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Potential Role of Branched-Chain Amino Acids in Glucose Metabolism Through the Accelerated Induction of the Glucose-Sensing Apparatus in the Liver

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ABSTRACT

Branched-chain amino acids (BCAAs) have a potential to improve glucose metabolism in cirrhotic patients; however, the contribution of liver in this process has not been clarified. To estimate the effect of BCAA on glucose metabolism in liver, we evaluated the mRNA expression levels of glucose-sensing apparatus genes in HepG2 cells and in rat liver after oral administration of BCAA. HepG2 cells were cultured in low glucose (100 mg/dl) or high glucose (400 mg/dl) in the absence or presence of BCAA. The mRNA expression levels and protein levels of GLUT2 and liver-type glucokinase (L-GK) were estimated using RT-PCR and immunoblotting. The expression levels of transcriptional factors, including SREBP-1c, ChREBP, PPAR-γm and LXRα, were estimated. The mRNA expression levels of transcriptional factors, glycogen synthase, and genes involved in gluconeogenesis were evaluated in rat liver at 3 h after the administration of BCAA. BCAA accelerated the expression of GLUT2 and L-GK in HepG2 cells in high glucose. Expression levels of ChREBP, SREBP-1c, and LXRα were also increased in this condition. BCAA administration enhanced the mRNA expression levels of L-GK, SREBP-1c, and LXRα and suppressed the expression levels of G-6-Pase in rat liver, without affecting the expression levels of glycogen synthase or serum glucose concentrations. BCAA administration enhanced the bioactivity of the glucose-sensing apparatus, probably via the activation of a transcriptional mechanism, suggesting that these amino acids may improve glucose metabolism through the accelerated utility of glucose and glucose-6-phosphate in the liver. J. Cell. Biochem. 112: 30–38, 2011.

KEY WORDS: BCAA; CIRRHOSIS; GLUCOSE INTOLERANCE; GLUT2; L-GK

Patients with liver cirrhosis show hypoproteinemia, ascites, and hepatic encephalopathy, which are induced by the damage of liver cells and portal hypertension. Hypoproteinemia, particularly hypoalbuminemia, is caused by reduced synthesis of albumin in the liver and a deteriorated amino acid balance due to increased protein catabolism [Charlton, 2006]. The primary protein imbalance seen in cirrhosis is characterized by reduced levels of branched-chain amino acids (BCAAs) including valine, leucine, and isoleucine, and elevated levels of aromatic amino acids [Blonde-Cynober et al., 1999]. Oral administration of a BCAA preparation has been proved to improve event-free survival, serum albumin concentration, and quality of life in patients with decompensated cirrhosis [Marchesini et al., 2003; Muto et al., 2005].

In addition to the effects of BCAA on the prevention of protein imbalance in cirrhosis, therapeutic potentials of BCAA to improve glucose metabolism have been reported. In patients with chronic liver disease, BCAA supplementation improved homeostasis model assessment of insulin resistance (HOMA-IR) [Kawaguchi et al., 2008] and decreased plasma glucose levels in glucose tolerance test [Korenaga et al., 2008]. In animal models, BCAA supplementation improved the response in glucose tolerance tests in rats with liver cirrhosis [Nishitani et al., 2005] and in normal and diabetic mice [Ikehara et al., 2008]. It is believed that the effect of BCAA on glucose metabolism is mainly due to enhanced glucose uptake in skeletal muscles through the accelerated translocation of glucose transporters to plasma membrane [Nishitani et al., 2002, 2005].

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However, the contribution of the liver in this process has not been clarified.

The liver plays a role in the uptake of blood glucose as by skeletal muscles. The primary transporter for glucose uptake in the liver is GLUT2. Glucose is taken up into the hepatocytes through GLUT2, and liver-type glucokinase (L-GK) traps glucose in the cytoplasm by phosphorylation. Therefore, GLUT2 and L-GK play important roles in the liver as the glucose-sensing apparatus [Kim and Ahn, 2004]. Glucose and glucose-6-phosphate are known to regulate the transcription of genes involved in the major metabolic pathways in the liver [Girard et al., 1997]. The glucose-sensing apparatus enables glucose to regulate the expression of glucose-responsive genes such as L-type pyruvate kinase, S14, fatty acid synthase (FAS) and GLUT2 [Leturque et al., 2005]. Thus, the glucose-sensing apparatus exerts a strong influence on glucose utilization and glycogen synthesis.

Therefore, we hypothesized that BCAA may affect glucose metabolism by improving the bioactivity of the glucose-sensing apparatus in the liver. From this perspective, we determined the effects of BCAA on the expression levels of GLUT2 and L-GK in HepG2 cells. The mRNA expression levels of transcriptional factors, which are known to regulate the expression of GLUT2 and L-GK, were also estimated. The effects of BCAA on the hepatic mRNA expression levels of the glucose-sensing apparatus, transcriptional factors, glycogen synthase, and genes involved in gluconeogenesis were also estimated in rats that were orally administered with BCAA. Our results showed that BCAA strongly accelerated GLUT2 and L-GK mRNA expression in HepG2 cells in a glucose-dependent manner, and dose-dependently enhanced the mRNA levels of L-GK in rat liver. The enhanced bioactivity of the liver glucose-sensing apparatus by BCAAs may have a role in the improvement of systemic glucose metabolism.

