Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease

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Abstract. Increased insulin resistance is a therapeutic target in patients with chronic liver disease. Branched-chain amino acids (BCAA) have been reported to improve insulin resistance in *in vivo* experiments. Thus, we investigated the effects of BCAA on insulin resistance in patients with chronic liver disease. Twelve patients with chronic liver disease were enrolled. Each patient was given one sachet of a BCAA-enriched supplement after breakfast and another at bedtime. The effects of the BCAA-enriched supplementation on insulin resistance were examined 30, 60 and 90 days after administration by the homeostasis model assessment method for insulin resistance (HOMA-IR) and for ß cell function (HOMA-%B). The HOMA-IR and HOMA-%B values were elevated at baseline, however, these parameters showed no significant changes after administration of the BCAA-enriched supplement in the overall patient population. By stratification via gender, patients in the male group showed a significantly greater elevation in the HOMA-IR value compared to the female patients at baseline. After the administration, the HOMA-IR and HOMA-%B values were significantly decreased only in the male group (9.4±4.8 vs. 2.4±0.7, 657±345 vs. 126±36, respectively; P<0.05). We found that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, a BCAA-enriched supplement improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, a BCAA-enriched supplement may be a useful therapeutic agent for decreasing insulin resistance in male patients with chronic viral liver disease.

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Introduction

The liver is one of the major target organs of insulin and chronic liver disease is associated with insulin resistance (1-3). Increased insulin resistance is related to the progression of hepatic fibrosis (4), development of hepatocellular carcinoma (HCC) (5,6) and reduction in long-term survival (6,7). Thus, the increase in insulin resistance is an important therapeutic target in patients at any stage of chronic liver disease.

Insulin resistance is treated by dietary modification, physical activity and/or drugs (8). However, a sufficient energy intake is required for patients with liver cirrhosis and dietary restrictions may lead to a decrease in liver function (9,10). Although physical activity is not restricted in compensated cirrhotic patients (11), such individuals often complain of fatigue, thus adequate exercise is not always possible. Biguanides and thiazolidinediones are insulin sensitizing agents and are currently utilized for the reduction of insulin resistance (12,13). However, it is not always possible to use these drugs in cirrhotic patients due to adverse effects, including lactic acidosis, fluid retention and severe hepatotoxicity (14,15).

Decreases in serum branched-chain amino acids (BCAA) levels are often seen in patients with chronic liver disease and lead to a decline of detoxified ammonia and albumin production. Therefore, BCAA are used for the treatment of hepatic encephalopathy and hypoalbuminemia (16,17). Previously, BCAA have been reported to modulate insulin signaling in an *in vivo* study. BCAA cause glucose up-take in the skeletal muscle, adipocytes and hepatocytes in rodents and in a rat model of liver cirrhosis (18-21). In addition, BCAA are known to up-regulate the mammalian target of rapamycin (mTOR), which cross-talks with intracellular insulin signaling (22,23). Taken together, these previous studies imply that BCAA improve glucose metabolism through the reduction of insulin resistance. In this study, we examined the effects of BCAA on insulin resistance in patients with chronic viral liver disease.

Materials and methods

A prospective, consecutive-patient entry study was conducted. Eligibility criteria were chronic viral liver disease with

Table I. Contents of one sachet of BCAA-enriched supplement (Aminofeel®).

Substance	Amount
BCAA	3200.0 mg
Valine	800.0 mg
Leucine	1600.0 mg
Isoleucine	800.0 mg
Calcium	22.1 mg
Magnesium	12.6 mg
Zinc	5.0 mg
Copper	0.2 mg
Selenium	49.6 μg
Chromium	14.4 μg
Pantothenic acid calcium	6.8 mg
Vitamin A	315.0 μg
Vitamin B1	2.4 mg
Vitamin B2	2.6 mg
Vitamin B6	2.4 mg
Vitamin B12	10.0 μg
Folic acid	0.2 mg
Vitamin C	40.0 mg
Vitamin D3	3.0 μg
Vitamin E	6.4 mg
Vitamin K2	29.6 μg
Niacin	12.0 mg

Table II. Characteristics of patients.

