Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application

Branched-Chain Amino Acid Catabolism in Exercise and Liver Disease[1–3]

Yoshiharu Shimomura,[4] Takashi Honda,[5] Makoto Shiraki,[6] Taro Murakami,[7] Juichi Sato,* Hisamine Kobayashi,† Kazunori Mawatari,† Mariko Obayashi,** and Robert A. Harris**

*Department of Materials Science and Engineering, Nagoya Institute of Technology, Nagoya 466-8555, Japan; *Department of General Medicine, Nagoya University Hospital, Nagoya 466-8560, Japan, †Ajinomoto Co., Tokyo 104-8315, Japan; and **Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202-5122*

ABSTRACT Branched-chain α-keto acid dehydrogenase (BCKDH) complex, the enzyme catalyst for the second step of the BCAA catabolic pathway, plays a central role in the regulation of BCAA catabolism. The activity of the complex is regulated by a covalent modification cycle in which phosphorylation by BCKDH kinase inactivates and dephosphorylation by BCKDH phosphatase activates the complex. Many studies suggest that control of the activity of the kinase is a primary determinant of the activity of the complex. The kinase exists at all times in the mitochondrial matrix space in two forms, with a large amount being free and a smaller amount bound rather tightly to the BCKDH complex. Only the bound form of the kinase appears to be catalytically active and, therefore, responsible for phosphorylation and inactivation of the complex. α-Ketoisocaproate, the transamination product of leucine and the most important known physiological inhibitor of BCKDH kinase, promotes release of the kinase from the complex. α-Chloroisocaproate, the analogue of leucine and the most potent known inhibitor of the kinase, is more effective than α-ketoisocaproate in promoting release of BCKDH kinase from the complex. Exercise and chronic liver disease (liver cirrhosis) likewise decrease the amount of the kinase bound to the complex in rat liver. The resulting activation of the BCKDH complex appears responsible for the increase in BCAA catabolism caused by exercise and liver cirrhosis. Our findings support the use of BCAA supplements for patients with liver cirrhosis. J. Nutr. 136: 250S–253S, 2006.

KEY WORDS: • *branched-chain amino acids* • *BCKDH complex* • *BCKDH kinase* • *leucine, α-ketoisocaproate* • *α-chloroisocaproate* • *exercise* • *liver cirrhosis* • *TNFα*

BCAAs leucine, isoleucine, and valine are essential amino acids in animals. They account for ~35% of the indispensable amino acids in muscle proteins and ~40% of the preformed amino acids required by mammals (1). Since the muscle mass of humans is ~40% of total body weight, a large amount of BCAAs is present in muscle proteins. Animals have a limited free amino acid pool that remains quite constant, and skeletal muscle contains the largest free amino acid pool of the body (2), corresponding to 3–5 g of total free amino acids/kg tissue. In the free amino acid pool, the BCAAs account for only ~0.1 g (0.6–1.2 mmol)/kg muscle (2). The pool size of free BCAAs is therefore quite small compared with the total BCAA content of muscle proteins. This is important because the free BCAAs, especially leucine, play an important role in protein metabolism. Leucine promotes protein synthesis and inhibits protein degradation via mechanisms involving the mammalian target of rapamycin (3,4). Furthermore, the transamination product of leucine (α-ketoisocaproate) is a potent regulator of BCAA catabolism (5) via strong inhibition of branched-chain α-keto acid dehydrogenase (BCKDH)[8] kinase. These findings suggest that leucine has functions beyond its role as an essential amino

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the conference "Symposium on Branched-Chain Amino Acids," held May 23–24, 2005 in Versailles, France. The conference was sponsored by Ajinomoto USA, Inc. The organizing committee for the symposium and guest editors for the supplement were Luc Cynober, Robert A. Harris, Dennis M. Bier, John O. Holloszy, Sidney M. Morris, Jr., and Yoshiharu Shimomura. Guest editor disclosure: L. Cynober, R. A. Harris, D. M. Bier, J. O. Holloszy, S. M. Morris, Y. Shimomura: expenses for travel to BCAA meeting paid by Ajinomoto USA; D. M. Bier: consults for Ajinomoto USA; S. M. Morris: received compensation from Ajinomoto USA for organizing BCAA conference.

[2] This work was supported in part by a grant-in-aid for scientific research (14370022; Y.S.) from the Ministry of Education, Science, Sports, and Culture, Japan, and a grant from the U.S. Public Health Service (National Institutes of Health DK 19259; R.A.H.).