MATERIALS AND METHODS

MATERIALS

The BCAA mixture (2:1:1.2 leucine/isoleucine/valine) and Dulbecco's modified Eagle's minimum essential medium (DMEM) and BCAA-free DMEM (ΔBCAA-DMEM) were generously supplied by Ajinomoto Co., Ltd (Tokyo, Japan). The concentration of each amino acid in ΔBCAA-DMEM was as follows (in mM): L-glycine, 0.40; L-alanine, 0.40; L-serine, 0.40; L-threonine, 0.80; L-cystine 2HCl, 0.20; L-methionine, 0.20; L-glutamine, 4.00; L-asparagine, 0.40; L-glutamic acid, 0.40; L-aspartic acid, 0.40; L-phenylalanine, 0.40; L-tyrosine, 0.40; L-tryptophan, 0.08; L-lysine HCl, 0.80; L-arginine HCl, 0.40; L-histidine HCl–H_2O, 0.20; L-proline, 0.40. Serum glucose was estimated using QuantiChrom glucose assay kit (BioAssay Systems, Hayward, CA). Anti-GLUT2 and anti-L-GK antibodies were purchased from Chemicon International, Inc. (Temecula, CA). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

CELL CULTURE AND TREATMENT

The human hepatoblastoma derived cell line HepG2 was cultured in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were seeded at 10,000 cells/cm^2 on Costar six-well plates or 10-cm dishes. After an overnight culture with serum-deprived medium, HepG2 cells were divided into four different conditions; in the absence of BCAAs (ΔBCAA-DMEM) with 100 or 400 mg/dl glucose, in the presence of BCAAs (ΔBCAA-DMEM supplemented with BCAA mixture) with 100 or 400 mg/dl glucose. The final concentration of each BCAA was 2 mM after supplementation with the BCAA mixture. The cells were incubated for 48 h and used for real-time PCR (RT-PCR) or Western blotting.

RT-PCR

Total RNA was prepared with TRIzol reagent (Invitrogen, CA) and cDNA was synthesized from 1.0 μg RNA with GeneAmp RNA PCR (Applied Biosystems, NJ) using random hexamers. RT-PCR was performed using LightCycler-FastStart DNA Master SYBR Green 1 (Roche, Switzerland) according to the manufacturer's instructions. The reaction mixture (20 μl) contained LightCycler-FastStart DNA Master SYBR Green 1, 4 mM $MgCl_2$, 0.5 mM of the upstream and downstream PCR primers, and 2 μl of first-strand cDNA as a template. To control for variations in the reactions, all PCR data were normalized against β-actin expression.

WESTERN BLOTTING OF THE GLUCOSE-SENSING APPARATUS

Protein samples were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under standard reducing conditions. The separated proteins were transferred by electrophoresis onto Immobilon-P polyvinylidene difluoride transfer membranes (Millipore Corp., Billerica, MA). The membranes were incubated with antibodies for GLUT2 or L-GK. Horseradish-peroxidase-conjugated anti-rabbit IgG antibody was used as the secondary antibody (GE Healthcare Bio-Science Corp., Piscataway, NJ), and signals were detected using the ECL Plus system (GE Healthcare Bio-Science Corp., Uppsala, Sweden).

ANIMAL TREATMENT

Eight- to 10-week-old male Wister rats weighing 175–225 g were obtained from Charles River Japan (Yokohama, Japan). They were maintained under controlled conditions with free access to standard chow and water. During all experimental procedures, the animals were given human care in compliance with institutional guidelines according to the "*Guide for the Care and Use of Laboratory Animals*" published by the National Institutes of Health. After fasting for 17 h, the rats were given a compulsory oral 2 g/kg glucose load by gavage administration and, 1 h later, administration of 0.75 or 1.5 g/kg BCAA mixture (2:1:1.2 leucine/isoleucine/valine) or saline. The composition of amino acids in the BCAA mixture was chosen by referring to BCAAs granules, which are used to treat cirrhotic patients in Japan and have been proved to improve glucose metabolism in cirrhotic rat [Nishitani et al., 2005]. Blood was taken from the tail vain to estimate glucose levels at the beginning, and at 1, 2, and 3 h after glucose administration. At 3 h after the BCAA administration, total RNA was isolated from the liver, and RT-PCR was performed. Eight rats were examined in each group.

