Beneficial effect of branched-chain amino acid supplementation on glycemic control in chronic hepatitis C patients with insulin resistance: Implications for type 2 diabetes

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Beneficial effect of branched-chain amino acid supplementation on glycemic control in chronic hepatitis C patients with insulin resistance: implications for type 2 diabetes

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Clinical Trial Registry Number: UMIN000001093

Registry name: Effects of branched-chain amino acid (Livact) on glucose tolerance in patients with chronic hepatitis C

Abstract

Objective: Branched-chain amino acids (BCAAs) improve disorders of albumin metabolism, quality of life, subjective symptoms, and prognosis in patients with chronic hepatitis. However, it remains unclear whether they improve insulin resistance. We examined the effects of BCAAs on glucose tolerance and insulin sensitivity in patients with chronic hepatitis C and insulin resistance. **Methods:** Individuals with a definitive diagnosis of chronic hepatitis C and insulin resistance were eligible for participation. Eligible participants were randomly assigned to the BCAA group or a control group. Participants were then crossed over to the other treatment for a further 12 weeks. Baseline clinical features, laboratory markers, fatty acid levels, and insulin sensitivity, assessed with oral glucose tolerance tests and a hyperinsulinemic euglycemic clamp, were also examined before and 12 and 24 weeks after the beginning of the study. **Results:** Of the 27 patients who completed the study, 14 began in the BCAA group and 13 began as controls. There were no significant differences in glucose metabolism parameters or lipid profiles between the groups. HbA1c values were improved in 10 patients and worsened or remained unchanged in 17 patients. The only predictive variable for change in HbA1c was the baseline Matsuda index: the lower the index, the greater the improvement in HbA1c values. **Conclusions:** BCAA therapy did not have

adverse effects on glucose tolerance or insulin sensitivity in patients with chronic hepatitis C and insulin resistance. Moreover, it had a therapeutic effect on HbA1c values in patients with marked peripheral (primarily muscle) insulin resistance.

Keywords: BCAA, glucose tolerance, insulin sensitivity

Abbreviations: HCC, hepatocellular carcinoma; BCAAs, branched-chain amino acids; PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; OGTT, oral glucose tolerance test; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; H-IR, hepatic insulin resistance index; AUCs; areas under the curve; MCR, glucose metabolic clearance rate; BMI, body mass index; FPG, fasting plasma glucose; HPLC, high-performance liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; SIM, selected ion monitoring;

Introduction

The liver is a key organ in glucose homeostasis and one of the major target organs of insulin. Insulin resistance increases in chronic liver disease [1] and is a risk factor for the progression of liver pathology, the development of hepatocellular carcinoma (HCC), and a decrease in long-term survival [1-3]. Therefore, insulin resistance is an important therapeutic target in patients at any stage of chronic liver disease. However, the use of pharmacological agents targeting to insulin resistance, such as biguanides and thiazolidinediones, is often limited in patients with advanced chronic hepatitis due to adverse effects, including lactic acidosis and severe hepatotoxicity.

Branched-chain amino acid (BCAA) supplementation improves disorders of albumin metabolism, quality of life, subjective symptoms, and prognosis in patients with chronic hepatitis [4, 5]. It remains unclear whether BCAAs improve insulin resistance in humans. BCAAs have been reported to modulate glucose metabolism in *in vivo* studies [6-9]. In addition, they promote glucose uptake in skeletal muscle in a rat model of liver cirrhosis [6] and adipocytes from diabetic db/db mice [7] and reduce hepatic glucose production in insulin-resistant Zucker fa/fa rats [8]. In skeletal muscle isolated from non-diabetic rats [9], BCAAs promote glucose uptake by enhancing translocation of glucose the transporter 4 to the plasma membrane via phosphatidylinositol 3-kinase

(PI3K) and protein kinase C pathways independent of mammalian target of rapamycin (mTOR). However, BCAAs/amino acids up-regulate mTOR in hepatocytes and the cirrhotic liver [10, 11]. mTOR negatively regulates insulin-mediated PI3K activity by degrading insulin receptor substrates in skeletal muscle cells during long-term insulin treatment [12]. In addition, human metabolomic profiling has shown that plasma BCAA levels are higher in obese humans than in thinner individuals and are significantly correlated with insulin resistance [13]. Furthermore, recent large-scale metabolomic analyses have shown that plasma BCAA levels are highly significantly associated with future diabetes, especially in subjects with insulin resistance [14]. These findings suggest that BCAAs play a dual role in glucose metabolism in skeletal muscle, enhancing glucose uptake under normoinsulinemic conditions while causing insulin resistance under hyperinsulinemic conditions. In this regard, the effects of BCAA administration on glucose metabolism depend on the balance between insulin-dependent and -independent BCAA signaling pathways [6].

