Branched-Chain Amino Acids as Pharmacological Nutrients in Chronic Liver Disease

Takumi Kawaguchi,[1] Namiki Izumi,[2] Michael R. Charlton,[3] and Michio Sata[1]

Branched-chain amino acids (BCAAs) are a group of essential amino acids comprising valine, leucine, and isoleucine. A low ratio of plasma BCAAs to aromatic amino acids is a physiological hallmark of liver cirrhosis, and BCAA supplementation was originally devised with the intention of normalizing amino acid profiles and nutritional status. However, recent studies on BCAAs have revealed that, in addition to their role as protein constituents, they may have a role as pharmacological nutrients for patients with chronic liver disease. Large-scale, multicenter, randomized, double-blinded, controlled trials on BCAA supplementation have been performed in Italy and Japan, and results demonstrate that BCAA supplementation improves not only nutritional status, but also prognosis and quality of life in patients with liver cirrhosis. Moreover, accumulating experimental evidence suggests that the favorable effects of BCAA supplementation on prognosis may be supported by unforeseen pharmacological actions of BCAAs. This review summarizes the possible effects of BCAAs on albumin synthesis and insulin resistance from clinical and basic viewpoints. We also review the newly discovered clinical impact of BCAAs on hepatocellular carcinoma and the prognosis and quality of life of patients with liver cirrhosis. (HEPATOLOGY 2011;54:1063-1070)

The liver is a central organ for regulating metabolism, and a variety of metabolic disorders are frequently seen in patients with chronic liver disease.[1,2] Decreased serum ratio of branched-chain amino acids (BCAAs) to aromatic amino acids (AAAs) is a hallmark of liver cirrhosis and is caused by several factors, including reduced nutritional intake, hypermetabolism, and ammonia detoxification in skeletal muscle.[3] Low serum BCAA/AAA ratio reduces biosynthesis and secretion of albumin in hepatocytes,[4] and is also associated with the prognosis of patients with chronic liver disease.[5]

BCAAs have aliphatic side chains with a branch point, and comprise valine (Val), leucine (Leu), and isoleucine (Ile) (Fig. 1). BCAAs are not only a constituent of protein, but also a source of glutamate, which detoxifies ammonia by glutamine synthesis in skeletal muscle.[3] Clinical studies have demonstrated that intravenous administration of BCAA improves hepatic encephalopathy with hyperammonemia.[6] Although dairy products and vegetables contain high BCAA content, increased consumption of these foods does not affect plasma BCAA levels in patients with cirrhosis.[7] The guidelines of the American Society for Parenteral and Enteral Nutrition and the European Societies for Clinical Nutrition and Metabolism currently recommend BCAA supplementation only for patients with cirrhosis with chronic hepatic encephalopathy unresponsive to pharmacotherapy.[8,9] A series of subsequent clinical trials and *in vitro* and *in vivo* studies suggest the possibility of more expansive utility of BCAA supplementation in liver disease.

Abbreviations: BCAA, branched-chain amino acid; BCATm, mitochondrial BCAA aminotransferase; DC, dendritic cell; GLUT, glucose transporter; IGF, insulin-like growth factor; IL, interleukin; Ile, isoleucine; Leu, leucine; MAPK, mitogen-activated protein kinase; mRNA, messenger RNA; MSUD, maple syrup urine disease; mTOR, mammalian target of rapamycin; NK, natural killer; PI3K, phosphatidylinositol 3-kinase; QOL, quality of life; Val, valine.

From the [1]Department of Digestive Disease Information and Research and Department of Medicine, Kurume, Japan; [2]Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; and [3]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN.

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Address reprint requests to: Takumi Kawaguchi, M.D., Ph.D., Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. E-mail: takumi@med.kurume-u.ac.jp; fax: +81-942-31-7820.