	Normal range	All patients
n		12
Age	n/a	64.3±2.4
BMI (kg/m^2)	18.5-25.0	24.3±0.6
Fat%BW (%)	20-27	32.8±2.4
Muscle%BW (%)	40-50	36.0±1.6
Visceral fat area (cm^2)	<100	118.2±14.9
Hemoglobin (g/dl)	14.0-18.0	13.6±0.4
White blood cell (μl)	4000-9000	4358±414
Platelet count (x10^4/μl)	13-36	15.3±1.6
AST (U/l)	12-33	43.1±5.6
ALT (U/l)	8-42	39.8±7.3
LDH (U/l)	119-229	190.8±13.0
γ-GTP (U/l)	10-47	49.7±8.3
Total protein (g/dl)	6.7-8.3	7.7±0.2
Albumin (g/dl)	4.0-5.0	3.8±0.1
BTR	4.4-10.1	5.1±0.4
BCAAs (μmol/l)	344-713	435.6±27.5
Tyrosine (μmol/l)	51-98	90.1±7.9
Cholinesterase (U/l)	214-466	155.8±16.1
Total bilirubin (mg/dl)	0.3-1.5	0.9±0.1
Total cholesterol (mg/dl)	128.0-220.0	178.5±9.1
Fasting glucose (mg/dl)	80.0-109.0	104.5±6.4
HbA1c (%)	4.3-5.8	5.5±0.2
IRI (μU/ml)	5.0-20.0	22.8±9.7
HOMA-IR	<5.4	5.5±2.1
HOMA-%B	>156.5	326.4±159.1
Zinc (μg/dl)	80-130	82.9±6.1

The values are expressed as mean ± standard error. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

sufficient food intake and serum albumin concentration >3.5 g/dl and <4.0 g/dl. Patients with hepatic encephalopathy, ascites, HCC or renal failure were excluded. A total of 12 patients with HCV-related chronic liver disease (n=11), or HBV-related chronic liver disease (n=1) were enrolled in this study from August 2006 to June 2007 at Kurume University Hospital. The diagnosis of liver disease was based on clinical, serological, imaging and/or histological evidence. The patients were treated as outpatients and no therapeutic interventions such as changes in eating habits and physical activity were made in the patients' life-style after entering the study. Informed consent for participation in the study was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the subjects were institutionalized.

Study design. Each patient was given one sachet of a BCAA-enriched supplement (Aminofeel®, Seikatsu Bunkasya Co. Inc, Chiba, Japan) after breakfast and another at bedtime. The contents of this supplement are summarized in Table I. Using blood biochemical tests, effects on liver function including glucose metabolism, derived from the administration of the BCAA-enriched supplement were examined at 30, 60 and 90 days.

Measurements of body composition and visceral fat area. Body fat and skeletal muscle were evaluated by an eight-polar direct segmental multifrequency-bioelectrical impedance analyzer (DSM-BIA; InBody 3.2, Biospace, Tokyo, Japan) before and after 90 days of administration of the BCAA-enriched supplement and were expressed as fat%body weight (fat%BW) and muscle%body weight (muscle%BW), respectively. Visceral fat area was measured by a DSM-BIA

Table III. Effects of BCAA-enriched supplementation on body composition, protein, lipid and glucose metabolism.

	Before	Administration of BCAA-enriched supplement		
		30 days	60 days	90 days
BMI	24.3±0.6	n/a	n/a	24.2±0.8
Fat%BW	32.8±2.4	n/a	n/a	32.6±2.4
Muscle%BW	36.0±1.6	n/a	n/a	36.1±1.6
Visceral fat area	118.2±14.9	n/a	n/a	110.9±6.2
BCAAs (μmol/l)	435.6±27.5	512.5±45.3	506.6±24.3	527.3±47.1
Tyrosine (μmol/l)	90.1±7.9	84.2±7.0	77.0±7.0[a]	89.1±7.1
BTR	5.1±0.4	6.6±0.8[a]	7.2±0.9[b]	6.4±0.7
Zinc (μg/dl)	82.9±6.1	108.3±6.9[a]	106.8±5.6[a]	103.3±6.9[a]
ALT (U/l)	39.8±7.3	43.8±7.0	42.5±7.5	42.1±6.1
Total protein (g/dl)	7.8±0.2	7.7±0.2	7.6±0.1	7.6±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1[a]	3.9±0.1
Cholinesterase (U/l)	155.8±16.1	154.1±13.8	170.7±18.3	170.1±18.5
Fasting glucose (mg/dl)	104.5±6.4	103.9±6.1	101.8±5.3	102.8±5.4
HbA1c (%)	5.5±0.2	5.5±0.2	5.5±0.2	5.4±0.3
IRI (μU/ml)	22.8±9.7	12.7±1.4	10.8±1.5	13.3±1.9
HOMA-IR	5.5±2.1	3.3±0.4	2.8±0.5	3.5±0.6
HOMA-%B	326.4±6.4	127.5±17.6	112.0±16.8	140.8±28.8
Total cholesterol (mg/dl)	178.5±9.1	172.8±9.4	178.6±10.0	173.1±7.2
Total bilirubin (mg/dl)	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05 and [b]P<0.01. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

before and after 90 days of administration of the BCAA-enriched supplement. The accuracy of the DSM-BIA analyzer has been reported (24).