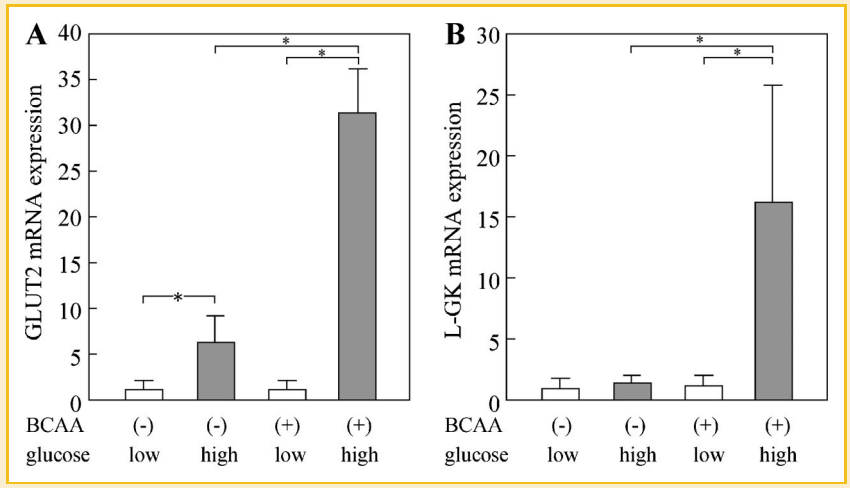

Fig. 1. RT-PCR analyses of mRNA expression levels of the glucose-sensing apparatus in HepG2 cells in the presence or absence of BCAA. GLUT2 (A) and L-GK (B). Total RNA was extracted from HepG2 cells cultured for 48 h in the absence of BCAA (ΔBCAA-DMEM) with low (100 mg/dl) or high glucose (400 mg/dl), or in the presence of BCAA (ΔBCAA-DMEM supplemented with BCAA mixture) with low or high glucose. The final concentration of each BCAA (valine, leucine, and isoleucine) was 2 mM in ΔBCAA-DMEM supplemented with the BCAA mixture. Samples were analyzed by RT-PCR and all PCR reactions were normalized for β-actin expression. The ratio of the mean expression of cells cultured in ΔBCAA-DMEM with low glucose was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 4$).

STATISTICAL ANALYSIS

The results are expressed as means ± standard deviation. Significant differences between the two groups were assessed using unpaired two-tailed *t*-tests. A value of $P < 0.05$ was considered significant.

RESULTS

BCAA ACCELERATED THE mRNA EXPRESSION OF GLUT2 AND L-GK IN A GLUCOSE-DEPENDENT MANNER

To analyze the effects of BCAA on the bioactivity of glucose sensing apparatus in HepG2 cells, the mRNA expression levels of GLUT2 and L-GK were estimated by RT-PCR. To control for variations in the reactions, all PCR data were normalized against β-actin expression, which expression is thought to be stable in nutritionally different condition, but is reported to be modified in the change of osmolarity [Husson et al., 1996]. Therefore, the mRNA expression levels of β-actin in HepG2 cells in the presence or absence of BCAA with low or high glucose were evaluated. The expression levels of β-actin among these groups were almost same and significant differences were not observed in these conditions (data not shown). In the absence of BCAA, the expression levels of GLUT2 were significantly higher in the high glucose condition (400 mg/dl) than in the low glucose condition (100 mg/dl) by more than fivefold. The addition of BCAA did not affect the expression levels of GLUT2 in the low glucose condition; however, BCAA strongly increased the expression of GLUT2 by more than 30-fold in the high glucose condition (Fig. 1A). The expression levels of L-GK were not increased in the high glucose condition in the absence of BCAA; however, the addition of BCAAs strongly increased the expression of L-GK by more than 15-fold (Fig. 1B). The effects of BCAA on the expression of the glucose-sensing apparatus were confirmed by immunoblotting (Fig. 2). The protein levels of both GLUT2 and L-GK were enhanced in the presence of BCAA in the high glucose condition. These results suggest that the presence of BCAA in the high glucose condition may be necessary to promote the maximal expression of the glucose-sensing apparatus in HepG2 cells.

BCAA ENHANCED THE mRNA EXPRESSION LEVELS OF SREBP-1C, ChREBP, AND LXRα IN A GLUCOSE-DEPENDENT MANNER

To study the transcriptional regulation of the glucose-sensing apparatus, the expression levels of transcriptional factors, which were predicted to regulate the expression of GLUT2 and L-GK, were estimated. Carbohydrate responsive element binding protein (ChREBP) is a glucose-signaling transcriptional factor by which glycolytic and lipogenic genes are reciprocally regulated by glucose and fatty acids in the liver [Dentin et al., 2006]. Sterol response element binding protein (SREBP)-1c regulates the transcription of

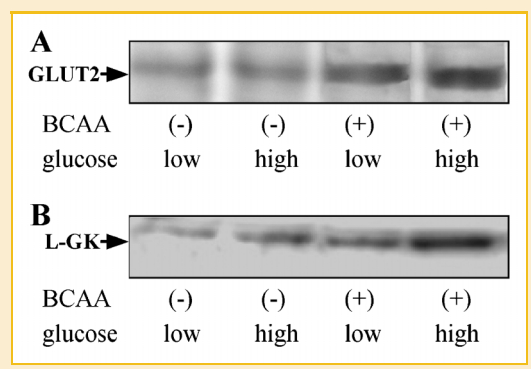

Fig. 2. Immunoblotting analyses of the glucose-sensing apparatus in the presence or absence of BCAA in HepG2 cells. Western blotting using the anti-GLUT2 antibody (A) or anti-L-GK antibody (B) was performed using cell lysates from cells cultured in the absence of BCAA (ΔBCAA-DMEM) with low or high glucose, and in the presence of BCAA (ΔBCAA-DMEM supplemented with BCAA mixture) with low or high glucose. The data shown are representative of three independent experiments.

