08	5.92 ± 0.55*	3.79 ± 0.96*,#
Leucine (nmol/ml)	144.7 ± 3.7	146.1 ± 3.9	145.6 ± 5.6	167.6 ± 5.6*,#
Isoleucine (nmol/ml)	82.2 ± 2.1	84.1 ± 2.1	82.4 ± 3.4	94.0 ± 4.0*,#
Valine (nmol/ml)	196.9 ± 5.4	187.4 ± 6.3	190.5 ± 9.8	216.1 ± 9.3*,#
BCKA (nmol/ml)	53.7 ± 1.5	53.5 ± 1.9	60.7 ± 1.5*	65.7 ± 3.6*

Values are means ± SE for nine rats.
* Values are significantly different from the group of LETO rats fed the same diet ($P < 0.05$).
# Values are significantly different from the same rats fed the control diet ($P < 0.05$).

the BCAA diet were significantly higher in OLETF than in LETO rats. Concentrations of total plasma BCKAs in both LETO and OLETF rats were similar between the two diet groups. When compared between LETO and OLETF rats, BCKA levels in both diet groups were significantly higher in OLETF than in LETO rats.

Some studies have shown that plasma BCAA concentrations are higher in obese Zucker rats than in lean rats [12,13]. This phenomenon may depend on the higher food intake of obese rats, as the difference in plasma BCAA concentrations was not observed in rats starved overnight [8]. In our study, OLETF rats had clear insulin resistance but were not in a severe diabetic condition, as determined by blood glucose concentrations. Rats were deprived of food for 6 h before dissection, presumably resulting in no differences in plasma BCAA concentrations between LETO and OLETF rats fed the control diet. However, intake of the BCAA diet significantly increased BCAA concentrations in OLETF rats, suggesting that the BCAA catabolic capacity is lower in OLETF rats than in LETO rats. Therefore, it was important to measure hepatic BCKDH complex activity.

Table 2
Activities of hepatic BCKDH complex and kinase, and citrate synthase in LETO and OLETF rats

Liver enzyme	LETO		OLETF	
	Control diet	BCAA diet	Control diet	BCAA diet
BCKDH complex				
Total activity (U/g tissue)	0.95 ± 0.03	1.11 ± 0.03[#]	0.33 ± 0.03[*]	0.48 ± 0.05[*,#]
Activity state (%)	73.5 ± 5.3	85.5 ± 4.6	6.9 ± 2.6[*]	58.9 ± 7.6[*,#]
BCKDH kinase (min^{-1})	0.72 ± 0.04	0.55 ± 0.03[#]	0.98 ± 0.08[*]	0.69 ± 0.03[*,#]
Citrate synthase (U/g tissue)	11.9 ± 0.7	11.8 ± 0.8	10.7 ± 0.8	12.2 ± 0.6

Values are means ± SE for nine rats.
[*] Values are significantly different from the group of LETO rats fed the same diet ($P < 0.05$).
[#] Values are significantly different from the same rats fed the control diet ($P < 0.05$).

Activities of hepatic BCKDH complex, BCKDH kinase, and citrate synthase in OLETF and LETO rats

The total activity of the BCKDH complex in the LETO/control diet group was ~1 U/g tissue (Table 2), which is comparable to the levels seen in other studies [2,7]. Total BCKDH complex activity in the rat liver was slightly, but significantly higher in the BCAA diet than in the control diet group. On the other hand, the total activity in the OLETF/control diet group was markedly lower than that in the corresponding LETO group, and the activity levels in this group were comparable to the activity of the hepatic enzyme in low-protein fed rats [14]. Total activity of the BCKDH complex in OLETF rats was significantly higher in the BCAA diet than in the control diet group, whereas the elevated activity in the OLETF/BCAA diet group was still less than half that in the corresponding LETO group.

The activity state of the BCKDH complex in LETO rats showed that more than 70% of the total enzyme was in its active form in both diet groups (Table 2). In OLETF rats, only ~7% of the enzyme was in active form in the control diet group, and the enzyme was considerably activated to ~60% by the BCAA diet.