The present study examined the effects of BCAA therapy on glucose tolerance and insulin sensitivity in patients with chronic hepatitis C and insulin resistance using a glucose clamp technique and measuring changes in blood glucose levels after an oral glucose tolerance test (OGTT). This is the first randomized controlled clinical study to

address the effects of BCAAs on glucose metabolism.

Methods

Study overview

A randomized, controlled, crossover trial of BCAAs in patients with chronic hepatitis C and insulin resistance was performed at Kanazawa University Hospital in accordance with the Declaration of Helsinki. The participants provided signed, written informed consent. This trial was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (number UMIN000001093).

Study protocol

Individuals (aged 31–78 years) were recruited from outpatients at Kanazawa University Hospital. Those with a definitive diagnosis of hepatitis C virus-associated chronic hepatitis and insulin resistance, as determined by a level of fasting immunoreactive insulin (IRI) during the observation period ≥10 μU/mL, were eligible for participation. All drugs were allowed to be taken during the study. Patients were excluded from the study if they met any of the following criteria: 1) continuous serum albumin level ≤2.5 g/dL; 2) regular treatment with an albumin preparation (at least once a week for 1

month or longer); 3) treatment with a BCAA preparation within 4 weeks prior to enrolling in the study; 4) coma of grade III or higher due to hepatic encephalopathy; 5) total bilirubin level ≥3.0 mg/dL; 6) concurrent hepatocellular carcinoma, as determined by diagnostic imaging; 7) alcoholic cirrhosis and alcohol dependence; 8) high-risk esophageal varices most likely requiring sclerotherapy in the near future; 9) concurrent renal failure most likely requiring dialysis in the near future; 10) congenital abnormality of BCAA metabolism; 11) poorly controlled diabetes with a fasting blood glucose level ≥150 mg/dL, or diabetes treated with sulfonylureas, biguanides, thiazolidinediones, or insulin; 12) concomitant corticosteroid therapy; 13) concomitant interferon therapy; and 14) inadequacy to participate in the study, as assessed by the investigators.

Randomization

Eligible participants were randomly assigned to the BCAA group or the control group. Those in the BCAA group were then prescribed oral supplementation with three packs of a BCAA nutrient (Livact (4.15 g/pack); Ajinomoto Pharma, Tokyo, Japan), taken three times a day: after breakfast, after dinner, and before sleep. The participants were then crossed over, without any washout, to the other treatment for a further 12 weeks. A random allocation sequence was computer-generated elsewhere. Neither the individuals

conducting the study nor the patients had access to the allocation sequence until the end of the study, when the final results were analyzed. The nutritional balance of the diet was monitored by a nationally registered dietitian. The composition of the diet of each patient was based on the following conditions: 30–35 kcal/kg per day total calories for ideal body weight (body mass index of 22), 1–1.5 g protein/kg per day, and a fat content equivalent to 25% of the total calories.

Baseline clinical features, laboratory markers, and plasma amino acid and fatty acid profiles were also examined before and 12 and 24 weeks after the beginning of the study. Venous blood samples were obtained in the morning after an overnight fast.