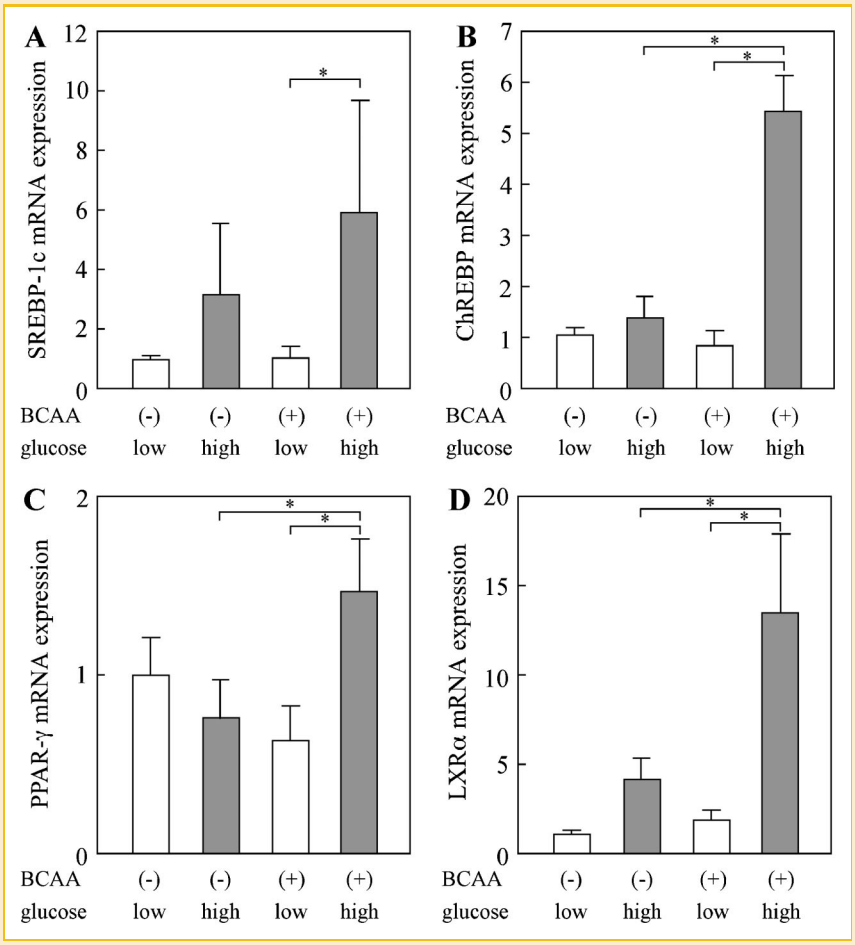

Fig. 3. RT-PCR analyses of mRNA expression levels of transcriptional factors in HepG2 cells in the presence or absence of BCAA. SREBP-1c (A), ChREBP (B), PPAR-γ (C), and LXRα (D). Total RNA was extracted from HepG2 cells cultured for 48 h in the absence of BCAA (ΔBCAA-DMEM) with low or high glucose, or in the presence of BCAA (ΔBCAA-DMEM supplemented with BCAA mixture) with low or high glucose. Samples were analyzed by RT-PCR and all PCR reactions were normalized for β-actin expression. The ratio of the mean expression in cells cultured in ΔBCAA-DMEM with low glucose was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 4$).

many genes involved in cholesterol and fatty acid synthesis [Horton et al., 2002]. The promoter regions of both GLUT2 and L-GK contain functional SREBP-1c binding sites, and SREBP-1c activates the mRNA expression of GLUT2 and L-GK [Iynedjian et al., 1988; Magnuson et al., 1989]. Peroxisome proliferator-activated receptor (PPAR)-γ regulates insulin sensitivity either by systemic insulin sensitization or by direct action on the transcription of genes involved in glucose disposal [Im et al., 2005]. PPAR response element (PPRE) binding sites exist in both the GLUT2 and L-GK promoter region, and PPAR-γ is involved in the upregulation of these genes [Kim and Ahn, 2004]. In the absence of BCAA, the expression levels of SREBP-1c were weakly increased in the high glucose condition by threefold. In the presence of BCAA, greater expression levels of SREBP-1c (by more than fivefold) were observed in the high glucose condition (Fig. 3A). The expression levels of ChREBP were not affected by glucose in the absence of BCAAs; however, the addition of BCAA accelerated the expression by more than fivefold in the high glucose condition (Fig. 3B). The expression levels of PPAR-γ were not affected by glucose in the absence of BCAA, and the addition of BCAA weakly increased the expression of PPAR-γ in the high glucose condition (Fig. 3C). The expression pattern of liver X receptor (LXR) α, which is thought to be a master regulator for glucose and lipid metabolism, was similar to the expression pattern of ChREBP; the greatest expression was observed in the presence of BCAA in the high glucose condition (Fig. 3D). These results suggest that upregulation of transcriptional factors may be involved in the increased bioactivity of the glucose-sensing apparatus.

BCAA ENHANCED THE EXPRESSION LEVELS OF LIPOGENIC GENES IN A GLUCOSE-DEPENDENT MANNER

The effects of BCAA on the upregulation of transcriptional factors involved in glucose and lipid metabolism, suggest that BCAA may influence the expression levels of lipogenic genes. Therefore, we analyzed the expression levels of acetyl-CoA carboxylase (ACC) 1 and FAS, which are known downstream targets of SREBP-1c and/or ChREBP (Fig. 4). The expression of ACC1 did not change in response to glucose in the absence of BCAA. However, the addition of BCAA increased the expression levels of ACC1 in the high glucose condition (Fig. 4A). The expression levels of FAS were increased by

