European Journal of Nutrition (2021) 60:1769–1780
https://doi.org/10.1007/s00394-020-02370-6

ORIGINAL CONTRIBUTION

Dietary intake of specific amino acids and liver status in subjects with nonalcoholic fatty liver disease: fatty liver in obesity (FLiO) study

Cristina Galarregui[1] · Irene Cantero[1] · Bertha Araceli Marin-Alejandre[1] · J. Ignacio Monreal[2,3] · Mariana Elorz[2,4] · Alberto Benito-Boillos[2,4] · José Ignacio Herrero[2,5,6] · Víctor de la O[2,7] · Miguel Ruiz-Canela[2,7,8] · Helen Hermana M. Hermsdorff[9] · Josefina Bressan[9] · Josep A. Tur[8,10] · J. Alfredo Martínez[1,2,8] · M. Angeles Zulet[1,2,8] · Itziar Abete[1,2,8]

Received: 10 April 2020 / Accepted: 19 August 2020 / Published online: 28 August 2020

Abstract

Purpose Identification of dietary factors involved in the development and progression of nonalcoholic fatty liver disease (NAFLD) is relevant to the current epidemics of the disease. Dietary amino acids appear to play a key role in the onset and progression of NAFLD. The aim of this study was to analyze potential associations between specific dietary amino acids and variables related to glucose metabolism and hepatic status in adults with overweight/obesity and NAFLD.

Methods One hundred and twelve individuals from the Fatty Liver in Obesity (FLiO) study were evaluated. Liver assessment was carried out by ultrasonography, magnetic resonance imaging and analysis of biochemical parameters. Dietary amino acid intake (aromatic amino acids (AAA); branched-chain amino acids (BCAA); sulfur amino acids (SAA)) was estimated by means of a validated 137-item food frequency questionnaire.

Results Higher consumption of these amino acids was associated with worse hepatic health. Multiple adjusted regression models confirmed that dietary AAA, BCAA and SAA were positively associated with liver fat content. AAA and BCAA were positively associated with liver iron concentration. Regarding ferritin levels, a positive association was found with BCAA. Dietary intake of these amino acids was positively correlated with glucose metabolism (glycated hemoglobin, triglyceride and glucose index) although the significance disappeared when potential confounders were included in the model.

Conclusion These findings suggest that the consumption of specific dietary amino acids might negatively impact on liver status and, to a lesser extent on glucose metabolism in subjects with overweight/obesity and NAFLD. A control of specific dietary amino acid composition should be considered in the management of NAFLD and associated insulin resistance. NCT03183193; June 2017.

Keywords Branched-chain amino acids · Sulfur amino acids · Aromatic amino acids · Fatty liver · Type 2 diabetes · Protein metabolism

Abbreviations

AAA	Aromatic amino acids
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAT	Brown adipose tissue
BCAA	Branched-chain amino acids
BMI	Body mass index
CMIA	Chemiluminescent microparticle immunoassay
CVD	Cardiovascular diseases
ELISA	Enzyme-linked immunosorbent assay
FFA	Free fatty acids
FFQ	Food frequency questionnaire
GGT	Gamma glutamyl transferase

M. Angeles Zulet and Itziar Abete contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00394-020-02370-6) contains supplementary material, which is available to authorized users.

✉ M. Angeles Zulet
mazulet@unav.es

✉ Itziar Abete
iabetego@unav.es

Extended author information available on the last page of the article

HbA1c	Glycated hemoglobin
HDL-c	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IDF	International Diabetes Federation
IR	Insulin resistance
IRS-1	Insulin receptor substrate-1
LDL-c	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
SAA	Sulfur amino acids
T2D	Type 2 diabetes
TG	Triglycerides
TyG index	Triglyceride–glucose index
WAT	White adipose tissue

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a condition of excessive lipid accumulation in the absence of alcohol abuse [1]. Its spectrum ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma [2, 3]. NAFLD is a highly widespread cause of chronic liver disease worldwide and its global prevalence is approximately 25%, with the highest incidence in the Middle East and South America [4]. NAFLD potentially contributes to an important burden of extra-hepatic complications. It frequently appears in combination with the metabolic syndrome (MetS) and cardiovascular diseases (CVD) [5, 6]. Likewise, NAFLD often coexists with insulin resistance (IR). Current evidences suggest that NAFLD is a recognized risk factor for the development of type 2 diabetes (T2D), and vice versa, individuals with T2D have an increased risk of developing NAFLD [7, 8].