[3] Author Disclosure: R. A. Harris and Y. Shimomura's travel expenses to BCAA meeting were paid by Ajinomoto USA.

[4] To whom correspondence should be addressed. E-mail: shimomura.yoshiharu@nitech.ac.jp.

[5] Present address: Department of Therapeutic Medicine, Nagoya University School of Medicine, Nagoya 466-8560, Japan.

[6] Present address: Department of Internal Medicine, Gifu University School of Medicine, Gifu 501-1194, Japan.

[7] Present address: Department of Nutrition, Faculty of Wellness, Chukyo Women's University, Ohbu 474-8651, Japan.

[8] Abbreviations used: BCAT, branched-chain aminotransferase; BCKA, branched-chain α-keto acid; BCKDH, branched-chain α-keto acid dehydrogenase; KIC, α-ketoisocaproate; TNFα, tumor necrosis factor-α.

acid. BCAA catabolism is greatly affected by a number of physiological conditions in mammals (6), making it important to elucidate the mechanisms responsible for alterations in the BCAA catabolism under such conditions. We describe here mechanisms responsible for regulation of BCAA catabolism during exercise and liver disease (acute failure and cirrhosis). The rationale for administration of BCAAs as therapy for liver disease is briefly discussed.

Regulation of BCAA catabolism

Mammalian cells have a high capacity system for oxidative disposal of BCAAs. In contrast with other essential amino acids, which are oxidized primarily in the liver, the most active system for oxidation of BCAAs per se is located in skeletal muscle cells. Although the liver cannot directly catabolize BCAAs, it has a very active system for degradation of the branched-chain α-keto acids (BCKAs) derived from the corresponding BCAAs (1,7). This organ specificity for BCAA catabolism is the result of a unique distribution of the first two enzymes of the catabolic pathway in animals, the branched-chain aminotransferase (BCAT) and BCKDH complex (8) (**Fig. 1**). This feature of the enzyme distribution is typical in rat tissues; the skeletal muscle has quite high and low activities of BCAT and BCKDH complex, respectively. The opposite distribution of these enzymes holds for the liver (8,9).

The first two steps in BCAA catabolism are common to the three BCAAs (Fig. 1). BCAT catalyzes transamination of BCAAs, which is a reversible reaction to form BCKAs, and the BCKDH complex catalyzes the oxidative decarboxylation of BCKAs, an irreversible reaction. Since the BCKDH complex commits BCAAs to degradation and the BCKDH complex is subject to regulation by covalent modification, it is believed that the BCKDH complex catalyzes the rate-limiting step in the overall catabolism of the BCAAs (1,7).

The BCKDH complex is regulated by a phosphorylation/dephosphorylation cycle. BCKDH kinase is responsible for inactivation of the complex by phosphorylation of the E1α-subunit of the complex (11,12), and BCKDH phosphatase is responsible for activation of the complex by dephosphorylation of E1α (13). Many reports suggest that the BCKDH kinase plays an important role in the regulation of the complex activity (6). The BCKDH phosphatase undoubtedly performs a significant role in the regulation of BCKDH complex activity, but little is known about the phosphatase, although it has been purified from bovine kidney (13). Thus far, our work has focused primarily on the role of BCKDH kinase in regulation of BCKDH complex activity.

The bound form of BCKDH kinase appears responsible for phosphorylation and inactivation of BCKDH complex

Recent studies (14) show that the BCKDH kinase exists in bound and free (nonbound) forms. The bound form of the kinase can be immunoprecipitated from extracts of rat liver mitochondria with antibodies against the E1 subunits (E1α and E1β) of the BCKDH complex. Supernatants obtained by immunoprecipitation of E1 contain significant amounts of the kinase corresponding to its free form. Under most conditions, the amount of free kinase exceeds the amount of kinase bound to the BCKDH complex in rat liver mitochondrial extracts. In the routine method for measurement of BCKDH kinase activity in rat liver (15), the BCKDH complex and its kinase are coprecipitated with 9% polyethylene glycol prior to assay of kinase activity. Although free and bound BCKDH kinase are both completely recovered in 9% polyethylene glycol precipitates, kinase activity against the recovered BCKDH complex correlates with the amount of the bound form of the kinase rather than the total amount of the kinase. Furthermore, the amount of total kinase is little affected by acute changes in physiological conditions (14,16), suggesting that the bound rather than the free form of the kinase is responsible for phosphorylation and inactivation of the complex. **Table 1** lists various treatments and conditions in which an inverse relationship between the amount of bound kinase and the activity state of the BCKDH complex has been found. The molecular basis for why only part of the kinase binds to the complex in these conditions and why only the bound form of the kinase is active is not known at this time.

