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Effect of the *BCAT2* polymorphism (rs11548193) on plasma branched-chain amino acid concentrations after dietary intervention in subjects with obesity and insulin resistance

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Abstract

Branched-chain amino acids (BCAA) are considered markers of insulin resistance (IR) in subjects with obesity. In this study, we evaluated whether the presence of the SNP of the branched-chain aminotransferase 2 (*BCAT2*) gene can modify the effect of a dietary intervention (DI) on the plasma concentration of BCAA in subjects with obesity and IR. A prospective cohort study of adult subjects with obesity, BMI ≥ 30 kg/m^2, homeostatic model assessment-insulin resistance (HOMA-IR ≥ 2·5) no diagnosed chronic disease, underwent a DI with an energy restriction of 3140 kJ/d and nutritional education for 1 month. Anthropometric measurements, body composition, blood pressure, resting energy expenditure, oral glucose tolerance test results, serum biochemical parameters and the plasma amino acid profile were evaluated before and after the DI. SNP were assessed by the TaqMan SNP genotyping assay. A total of eighty-two subjects were included, and fifteen subjects with a *BCAT2* SNP had a greater reduction in leucine, isoleucine, valine and the sum of BCAA. Those subjects also had a greater reduction in skeletal muscle mass, fat-free mass, total body water, blood pressure, muscle strength and biochemical parameters after 1 month of the DI and adjusting for age and sex. This study demonstrated that the presence of the *BCAT2* SNP promotes a greater reduction in plasma BCAA concentration after adjusting for age and sex, in subjects with obesity and IR after a 1-month energy-restricted DI.

Key words: **Branched-chain amino acids: Insulin resistance: Polymorphism: Obesity: Branched-chain aminotransferase 2**

Branched-chain amino acids (BCAA) – leucine, isoleucine and valine – are essential amino acids since the human body does not synthesise them, and they are obtained mainly through the intake of foods of animal origin such as meat, fish, dairy products and eggs; and also from foods of vegetable origin such as legumes, soyabeans and nuts[1,2]. The first reaction of BCAA catabolism is a reversible transamination catalysed by the mitochondrial isoform of branched-chain aminotransferase (BCATm), encoded by the branched-chain aminotransferase 2 (*BCAT2*) gene[3]. This process occurs mainly in skeletal muscle through a reversible transamination reaction that generates branched-chain keto acids (BCKA) such as leucine-derived α-ketoisocaproic acid, isoleucine-derived

Abbreviations: BCAA, branched-chain amino acid;; BCAT2, branched-chain aminotransferase 2;; IR, insulin resistance;; REE, resting energy expenditure;; DI, dietary intervention;; BCKA, branched-chain keto acid.

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α-keto-β-methylvalerate and valine-derived α-ketoisovaleric acid[3,4]. Several studies have shown a strong association between plasma concentrations of BCAA and various metabolic disorders, such as insulin resistance (IR), type 2 diabetes and CVD in subjects with obesity[3–6]. In 1969, Felig *et al.*[7] reported for the first time that subjects with obesity presented higher concentrations of circulating BCAA[1]. Likewise, metabolomic studies have revealed that increases in BCAA, phenylalanine and tyrosine concentrations are associated with up to a five times higher risk of developing type 2 diabetes[8]. However, there is controversy as to whether increase in plasma BCAA is a consequence of IR or its cause. Evidence for the latter is the fact that high intake of BCAA increases the mammalian target of rapamycin (mTOR) phosphorylation and serine phosphorylation of insulin receptor substrate-1 in high-fat fed rats, which also presented glucose intolerance, whereas administration of rapamycin, a well-known mTOR inhibitor, improved glucose intolerance in those rats[5]. On the other hand, the decrease in glycolysis, frequently present during IR, causes an impairment of BCATm activity due to a decrease in amino group acceptors[9,10], suggesting that IR would affect BCAA metabolism and therefore increase plasma BCAA.

Alterations in BCATm activity could also be the result of genetic variations. Previous studies have shown that individuals with the genetic variant in *BCAT2* (rs11548193) have lower serum BCAA concentrations compared with subjects with common allele[11]. This genetic variant in *BCAT2*, which corresponds to a non-synonymous SNP found on chromosome 19q13·33 and changes the amino acid threonine to arginine, is reported to have a worldwide frequency of 9·6 % and presents the following genotypes: GG, common homozygous; GC, heterozygous; and CC, uncommon homozygous[12].

Given that the genetic variant in the *BCAT2* gene modifies amino acid concentration, it is possible that the variant also modifies dietary treatment response. A reduction in energy intake is the main component of dietary treatments of subjects with obesity to achieve safe and adequate weight loss. A weight loss of approximately 3–5 % can improve clinical parameters, such as reducing triglyceride and glucose levels[13]. Many dietary approaches exist to reduce energy intake, and a restriction of 2093-4186 kJ/d or 3140 kJ/d is recommended to achieve a loss of 0·5–1 kg/week[13,14]. However, to our knowledge, there are no studies that evaluate the effect of reduced energy intake on plasma BCAA concentration and whether this effect is impacted by a genetic variant in the *BCAT2* gene. Our hypothesis is that the presence of the *BCAT2* polymorphism combined with the effect of an energy restriction intervention decreases the concentration of BCAA in subjects with obesity and IR. Therefore, the main objective of this study was to evaluate whether the presence of the rs11548193 polymorphism in the *BCAT2* gene can modify the effect of an energy restriction dietary intervention (DI) on the plasma concentration of BCAA in subjects with obesity and IR.

Experimental methods

Study design

A prospective cohort study was conducted at the Department of Nutrition Physiology at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) in Mexico between March 2018 and December 2019. The participants for the study were selected from a cohort of subjects with obesity underwent DI with an energy restriction of 3140 kJ/d based on total energy expenditure for 4 weeks, as recommended by the American Heart Association[13]. The study consisted of five weekly visits. Before the first visit, medical history and anthropometric and biochemical measurements were evaluated to determine whether subjects met the inclusion criteria. During the first and last visits, we performed a 24-h food recall and the International Physical Activity Questionnaire (IPAQ) and determined anthropometric measurements, body composition, resting energy expenditure (REE) and biochemical parameters in serum and plasma. In addition, a 2-h oral glucose tolerance test was performed to evaluate the AUC of glucose and insulin.