Evaluation of insulin sensitivity by OGTT

After an overnight fast (10–12 h), a 75-g OGTT was performed at 8:30 a.m. Blood samples were obtained 0, 30, 60, 90, and 120 min after the glucose load for the measurement of plasma glucose and serum insulin concentrations. From the OGTT data, the Matsuda index, an index of whole-body insulin sensitivity (10,000/√ [fasting glucose × fasting insulin] × [mean glucose × mean insulin during OGTT]) that is highly correlated with the rate of whole-body glucose disposal during a euglycemic insulin clamp [15], was calculated. The homeostasis model assessment of insulin resistance

(HOMA-IR) was calculated using the formula HOMA-IR = (fasting insulin [μU/mL] × fasting plasma glucose [mg/dl]) / 405 [16]. The hepatic insulin resistance index (H-IR) was calculated as the product of the total areas under the curve (AUCs) for glucose and insulin during the first 30 min of the OGTT (glucose 0–30 [AUC] × insulin 0–30 [AUC]). This index was strongly correlated with hepatic insulin resistance in a euglycemic insulin clamp study (fasting plasma insulin × basal endogenous glucose production) [17]. In any given individual, the HOMA-IR, H-IR (primarily liver), and Matsuda index (muscle plus liver) provide different information [15, 17].

Evaluation of insulin sensitivity through a hyperinsulinemic euglycemic clamp

The insulin sensitivity of 11 of the 27 patients was also evaluated in a hyperinsulinemic euglycemic clamp study [18]. The patients did not receive any medication on the morning of the examination. At approximately 9 a.m., after an overnight fast of at least 10 h, an intravenous catheter was placed in an antecubital vein in each subject for infusion, and a second catheter was placed in the contralateral hand for blood sampling. The euglycemic hyperinsulinemic clamp technique was performed using an artificial pancreas (model STG-22; Nikkiso, Tokyo, Japan), as described previously [19]. A solution of 0.8 U/mL insulin (Novolin R: Novo Nordisk, Copenhagen, Denmark) in

normal saline was allowed to remain in the intravenous lines for at least 15 min, and the lines were then flushed before starting the insulin infusion. Insulin was infused at a rate of 3.0 mU/kg/min, resulting in a steady-state insulin concentration of 290.3±61.7 μU/mL (mean±SD). Blood glucose levels were continuously determined during the clamp study and maintained with a variable-rate infusion of 20% glucose at fasting levels or 100 mg/dL, whichever was higher. The steady-state period was maintained for 30 min or longer, during which the coefficients of variation for blood glucose and the glucose infusion rate were each less than 5%. The glucose levels reached during the clamp study were 91.3±15.5 mg/dL. Insulin sensitivity was expressed as the glucose metabolic clearance rate (MCR) in mg/kg per minute. The mean MCR in healthy subjects (*n*=9; age, 26.6±2.9 years; body mass index [BMI], 22.3±2.1 kg/m^2) was 13.5±3.4 mg/kg/min [20].

Statistical analysis

All of the data are expressed as the mean±standard error. The differences between the two groups were analyzed with a Mann-Whitney U test. We used the multilevel model, as recommended by Mills and colleagues for analyzing crossover studies [21]. This model was used specifically to allow for the assessment of whether there were any

effects with time or any carryover effect from one treatment phase to another. Therefore, 3 months after a 3-month administration of BCAA are regarded as a washout period. To assess the treatment effect on the parametric data, these results were double checked with a paired t test after the exclusion of carryover effects [21]. Both the BCAA and control groups shared the baseline clinical characteristics of all of the subjects at the beginning of this study. Statistical comparisons between data from before the administration of the BCAAs and the data obtained after 12 and 24 weeks were performed using a Wilcoxon matched-pairs signed rank test. *P*-values of <0.05 were considered to be significant.

Results

Baseline metabolic parameters

Figure 1 shows the trial profile and Table 1 shows the baseline characteristics of the participants. All of the patients were Japanese and lived in Ishikawa, Japan. The patients were recruited between March 2008 and March 2010, with follow-up continuing for 6 months. Three participants withdrew before completion of the study. The first dropout case in the BCAA-first group, aged 63 years, voluntarily did not take the BCAA nutrient without any reasons and withdrew consent 1 day after the first treatment; the

second case in the control-first group, aged 55 years, declined to attend the study and withdrew consent 8 weeks after the first treatment started; and the third case in the control-first group, aged 59 years, did not attend the outpatient clinic 4 weeks after the first treatment started. Of the remaining 27 patients, 14 began in the BCAA group and 13 began as controls. All of the patients who withdrew were excluded from the analysis because none of them completed their first course of treatment, and they only had baseline OGTT data. We performed a completed case analysis rather than an intention-to-treat analysis because there were few dropouts, and their reasons for dropping out were unrelated to baseline values or their response. No adverse effects of treatment were reported.