Laboratory determinations. Venous blood samples were obtained in the morning after an overnight fast. Complete blood cell counts and levels of serum aspartate amino-transferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase, total protein, albumin, BCAA, tyrosine, cholinesterase, total bilirubin, total cholesterol, immunoreactive insulin (IRI), zinc, plasma glucose and HbA1c were measured by standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described (25,26). BCAA tyrosine ratio (BTR) was calculated as BCAA/tyrosine.

Evaluation for insulin resistance and ß cell function. Insulin resistance and ß cell function were evaluated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method (27). The formulas used for the HOMA model are as follows: Insulin resistance (HOMA-IR) = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405; ß cell function (HOMA-%B) = fasting insulin (μU/ml) x 360/[fasting glucose (mg/dl) - 63].

Statistical analysis. All data are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. Statistical comparisons between before administration of the BCAA-enriched supplement and after 30, 60, or 90 days, were performed by Wilcoxon's test. P-values <0.05 were considered significant.

Results

Patient characteristics. Patient characteristics prior to BCAA-enriched supplementation administration are summarized in Table II. Serum aspartate aminotransferase and γ-glutamyl transpeptidase levels were elevated in comparison to normal limits. Serum albumin and cholinesterase levels were decreased. Although the BMI value and the levels of fasting plasma glucose and HbA1c were within normal limits, the values of fat%BW, visceral fat area, serum IRI, HOMA-IR, and HOMA-%B were elevated.

Effects of BCAA-enriched supplement on body composition, protein, lipid and glucose metabolism. The effects on body composition, protein, lipid and glucose metabolism as a result of administering the BCAA-enriched supplementation are summarized in Table III. There were no significant changes on body composition between before and after 90 days

Table IV. Characteristics of male and female groups.

	Normal range	Male group	Female group	P
n		5	7	
Age	n/a	61.2±3.4	66.4±3.3	0.29
BMI (kg/m^2)	18.5-25.0	24.8±1.1	23.9±0.8	0.29
Fat%BW (%)	20-27	23.2±3.0	35.5±1.6	0.002
Muscle%BW (%)	40-50	42.9±1.7	34.0±0.9	0.002
Visceral Fat Area (cm^2)	>100	111.0±12.9	120.3±18.4	0.66
Hemoglobin (g/dl)	14.0-18.0	14.4±0.7	13.0±0.5	0.17
White blood cell (/μl)	4000-9000	4040±738	4585±508	0.46
Platelet count (x10^4/μl)	13-36	11.8±1.5	17.9±2.0	0.06
AST (U/l)	12-33	43.6±6.5	42.7±8.8	0.46
ALT (U/l)	8-42	51.8±15.7	31.8±4.5	0.46
LDH (U/l)	119-229	177.0±27.4	205.2±10.8	0.56
γ-GTP (U/l)	10-47	50.6±8.1	49.0±13.5	0.68
Total protein (g/dl)	6.7-8.3	7.9±0.3	7.6±0.2	0.46
Albumin (g/dl)	4.0-5.0	3.8±0.1	3.8±0.1	0.57
BTR	4.4-10.1	4.6±0.6	5.5±0.5	0.29
BCAAs (μmol/l)	344-713	509.1±38.7	383.1±23.7	0.03
Tyrosine (μmol/l)	51-98	115.0±38.7	72.3±4.8	0.02
Cholinesterase (U/l)	214-466	171.6±36.4	144.6±11.5	0.80
Total bilirubin (mg/dl)	0.3-1.5	1.0±0.3	0.8±0.1	0.80
Total cholesterol (mg/dl)	128.0-220.0	160.2±9.9	191.5±12.0	0.10
Fasting glucose (mg/dl)	80.0-109.0	105.2±14.9	104.0±5.0	0.51
HbA1c (%)	4.3-5.8	6.0±0.4	5.2±0.1	0.17
IRI (μU/ml)	5.0-20.0	40.4±22.0	10.2±1.8	0.04
HOMA-IR	<5.4	9.4±4.8	2.7±0.6	0.03
HOMA-%B	>156.5	657.6±345.8	89.8±14.4	0.17
Zinc (μg/dl)	80-130	81.8±10.9	83.7±7.8	0.46

The values are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

of the administration of the BCAA-enriched supplement. Although serum BCAA levels were not significantly increased, the BTR value was significantly increased 30 and 60 days after administration of the BCAA-enriched supplement. The serum zinc level was significantly increased 30, 60 and 90 days after the administration and the serum albumin level was also significantly increased 60 days after the administration. There were no significant changes in fasting plasma glucose, HbA1c, IRI levels, HOMA-IR or HOMA-%B values after administration of the BCAA-enriched supplement.