Participants

Mestizo Mexican adults between 18 and 60 years old with a BMI ≥ 30 kg/m^2 and insulin resistance (HOMA-IR ≥ 2·5) were included. Subjects with a history of weight loss ≥ 3 kg in the last 3 months, pregnant women, smokers, individuals with a history of amino acid supplement use, those with a diagnosis of chronic diseases such as type 2 diabetes, high blood pressure, CVD, cancer, autoimmune diseases, or liver or kidney diseases, and patients undergoing pharmacological treatment were excluded. The study was conducted according to the guidelines set forth in the Declaration of Helsinki, and all procedures involving human subjects were approved by the INCMNSZ Ethics Committee (reference: 2373). Written informed consent was obtained from all subjects.

Dietary intervention

The study participants received menus and a 30-d prescription with indications for a diet with a 3140 kJ/d energy restriction based on their habitual total energy expenditure[13]. Total energy expenditure was calculated by multiplying the REE determined by indirect calorimetry and the physical activity factor according to WHO/FAO recommendations[15]. The DI contained the following macronutrient distribution: protein 0·8–1 g/kg of body weight, 50 % energy content from carbohydrates and 25–30 % energy content from fat. The timing of protein intake was evenly distributed over the three meals (breakfast–lunch–dinner) with a mean (±SD) protein intake of 22·7g ± 6·70 per mealtime. They were also given a weekly food supply with 80 % of the foods contained in the menu to improve compliance with the diet. Compliance with the diet was evaluated weekly using a 24-h diet recall and a 3-d food record (food log). Previously, the participants were trained by a nutrition expert to correctly complete the 3-d food records. Data were processed and analysed in grams using Food Processor software (Version 11.6.522 2018, ESHA Research).

Anthropometric measurements and body composition

The anthropometric assessment included the determination of body weight, height and waist circumference using a standard calibrated electronic balance BSM 370 (Biospace Co. Ltd.) and

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a flexible tape measure (SECA 201, Hamburgo). The body composition assessment included the determination of fat-free mass, skeletal muscle mass, lean mass and fat mass by Inbody 720 multifrequency bioimpedance analysis (Biospace, Co.). Weight, height and waist circumference determinations were performed according to Lohman's method[16]. Body composition was performed with patients wearing light clothing and barefoot on the electrodes of the analyser platform and with their arms holding the upper electrodes at a 15-degree angle to their sides. All measurements were performed by a trained nutritionist.

Biochemical and clinical parameters

At the first and last visits, 5 ml of blood was collected after the participants fasted for 12 h. The blood sample was centrifuged at 3000 rpm for 10 min, and then the serum was separated and stored at –80°C until analysis. Serum concentrations of glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, TAG, aspartate aminotransferase, alanine aminotransferase, C-reactive protein, albumin, creatinine and urea were analysed by an enzymatic colorimetric method using the Cobas C111 analyser (Roche Diagnostic. Indianapolis. IN). Insulin (80-INSHU-E01 ALPCO), adiponectin (80-ADPHU-E01 ALPCO) and leptin (EZHL-80SK Millipore) were measured by ELISA. An oral glucose tolerance test was performed after the participants fasted for 12 h, and 0-, 15-, 30-, 45-, 60-, 90-, and 120-min glucose and insulin concentrations were determined after the participants consumed 75 g of glucose dissolved in water. Then, the AUC of glucose and insulin was determined using the trapezium rule[17]. HOMA-IR was calculated using the following equation: fasting glucose (mg/dl) × insulin (μU/ml)/405[18]. Blood pressure was measured at the beginning and end of the intervention using a digital sphygmomanometer; three measurements were taken at an interval of 5 min, and the average of the last two measurements was considered (Omron, HEM-781 INT).

Resting energy expenditure

REE was determined by the Quark PFT calorimeter (Cosmed). The measurements were conducted in a quiet, thermoneutral environment (20–25°C) with controlled humidity (45–55 %) in the morning (07.00 and 09.30 h) and after 10–12 h of fasting. Participants were instructed to refrain from engaging in vigorous resistance exercise, drinking alcohol or consuming caffeine for at least 12 h before attending their laboratory visit. The REE was determined according to the Weir equation without using the urinary urea nitrogen level[19].

Amino acid profile

The plasma amino acid profile was determined by HPLC. First, 150 μl of plasma was added to 38 μl of 10 % sulfosalicylic acid to deproteinise the sample. The samples were then incubated for 30 min at 4°C and centrifuged at 14 000 rpm for 10 min to separate the supernatant. Then, 100 μl of the supernatant was taken, and 1 μl of the internal standard (norvaline; 15 mM) was added; the sample was derivatised and injected. The procedure was performed using a sampling device (Agilent; G1367F) coupled to an HPLC system (Agilent 1260 Infiniti) and a fluorescence detector (Agilent; G1321B). A ZORBAX Eclipse AAA column was used and maintained at 40°C. Chromatographic conditions were maintained according to the column's technical instructions.

Branched-chain ketoacids

The concentration of BCKA present in serum was carried out by separating and quantifying them using an Exion LC AD system coupled to a SCIEX QTRAP 6500+ system. Serum was centrifuged at 14 000 g for 10 min at 4°C. Then, 100 μl of serum were deproteinised with 800 μl of 100 % cold methanol (–80°C), incubated for 30 min on ice and centrifuged at 14 000 g for 10 min at 4°C. The supernatant was dried in a Speedvac concentrator (Savant SPD121P) and the sample was then resuspended in 200 μl of water. Then, 1 μl of the extracted sample was injected and the BCKA (α-ketoisocaproate; α-keto-β-methylvalerate and α-ketoisovalerate) were separated using a chromatographic method previously reported[20], using a Zorbax Eclipse Plus C18 column, 4·6 × 50 mm, 1·8 μm (Agilent) at 30°C and a flow rate of 0·3 ml/min. Buffer A consisted of 10 mM of ammonium acetate in water and Buffer B of acetonitrile. The 10-min separation gradient consisted of 5 % B as starting conditions; 0–3 min a linear gradient from 5–30 % B; 3–3·5 min from 30–90 % B; 3·5–6·5 min from 90 % B; 6·5–7 min from 90–5 % B and 7–10 min from 5 % B. The BCKA were identified according to multiple reaction monitoring transitions and retention times. The compound parameters were optimised according to the metabolite: α-ketoisocaproate (Q1/Q3:129–> 129; DP: –55 V; EP: –10V; CE: –5 V; CXP: –40V); α-keto-β-methylvalerate (Q1/Q3:129–> 56·9; DP: –60V; EP: –10V; CE: –16V; CXP: –27V) and α-ketoisovalerate (Q1/Q3:115–> 115; DP: –55V; EP: –10V; CE: –5V; CXP: –40V). The ion source settings: curtain gas: 20 psi; voltage ionspray (V): –4500; ion source temperature: 200°C; nebuliser gas (GS1) and drying gas (GS2) were 20 and 40 psi, respectively.