There were no significant differences in any of the measured parameters between the groups before randomization, except for waist circumference and fasting plasma glucose (FPG). The BCAA-first group had a significantly lower mean waist circumference and a significantly lower mean FPG level than the BCAA-second group. For all of the subjects, the mean age was 61.3±2.1 years and the mean BMI was 24.6±2.0 kg/m^2. According to the inclusion criteria, all of the subjects were insulin resistant and the mean fasting IRI was as high as 18.4±4.0 IU/L. There were no abnormalities in the serum levels of total or direct bilirubin, alkaline phosphatase, total

protein, or creatinine in either group (data not shown).

Effects of BCAAs on body composition and lipid, protein, and glucose metabolism

The effects of administering BCAAs on body composition and lipid, protein, and glucose metabolism are summarized in Table 2. There were no significant differences between any of the glucose metabolism parameters (FPG, HbA1c, IRI, HOMA-IR, Matsuda index, H-IR, or MCR) or lipid profiles between the two groups. However, the BCAA group had significantly higher AST and ALT levels than the controls. The investigators were not given access to these results to maintain treatment blinding. The differences in the concentrations of hemoglobin, creatinine, and hepatic reserve were not significant between the two groups. These trends were similar in subgroups stratified by glucose tolerance (as diabetes, borderline glucose intolerance, and normal glucose tolerance) (Supplementary Table 1A-C). Additionally, unlike previous reports that suggested that BCAA-mediated improvements in insulin resistance are only observed in male patients [22, 23], there were no significant sex differences in the effects of BCAA in the present study (Supplementary Table 2A, B).

Effects of BCAAs on amino acid metabolism

We measured plasma amino acid concentrations with high-performance liquid chromatography (HPLC)-electrospray ionization (ESI)-mass spectrometry (MS), followed by derivatization. An MSQ Plus LC/MS system (Thermo Fischer Scientific, Waltham, MA, USA) equipped with an ESI source was used in positive-ionization mode for selected ion monitoring (SIM). Xcalibur version 1.4 SR1 software (Thermo Fisher Scientific, Yokohama, Japan) was used for data collection and processing. The HPLC separation system consisted of an L-2100 pump, an L-2200 autosampler, and an L-2300 column oven (Hitachi High-Technologies Corporation, Tokyo, Japan). A Wakosil-II 3C8-100HG column (100, 2.1, 3 mm; Wako Pure Chemical Industries, Osaka, Japan) was used for separation, and the mobile phase consisted of eluent A (25 mM ammonium formate in water, pH 6.0) and eluent B (water:acetonitrile=40:60). The plasma amino acid concentration profiles (aminograms) were analyzed for differences between the BCAA and control groups (Table 3). Remarkably, the component that showed the strongest difference between the groups was the combination of leucine, valine, phenylalanine, threonine, and proline (P<0.05).

Baseline clinical features and laboratory markers associated with changes in HbA1c

HbA1c values improved in 10 patients (37.0%), and worsened or remained unchanged in 17 patients (63.0%). As shown in Table 4, the only predictive variable for change in HbA1c was the baseline Matsuda index (P=0.014): the lower the index, the greater the improvement in HbA1c values. Furthermore, the rate of change of HbA1c tended to be correlated with the rate of change of the Matsuda index (r=-0.405, P=0.069). Other glucose metabolism markers, such as baseline IRI, HOMA-IR, H-IR, and MCR did not significantly predict changes in HbA1c values.

Discussion

Chronic liver disease, especially hepatitis C, is associated with insulin resistance and diabetes [1]. Indeed, liver disease is found in approximately 70% of patients with glucose intolerance and in approximately 40% of those with diabetes [24]. Conversely, diabetes and obesity affect the pathology of chronic liver diseases [25, 26]. We previously reported that diabetes and obesity accelerated histological prognosis, the development of liver cirrhosis and HCC, the recurrence of HCC after surgical treatment, and liver-related death in patients with hepatitis C [1-3]. Thus, insulin resistance is an important therapeutic target in patients with chronic hepatitis C of any stage.