In addition to genetic predisposition, unhealthy lifestyles based on a sedentary behaviour together with high energy density diets, are considered important contributors to the disease [9–11]. Low-grade inflammatory processes as well as oxidative stress are the main metabolic mediators involved in the onset/evolution of NAFLD [12, 13]. Therapeutic approaches focus on lifestyle modification remain as the first line of therapy, aiming mainly at controlling body weight and cardio-metabolic risk factors related to metabolic syndrome [14–16].

It is known that nutrients are not consumed in isolation in the daily diet; however, some dietary components might have a key role within a dietary pattern since they might trigger inflammation and oxidative stress [17, 18]. In this context, dietary amino acids may be important factors to be considered in the relationship between dietary protein and chronic diseases [19].

An adequate intake of amino acids is necessary for protein synthesis and maintenance of long-term balance. However, research findings suggested that a dietary amino acid pattern, rich in Branched-Chain amino acids (BCAA, leucine, isoleucine and valine) and Sulfur amino acids (SAA, methionine and cystine), among others could increase the risk of hypertension [20]. More recently, individual or cluster of amino acids have been associated with the incident cardiovascular disorders, suggesting their significant role in the pathogenesis of CVD. High concentrations of BCAA have been observed in individuals with CVD risk [21].

Recent studies also suggest an association between specific dietary amino acids and plasma concentrations, specifically BCAA, with increased risk of other metabolic disturbances (obesity, T2D and hepatic lipid accumulation) [22, 23]. Likewise, some investigations have found increased Aromatic amino acids (AAA, tyrosine and phenylalanine) [23] and SAA [24] in liver disease and IR.

The aim of this research was to analyze potential associations between specific dietary amino acids (AAA; BCAA; SAA) and variables related to glucose metabolism and hepatic health in subjects with overweight/obesity and NAFLD.

Materials and methods

Participants

The current cross-sectional study included 112 (65 male and 47 female) adults between 40–80 years old with overweight/obesity and ultrasound-confirmed liver steatosis [diagnosis made by professional hepatologists using an ultrasonography equipment (Siemens ACUSON S2000 and S3000, Erlangen, Germany)] [25]. Exclusion criteria included the presence of known liver disease (other than NAFLD), ≥ 3 kg body weight loss in the last 3 months, high alcohol consumption (>21 and >14 units of alcohol per week for men and women, respectively) [26], endocrine disorders (hyperthyroidism or uncontrolled hypothyroidism), pharmacological treatment with immunosuppressants, cytotoxic agents, systemic corticosteroids, or other drugs that could potentially cause hepatic steatosis or alteration of liver tests, the presence of active autoimmune diseases or requiring pharmacological treatment, acute infections, the use of weight modifiers, the presence of severe psychiatric disorders and inability to follow the diet (food allergies, intolerances) as well as difficulties to follow scheduled visits.

All the procedures performed were in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was appropriately registered (www.clinicaltrials.gov; NCT03183193). All participants gave their informed consent prior to their inclusion in the study.

The study protocol and informed consent document were approved by the Research Ethics Committee of the University of Navarra on 24 April 2015 (ref. 54/2015).