Genotyping

At the first visit, an additional 5-ml blood sample was obtained, and DNA was extracted from the leucocytes using the QIAamp DNA Blood Mini Kit (QIAGEN) as described by Miller *et al.*[21] The presence of the *BCAT2* gene SNP (rs11548193) was evaluated by real-time PCR using predesigned TaqMan assays (Roche) on a LightCycler 480 instrument (Roche). Genotypes were in Hardy–Weinberg equilibrium according to the χ^2 test.

Statistical analysis

The sample size was calculated with Quanto software according to a β-type error of 0·2, an α type error of 0·05, a polymorphism frequency of 10–26 % and a difference in the concentrations of BCAA of 22·8 ± 63·3[22]. Continuous variables are expressed as the mean and standard error, and dichotomous variables are expressed as frequencies and percentages. The distribution of variables was evaluated using the Kolmogorov–Smirnov test; variables with non-normal distribution were log-transformed prior to analysis. To analyse the difference in the baseline

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variables between those who presented the *BCAT2* polymorphism and those who did not, Student's independent sample *t* test was used. Baseline and final (after 1 month) anthropometric, clinical, biochemical and amino acids parameters were compared among groups using two-way ANOVA (time × genotype) adjusted for age and sex. To analyse the differences in absolute values of the anthropometric, clinical, biochemical variables and plasma amino acid profiles between those with the *BCAT2* polymorphism and those without it, Student's *t* test for independent samples was used. Mann–Whitney U statistical analysis was used to analyse whether there were differences in diet adherence between those with the *BCAT2* polymorphism and those without it. The significance level was set at *P*-value < 0·05. The data were analysed using SPSS for Mac (version 25, SPSS Inc.).

Results

Participants

A total of 82 subjects with obesity (BMI 38·1 ± 5·89 kg/m^2) and IR (HOMA-IR 6·62 ± 4·32) were included in the study. The genotypic frequency of the *BCAT2* SNP was 81·7 % for common homozygosity (GG), while for heterozygosity plus uncommon homozygosity (GC + CC), it was 18·3 %. Of the 82 participants, 75 completed the study (GG = 60 participants, GC + CC = 15 participants). Baseline anthropometric, biochemical and clinical characteristics according to the presence and absence of SNP were similar among the participants and are presented in Table 1. Of the participants who did not complete the study, three subjects did not finish for family reasons, two for a change of address, one due to an accident and one because of hypolipemic treatment initiation.

Energy and nutrient intake

The subjects were instructed to follow a diet with an energy restriction of 3140 kJ/d for 1 month. The median percentage of compliance with the dietary treatment was 100 % for the two groups, with no significant differences. The characteristics of composition of the diet consumed by the subjects are shown in Supplementary Table 1.

Anthropometric and clinical variables

During the follow-up, we evaluated the changes in body composition variables. All the subjects had decreased weight, regardless of the presence of the *BCAT2* polymorphism (3·18 ± 0·25 kg *v.* 3·76 ± 0·55 kg). Interestingly, the group that presented the *BCAT2* polymorphism had a greater decrease in skeletal muscle mass (0·39 ± 0·10 kg *v.* 0·96 ± 0·27 kg), fat-free mass (0·67 ± 0·16 kg *v.* 1·60 ± 0·47 kg) and total body water (1·37 ± 0·32 lt *v.* 3·12 ± 0·91 L) compared with the changes in the group that did not present the polymorphism; however, no significant changes were observed. Interestingly, a significant interaction when adjusting for age and sex was observed in weight, skeletal muscle mass, fat-free mass and total corporal water ($P < 0·0001$) (Table 2). Regarding blood pressure, it was observed that after the intervention, both groups presented similar decreases. On the other hand, the dynamometry-measured muscle strength presented a greater reduction in the group with the polymorphic variant of *BCAT2* (1·52 ± 0·89 kg) than in the group without the polymorphic variant (0·01 ± 0·43 kg); however, this difference was not statistically significant. There was no statistically significant change in the REE or RQ. Interestingly, when adjusting for age and sex, a significant interaction was observed in systolic blood pressure, diastolic blood pressure, muscle strength and REE ($P < 0·05$) (Table 3).

Table 1. Baseline anthropometric, biochemical and clinical characteristics according to the presence and absence of the *BCAT2* polymorphism