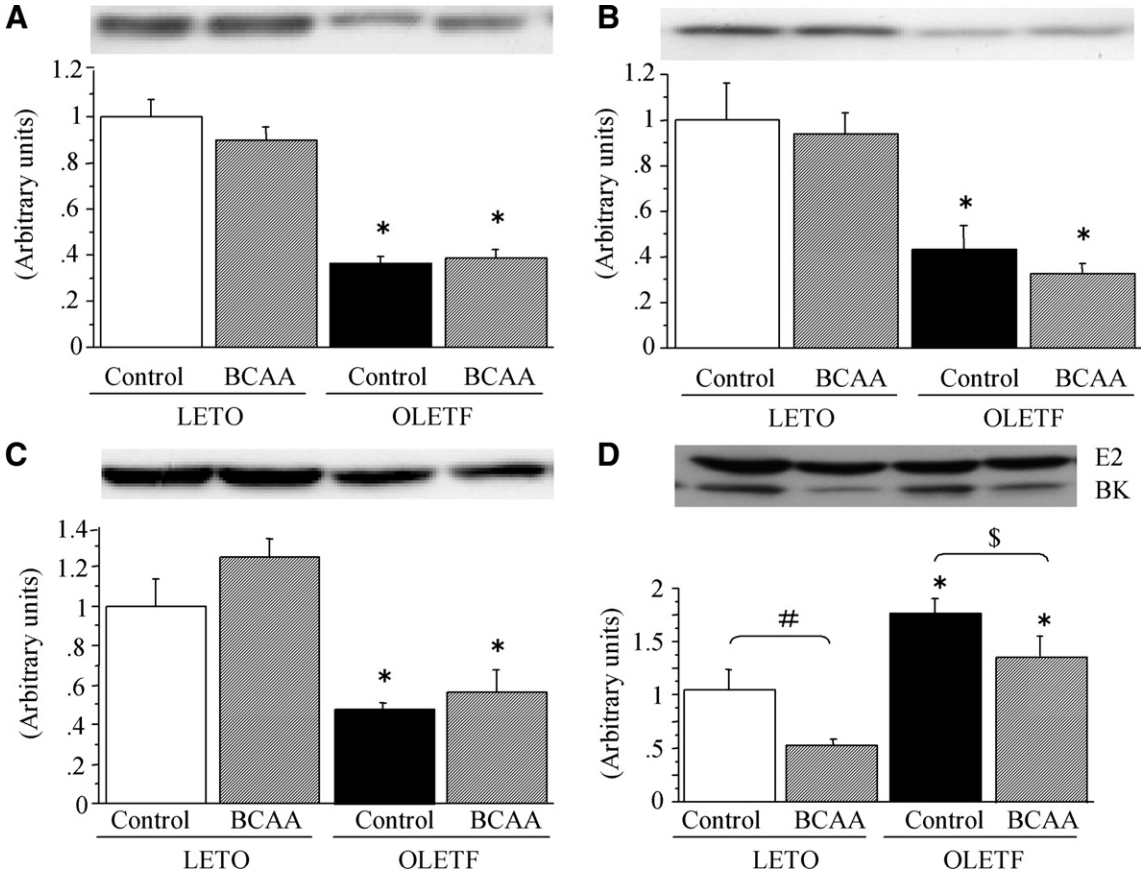

Fig. 1. Abundance of hepatic BCKDH complex subunits (E1α (A), E1β (B), and E2 (C)) and bound BCKDH kinase (BK) protein (D) in OLETF and LETO rats. Band intensities are presented relative to mean values of the LETO/control diet group. Typical images of Western blots are shown above each bar. Values are means ± SE for nine rats. [*]Values are significantly different from the group of LETO rats fed the same diet ($P < 0.05$). [#] $P < 0.05$, [$] $P < 0.1$.

The BCKDH kinase activities in both diet groups were significantly higher in OLETF than in LETO rats (Table 2). However, kinase activities in the BCAA diet groups were lower than those in the control diet groups.

The findings regarding hepatic BCKDH complex activities strongly suggest that OLETF rats have a very low capacity to catabolize BCKAs. This phenomenon cannot be attributed to a decrease in the mitochondrial oxidative capacity, as citrate synthase activity was almost the same in OLETF and LETO rats (Table 2). BCKDH kinase activity was high in OLETF rats, being responsible for the low activity state of the complex. However, intake of the BCAA diet decreased the kinase activity in OLETF rats, resulting in higher activity of the BCKDH complex. Therefore, BCAA supplementation promoted BCKA catabolism.