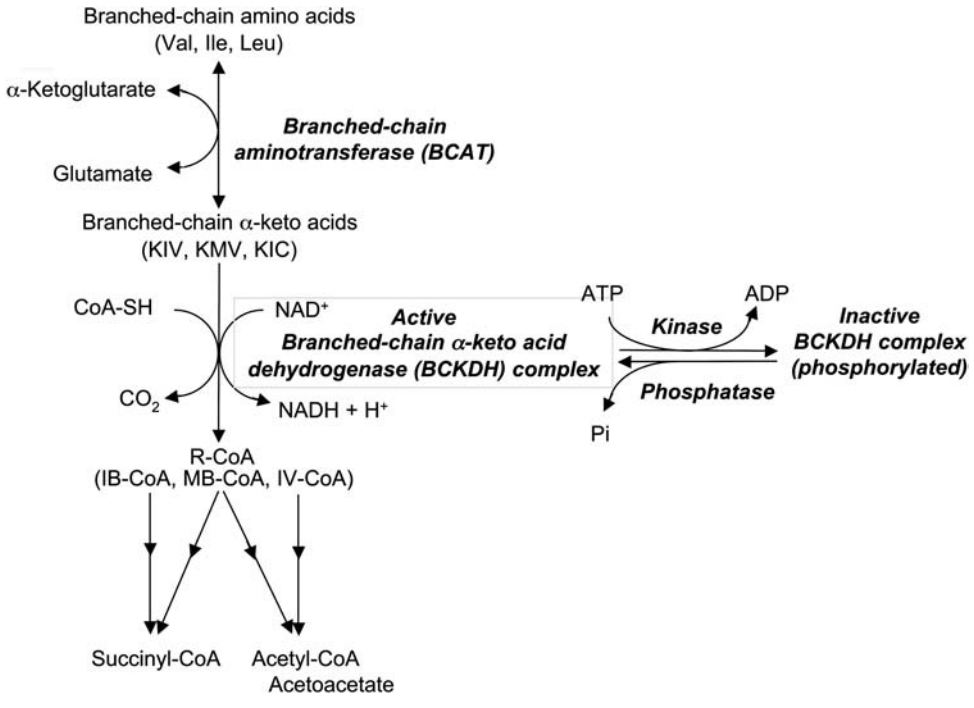

FIGURE 1 BCAA catabolism: the first two steps of the catabolic pathway and regulation of the BCKDH complex. KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; KIC, α-ketoisocaproate; CoA-SH, coenzyme A, reduced form; IB-CoA, isovaleryl-CoA; MB-CoA, α-methylbutyryl-CoA; IV-CoA, isovaleryl-CoA; R-CoA, acyl-CoA. This figure is a modified version of a previously published figure by Shimomura et al. (10).

TABLE 1

Effect of various treatments and conditions on the relative amount of bound BCKDH kinase versus activity state of the BCKDH complex in rat liver and muscle[1]

Treatment/Condition	Bound kinase	BCKDH activity state	Reference
[Rat liver]			
High protein feeding[2]	Low	High	17, 18
Low protein feeding[3]	High	Low	17, 18
Postprandial (female)[4]	Low	High	14
Postabsorptive (female)[4]	High	Low	14
Exercising	Low	High	16
Clofibric acid	Low	High	18
TNFα	Low	High	19
KIC	Low	ND	20
α-Chloroisocaproate	Low	ND	20
Liver cirrhosis	Low	High	21
[Rat muscle]			
Resting	High	Low	16
Exercising	Low	High	16

[1] TNFα, tumor necrosis factor-α; ND, not determined.
[2] A stock diet (CE-2 [CLEA Japan], LM-485 Mouse/Rat Sterilizable Diet 7912 [Harlan Teklad], or Purina Rodent Laboratory Chow 5002 [Harlan Industries]) or semipurified diet containing 50% protein (ICN Nutritional Biochemicals) was used.
[3] A semipurified diet containing 0% protein (17) or 8% protein (ICN Nutritional Biochemicals) was used.
[4] This phenomenon was tested only in female rats because hepatic BCKDH complex and kinase activities show the typical diurnal variation only in female rats but not in male rats (14, 22).