	BCAT2				
	GG *n* 60		GC + CC *n* 15		
	Mean	SE	Mean	SE	*P*
Age (years)	39·2	1·31	34·6	2·91	0·12
Sex (n, %)					
Female					
n	41		13		0·20
%	68		87		
Male					
n	19		2		
%	32		13		
Anthropometric and body composition parameters					
Weight (kg)	98·6	2·72	98·6	4·76	0·99
BMI (kg/m^2)*	38·0	0·75	37·7	1·64	0·88
Waist (cm)*	117	1·87	113	3·99	0·31
Fat mass (%)	46·7	0·76	45·7	1·45	0·55
Skeletal muscle mass (%)	29·6	0·45	30·3	0·83	0·50
Fat-free mass (%)	53·2	0·75	54·3	1·47	0·51
Visceral fat area (cm^2)	158	3·78	153	6·02	0·51
TBW (L)*	38·3	1·04	38·9	1·47	0·80
Blood pressure, REE and physical activity					
SBP (mmHg)	111	1·54	111	3·81	0·89
DBP (mmHg)*	78·8	1·28	77·2	3·05	0·65
REE (kJ)*	7708	256	7503	271	0·73
TEE (kJ)	9898	2458	9751	1361	0·73
Muscular strength (kg)*	26·5	1·22	26·8	2·35	0·79
Biochemical parameters					
Glucose (mg/dl)	104	1·63	107	3·51	0·54
Total cholesterol (mg/dl)	177	4·11	181	9·76	0·71
TAG (mg/dl)*	195	15·8	188	23·5	0·84
HDL-cholesterol (mg/dl)	32·4	1·12	33·3	3·41	0·74
LDL-cholesterol (mg/dl)	132	4·13	131	9·52	0·93
AST (U/L)*	27·5	1·93	31·8	7·44	0·42
ALT (U/L)*	35·2	2·86	35·7	8·50	0·93
Albumin (g/l)	41·0	0·35	41·0	0·71	0·96
Creatinine (mg/dl)*	0·66	0·02	0·64	0·02	0·60
CRP (mg/l)*	4·91	0·42	4·26	0·66	0·49
Leptin (ng/ml)	48·4	3·04	50·6	6·32	0·74
Adiponectin (μg/ml)*	6·42	0·33	6·97	1·14	0·53
Insulin (μU/ml)*	24·2	2·09	27·8	3·83	0·43
IR-HOMA*	6·21	0·51	7·24	1·01	0·37
BCAA (nmol/ml)	339	9·08	355	9·83	0·26

BCAT2, branched-chain aminotransferase 2; TBW, total body water; SBP, systolic blood pressure; DBP, diastolic blood pressure; REE, resting energy expenditure; TEE, total energy expenditure; AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein; IR-HOMA, insulin resistance homeostatic model assessment; BCAA, branched-chain amino acids.
Differences between the groups were tested with independent sample Student's *t* tests. All variables were logarithmically transformed prior to analysis.
* Variables with non-parametric distribution.

Biochemical variables

After the intervention, a decrease in glucose, total cholesterol, TAG, LDL-cholesterol, aspartate aminotransferase, alanine

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Table 2. Baseline and final anthropometric and body composition parameters according to the presence and absence of the *BCAT2* polymorphism (Mean values and standard errors; all variables were logarithmically transformed prior to analysis)

	Baseline		1 month		Δ			
	Mean	SE	Mean	SE	Mean	SE	*P**	*P***
Weight, kg								
GG	98·6	2·72	95·4	2·63	–3·18	0·25	0·94	<0·0001
GC + CC	98·6	4·76	94·9	4·55	–3·76	0·55		
BMI, kg/m^2†								
GG	38·0	0·75	36·8	0·75	–1·19	0·08	0·90	0·99
GC + CC	37·7	1·64	36·3	1·58	–1·45	0·19		
Waist, cm†								
GG	117	1·87	113	1·79	–4·20	0·36	0·91	0·16
GC + CC	113	3·99	108	3·96	–4·51	0·65		
Fat mass, kg								
GG	46·4	1·67	43·9	1·66	–2·48	0·26	0·94	0·96
GC + CC	45·6	3·44	43·5	3·20	–2·10	0·55		
SMM, kg†								
GG	29·1	0·83	28·7	0·82	–0·39	0·10	0·79	<0·0001
GC + CC	29·6	1·18	28·6	1·17	–0·96	0·27‡		
FFM, kg†								
GG	52·2	1·40	51·5	1·38	–0·67	0·16	0·80	<0·0001
GC + CC	53·0	1·99	51·4	1·97	–1·60	0·47‡		
VFA, cm^2								
GG	158	3·78	153	3·93	–5·27	0·96	0·82	0·17
GC + CC	153	6·02	150	6·27	–3·26	1·46		
TCW, L†								
GG	38·3	1·04	37·7	1·01	–1·37	0·32	0·81	<0·0001
GC + CC	38·9	1·47	37·6	1·45	–3·12	0·91‡		

BCAT2, branched-chain aminotransferase 2; SMM, skeletal muscle mass; FFM, fat-free mass; VFA, visceral fat area; TCW, total corporal water.
* Statistical analysis was with two-way ANOVA (time × genotype) test.
** Two-way ANOVA adjusted for age and sex.
† Variables with non-parametric distribution.
‡ *P*-value < 0·05 with Student's *t* test for independent samples.

Table 3. Baseline and final blood pressure, REE and muscle strength according to the presence and absence of the *BCAT2* polymorphism (Mean values and standard errors; all variables were logarithmically transformed prior to analysis)

	Baseline		1 month		Δ			
	Mean	SE	Mean	SE	Mean	SE	*P**	*P***
SBP, mmHg								
GG	111	1·54	106	1·51	–4·48	1·06	0·80	0·026
GC + CC	111	3·81	104	3·19	–5·56	2·15		
DBP, mmHg†								
GG	78·8	1·28	74·4	1·22	–4·40	0·95	0·91	0·005
GC + CC	77·2	3·05	72·6	2·79	–4·67	1·81		
Muscle strength, kg†								
GG	26·5	1·22	26·4	1·16	–0·01	0·43	0·92	<0·0001
GC + CC	26·8	2·35	25·3	1·86	–1·52	0·89		
REE, kJ†								
GG	7708	255	7410	199	–296	166	0·47	<0·0001
GC + CC	7503	270	6916	347	–594	261		
RQ								
GG	0·83	0·01	0·85	0·01	0·02	0·01	0·51	0·33
GC + CC	0·81	0·02	0·81	0·03	–0·01	0·02		

BCAT2, branched-chain aminotransferase 2; SBP, systolic blood pressure; DBP, diastolic blood pressure; REE, resting energy expenditure.
Muscle strength, 55 subjects in the GG group and 13 subjects in the GC + CC group. REE, 56 subjects in the GG group and 14 subjects in the GC + CC group.
* Statistical analysis was with two-way ANOVA (time × genotype) test.
** Two-way ANOVA adjusted for age and sex.
† Variables with non-parametric distribution.

aminotransferase, C-reactive protein, leptin, adiponectin and insulin concentration was observed in both groups, and no significant difference was observed between those who presented the *BCAT2* polymorphism variant and those who did not. Likewise, as for the HDL cholesterol concentration, a decrease of 2·60 ± 1·30 mg/dl was observed in the group with the *BCAT2* polymorphism compared with an increase of 2·14 ± 0·67 mg/dl in the group that did not present the polymorphism without the polymorphism, but no significance was observed. Nevertheless, when adjusting for age and sex, a significant interaction was