In the present open-label, randomized, controlled cross-over intervention trial, we

found that an increase in plasma levels of BCAAs after 12 weeks of oral administration of a BCAA preparation did not change the glycemic levels or insulin sensitivity in patients with chronic hepatitis C. However, when we stratified the patients regarding changes in glycemic control (HbA1c improved and non-improved), BCAAs improved glycemic control in patients with lower Matsuda index. Furthermore, the percentage change in HbA1c tended to be correlated with the percentage change in the Matsuda index (r=-0.405, *P*=0.069). However, serum IRI, H-IR, and HOMA-IR, which are thought to be hepatic insulin resistance indices, did not significantly predict changes in HbA1c values. BCAAs may only ameliorate glucose metabolism in patients with marked insulin resistance in the skeletal muscle. Therefore, contrary to our initial hypothesis, these findings suggest that BCAAs are effective in glycemic control in patients with type 2 diabetes, which is characterized by skeletal muscle insulin resistance.

The limitations of our study include the short period of treatment of 12 weeks and the small numbers of patients investigated. To confirm the significance of BCAA therapy on insulin resistance in patients with chronic hepatitis C, a large-scale, long-term study is required to confirm our conclusion.

In summary, BCAA supplementation therapy did not have adverse effects on glucose

tolerance or insulin sensitivity in patients with chronic hepatitis C or insulin resistance. BCAAs did not significantly improve overall glycemic control in the present study. However, notably, BCAA therapy may exert a beneficial effect on HbA1c values in patients with marked insulin resistance in the skeletal muscle. Currently, in Japan, BCAA supplementation is covered by insurance only for patients with chronic liver diseases. Based on our preliminary observations, future clinical trials should also evaluate the effect of BCAAs in patients with type 2 diabetes.

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Conflict of Interest None of the authors have any financial or other interest in the dissemination of this article.

Author contributions: Yumie Takeshita: designing and conducting the study, analyzing and interpreting the data, and writing the manuscript. Toshinari Takamura: analyzing and interpreting the data and editing the manuscript. Yuki Kita: analyzing and interpreting the data. Hitoshi Ando: designing and conducting the study. Teruyuki Ueda,

Kenichiro Kato, Hirofumi Misu, Hajime Sunagozaka, Yoshio Sakai, Tatsuya Yamashita, Eishiro Mizukoshi and Masao Honda: collecting clinical information for chronic hepatitis C and insulin resistance. Shuichi Kaneko: initiating and organizing the study. All authors read and approved the final manuscript.

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infection. *Metabolism* 2010; 59: 486-491.

Figure legends

Figure 1. Trial profile and protocol.

(1) Clinical features (including body composition) and laboratory markers. (2) Evaluation of insulin sensitivity based on OGTT results. (3) Evaluation of insulin sensitivity based on euglycemic insulin clamp results.