Activation of the BCKDH complex by exercise

Exercise greatly increases energy expenditure, resulting in promotion of amino acid catabolism in general and especially the oxidation of BCAAs (2). Consistent with this finding, it is well established that endurance exercise activates the BCKDH complex in human and rat skeletal muscle (9,23) as well as rat liver (23). By contrast, endurance exercise decreases BCKDH kinase activity in liver (16,24), which surely accounts at least in part for activation of the hepatic BCKDH complex during exercise. It is unlikely that altered gene expression of the kinase can be responsible for the decrease in kinase activity caused by short periods of exercise. In our study using an electrically stimulated muscle contraction model (25), increases in leucine and α-ketoisocaproate (KIC) concentrations in the muscle were suggested to be one factor responsible for muscle BCKDH activation because KIC is a potent inhibitor of the kinase (5). We have further demonstrated that the amount of BCKDH kinase bound to the complex is reduced by exercise (16). In an in vitro study designed to extend this work, we found KIC promotes dissociation of the BCKDH kinase from the BCKDH complex (20) (**Fig. 2**), consistent with activation of BCKDH complex by increasing the KIC concentration during muscle exercise. These findings suggest that a change in conformation induced by KIC that modifies BCKDH kinase binding to the BCKDH complex may be involved in regulation of BCKDH complex activity (**Fig. 3**).

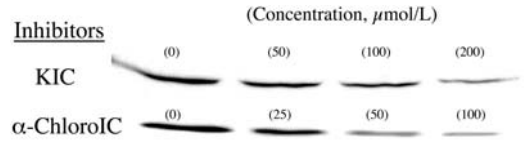

FIGURE 2 KIC and α-chloroisocaproate (α-ChloroIC) inhibit binding of BCKDH kinase to the BCKDH complex. The inhibitors were added to the rat liver extract before immunoprecipitation with antiserum against the E2 subunit of the BCKDH complex. The BCKDH kinase in the precipitate (the bound form) was detected by Western blot analysis with antiserum against the kinase. Data presented in this figure were published previously by Murakami et al. (20).

Regulation of hepatic BCKDH complex activity in rats with acute or chronic liver failure

The possibility of beneficial nutraceutical effects of BCAAs are of great interest. Indeed, BCAA supplements are given in liver cirrhotic patients in Japan to improve serum albumin concentrations (26,27). However, administration of BCAAs to patients with acute liver failure may cause nitrogen overload and accelerate hyperammonemia and hepatic encephalopathy. It was, therefore, of interest to compare responses of the BCKDH complex to acute and chronic liver failure (21). Rats with acute liver failure were produced by injection of a single dose (2 mL/kg body weight) of carbon tetrachloride. Liver cirrhotic rats were produced by repeated injection of this toxic compound (twice a week at 0.5 mL/kg body weight) for 21 weeks. Acute liver failure decreased hepatic BCKDH complex total activity by 50% and elevated the concentrations of plasma BCAAs and BCKAs by 2-fold compared with those of control rats, suggesting that oxidation of BCKAs (and therefore BCAAs) was suppressed in the rats. By contrast, liver cirrhosis did not suppress hepatic BCKDH enzyme activity but rather increased actual activity of the complex ~2-fold and significantly decreased the concentrations of plasma BCAAs and BCKAs, suggesting BCKA catabolism is enhanced in the cirrhotic liver in comparison with the normal liver. In livers of cirrhotic rats, the bound form of BCKDH kinase was significantly decreased by ~30% compared with that of the control rats, suggesting that activation of the BCKDH complex in the cirrhotic livers may be attributed to a decrease in the bound form of the kinase.

We also recently found that injection of rats with TNFα (25 or 50 μg/kg body weight) promotes dissociation of the BCKDH kinase from the complex in rat liver, resulting in activation of the complex (19). Cytokine levels are increased in cirrhotic liver induced by carbon tetrachloride (28). Therefore, the dissociation of BCKDH kinase from the complex induced by TNFα may be involved in the mechanisms responsible for activation of the BCKDH complex in cirrhotic liver.