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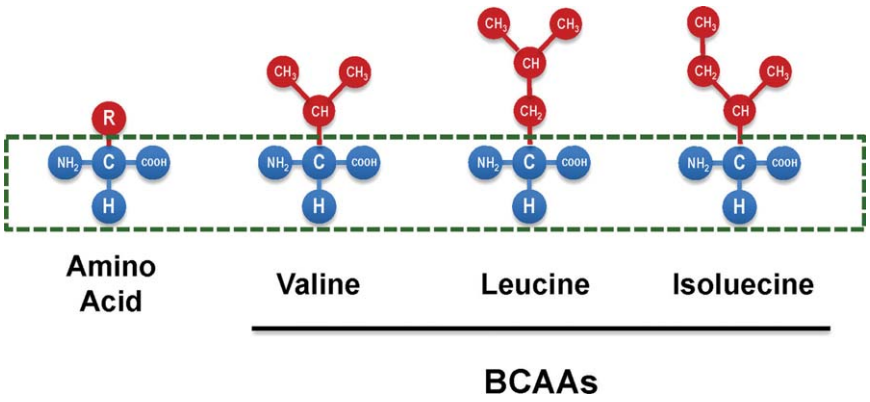

Fig. 1. Chemical structure of BCAAs. The dotted rectangle indicates the basic amino acid structure. The generic BCAA has an aliphatic side chain with a branch point. R, residue.

The liver carries out four main functions in protein metabolism: formation of plasma proteins, amino acid interconversion, deamination of amino acids, and urea synthesis (for ammonia excretion). Among the many other functions of the liver, it is responsible for the metabolism of hormones that have discordant effects on protein metabolism, including insulin, androgens, and glucagon. It is thus not surprising that cirrhosis is associated with altered circulating amino acid profiles, with decreased serum BCAA levels seen in patients even with compensated cirrhosis.[10] It is widely believed that the changes in amino acid metabolism not only occur as an epiphenomenon of liver disease but also play a role in the pathogenesis of many of the complications of cirrhosis, such as encephalopathy,[11] hypoalbuminemia with edema, and insulin resistance.[12-14] The potential of BCAA supplementation to alter the metabolic basis and frequency of complications of cirrhosis is suggested by studies indicating that BCAAs may inhibit hepatocarcinogenesis and improve immune function and oxidative stress *in vitro* and *in vivo*.[15-19] Clinical studies have further demonstrated that BCAA supplementation may improve the quality of life (QOL) and prognosis in patients with liver cirrhosis.[16,20,21]

Nutritional aspects of BCAAs on hepatic encephalopathy, liver regeneration, or hepatic cachexia have been well reviewed.[22,23] In this article, we review the recently identified pharmaceutical aspects of BCAAs on pathological conditions and complications associated with chronic liver disease from both the clinical and basic research viewpoints. We also summarize side effects of BCAA supplementation (Supporting Text).

Albumin Synthesis

BCAAs, particularly Leu, activate the mammalian target of rapamycin (mTOR) and subsequently up-regulates the downstream eukaryotic initiation factor 4E-binding protein-1 and 70-kDa ribosomal protein S6 kinase, which regulate messenger RNA (mRNA) translation and synthesis of albumin in cultured rat hepatocytes (Fig. 2).[4,12,24] Leu also stimulates the nuclear import of polypyrimidine-tract–binding protein, which binds to albumin mRNA and increases its translation in HepG2 cells (Fig. 2).[25] Consistent with these *in vitro* studies, BCAA supplementation has been found to activate the mTOR signaling cascade and increase albumin synthesis in animal models of cirrhosis.[26]

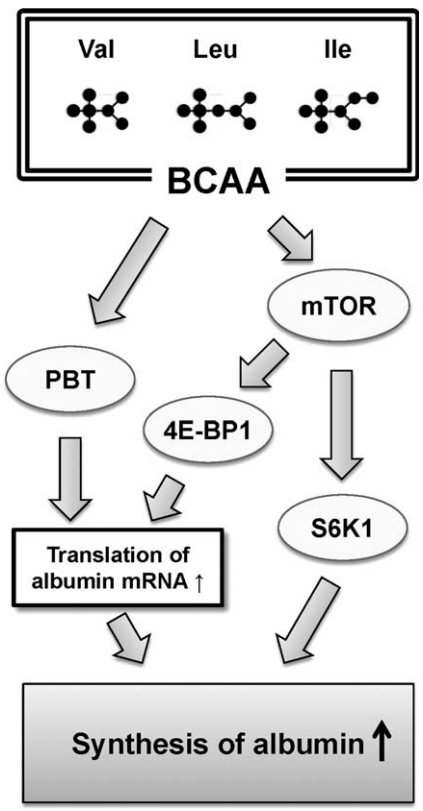

Fig. 2. Molecular mechanisms for BCAA-induced albumin synthesis. BCAA activates the mTOR and subsequently up-regulates the downstream molecules, eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and 70-kDa ribosomal protein S6 kinase (S6K1), which regulate mRNA translation and synthesis, respectively. BCAAs also stimulate the nuclear import of polypyrimidine-tract–binding protein (PBT), which binds with albumin mRNA and increases albumin translation.