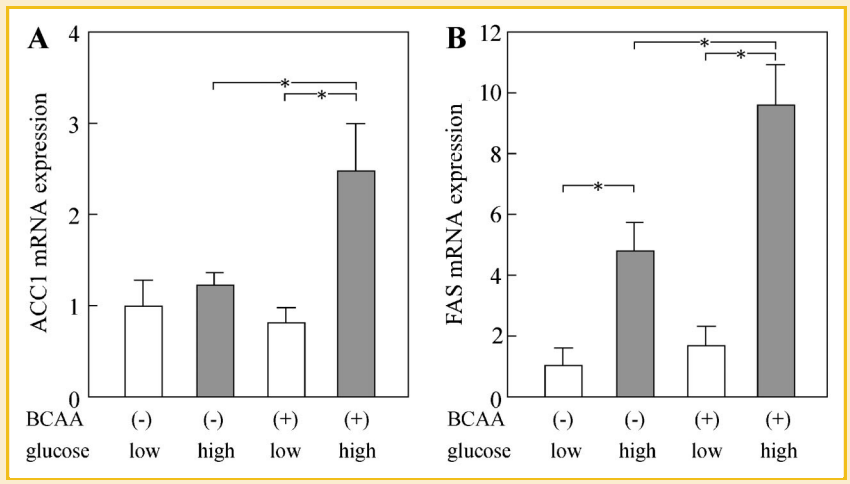

Fig. 4. RT-PCR analyses of mRNA expression levels of lipogenic genes in HepG2 cells in the presence or absence of BCAA. Lipogenic genes, whose expression levels are regulated by SREBP-1c and ChREBP, were analyzed. ACC1 (A) and FAS (B). Total RNA was extracted from HepG2 cells cultured in the absence of BCAAs (ΔBCAA-DMEM) with low or high glucose, or in the presence of BCAAs (ΔBCAA-DMEM supplemented with BCAA mixture) with low or high glucose. Samples were analyzed by RT-PCR and all PCR reactions were normalized for β-actin expression. The ratio of the mean expression in cells cultured in ΔBCAA-DMEM with low glucose was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 4$).

more than fourfold in the high glucose condition in the absence of BCAA, and the addition of BCAA induced maximal expression of FAS (ninefold greater expression) in the high glucose condition (Fig. 4B). These results suggest that BCAA-induced upregulation of transcriptional factors may be physiologically active to transactivate lipogenic genes.

ORAL BCAA ADMINISTRATION INCREASED THE EXPRESSION LEVELS OF L-GK, BUT NOT GLUT2 IN RAT LIVER

To estimate the effects of BCAA in vivo, we analyzed the mRNA expression levels of GLUT2 and L-GK in rat liver after oral administration of glucose and BCAA. Because the presence of abundant glucose seemed to be necessary for the maximal effects of BCAA, 2 g/kg of glucose was administered 1 h before BCAA administration. The hepatic expression levels of L-GK were increased dose-dependently by BCAA (Fig. 5A). However, BCAA did not increase the hepatic expression levels of GLUT2 (Fig. 5B).

BCAA ENHANCED THE mRNA EXPRESSION LEVELS OF SREBP-1c AND LXRα IN RAT LIVER

The mRNA expression levels of transcriptional factors were estimated in rat liver. In accordance with the observations in HepG2 cells, the expression levels of SREBP-1c were significantly increased by BCAA administration (Fig. 6A). The expression levels

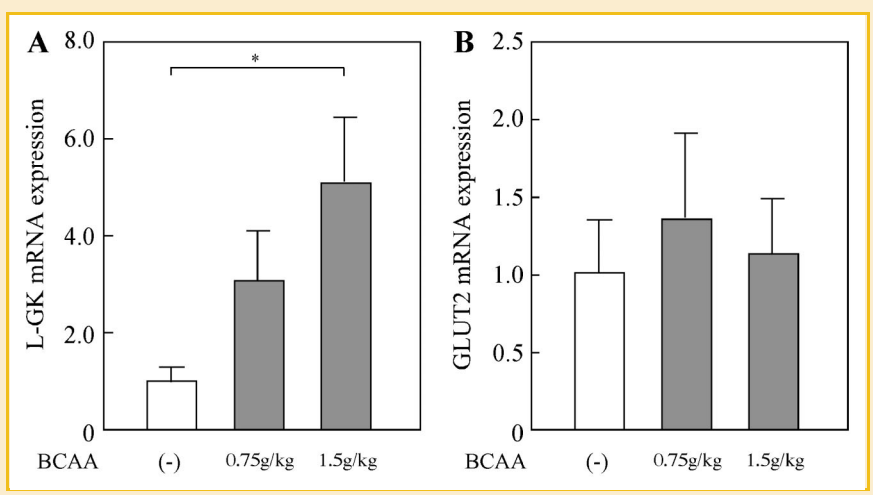

Fig. 5. RT-PCR analyses of hepatic mRNA expression levels of the glucose-sensing apparatus in rats administered with BCAA. Eight- to 10-week-old male Wister rats were orally administrated with 0.75 or 1.5 g/kg of BCAA mixture (2:1:1.2 leucine/isoleucine/valine) 1 h after the administration of 2.0 g/kg glucose. Control rats were given saline instead of BCAA. Total RNA was isolated 3 h after the BCAA challenge, and RT-PCR analyses were performed. L-GK (A), and GLUT2 (B). All PCR reactions were normalized for β-actin expression. The ratio of the mean expression in animals without BCAA was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 8$).