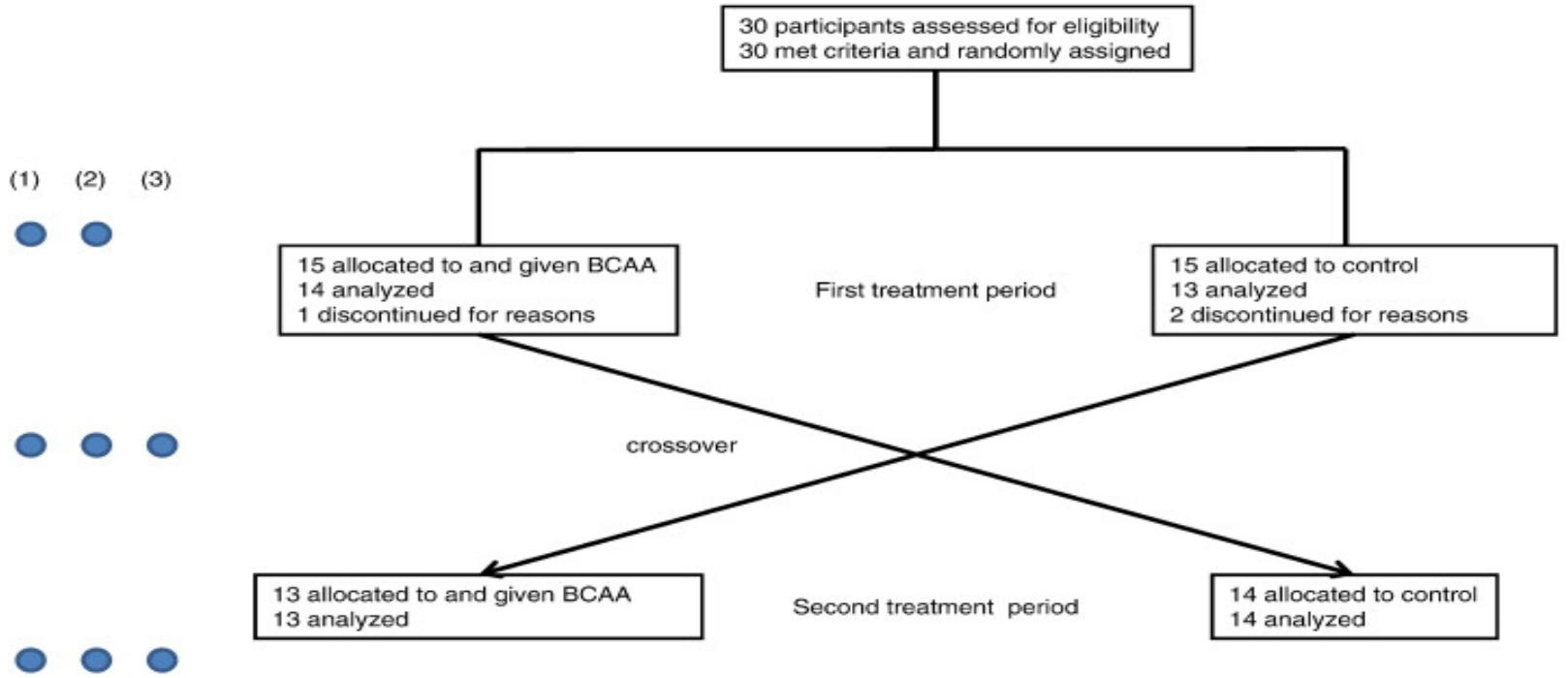

Fig.1 Trial profile and protocol. (1) Clinical features (including body composition) and laboratory markers. (2) Evaluation of insulin sensitivity based on OGTT results. (3) Evaluation of insulin sensitivity based on euglycemic insulin clamp res...

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Table 1. Baseline characteristics of participants.

	All (N = 27)	BCAA first (N = 14)	Control first (N = 13)	P value
Ages (years)	61.3 ± 2.1	58.6 ± 2.9	64.2 ± 3.0	N.S.
Sex (Men Women)	8 : 19	5 : 9	3 :10	
Body Weight (kg)	60.1 ± 1.6	59.7 ± 2.2	60.5 ± 2.5	N.S.
Waist circumference (cm)	87.9 ± 6.2	85.3 ± 1.6	90.9 ± 1.4	P = .016*
BMI (kg/m^2)	24.6 ± 2.0	24.1 ± 0.5	25.0 ± 0.6	N.S.
FPG (mg/dL)	96.2 ± 1.9	92.1 ± 2.1	100.6 ± 2.9	P = .025*
HbA1c (%)	4.9 ± 0.4	5.0 ± 0.1	4.9 ± 0.1	N.S.
IRI (IU/L)	18.4 ± 4.0	13.8 ± 1.6	23.3 ± 8.0	N.S.
HOMA-IR	4.6 ± 1.1	3.2 ± 0.4	6.1 ± 2.2	N.S.
TC (mg/dL)	155.9 ± 7.9	159.1 ± 12.1	152.7 ± 10.6	N.S.
TG (mg/dL)	84.3 ± 6.6	78.0 ± 8.4	90.5 ± 10.2	N.S.
HDL-C (mg/dL)	47.5 ± 3.0	48.5 ± 5.4	46.5 ± 3.0	N.S.
AST (IU/L)	53.3 ± 4.1	56.9 ± 6.9	49.4 ± 4.1	N.S.
ALT (IU/L)	50.0 ± 7.1	57.4 ± 13.0	41.9 ± 4.4	N.S.
BUN (mg/dL)	13.6 ± 0.8	12.6 ± 1.1	14.7 ± 1.3	N.S.
HpT (%)	87.5 ± 4.3	89.4 ± 6.6	84.8 ± 4.8	N.S.
Total protein (g/dL)	7.5 ± 0.1	7.6 ± 0.2	7.5 ± 0.2	N.S.
Albumin (g/dL)	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	N.S.
BCAA (mmol/L)	412.5 ± 18.9	425.4 ± 29.7	398.5 ± 23.5	N.S.
Matsuda index	3.0 ± 0.4	3.3 ± 0.7	2.7 ± 0.4	N.S.
H-IR × 10^6	5.8 ± 0.5	6.1 ± 0.7	5.5 ± 0.7	N.S.