Muto et al. conducted a multicenter, randomized, controlled trial in which 622 patients with cirrhosis were administered BCAAs at 12 g/day for 2 years. In that study, serum albumin levels in the BCAA group were significantly higher than in the nutrient intake-matched control group.[16] However, in another randomized, controlled study by Marchesini et al., BCAA treatment did not result in a significant increase in serum albumin levels.[15] Although the reason for this discrepancy remains unclear, a possible explanation is the difference in the BCAA/AAA ratio among the participants in the two studies. Approximately 45% of enrolled patients were Child-Pugh class A in the former study,[16] whereas all the patients were Child-Pugh class B or C in the latter study.[15] The BCAA/AAA ratio decreases along with progression of liver cirrhosis.[27] Because the BCAA/AAA ratio is positively correlated with the synthesis and secretion of albumin,[4] and the response to BCAA treatment,[27] a low BCAA/AAA ratio may be a reason for the discrepancy in results between the studies. In addition, the majority of other randomized, controlled trials have demonstrated that BCAA supplementation results in a significant increase in serum albumin levels in patients with cirrhosis (Supporting Table 1). The aggregate of the evidence suggests that BCAA administration may increase serum albumin levels in patients with liver cirrhosis.

Insulin Resistance

BCAAs are thought to affect glucose metabolism.[28] Recently, She et al. knocked out the gene of mitochondrial BCAA aminotransferase (BCATm), which catalyzes the first step of BCAA catabolism, leading to a significant elevation in the serum BCAA level. In $BCATm^{-/-}$ mice, fasting blood glucose and fasting serum insulin levels were decreased by 33% and 67%, respectively, and the Homeostasis Model Assessment for Insulin Resistance index was significantly lower than that of wild-type mice.[14] Similarly, treatment with Leu or Ile has been reported to improve insulin sensitivity in mice fed a high-fat diet.[29,30]

Supplementation with BCAAs enhances glucose metabolism in skeletal muscle, adipose tissue, and liver; however, the molecular mechanisms in each organ are different. In skeletal muscle, BCAAs promote glucose uptake through activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C and subsequent translocation of glucose transporter 1 (GLUT1) and GLUT4 to the plasma membrane (Fig. 3).[13,31] In adipose tissue, Leu enhances insulin-induced phosphorylation of Akt (protein kinase B) on Ser473 and Thr308 and mTOR on Ser2448, ultimately increasing glucose uptake (Fig. 3).[32] In the liver, BCAAs up-regulate the liver X receptor α (LXRα)/sterol regulatory element binding protein-1c (SREBP1c) pathway and subsequently activate liver-type glucokinase and GLUT2. In addition, BCAA suppresses hepatic expression of glucose-6-phosphatase, which catalyzes the final steps of gluconeogenesis (Fig. 3).[33] Recently, BCAA supplementation has been reported to improve insulin resistance by increasing oxidation of free fatty acids. BCAAs increase peroxisome proliferator-activated receptor α