Table 4. Baseline and final biochemical parameters according to the presence and absence of the *BCAT2* polymorphisms (Mean values and standard errors; all variables were logarithmically transformed prior to analysis)

	Baseline		1 month		Δ			
	Mean	SE	Mean	SE	Mean	SE	*P**	*P***
Glucose, mg/dl								
GG	104	1·63	97·1	1·22	−7·20	1·60	0·14	0·7
GC + CC	107	3·51	92·8	2·14	−13·8	3·93		
Total cholesterol, mg/dl								
GG	177	4·11	162	4·04	−14·8	4·54	0·60	0·9
GC + CC	181	9·76	159	9·48	−21·6	7·23		
TAG, mg/dl†								
GG	195	15·8	136	7·74	−58·8	14·6	0·79	0·04
GC + CC	188	23·5	145	21·9	−43·4	22·3		
HDL-cholesterol, mg/dl								
GG	32·4	1·12	34·5	1·18	2·14	0·67	0·24	0·73
GC + CC	33·3	3·41	30·7	2·85	−2·60	1·30‡		
LDL-cholesterol, mg/dl								
GG	132	4·13	116	4·34	−16·2	3·50	0·87	0·61
GC + CC	131	9·52	113	8·88	−18·4	6·69		
AST, U/L†								
GG	27·5	1·93	23·6	1·00	−3·94	1·21	0·64	0·5
GC + CC	31·8	7·44	25·3	5·10	−6·48	3·08		
ALT, U/L†								
GG	35·2	2·86	31·9	2·24	−3·28	1·25	0·74	0·003
GC + CC	35·7	8·50	30·0	7·30	−5·68	2·48		
Albumin, g/l								
GG	41·0	0·35	40·9	0·40	−0·05	0·28	0·15	<0·0001
GC + CC	41·0	0·71	39·2	0·78	−1·74	0·77‡		
Creatinine, mg/dl†								
GG	0·66	0·02	0·65	0·01	−0·01	0·01	0·14	<0·0001
GC + CC	0·64	0·02	0·56	0·03	−0·07	0·02‡		
CRP, mg/dl†								
GG	4·91	0·42	4·16	0·44	−0·75	0·42	0·75	0·74
GC + CC	4·26	0·66	3·11	0·52	−1·15	0·39		
Leptin, ng/ml								
GG	48·4	3·04	37·2	2·80	−11·1	1·42	0·92	<0·0001
GC + CC	50·6	6·32	38·2	5·58	−12·4	3·59		
Adiponectin, ug/ml†								
GG	6·42	0·33	5·99	0·30	−0·42	0·17	0·96	0·1
GC + CC	7·17	1·14	6·42	0·78	−0·75	0·48		
Insulin, UI/ml†								
GG	24·2	2·09	16·5	1·08	−7·63	2·24	0·75	0·51
GC + CC	27·8	3·83	20·1	3·71	−7·64	3·32		
AUC glucose								
GG	18 212	507	16 526	421	−1685	487	0·43	0·97
GC + CC	18 410	783	15 692	568	−2718	552		
AUC insulin								
GG	16 251	879	12 702	801	−3548	601	0·88	0·035
GC + CC	15 687	1751	12 414	1823	−3272	1617		
IR-HOMA†								
GG	6·21	0·52	3·95	0·26	−2·25	0·55	0·63	0·62
GC + CC	7·24	1·01	4·67	0·89	−2·57	0·90		
Matsuda index								
GG	1·83	0·10	2·74	0·18	0·90	0·17	0·43	0·039
GC + CC	1·69	0·19	3·30	0·61	1·60	0·54		

BCAT2, branched-chain aminotransferase 2; AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein; IR-HOMA, insulin resistance homeostatic model assessment.
* Statistical analysis was with two-way ANOVA (time × genotype) test.
** Two-way ANOVA adjusted for age and sex.
† Variables with non-parametric distribution.
‡ *P*-value < 0·05 with Student's *t* test for independent samples.

observed in the TAG, alanine aminotransferase, albumin, creatinine, leptin, AUC insulin and Matsuda index ($P < 0{\cdot}05$) (Table 4).

Amino acid profile

After the intervention, the group that presented the *BCAT2* polymorphism had a greater decrease in the concentrations of the following amino acids: proline (18·5 ± 11·8 nmol/ml *v.* 67·4 ± 19·0 nmol/ml), lysine (3·68 ± 3·00 nmol/ml *v.* 27·3 ± 7·19 nmol/ml) and alanine (46·3 ± 10·2 nmol/ml *v.* 120 ± 30·0 nmol/ml) ($P < 0{\cdot}05$) (Fig. 1). Interestingly, when adjusting for age and sex, a significant interaction was observed with the BCAA leucine, isoleucine, valine and with