All data are expressed as the mean and standard error. Differences between the two groups were analyzed by a Mann–Whitney U test. BMI, body mass index; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; H-IR, hepatic insulin resistance index.

■Difference between the BCAA-first group and the control-first group. P values of < .05 were considered significant.

Table 2. Effects of BCAA on body composition, protein, lipid and glucose metabolism.

	BCAA group	Control group	*P* value
Body weight (kg)	61.1 ± 1.9	60.5 ± 1.9	N.S
Waist circumference (cm)	87.8 ± 1.7	88.9 ± 1.7	N.S
BMI (kg/m^2)	24.9 ± 0.5	24.7 ± 0.4	N.S
FPG (mg/dL)	96.6 ± 2.1	96.2 ± 2.0	N.S
HbA1c (%)	4.9 ± 0.1	5.0 ± 0.1	N.S
IRI (IU/L)	17.8 ± 3.6	21.2 ± 4.6	N.S
HOMA-IR	4.5 ± 1.1	5.3 ± 1.3	N.S
TC (mg/dL)	157.6 ± 7.2	164.9 ± 7.3	N.S
TG (mg/dL)	78.3 ± 6.3	80.9 ± 5.3	N.S
HDL-C (mg/dL)	49.4 ± 2.9	50.0 ± 2.7	N.S
AST (IU/L)	61.2 ± 6.9	46.0 ± 3.0	*P* = .047*
ALT (IU/L)	68.3 ± 11.0	44.2 ± 4.8	*P* = .049*
BUN (mg/dL)	14.6 ± 1.0	12.9 ± 0.7	N.S
HpT (%)	86.8 ± 4.2	91.4 ± 5.7	N.S
Total protein (g/dL)	7.5 ± 0.1	7.6 ± 0.1	N.S
Albumin (g/dL)	4.0 ± 0.1	4.0 ± 0.1	N.S
BCAA (μmol/L)	472.6 ± 27.0	403.5 ± 21.0	*P* = .050*
Matsuda index	2.9 ± 0.3	2.5 ± 0.2	N.S
H-IR × 10^6	6.6 ± 0.1	6.2 ± 0.6	N.S
MCR (mg/kg/min)	9.8 ± 0.8	10.3 ± 1.0	N.S

All data are expressed as the mean and standard error. Statistical comparisons between data from before administration of the BCAAs and data obtained after 12 and 24 weeks were performed using a Wilcoxon matched-pairs signed rank test. BMI, body mass index; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; H-IR, hepatic insulin resistance index; MCR, glucose metabolic clearance rate.

■Difference between the BCAA group and control group. P values of < .05 were considered significant.

Table 3. Effects of BCAAs on amino acids metabolism.