It has been reported that BCAA administration improves protein turnover in rats and humans with liver cirrhosis (29–31), suggesting that BCAA supplements are appropriate for patients with liver cirrhosis. Our finding of activation of the capacity for BCKA oxidation and therefore BCAA oxidation in cirrhotic liver supports the use of BCAA supplements for

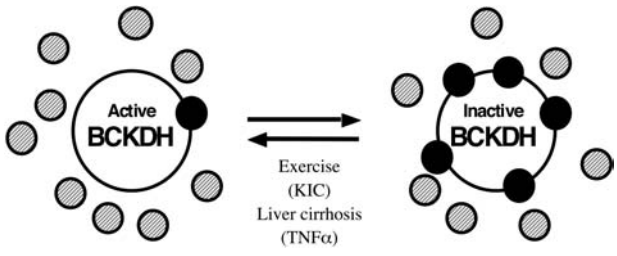

FIGURE 3 The activation of BCKDH complex by exercise and liver cirrhosis is associated with a decrease in the bound form of the BCKDH kinase. KIC and TNFα may be involved in the dissociation of BCKDH kinase from BCKDH complex. Hatched circles designate the free form of the kinase; filled circles designate the bound form of the kinase.

patients with liver cirrhosis. However, BCAA administration in patients with acute liver failure, especially those with high serum BCAA concentrations, may not be appropriate because of the likelihood of BCAA overload.