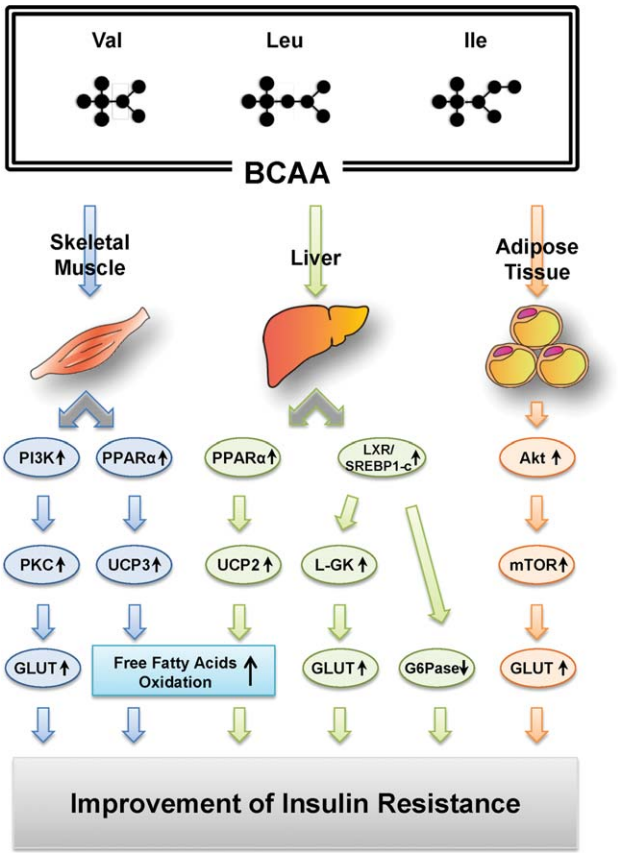

Fig. 3. Distinctive molecular pathway for BCAA-induced improvement of insulin resistance in insulin target organs. BCAAs improve glucose metabolism by acting on insulin target organs such as skeletal muscle, adipose tissue, and the liver. However, the molecular mechanisms in each organ differ. In the skeletal muscle, BCAAs promote glucose uptake through activation of PI3K and protein kinase C and subsequent translocation of GLUT1 and GLUT4 to the plasma membrane. In the adipose tissue, BCAAs, especially Leu, augment insulin-induced phosphorylation of Akt and mTOR, and consequently increase the glucose uptake. In the liver, BCAA activates the liver X receptor α (LXR)/sterol regulatory element binding protein-1c (SREBP1-c) pathway and subsequently up-regulates liver-type glucokinase (L-GK) and GLUT2. In addition, LXR/SREBP-1c activation suppresses hepatic expression of glucose-6-phosphatase (G6Pase), which catalyzes the final steps of gluconeogenesis. BCAAs also increase peroxisome proliferator-activated receptor (PPAR) α expression and subsequent uncoupling proteins 2 (UCP2) in liver and UCP3 in muscle. Up-regulation of UCP2 and UCP3 expression increases oxidation of free fatty acids and improves insulin resistance.

expression and subsequent expression of uncoupling proteins 2 in liver and uncoupling proteins 3 in muscle (Fig. 3).[34,35] These recent studies have revealed distinct cross-talk mechanisms between BCAAs and the insulin signaling cascade in insulin target organs.

Previous clinical studies have reported that BCAA infusion decreases plasma glucose levels in patients with advanced liver cirrhosis.[36] Furthermore, oral BCAA supplementation reduces both blood glucose[37,38] and insulin resistance in patients with chronic liver disease.[18,39] However, these studies had small sample sizes and/or were lacking in adequate controls. A randomized, controlled trial is required to definitively evaluate the effects of BCAA supplementation on insulin resistance in cirrhosis.

Hepatocellular Carcinoma

Clinical studies have reported that long-term oral supplementation with BCAAs is associated with decreased frequency of development of hepatocellular carcinoma (HCC) and HCC recurrence after treatment with radiofrequency ablation in patients with cirrhosis.[17,40] Recent animal studies have also suggested an antihepatocarcinogenic activity of BCAAs.[41,42] Animals used in these studies were, however, obese diabetic mice with insulin resistance.[41,42] Because insulin resistance is closely linked to hepatocarcinogenesis,[43] it is possible that BCAAs may inhibit hepatocarcinogenesis through amelioration of insulin resistance. Indeed, suppression of hepatocarcinogenesis is accompanied with significant reduction in insulin resistance in BCAA-treated animals.[41,42] A randomized, controlled trial demonstrated that BCAA supplementation reduces the frequency of development of HCC, but the effect was only evident in patients with cirrhosis who are obese and have hepatitis C virus infection (approximately 30% reduction in the development of HCC in 3 years).[17] Because patients who are obese and infected with hepatitis C virus frequently have insulin resistance,[44,45] these findings also support the hypothesis that BCAAs suppress hepatocarcinogenesis through amelioration of insulin resistance.