	Baseline	BCAA	Control	*P* value*
BCAA (μmol/L)	465.3 ± 99.4	461.4 ± 120.2	418.6 ± 99.6	N.S.
Phenylalanine	103.7 ± 10.9	112.8 ± 23.9	105.5 ± 14.7	*P* = .014
Threonine	139.7 ± 26.1	121.3 ± 31.6	132.3 ± 28.4	*P* = .016
Proline	193.5 ± 59.7	172.8 ± 49.1	200.7 ± 86.0	*P* = .024
Leucine	140.3 ± 28.6	144.2 ± 40.8	129.5 ± 31.4	*P* = .025
Valine	253.9 ± 56.0	247.5 ± 61.8	219.7 ± 48.6	*P* = .040
Glycine	334.5 ± 46.2	306.6 ± 49.7	376.6 ± 272.5	N.S
Tryptophan	64.9 ± 11.9	64.9 ± 13.9	62.2 ± 12.9	N.S
Arginine	135.5 ± 31.9	125.9 ± 36.5	136.6 ± 37.5	N.S
Ornithine	124.8 ± 27.2	114.0 ± 28.9	122.6 ± 37.1	N.S
Isoleucine	71.2 ± 17.4	69.7 ± 21.4	69.3 ± 23.8	N.S
Methionine	32.1 ± 11.5	35.5 ± 9.7	34.6 ± 7.5	N.S
Glutamine	277.2 ± 169.5	365.2 ± 191.2	406.5 ± 157.4	N.S
Lysine	20.8.7 ± 37.7	194.9 ± 41.2	193.3 ± 44.9	N.S
Histidine	96.3 ± 10.7	92.7 ± 13.4	90.8 ± 12.3	N.S
Citrulline	41.3 ± 11.9	40.9 ± 11.6	40.5 ± 11.1	N.S
Asparagine	37.5 ± 10.4	43.2 ± 11.2	44.1 ± 7.4	N.S
Alanine	485.5 ± 84.9	441.7 ± 78.5	463.4 ± 152.1	N.S
Threonine	139.7 ± 26.1	121.3 ± 31.6	132.3 ± 28.4	N.S
Tyrosine	131.5 ± 27.0	129.6 ± 24.9	130.3 ± 22.3	N.S

All data are expressed as the mean and standard error. Statistical comparisons between data from before administration of the BCAAs and data obtained after 12 and 24 weeks were performed using a Wilcoxon matched-pairs signed rank test.

■Difference between the BCAA group and control group. P values of < .05 were considered significant.

Table 4. Baseline clinical features and laboratory markers associated with changes in HbA1c in 27 patients.

	HbA1c non-improved (*N* = 17)	HbA1c improved (*N* = 10)	*P* value
Body weight (kg)	61.6 ± 2.4	57.6 ± 2.2	N.S
Waist circumference (cm)	87.7 ± 1.8	87.6 ± 1.5	N.S
BMI (kg/m^2)	24.7 ± 0.6	24.4 ± 0.3	N.S
FPG (mg/dL)	97.6 ± 2.7	97.9 ± 3.1	N.S
HbA1c (%)	4.9 ± 0.1	5.0 ± 0.1	N.S
IRI (IU/L)	14.0 ± 1.6	29.0 ± 11.1	N.S
HOMA-IR	3.5 ± 0.4	7.4 ± 3.1	N.S
TC (mg/dL)	160.7 ± 11.4	146.8 ± 7.7	N.S
TG (mg/dL)	91.6 ± 9.0	70.3 ± 7.2	N.S
HDL-C (mg/dL)	48.8 ± 4.1	45.1 ± 4.0	N.S
AST (IU/L)	49.1 ± 3.9	60.3 ± 8.7	N.S
ALT (IU/L)	43.9 ± 5.1	60.2 ± 17.1	N.S
BUN (mg/dL)	13.9 ± 1.1	13.1 ± 1.4	N.S
HpT (%)	89.5 ± 6.5	84.1 ± 3.8	N.S
Total protein (g/dL)	7.6 ± 0.2	7.4 ± 0.2	N.S
Albumin (g/dL)	4.1 ± 0.1	3.9 ± 0.1	N.S
BCAA (μmol/L)	412.3 ± 21.4	412.8 ± 40.0	N.S
Matsuda index	2.9 ± 0.3	1.8 ± 0.2	*P* = .014■
H-IR × 10^6	5.5 ± 0.6	6.7 ± 1.0	N.S
MCR (mg/kg/min)	10.1 ± 1.0	8.6 ± 1.3	N.S

All data are expressed as the mean and standard error. Differences between the two groups were analyzed by a Mann-Whitney U test. BMI, body mass index; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; H-IR, hepatic insulin resistance index ; MCR, glucose metabolic clearance rate.

■Difference between the HbA1c-non-improved group and the HbA1c-improved group. P values of < .05 were considered significant.