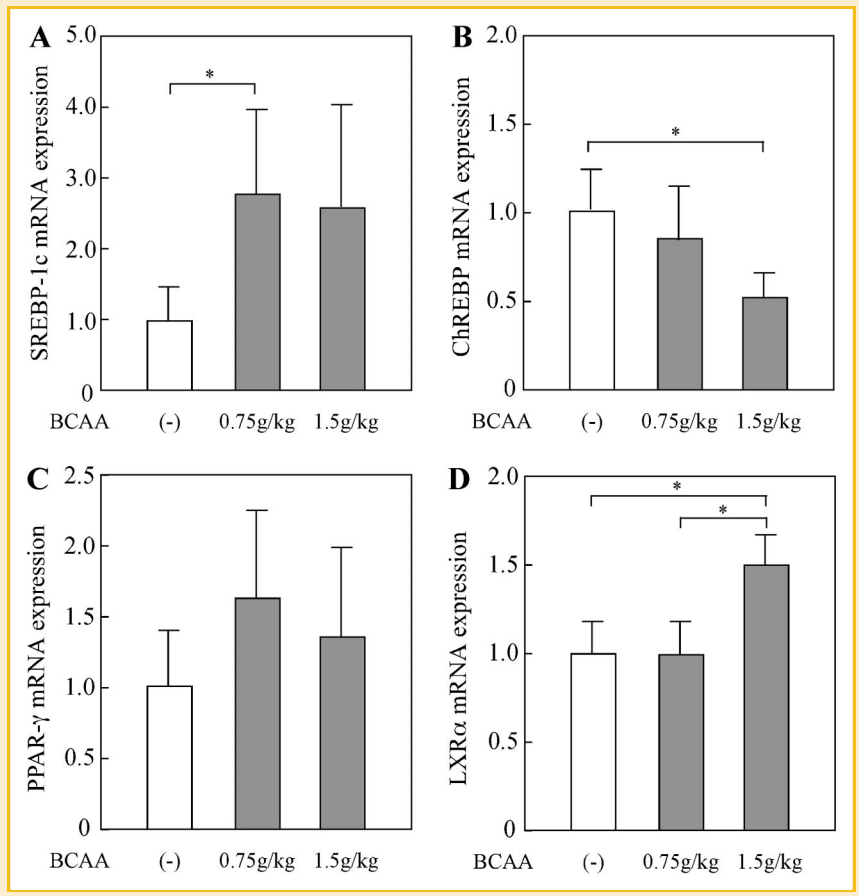

Fig. 6. RT-PCR analyses of hepatic mRNA expression levels of transcriptional factors in rats administered with BCAA. SREBP-1c (A), ChREBP (B), PPAR-γ (C), and LXRα (D). Wister rats were administered with 0.75 or 1.5 g/kg of BCAA mixture 1 h after the administration of 2.0 g/kg glucose. Control rats were given saline instead of BCAA. Total RNA was isolated 3 h after the BCAA challenge, and RT-PCR analyses were performed. All PCR reactions were normalized for β-actin expression. The ratio of the mean expression in animals without BCAA was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 8$).

of ChREBP, which were enhanced by BCAA in the high glucose condition in HepG2 cells, were not increased, but were actually decreased by BCAA administration (Fig. 6B). The expression levels of PPAR-γ were unaffected by BCAA administration. As observed in HepG2 cells, the expression levels of LXRα were significantly increased by 1.5 g/kg BCAA (Fig. 6D), but not by 0.75 g/kg BCAA.

ORAL BCAA ADMINISTRATION DID NOT AFFECT THE HEPATIC mRNA EXPRESSION LEVELS OF GLYCOGEN SYNTHASE OR SERUM GLUCOSE CONCENTRATIONS IN RATS

To estimate the effects of BCAA on glycogen synthesis, the hepatic mRNA expression levels of glycogen synthase, which catalyzes glucose residues into a polymeric chain for storage as glycogen, were analyzed in rat administered with BCAA. BCAA administration did not significantly affect the expression of glycogen synthase (data not shown). Then, the time course of serum glucose levels after BCAA administration was examined. All the animals were orally administered with 2 g/kg glucose, followed by the administration of 0.75 or 1.5 g/kg BCAA or saline 1 h after glucose administration. The serum glucose levels were not significantly different between the groups of rats without BCAA, or with 0.75 or 1.5 g/kg BCAA (Fig. 7).

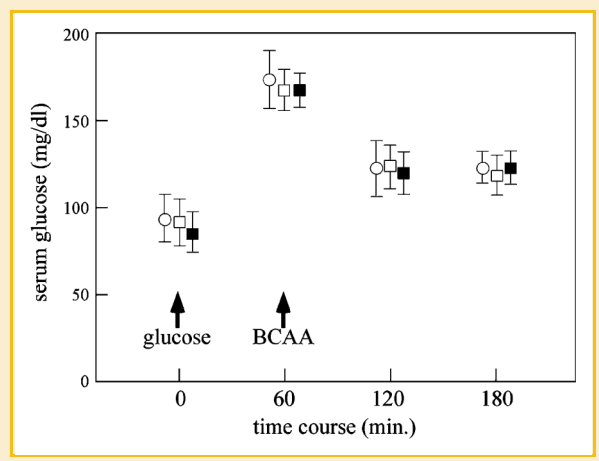

Fig. 7. Time course of serum glucose levels after the administration of BCAA. Wister rats were administered with 0.75 or 1.5 g/kg of BCAA mixture 1 h after the administration of 2.0 g/kg glucose. Control rats were given saline instead of BCAAs. Blood was taken from the tail vain for glucose measurements before glucose administration, and at 1 (before BCAAs administration), 2 and 3 h after glucose administration. The symbols used are: ○, serum glucose in rats without BCAA administration; □, with 0.75 g/kg of BCAA; ■, with 1.5 g/kg of BCAA ($n = 8$).