Insulin is a carcinogenic factor with mitogenic and cell proliferative effects through activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase pathway.[46] Insulin also cross-reacts with insulin-like growth factor 1 (IGF-1) receptor and further activates the Raf/MAPK kinase/MAPK cascade.[47] Moreover, excess insulin binds to IGF-binding proteins, resulting in increased levels of free serum IGF-1 (Fig. 4).[48] Thus, insulin resistance/hyperinsulinemia enhances hepatocarcinogenesis through multiple pathways. Possible mechanisms for BCAA-induced inhibition of HCC development include: (1) BCAA activation of the insulin signaling cascade through up-regulation of PI3K[2,13,18] with reduction of serum insulin levels (Fig. 4) and (2) inhibition of the IGF/IGF-1R axis by suppressing the expressions of IGF-1, IGF-2, and IGF-1 receptor mRNA (Fig. 4).[41]

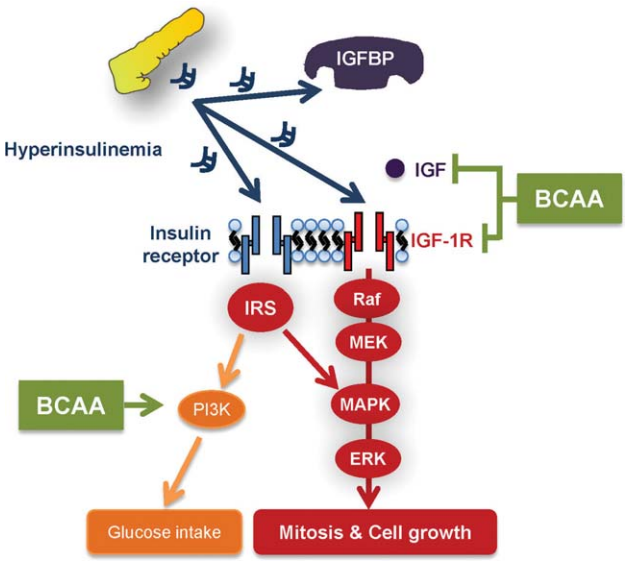

Fig. 4. Molecular mechanisms of the association between hyperinsulinemia and HCC and of BCAA-induced inhibition of hepatocarcinogenesis. As an adaptive response to insulin resistance, pancreatic beta cells secrete excess insulin. Insulin activates mitosis and cell growth through activation of the insulin receptor substrate (IRS)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway. Insulin also cross-reacts with IGF-1 receptor (IGF-1R) and further activates the Raf/MAPK kinase (MEK)/MAPK cascade. Furthermore, excess insulin binds to IGF-binding proteins (IGFBP), resulting in increase in the level of free serum IGF-1. BCAA activates the insulin signaling cascade via up-regulation of PI3K and improves glucose uptake and reduces the serum insulin levels. BCAA also suppresses the IGF/IGF-1R axis through down-regulation of IGF-1, IGF-2, and IGF-1R mRNA expressions, leading to inhibition of mitosis and cell growth.

Besides activation of intracellular insulin and IGF-1 signaling cascade, insulin causes angiogenesis,[42] migration of HCC,[49] and epithelial mesenchymal transition of hepatocytes.[50] Because BCAAs reduce insulin resistance, BCAAs may suppress angiogenesis, migration, and epithelial mesenchymal transition of hepatocytes. BCAAs are also known to attenuate insulin resistance-induced expression of endothelial growth factor and eventually suppress hepatic neovascularization.[42] Thus, the diverse effects of BCAAs on insulin resistance may suppress hepatocarcinogenic activity.

In addition, BCAAs are reported to affect immune function *ex vivo* and *in vivo* studies (Supporting Table

2). In patients with cirrhosis, BCAAs increase liver-associated lymphocyte counts and restore phagocytic function of neutrophils and natural killer activity of lymphocytes.[51] Moreover, BCAA treatment may suppress hepatic oxidative stress by modulating the redox state of albumin.[52,53] Serum albumin is divided into two forms, reduced and oxidized albumin, depending on the redox state at Cys34,[54,55] and the oxidized/reduced albumin ratio increases in patients with cirrhosis.[56,57] BCAA supplementation increases ratio of reduced albumin[52] and decreases iron-related oxidative stress in patients with cirrhosis,[53] suggesting that BCAAs may reduce the iron-induced oxidative stress through a qualitative alteration of serum albumin. Thus, BCAAs may suppress hepatocarcinogenesis partly by improvement of immune function and reduction of oxidative stress.

Mortality and Clinical Decompensation

Some reports suggest that oral BCAA supplementation improves survival in a rat model of cirrhosis and in decompensated patients with cirrhosis.[58-60] Marchesini et al. first performed a randomized, controlled trial exploring the usefulness of BCAAs in patients with cirrhosis.[15] One year of BCAA treatment significantly reduced the occurrence of the primary outcome (a composite of death, number of hospital admissions, and duration of hospital stay) compared to that in the lactalbumin-treated group.[15] Although this study shows the effectiveness of BCAA supplementation, the complications that contributed to the reduction of outcome incidence was not identified because of a small number of enrolled patients (n = 59 in BCAA group) and high dropout rate (15% in the BCAA group) due to poor compliance with the BCAA supplement.