LITERATURE CITED

1. Harper AE, Miller RH, Block KP. Branched-chain amino acid metabolism. Annu Rev Nutr. 1984;4:409–54.
2. Rennie MJ. Influence of exercise on protein and amino acid metabolism. In: Rowell, LB, Shepherd, JT, editors. Handbook of Physiology, Section 12. Exercise: Regulation and Integration of Multiple Systems. New York: Oxford University Press; 1996. p. 995–1035.
3. Bolster DR, Jefferson LS, Kimball SR. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc Nutr Soc. 2004;63:351–6.
4. Mordier S, Deval C, Bechet D, Tassa A, Ferrara M. Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. J Biol Chem. 2000;275:29900–6.
5. Paxton R, Harris RA. Regulation of branched-chain α-ketoacid dehydrogenase kinase. Arch Biochem Biophys. 1984;231:48–57.
6. Shimomura Y, Obayashi M, Murakami T, Harris RA. Regulation of branched-chain amino acid catabolism: nutritional and hormonal regulation of activity and expression of the branched-chain α-keto acid dehydrogenase kinase. Curr Opin Clin Nutr Metab Care. 2001;4:419–23.
7. Harris, RA, Zhang, B, Goodwin, GW, Kuntz, MJ, Shimomura, Y, Rougraff, P, Dexter, P, Zhao, Y, Gibson, R, Crabb, DW. Regulation of the branched-chain α-ketoacid dehydrogenase and elucidation of a molecular basis for maple syrup urine disease. Adv Enzyme Regul. 1990;30:245–63.
8. Suryawan A, Hawes JW, Harris RA, Shimomura Y, Jenkins AE, Hutson SM. A molecular model of human branched-chain amino acid metabolism. Am J Clin Nutr. 1998;68:72–81.
9. Shimomura Y, Fujii H, Suzuki M, Murakami T, Fujitsuka N, Nakai N. Branched-chain α-keto acid dehydrogenase complex in rat skeletal muscle: regulation of the activity and gene expression by nutrition and physical exercise. J Nutr. 1995;125(Suppl):1762S–5S.
10. Shimomura Y, Murakami T, Nakai N, Nagasaki M, Harris RA. Exercise promotes BCAA catabolism: effects of BCAA supplementation on skeletal muscle during exercise. J Nutr. 2004;134(Suppl):1583S–7S.
11. Shimomura Y, Nanaumi N, Suzuki M, Popov KM, Harris RA. Purification and partial characterization of branched-chain α-ketoacid dehydrogenase kinase from rat liver and rat heart. Arch Biochem Biophys. 1990;283:293–9.
12. Popov KM, Zhao Y, Shimomura Y, Kuntz MJ, Harris RA. Branched-chain α-ketoacid dehydrogenase kinase: molecular cloning, expression, and sequence similarity with histidine protein kinases. J Biol Chem. 1992;267:13127–30.
13. Damuni Z, Reed LJ. Purification and properties of the catalytic subunit of the branched-chain α-keto acid dehydrogenase phosphatase from bovine kidney mitochondria. J Biol Chem. 1987;262:5129–32.
14. Obayashi M, Sato Y, Harris RA, Shimomura Y. Regulation of the activity of branched-chain 2-oxo acid dehydrogenase (BCODH) complex by binding BCODH kinase. FEBS Lett. 2001;491:50–4.
15. Nakai N, Kobayashi R, Popov KM, Harris RA, Shimomura Y. Determination of branched-chain α-keto acid dehydrogenase activity state and branched-chain α-keto acid dehydrogenase kinase activity and protein in mammalian tissues. Methods Enzymol. 2000;324:48–62.
16. Xu M, Nagasaki M, Obayashi M, Sato Y, Tamura T, Shimomura Y. Mechanism of activation of branched-chain α-keto acid dehydrogenase complex by exercise. Biochem Biophys Res Commun. 2001;287:752–6.
17. Popov KM, Zhao Y, Shimomura Y, Jaskiewicz J, Kedishvili NY, Irwin J, Goodwin GW, Harris RA. Dietary control and tissue specific expression of branched-chain α-ketoacid dehydrogenase kinase. Arch Biochem Biophys. 1995; 316:148–54.
18. Kobayashi R, Murakami T, Obayashi M, Nakai N, Jaskiewicz J, Fujiwara Y, Shimomura Y, Harris RA. Clofibric acid stimulates branched-chain amino acid catabolism by three mechanisms. Arch Biochem Biophys. 2002;407:231–40.
19. Shiraki M, Shimomura Y, Miwa Y, Fukushima H, Murakami T, Tamura T, Tamura N, Moriwaki H. Activation of hepatic branched-chain α-keto acid dehydrogenase complex by tumor necrosis factor-α in rats. Biochem Biophys Res Commun. 2005;328:973–8.
20. Murakami T, Matsuo M, Shimizu A, Shimomura Y. Dissociation of branched-chain α-keto acid dehydrogenase kinase (BDK) from branched-chain α-keto acid dehydrogenase complex (BCKDC) by BDK inhibitors. J Nutr Sci Vitaminol (Tokyo). 2005;51:48–50.
21. Honda T, Fukuda Y, Nakao I, Katano Y, Goto H, Nagasaki M, Sato Y, Murakami T, Shimomura Y. Effects of liver failure on branched-chain α-keto acid dehydrogenase complex in rat liver and muscle: comparison between acute and chronic liver failure. J Hepatol. 2004;40:439–45.
22. Kobayashi R, Shimomura Y, Murakami T, Nakai N, Fujitsuka N, Otsuka M, Arakawa N, Popov KM, Harris RA. Gender difference in regulation of branched-chain amino acid catabolism. Biochem J. 1997;327:449–53.
23. Wagenmakers AJM, Brookes JH, Coakley JH, Reilly T, Edwards RH. Exercise-induced activation of the branched-chain 2-oxo acid dehydrogenase in human muscle. Eur J Appl Physiol. 1989;59:159–67.
24. Kobayashi R, Shimomura Y, Murakami T, Nakai N, Otsuka M, Arakawa N, Shimizu K, Harris RA. Hepatic branched-chain α-keto acid dehydrogenase complex in female rats: activation by exercise and starvation. J Nutr Sci Vitaminol (Tokyo). 1999;45:303–9.
25. Shimomura Y, Fujii H, Suzuki M, Fujitsuka N, Naoi M, Sugiyama S, Harris RA. Branched-chain 2-oxo acid dehydrogenase complex activation by titanic contractions in rat skeletal muscle. Biochim Biophys Acta. 1993;1157:290–6.
26. Muto Y, Yoshida T, Sato S, Suzuki K, Watanabe A, Ogawa N. Effect of oral administration with branched-chain amino acid granules (BCAA-G) in patients with liver cirrhosis: dose finding study (in Japanese). JJPEN. 1989;14:172–96.
27. Kato M, Yoshida T, Moriwaki H, Muto Y. Effect of branched-chain amino acid (BCAA) enriched-nutrient mixture on albumin metabolism in cirrhotic patients. Acta Hepatol Jpn. 1991;32:692–9.
28. Orfila C, Lepert JC, Alric L, Carrera G, Beraud M, Vinel JP, Pipy B. Expression of TNF-alpha and immunohistochemical distribution of hepatic macrophage surface markers in carbon tetrachloride-induced chronic liver injury in rats. Histochem J. 1999;31:677–85.
29. Usui T, Moriwaki H, Hatakeyama H, Kasai T, Kato M, Seishima M, Okuno M, Ohnishi H, Yoshida T, Muto Y. Oral supplementation with branched-chain amino acids improves transthyretin turnover in rats with carbon tetrachloride-induced liver cirrhosis. J Nutr. 1996;126:1412–20.
30. Okuno M, Moriwaki H, Kato M, Muto Y, Kojima S. Changes in the ratio of branched-chain to aromatic amino acids affect the secretion of albumin in cultured rat hepatocytes. Biochem Biophys Res Commun. 1995;214:1045–50.
31. Marchesini G, Bianchi G, Merli M, Amodio P, Panella C, Loguercio C, Rossi Fanelli F, Abbiati R. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology. 2003;124:1792–801.