BCAA REDUCED THE mRNA EXPRESSION LEVELS OF G-6-PASE, BUT NOT PEPCK IN RAT LIVER

The mRNA expression levels of the key enzymes regulating gluconeogenesis were estimated in rat liver. Phosphoenolpyruvate carboxykinase (PEPCK) decarboxylates and phosphorylates oxaloacetate into phosphoenolpyruvate, one of the earliest, rate-limiting steps in gluconeogenesis. Glucose-6-phosphatase (G-6-Pase) catalyzes the final steps of gluconeogenesis, the production of free glucose from glucose 6-phosphate. The administration of 0.75 and 1.5 g/kg of BCAA markedly suppressed the mRNA expression levels of G-6-Pase (Fig. 8A). However, BCAA did not affect the expression levels of PEPCK (Fig. 8B).

DISCUSSION

It is generally accepted that one of the features of BCAA administration is modification of glucose metabolism. The blood glucose-lowering effects of BCAA in patients with chronic liver disease have been reported [Kawaguchi et al., 2008; Korenaga et al., 2008]. However, the detailed mechanism of BCAA in glucose metabolism is not fully understood. Previous reports using animal models suggest that the promotion of glucose uptake in skeletal muscles by BCAA is one of the main mechanisms responsible for the decrease in blood glucose [Nishitani et al., 2002, 2005]. However, the contribution of the liver to the BCAA-induced glucose lowering effects has not been clarified. In this study, we showed elevated bioactivity of the glucose-sensing apparatus, particularly L-GK, in HepG2 cells and in rat liver, presenting a clue to explore the effects of BCAA in the liver.

The glucose-sensing apparatus, particularly GLUT2 and glucokinase, recognizes the carbohydrate environment and regulates cellular glucose metabolism. In particular, glucokinase in the liver and in pancreatic β-cells is involved in the regulation of glucose metabolism. In β-cells, glucokinase plays a role as a glucose sensor to stimulate glucose-dependent insulin secretion. In the liver, which contains 99.9% of the total glucokinase in the body, L-GK regulates hepatic glycolysis, glucose oxidation, the pentose phosphate shunt, glycogen synthase, glycogenolysis, gluconeogenesis, and the synthesis of triacylglycerols [Matschinsky, 2009]. This regulatory activity of glucokinase has prompted the development of glucokinase activators to treat type 2 diabetes mellitus (T2DM). Compounds analyzed as candidates for glucokinase activators show their activities to stimulate insulin secretion, increase glucose uptake, augment hepatic glycogen synthesis, and reduce hepatic glucose output. From this aspect, the upregulated expression of L-GK by BCAA may play a role on the reduction of blood glucose by enhanced glycogen synthesis or reduced gluconeogenesis. In our experiments, reduced expression of G-6-Pase in the liver was observed in the BCAA treated animals, suggesting that BCAA may play a role in the suppression of gluconeogenesis. However, the hepatic expression of glycogen synthase was not increased by BCAA administration, which may explain why the blood glucose concentrations were not affected. It has been reported that chronic BCAA administration increases muscle and hepatic glycogen content in the rats supplemented with BCAA for 6 weeks [de Araujo et al., 2006]; therefore, long-term administration of BCAA may be necessary to increase hepatic glycogen synthesis and lower the blood glucose.

The biological activity of the glucose-sensing apparatus is tightly regulated by transcriptional mechanisms. The transcriptional factors SREBP-1c and PPAR-γ are thought to be involved in the transcriptional regulation of the glucose-sensing apparatus because the functional binding sites for SREBP-1c and PPAR-γ were identified in the GLUT2 and L-GK promoter regions, and the mRNA expression of these genes are upregulated by SREBP-1c and PPAR-γ [Iynedjian et al., 1988; Magnuson et al., 1989; Kim and Ahn, 2004; Im et al., 2005]. In our study, BCAA markedly upregulated expression of SREBP-1c, ChREBP, and LXRα in HepG2 cells, and