Characteristics of the male and female groups. Characteristics of the male and female groups before administration of the BCAA-enriched supplement are summarized in Table IV. Fat%BW value was significantly higher in the female group than that in the male group. Muscle%BW value was

significantly higher in the male group than that in the female group. Serum BCAAs and tyrosine levels were significantly higher in the male group than those in the female group. Although fasting plasma glucose and HbA1c levels were not significantly different between the two groups, serum IRI level and HOMA-IR values were significantly higher in the male group than those in the female group prior to supplement administration.

Effects of BCAA-enriched supplement on body composition, protein and lipid metabolism in the male and female groups. The effects of administering the BCAA-enriched supplement on body composition, protein and lipid metabolisms in the male and female groups are summarized in Tables V and VI, respectively. There were no significant changes on body composition between before and after 90 days of administration of the BCAA-enriched supplement.

Table V. Effects of BCAA-enriched supplementation on protein and lipid metabolism in the male group.

| | | Administration of BCAA-enriched supplement | | |
	Before	30 days	60 days	90 days
BMI	24.8±1.1	n/a	n/a	24.4±2.1
Fat%BW	23.2±3.0	n/a	n/a	23.1±3.1
Muscle%BW	42.9±1.7	n/a	n/a	42.8±1.9
Visceral fat area	111.0±12.9	n/a	n/a	109.1±14.1
BCAA (μmol/l)	509.1±38.7	569.4±98.0	488.9±35.4	437.4±44.0
Tyrosine (μmol/l)	115.0±10.0	103.7±6.8	94.2±11.5[a]	102.0±9.8
BTR	4.6±0.6	5.7±1.2	5.5±0.7[a]	4.4±0.5
Zinc (μg/dl)	81.8±10.9	116.0±15.0[a]	97.4±4.0	87.4±6.6
ALT (U/l)	51.8±15.7	55.6±14.6	54.0±16.7	53.2±13.2
Total protein (g/dl)	7.9±0.3	7.7±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1	4.0±0.1[a]
Cholinesterase (U/l)	171.6±36.4	166.0±31.3	201.8±38.8	203.2±39.0
Total cholesterol (mg/dl)	160.2±9.9	157.2±13.9	162.4±12.7	163.0±11.8
Total bilirubin (mg/dl)	1.0±0.3	1.2±0.3	1.0±0.3	1.1±0.3

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Table VI. Effects of BCAA-enriched supplement on protein and lipid metabolism in the female group.