Since 1996, a BCAA supplement formulation (L-Val:L-Leu:L-Ile = 1.2:2:1; Ajinomoto Pharmaceuticals, Tokyo, Japan) has been approved for use in cirrhosis in Japan. The supplement is in the form of small uniform granules, which reduces BCAA-induced stimulation of taste buds and contributes to improved compliance. Using these BCAA granules, Muto et al. performed a large (n = 314 in the BCAA group) randomized, controlled trial.[16] None of the patients discontinued the study because of poor compliance. A preplanned safety analysis revealed that BCAA granules significantly reduced the occurrence of the overall primary outcome (hepatic failure, variceal bleeding, development of liver cancer, and death from any cause) compared to that in the control diet group. Among individual events of primary outcome, the occurrence of hepatic failure was significantly less in the BCAA group compared to the control diet group (hazard ratio 0.45; 95% confidence interval 0.23-0.88; $P = 0.016$). On the basis of the results, the Data and Safety Monitoring Board concluded that the harm associated with the increased occurrence of primary outcome in the control diet group outweigh any potential benefits and the study was discontinued 10 months early due to safety concerns. Beneficial effects of BCAAs on clinical decompensation, including development of hepatic failure, are also reported in patients with cirrhosis accompanied with HCC.[61-63] Thus, the treatment with BCAA supplementation is now recommended in the guidelines for the treatment of liver cirrhosis by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis from the Ministry of Health, Labour and Welfare of Japan.[64]

Quality of Life

Generally, the overall health status and QOL of patients with liver cirrhosis is poor.[65,66] Patients with cirrhosis frequently complain of fatigue and sleep disturbances. There is, however, no standard approach to the management of these symptoms in the absence of overt hepatic encephalopathy.[67] In a randomized study, BCAA-enriched supplements have been reported to improve weakness and easy fatigability compared to ordinary food.[20] BCAA-enriched supplementation has also been reported to improve the Epworth Sleepiness Scale score.[21] In large-scale randomized controlled trials, BCAA supplementation was found to significantly improve the Short Form-36 scores of general health perception compared to control groups.[15,16]

Although it is still unclear how BCAA supplementation provides relief from fatigue and sleep disturbances in patients with cirrhosis, there are at least three possible mechanisms. First, fatigue and sleep disturbances could be caused by minimal hepatic encephalopathy, and BCAA may ameliorate these symptoms by improving this condition.[68] Second, increased serum tryptophan levels are known to impair the QOL in various conditions involving malnourishment, including liver cirrhosis.[69] Tryptophan is a precursor for the neurotransmitter 5-hydroxytryptamine, which is associated with fatigue and sleep disturbances.[70] Because BCAAs compete with tryptophan for transport into the brain, these symptoms may be alleviated by supplementation with BCAAs.[71] Third, impaired cerebral blood flow is associated with fatigue and sleep disturbance[72] and is decreased in patients with liver cirrhosis.[73,74] BCAA supplementation is known to improve cerebral blood flow, possibly resulting in lessened fatigue and sleep disturbances.[75,76]

Muscle cramps are also associated with poor QOL in patients with liver cirrhosis,[77] and the frequency of muscle cramps has been reported to be dramatically reduced by BCAA supplementation over a period of 3 months (7.4 ± 2.0 versus 0.3 ± 0.5 times/week).[78] Muscle cramps are caused by a variety of factors, including diuretic treatment, reduction of circulating volume, and deficiency of vitamin E and taurine.[79] Amino acid imbalance decreases taurine production, and therefore, BCAA may inhibit muscle cramps, possibly through improvement of the imbalance and consequent restoration of taurine production.[78,79]

Conclusion

In this article, we have reviewed evidence for potential pharmaceutical properties of BCAAs on various physiological and clinical events associated with chronic liver disease. Evidence for beneficial effects of BCAA supplementation has yet to be fully validated, and improvement for low compliance of BCAA supplementation is still required. However, there is substantial evidence that depletion of serum BCAA levels is involved in the progression of liver disease and the development of clinically important sequelae. Pharmacological supplementation with BCAAs may be a promising therapeutic strategy for patients with liver cirrhosis.

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