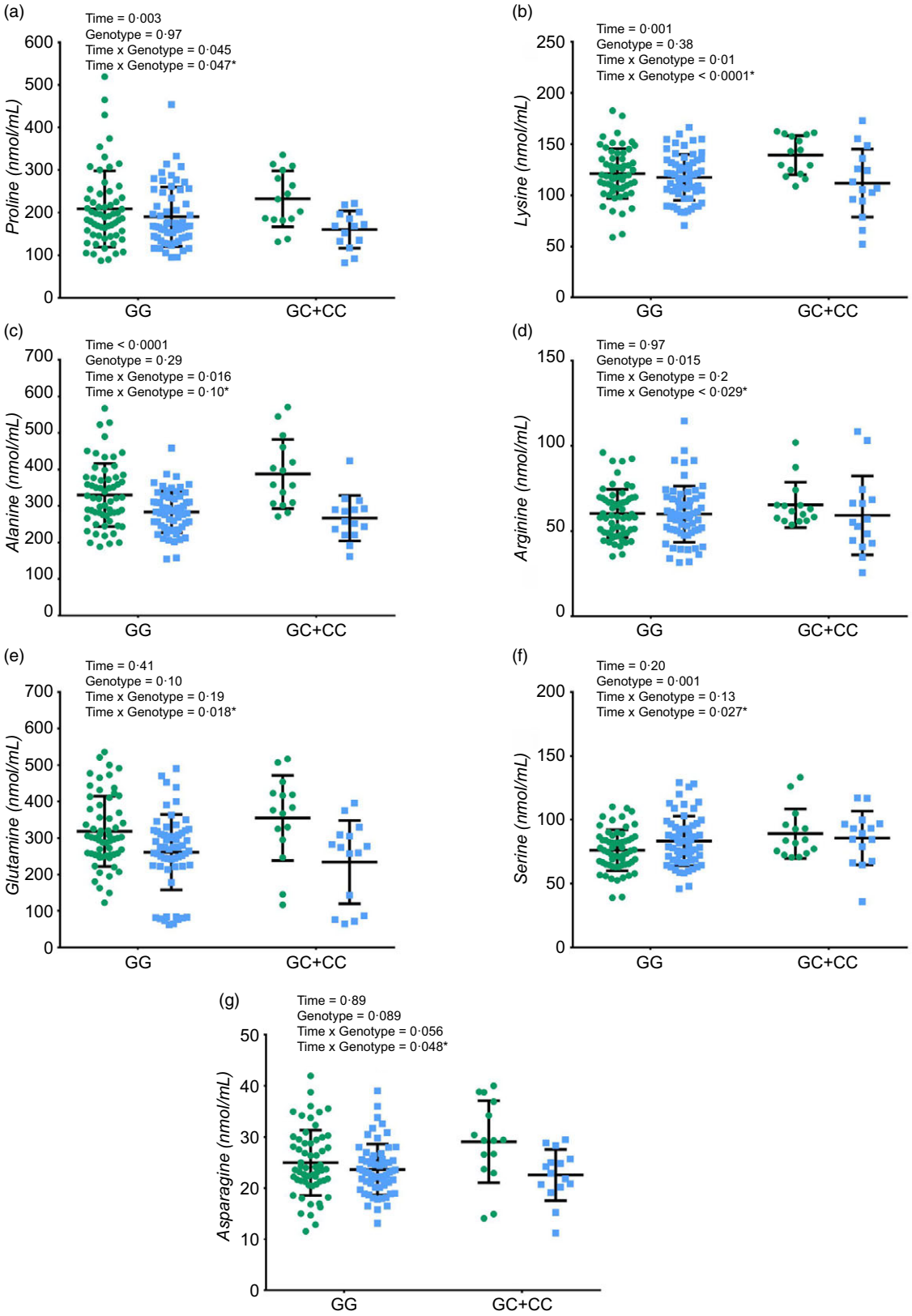

Fig. 1. Baseline and final concentration of a, proline; b, lysine; c, alanine; d, arginine; e, glutamine; f, serine; and g, asparagine. Statistical analysis was performed using two-way ANOVA. *Adjusted for age and sex. ●, Baseline; ■, Final

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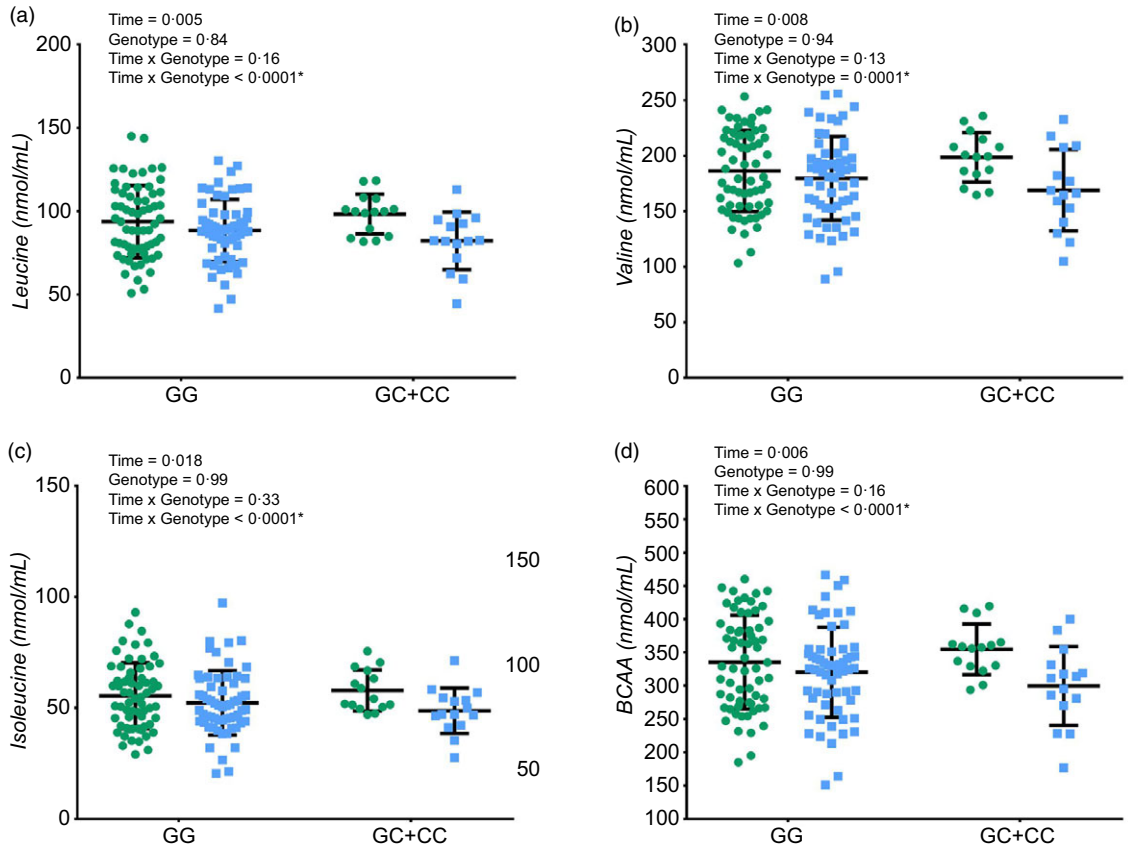

Fig. 2. Baseline and final concentration of a, leucine; b, valine; c, isoleucine; and d, sum of BCAA. Statistical analysis was performed using two-way ANOVA. *Adjusted for age and sex. ●, Baseline; ■, Final. BCAA, branched-chain amino acid.

the total sum of BCAA ($P < 0{\cdot}001$) (Fig. 2), and also with the amino acids arginine, glutamine, serine and asparagine ($P < 0{\cdot}05$). There was no significant interaction with the rest of the amino acids (Table 5).