| | | Administration of BCAA-enriched supplement | | |
	Before	30 days	60 days	90 days
BMI	23.9±0.8	n/a	n/a	24.3±1.8
Fat%BW	35.5±1.6	n/a	n/a	35.3±1.4
Muscle%BW	34.0±0.9	n/a	n/a	34.1±1.0
Visceral fat area	120.3±18.4	n/a	n/a	111.4±4.2
BCAAs (μmol/l)	383.1±23.7	471.9±34.8[a]	519.1±34.6[a]	591.6 ± 66.3[a]
Tyrosine (μmol/l)	72.3±4.8	70.3±7.3	66.2±6.0	79.9±8.9
BTR	5.5±5.0	7.3±1.0	8.5±1.3[a]	7.7±9.0
Zinc (μg/dl)	83.7±7.8	102.9±5.7	113.6±8.5	114.6±8.8[a]
ALT (U/l)	31.1±4.5	35.3±5.0	34.3±4.0	34.1±4.9
Total protein (g/dl)	7.6±0.2	7.6±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±4.7	3.9±2.4	3.9±0.1
Cholinesterase (U/l)	144.6±11.5	145.6±10.1	148.4±11.5	146.4±11.3
Total cholesterol (mg/dl)	191.6±12.0	183.9±11.5	190.1±13.5	180.3±8.7
Total bilirubin (mg/dl)	0.8±0.1	0.8±0.1	0.8±4.2	0.7±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Significant increases in serum BTR value and zinc level were seen in the two groups after the supplement administration (Tables V and VI). In addition, the serum BCAA level was significantly increased in the female group (Table VI). The serum albumin level was also significantly increased, though only in the male group (Table V).
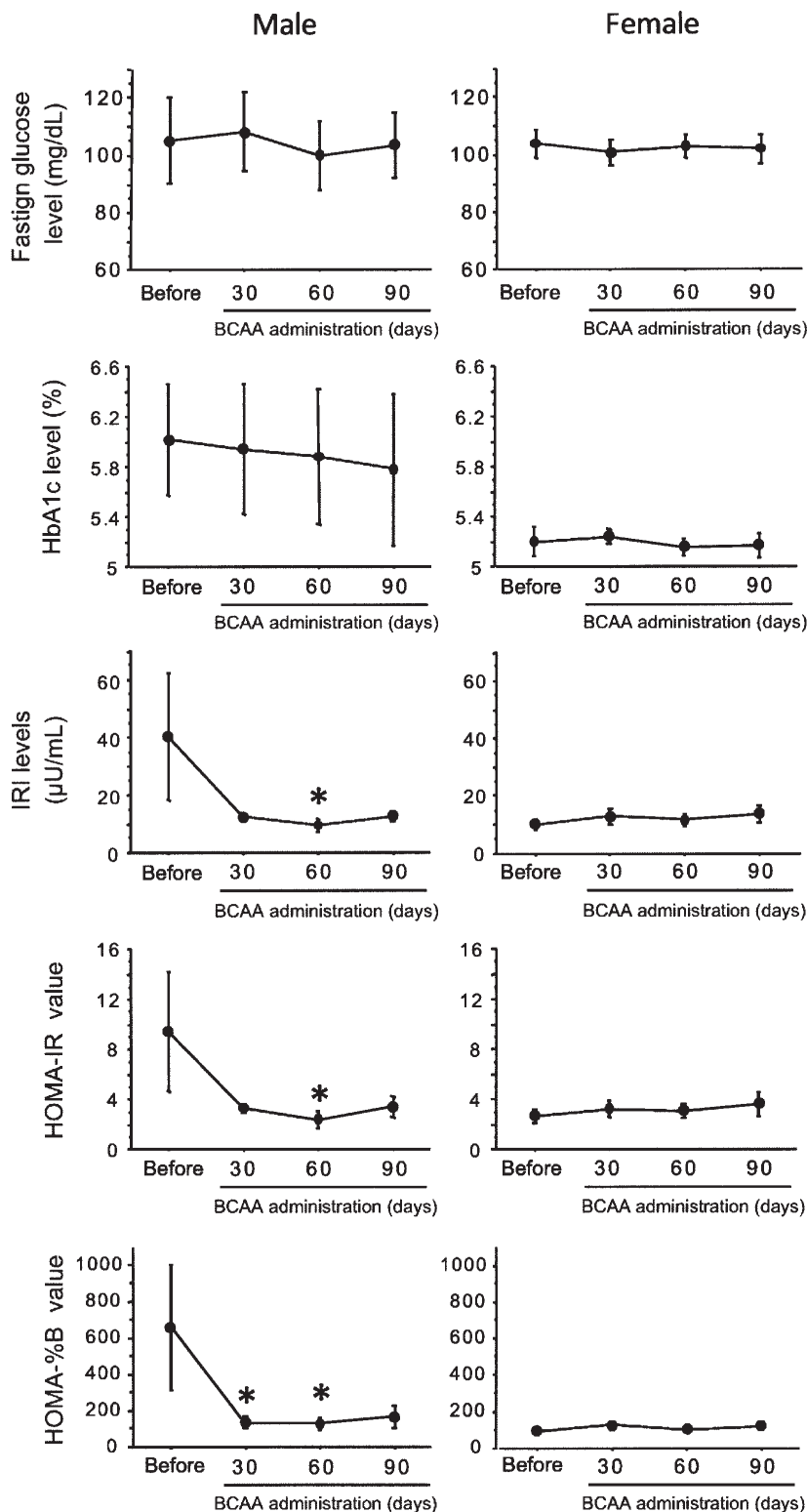
Figure 1. The effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Glucose metabolism was evaluated by the fasting glucose level, HbA1c value, serum IRI level, HOMA-IR and HOMA-%B values. The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration was performed by Wilcoxon's test. *P<0.05. HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function.

Effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Although there were no significant changes in the fasting plasma glucose and HbA1c levels during the administration of the BCAA-enriched supplement in the two groups, the serum IRI level was significantly decreased in the male group after the supplement administration (Fig. 1). Similarly, a significant decrease was seen in the HOMA-IR and HOMA-%B values in the male group, though not in the female group (Fig. 1).

Discussion

In this study, we demonstrated that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, BCAA-enriched supplementation caused decreases in the serum IRI level, the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat, suggesting the direct effects of the BCAA-enriched supplement on glucose metabolism among the male patients with chronic viral liver disease.

Increased insulin resistance is known to exist in pre-cirrhotic patients (2) and is a risk factor for the progression of hepatic fibrosis (4), development of HCC (5,6) and a reduction in long-term survival (6,7). Similar to previous reports (2,6,25,26,28), the enrolled patients in this study exhibited chronic hepatitis with increased insulin resistance. Since BCAA are known to modulate insulin signaling (18-21), we examined the effects of the BCAA-enriched supplementation on insulin resistance. In our study, the serum IRI level and the HOMA-IR and HOMA-%B values were all reduced after the supplement administration. However, the reduction in these parameters was not statistically significant. We previously reported on a gender difference in chronic viral liver disease-related insulin resistance (6). Therefore, we examined the effects of the BCAA-supplement on insulin resistance by stratification via gender.

Male cirrhotic patients show a significantly greater increase in insulin resistance compared to female cirrhotic patients (6). Similarly, increased IRI levels and HOMA-IR values were seen only in the male group in this study. Although the reason for the gender difference in insulin resistance is unclear, one possibility is that tyrosine is involved in the development of insulin resistance. The serum tyrosine level in our study of the male group was significantly increased compared to the female group. A synthetic enzyme of tyrosine, henylalanine hydroxylase, is known to be regulated by testosterone, a sex hormone (29). Tyrosine is the precursor of epinephrine, which causes peripheral and hepatic insulin resistance (30). The serum tyrosine level has a positive correlation with insulin resistance (31). Thus, the gender difference in the tyrosine production pathway and the metabolite of tyrosine may have been responsible for the increased insulin resistance in the male group among our patients.

BCAA-enriched supplementation decreased the serum IRI level and the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat area. These data suggest that the BCAA-enriched supplementation improves insulin resistance and ß cell function. In this study, insulin resistance was significantly reduced 60 days after administration of the BCAA-enriched supplement. It remains unclear, however, how the supplement improved insulin resistance. Since the supplement used in this study contains trace elements such as zinc, chromium and selenium, which are known to decrease insulin sensitivity (32-34), it is reasonable to assume that these trace elements may contribute to improve insulin resistance. Alternatively, changes in the constitution of amino acids may be involved in the improvement in insulin resistance. Moreover, tyrosine is the precursor of epinephrine, which causes peripheral and hepatic

insulin resistance (30). In this study, changes in serum tyrosine level and BTR reflected changes in insulin resistance. Insulin resistance was significantly reduced 60 days after the administration of the BCAA-enriched supplement. Likewise, the serum tyrosine level was significantly decreased and BTR was significantly increased 60 days after the administration of the BCAA-enriched supplement. Thus, a decrease in the serum tyrosine level and changes in the constitution of amino acids may contribute to a reduction in insulin resistance. In good agreement with our results, Vlasakova et al reported that BTR shows a negative correlation with insulin resistance (31). Although the reason for changes in the constitution of amino acids remains uncertain, a possibility is that BCAA activate mTOR, which in turn, promotes protein synthesis (35). Desai et al reported that BCAA cause the incorporation of tyrosine into protein as well as albumin renewal (36). In our study, a significant increase in serum albumin level was seen following the decrease in serum tyrosine level and an increase in BTR. Taken together, these findings suggest that the BCAA-enriched supplementation seems to lead to a dramatic reduction of insulin resistance through both changes in the constitution of amino acids and effects of trace elements.

A limitation of this study was the small sample size. However, leucine is reported to improve insulin resistance in *in vivo* experiments (37). In human subjects, two cases which showed BCAA improved insulin resistance were recently reported (38). These previous reports suggest the effects of the BCAA-enriched supplement on glucose metabolism. In order to confirm the significance of the BCAA-enriched supplement on insulin resistance in patients with LC, a large-scale multicenter clinical study is required.

This study revealed that the BCAA-enriched supplementation improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, the BCAA-enriched supplementation may be one of the useful therapeutic agents for insulin resistance in male patients with chronic viral liver disease.

Acknowledgements

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