Abundance of hepatic BCKDH complex subunits and BCKDH kinase in OLETF and LETO rats

The abundance of E1α, E1β, and E2 subunits in the BCKDH complex in the liver was analyzed by Western blotting (Fig. 1A–C). Protein levels of the three subunits in LETO and OLETF rats corresponded to the total activities of the BCKDH complex; the amounts of these subunits in OLETF rats were less than half of those in LETO rats, although there were no differences between the control and BCAA diet groups in either LETO or OLETF rats. Our results are consistent with observations in fatty Zucker rats reported by She et al. [8]. These findings suggest that expression of the hepatic BCKDH complex may be suppressed in type 2 diabetic rats.

It has been reported that the abundance of the bound form of BCKDH kinase is correlated to kinase activity [2]. As expected, the abundance of bound kinase corresponded with kinase activity (Fig. 1D); the amount of bound kinase in both diet groups was greater in OLETF than in LETO rats and tended to be less in the BCAA diet group than in the control diet group in both LETO and OLETF rats. This suggests that the bound form of the kinase plays an important role in the regulation of the BCKDH complex activity, as suggested previously [1]. It has been reported that insulin upregulates the expression of BCKDH kinase in cultured rat cells [15], but the bound form of the kinase was not determined in that study. In the present study, the abundance of total kinase was not greater in OLETF rats than in corresponding LETO rats (data not shown). Therefore, high levels of serum insulin in OLETF rats may have affected kinase status, resulting in its conversion from a free into a bound form. However, the detailed mechanisms responsible for the BCKDH kinase conversion remain unclear.

Table 3
Concentrations of blood components and liver enzyme activities in ZDF rats

Blood component or liver enzyme	ZDF rat	
	Control ($n = 5$)	Diabetic ($n = 4$)
Glucose (mg/dl)	169.0 ± 5.6	195.0 ± 25.7
Insulin (ng/ml)	3.36 ± 0.08	21.0 ± 3.7*
Leucine (nmol/ml)	127.6 ± 6.2	171.7 ± 2.9*
Isoleucine (nmol/ml)	75.7 ± 4.8	103.7 ± 0.4*
Valine (nmol/ml)	177.3 ± 9.7	261.4 ± 2.0*
BCKDH complex		
Total activity (U/g tissue)	1.37 ± 0.02	0.84 ± 0.02*
Activity state (%)	7.1 ± 4.6	9.9 ± 9.9
BCKDH kinase (min^{-1})	0.55 ± 0.04	0.75 ± 0.05*
Citrate synthase (U/g tissue)	17.0 ± 0.6	17.2 ± 0.3

Values are means ± SE.
* Values are significantly different from the control group rats ($P < 0.05$).

Concentrations of blood components and hepatic BCKDH complex and kinase activities in ZDF rats

In order to confirm the effects of type 2 diabetes on the activities of the hepatic BCKDH complex and kinase, ZDF rats were used as another type 2 diabetic model. Concentrations of plasma glucose tended to be higher in diabetic rats than in control rats. The concentrations of serum insulin and individual plasma BCAAs were significantly higher in diabetic rats than in control rats (Table 3). Total activity of hepatic BCKDH complex in ZDF diabetic rats was ~60% of that in the control rats, although the activity state was fairly low in both groups, with no significant differences seen between the groups (Table 3). BCKDH kinase activity was significantly higher in ZDF diabetic rats than in the control rats. The activities of citrate synthase did not differ between the two groups of rats. These results suggest that the BCAA catabolic capacity in ZDF diabetic rats is similar to that in OLETF rats.

Conclusions

The present study demonstrates that the enzyme activity (especially total activity) of the hepatic BCKDH complex in rat models for type 2 diabetes is significantly lower than those in control rats, thus suggesting the downregulation of BCAA catabolism in type 2 diabetic animals. Intake of the BCAA diet decreased both the activity and level of the bound form of hepatic BCKDH kinase, thus increasing complex activity in OLETF rats. These results suggest that BCAA catabolism in type 2 diabetes mellitus may be enhanced by dietary supplementation of BCAAs.

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