Branched-chain ketoacids

As BCAA changed significantly when adjusted for age and sex, we determined the concentration of the three BCKA derived from leucine, isoleucine and valine, α-ketoisocaproate, α-keto-β-methylvalerate and α-ketoisovalerate, respectively, which are the products of *BCAT2* activity. When adjusted for age and sex, a significant increase was observed with all three keto acids ($P < 0{\cdot}0001$) in the *BCAT2* polymorphism group (Table 6).

Discussion

This study investigated whether the presence of the rs11548193 SNP in the *BCAT2* gene can modify the effects of a DI on the concentration of BCAA in individuals with obesity and IR. Our results showed that an energy restriction of 3140 kJ/d based on total energy expenditure promoted a weight loss higher than 3 % in both intervention groups, similar to what was previously reported in other studies[13]. The change in body weight was not associated with changes in the REE or the type of oxidised substrate (RQ). Therefore, the decrease in body weight can be directly associated with dietary energy restriction since the intake of macronutrients was similar between the two groups. Likewise, in both groups, the intake of saturated fats was less than 10 % of the energy value, demonstrating a good-quality diet with low consumption of processed meats, following the recommendations of international guides[13,23].

Protein intake was similar and adequate; more than 20 % of energy was obtained from protein in both groups[13,23]. Although the amount of protein consumed was adequate in both groups, a decrease in skeletal muscle mass and fat-free mass in the *BCAT2* polymorphism (rs11548193) group was observed. In this sense, it has been suggested that muscle protein synthesis does not change or increase after exercise or high protein intake in individuals with obesity and IR[24]. Protein synthesis and degradation in muscle are regulated by BCAA through the activation of mTORC1 and ubiquitin proteasome pathways, respectively[3]. Our results showed that after adjusting for age and sex, the presence of the *BCAT2* SNP significantly decreased the concentration of leucine, valine and overall BCAA after the adoption of an energy-restricted normoprotein diet, which could decrease mTOR activation and, consequently, protein synthesis[25,26]. In addition, the decrease in BCAA concentration could be associated with a deficiency of BCATm metabolites such as α-ketoisocaproic acid and β-hydroxy-β-methylbutyrate, which

Table 5. Baseline and final plasma amino acid profile according to the presence and absence of the *BCAT2* polymorphism (Mean values and standard errors; all variables were logarithmically transformed prior to analysis)

	Baseline		1 month		Δ			
	Mean	SE	Mean	SE	Mean	SE	*P**	*P***
Proline, nmol/ml†								
GG	209	11·5	191	9·02	−18·5	11·8	0·045	0·047
GC + CC	228	17·4	161	11·7	−67·4	19·0		
Lysine, nmol/ml								
GG	121	3·14	118	2·89	−3·68	3·00	0·010	<0·0001
GC + CC	139	4·91	112	8·56	−27·3	7·19‡		
Leucine, nmol/ml								
GG	94·9	2·80	88·4	2·41	−6·48	2·56	0·16	<0·0001
GC + CC	98·3	3·08	82·2	4·46	−16·1	4·39		
Isoleucine, nmol/ml								
GG	56·2	1·92	52·3	1·87	−3·96	1·82	0·33	<0·0001
GC + CC	57·8	2·37	48·7	2·65	−9·11	2·95		
Phenylalanine, nmol/ml								
GG	41·9	0·97	38·3	1·19	−3·63	1·57	0·49	0·28
GC + CC	43·8	0·91	38·2	2·20	−5·58	2·16		
Tryptophan, nmol/ml								
GG	34·3	1·07	32·8	1·09	−1·59	1·27	0·10	0·08
GC + CC	39·9	1·72	32·1	1·94	−7·89	2·31‡		
Methionine, nmol/ml†								
GG	107	2·02	106	1·07	−0·97	2·90	0·27	0·09
GC + CC	113	1·89	104	3·84	−8·67	4·28		
Valine, nmol/ml								
GG	188	4·73	179	4·85	−8·84	4·25	0·13	0·001
GC + CC	198	5·73	169	9·46	−29·5	10·7‡		
Tyrosine, nmol/ml								
GG	58·6	1·74	52·2	1·49	−6·35	1·65	0·058	0·4
GC + CC	63·5	2·61	47·9	1·69	−15·5	3·00‡		
Alanine, nmol/ml								
GG	330	11·1	284	7·34	−46·3	10·2	0·016	0·1
GC + CC	387	24·4	266	16·1	−120	30·0		
Arginine, nmol/ml								
GG	60·3	1·83	59·9	2·12	−0·34	2·01	0·20	0·029
GC + CC	65·3	3·42	59·2	5·98	−6·15	6·20		
Threonine, nmol/ml†								
GG	186	4·62	165	5·25	−21·0	6·10	0·11	0·19
GC + CC	203	9·97	153	12·7	−50·6	17·0‡		
Glycine, nmol/ml†								
GG	160	7·79	142	6·77	−17·2	8·43	0·17	0·23
GC + CC	193	22·7	136	10·4	−56·7	25·6		
Histidine, nmol/ml†								
GG	28·6	1·71	63·7	2·97	35·0	12·2	0·44	0·7
GC + CC	38·4	2·72	68·7	17·3	30·3	16·0		
Glutamine, nmol/ml†								
GG	318	12·4	261	13·3	−57·2	20·4	0·19	0·018
GC + CC	355	30·1	234	29·5	−121	45·2		
Serine, nmol/ml								
GG	76·1	2·06	83·4	2·52	7·02	2·00	0·13	0·027
GC + CC	89·1	5·00	85·7	5·43	−3·41	6·23		
Asparagine, nmol/ml								
GG	24·9	0·82	23·6	0·64	−1·35	0·83	0·056	0·048
GC + CC	29·0	2·07	22·5	1·29	−6·51	2·27‡		
Glutamate, nmol/ml†								
GG	69·9	4·21	63·0	3·81	−6·98	5·58	0·37	0·33
GC + CC	82·0	13·5	58·8	6·94	−23·1	14·0		
Aspartate, nmol/ml†								
GG	5·17	0·16	5·71	0·42	0·61	0·46	0·11	0·14
GC + CC	6·08	0·85	5·03	0·48	−1·05	1·06		
ΣBCAA, nmol/ml								
GG	340	9·08	320	8·73	−19·2	7·96	0·16	<0·0001
GC + CC	354	9·83	299	15·2	−54·8	16·5‡		

BCAT2, branched-chain aminotransferase 2; BCAA, branched-chain amino acids.
* Statistical analysis was with two-way ANOVA (time × genotype) test.
** Two-way ANOVA adjusted for age and sex.
† Variables with non-parametric distribution.
‡ *P*-value < 0·05 with Student's *t* test for independent samples.