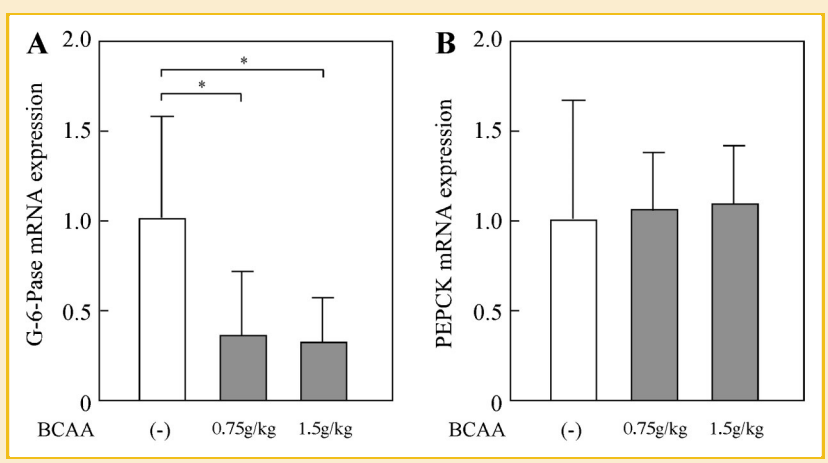

Fig. 8. RT-PCR analyses of hepatic mRNA expression levels of genes involved in gluconeogenesis in rats administered with BCAA. G-6-Pase (A), and PEPCK (B). Wister rats were administered with 0.75 or 1.5 g/kg of BCAA mixture 1 h after the administration of 2.0 g/kg glucose. Control rats were given saline instead of BCAA. Total RNA was isolated 3 h after the BCAA challenge, and RT-PCR analyses were performed. All PCR reactions were normalized for β-actin expression. The ratio of the mean expression in animals without BCAA was used as a control (onefold). $^{*}P < 0.05$; statistically significant differences ($n = 8$).

upregulated SREBP-1c and LXRα in rat liver. Because LXRα is known to transactivate SREBP-1c and ChREBP in the presence of glucose or glucose-6-phosphate, as direct agonists, the increased glucose or glucose-6-phosphate, through the activation of the glucose-sensing apparatus by BCAA, may have a role in the activation of LXRα. From these in vitro and in vivo results, it is speculated that the LXRα-induced SREBP-1c-dependent mechanism may be the main signaling pathway for BCAA-induced transactivation of the glucose-sensing apparatus. The increased expression of ACC and FAS, both are established to be transcriptionally induced by SREBP-1c, may reinforce the LXRα-induced SREBP-1c dependent mechanism is physiologically active to upregulate GLUT2 and L-GK in the presence of BCAA.

The mRNA expression levels of GLUT2 were upregulated by BCAA in HepG2 cells, but not in rat liver. The reason for this discrepancy in GLUT2 expression between HepG2 cells and rat liver is currently unknown. The hepatic levels of GLUT2 in states of impaired glucose metabolism reported so far are not consistent. Oka et al. [1990] reported increased hepatic GLUT2 in streptozocin-induced diabetic rats; however, Thorens et al. [1990] found little or no change in hepatic GLUT2 mRNA or protein levels in a similar diabetic rat model. It is predicted that the animal model, age or sensitivity to nutrition may account for the inconsistent GLUT2 expression and a further unknown mechanism may be involved in the regulation of GLUT2 expression. Therefore, we speculate that an additional mechanism, which regulates GLUT2 but not L-GK, is involved in GLUT2 expression in the liver. Another reason for the discrepancy in GLUT2 expression between HepG2 cells and rat liver may be induced by the metabolic features of HepG2 cells, which are derived from human hepatoblastoma. However, many studies investigating glucose and lipid metabolism in HepG2 cells have been reported and the metabolic reactions of HepG2 cells in these fields are comparable in other models [Kim and Ahn, 1998; Sugimoto et al., 2007; Luo et al., 2009]. Therefore, we believe that the metabolic reactions in HepG2 cells in this study are mostly physiological. The experiments using hepatocytes from primary culture might be useful to resolve the problems; however, the phenotypic instability of hepatocytes and the high batch-to-batch functional variability of hepatocyte preparations from the liver may reduce the reproducibility of results [Donato et al., 2008].

Unfortunately, we can not clarify the exact mechanism why the high concentration of glucose and BCAA induced the synergic action on the upregulation of glucose sensing apparatus. However, we could hypothesize for this action including the participation of PKB/Akt signaling to regulate the genes, especially for glucokinase. The upregulation of glucokinase is induced by Insulin/IGF-1 signaling through the rapid phosphorylation of PKB/Akt and FoxO1, a known target of Akt signaling [Yoshida et al., 2007]. High concentration of glucose is reported to induce the activation of ERK1/2 and PKB/Akt [Kim et al., 2006]. Therefore, high concentration of glucose may induce the upregulation of glucokinase through the activation of PKB/Akt signaling. Leucine, one of the amino acids of BCAA, is known to stimulate protein synthesis via the activation of the mammalian target of rapamycin (mTOR)/p70 ribosomal S6 kinase (p70S6K) axis independent of PKB/Akt signaling in muscle cells [Sanchez Canedo et al., 2010]. However, in some types of cells, leucine stimulates protein synthesis via the activation of PKB/Akt signaling [Perez de Obanos et al., 2006; Lee et al., 2008]. Taken together, if leucine can activate PKB/Akt signaling in hepatocytes or mTOR/p70S6K axis can directly stimulate the downstream of PKB/Akt, for example, FoxO1, leucine may induce the upregulation of L-GK, and this action of leucine may be synergistically reinforced by the presence of high concentration of glucose. Further investigation is necessary to clarify the mechanism regulating the synergic action of BCAA and high concentration of glucose on the expression of glucose sensing apparatus.

In this study, we demonstrated that BCAA administration upregulates the glucose-sensing apparatus in the liver. The increased utilization of glucose and glucose-6-phosphate in the liver may enhance the systemic glucose metabolism and energy balance. Further studies are needed to clarify the detailed mechanism of BCAA on glucose metabolism and the therapeutic significance of BCAA in patients with deteriorated glucose metabolism.

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