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Table 6. Baseline and final serum branched-chain ketoacids concentration according to the presence and absence of the *BCAT2* polymorphism (Mean values and standard errors; all variables were logarithmically transformed prior to analysis)

	Baseline		1 month		Δ			
	Mean	SE	Mean	SE	Mean	SE	*P**	*P***
KIC, μM								
GG	19·3	0·87	19·6	0·80	0·23	0·82	0·85	<0·0001
GC + CC	18·9	1·08	19·7	0·78	0·78	1·10		
KMV, μM								
GG	30·0	1·45	30·8	1·20	0·79	1·33	0·98	<0·0001
GC + CC	29·5	1·93	31·0	1·29	1·53	1·70		
KIV, μM								
GG	15·0	0·56	14·2	0·43	−0·75	0·50	0·63	<0·0001
GC + CC	14·4	0·69	15·0	0·59	0·60	0·74		

BCAT2, branched-chain aminotransferase 2; KIC, α-ketoisocaproic acid; KMV, α-keto-β-methylvalerate acid; KIV, α-ketoisovaleric acid.
* Statistical analysis was with two-way ANOVA (time × genotype) test.
** Two-way ANOVA adjusted for age and sex.

mediate signalling through mTORC1 activation to decrease protein breakdown[27]. However, our results showed that the concentration of these types of BCKA increased significantly when adjusted for age and sex, indicating that plasma BCKA concentration may not reflect BCAA concentrations, as it will also depend on the activity of downstream enzymes in their catabolism, including but not limited to branched-chain ketoacid dehydrogenase (BCKDH). In addition, the effect of BCAA and branched-chain ketoacids could be beyond modulation of mTOR activity. In fact, further research is needed to better understand the implications of the *BCAT2* polymorphism on BCAA and BCKA concentration, especially in tissues. For instance, it has been demonstrated that an increase in BCKA in skeletal muscle of mice with diet-induced obesity inhibited insulin-induced protein kinase B (AKT) phosphorylation and decreased glucose uptake, indicating a decrease in insulin signalling[28].

The *BCAT2* rs11548193 polymorphism is a non-synonymous C > G mutation, causing a change from threonine to arginine at position 186, which could alter *BCAT2* activity. In fact, structural crystallographic analysis shows that the Arg186 produced by the SNP might be repulsed by Lys59, leading to protein dimer destabilisation and altering enzyme[29]. The reduction in plasma BCAA and the increase in the BCKA in the subjects carrying the variant suggest that the rs11548193 polymorphism may increase *BCAT2* activity. However, further research is needed to determine the effect of the evaluated polymorphisms on *BCAT2* specificity and affinity to substrates, and therefore activity. In addition to the reduction in leucine in the group that presented the polymorphism, there was a decrease in the concentration of other amino acids such as proline, lysine, tryptophan, valine, tyrosine, alanine and serine and in the sum of BCAA. The decrease in alanine is particularly relevant as BCAA transamination is the main source of alanine, and thus an increase in alanine would have been expected as previously reported[30]. Further research is needed to establish whether the decrease in alanine is the result of an increase in amino acid oxidation, which could be assessed by measuring ammonia excretion or a specific increase in alanine utilisation in the gluconeogenic or glyceroneogenic pathways.

Moreover, in individuals with obesity, the increase in BCAA has been associated with diverse metabolic alterations, mainly IR[3]. Nevertheless, IR could also cause the increase in BCAA levels as has been previously reviewed[9,10]. In this regard, mitochondrial function plays a determinant role. Excess nutrients or macronutrients cause mitochondrial acetyl-CoA overload, which alters the catabolism of glucose by inhibiting pyruvate dehydrogenase, of fatty acids by inhibiting carnitine-palmitoyl transferase 1 and of BCAA by activating branched-chain ketoacid dehydrogenase kinase, which decreases BCAA catabolism. This mitochondrial blockage leads to accumulation of acylcarnitines of different chain lengths, which have emerged as inducers of mitochondrial dysfunction[31], responsible for IR[32] and biomarkers of metabolic diseases[33]. Likewise, several studies have found that modifications in the plasma amino acid profile can be used as an early indicator of the risk of metabolic alterations, mainly those involving glucose metabolism. In fact, one study in normoglycaemic individuals showed that plasma levels of isoleucine, leucine, valine, tyrosine and phenylalanine predicted the development of diabetes during a 12-year follow-up[8]. Another study showed that fasting glucose levels and HOMA-IR correlated positively with valine, glutamate and tyrosine levels but negatively with citrulline, glutamine and glycine levels. Additionally, it has been shown that β-cell function correlates positively with the levels of leucine, tryptophan, valine and glutamate but negatively with the levels of citrulline, glutamine, glycine and serine[34,35]. Therefore, the decrease in these amino acids raises the possibility that alterations in the levels of plasma metabolites may play an important role in the metabolic outcome of participants with *BCAT2* polymorphisms. This type of information allows metabolic profiles to be generated in patients to identify early pathophysiological changes.

One limitation of the study was the small sample size and the short intervention period. Thus, we need further studies to confirm the results with a larger sample size and a longer intervention duration.

Conclusions

This study showed that compared with the absence of the SNP, the presence of the rs11548193 *BCAT2* polymorphism was associated with decreased BCAA concentrations after 1 month of an energy-restricted DI, after adjusting for age and sex. These results represent a starting point for future intervention studies with a greater number of patients.

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There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114521002920

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