Anthropometric, body composition and biochemical measurements

Anthropometric measurements (body weight, height and waist circumference), body composition (DXA, Lunar iDXA, encore 14.5, Madison, WI, USA), and blood pressure (Intelli Sense. M6, OMRON Healthcare, Hoofddorp, The Netherlands) were determined in fasting conditions under previously described standardized procedures [27]. The body mass index (BMI) was calculated as the body weight divided by the squared height (kg/m^2). Fasting blood samples were properly collected, processed (15 min; 3500 rpm; 5 °C), and stored at − 80 °C until the analyses were performed. Blood glucose, glycated hemoglobin (HbA1c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) concentrations were determined on a suitable autoanalyzer (Pentra C-200; HORIBA ABX, Madrid, Spain) with specific kits and using standardized methods. Insulin concentrations were quantified using specific enzyme-linked immunosorbent assay (ELISA) kits (Demeditec; Kiel-Wellsee, Germany) in a Triturus auto-analyzer (Grifols, Barcelona, Spain). Ferritin serum levels were analyzed by an external certified laboratory (Eurofins Megalab S.A, Madrid, Spain) using a chemiluminescent microparticle immunoassay (CMIA) technology (Abbott Architect Ferritin Assay). The low-density lipoprotein (LDL-c) levels were calculated using the Friedewald formula [28]: LDL-c = TC—HDL-c—TG/5. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [29] whereas the triglyceride–glucose (TyG) index was determined as Ln [TG (mg/dL) × glucose (mg/dL)/2] [30]. HOMA-IR and TyG were used as indicators of IR.

Images techniques for the assessment of liver status

Hepatic assessment was determined under fasting conditions by qualified and experienced staff at the University of Navarra Clinic. The presence of hepatic steatosis was evaluated by Ultrasonography (Siemens ACUSON S2000 and S3000) in accordance with previously described methodology [25]. Magnetic Resonance Imaging (MRI) (Siemens Aera 1.5 T) was performed to quantify the fat and iron content of the liver as well as to determine the hepatic volume, as described elsewhere [3]. The technique used in the quantification of liver fat and iron content was the high-speed T2-corrected multiecho (HISTO) method. The HISTO Magnetic Resonance spectroscopic technique was developed to acquire multiple echoes in a single acquisition, which enables the quantification of water and lipid T2, and subsequently to provide a corrected measure of hepatic lipid content [31].

Dietary assessment

Dietary intake was collected by registered dietitians with a validated semiquantitative 137-item food frequency questionnaire (FFQ) [32]. Each item in the questionnaire included a typical portion size. For each food item, daily food consumption was estimated by multiplying the portion size by the consumption frequency and dividing as described elsewhere [33]. Dietary amino acids of the foods contained in each item were derived from accepted food composition and nutrition tables [34].

Statistical analyses

The normal distribution of the continuous variables was assessed using the Shapiro–Wilk test. The data were expressed as a mean ± standard deviation for continuous traits and percentage for categorical variables. Participants were classified according to amino acid consumption medians (AAA: 1240 mg/day; BCAA: 2905 mg/day; SAA: 649 mg/day). Differences between groups (< or ≥ the median) were assessed by the Student's *t* test and the Mann–Whitney *U* test for quantitative parametric and non-parametric variables, respectively. Regarding categorical variables, differences in the frequency distribution among groups were assessed by means of Chi squared test. Spearman correlations were performed to further assess the association between amino acid consumption and liver status and glucose metabolism-related variables. Multivariable quantile regression analyses were performed to investigate the influence of AAA, BCAA and SAA consumption on the variability of liver status and glucose metabolism variables after adjusting for potential confounders (Model 1: age, sex; Model 2: age, sex, body mass index, energy intake (kcal/day) and physical activity (METs-min/week); Model 3: age, sex, physical activity (METs-min/week), protein intake (%), carbohydrate intake (%) and fat intake (%); Model 4: age, sex, physical activity (METs-min/week) and protein intake (%); Model 5: age, sex, physical activity (METs-min/week), fruits (g/day) and vegetables (g/day)) when indicated. Statistical calculations were performed with Stata version 12.1 (StataCorp 2011, College Station, TX, USA). Graphs were generated using GraphPad Prism 6 (Graph-Pad Software, San Diego, CA, USA). All *p* values presented are two tailed, and differences were considered statistically significant at $p < 0.05$.

Results

At baseline, the average age of participants was 51 ± 9 years old and 42% were women. The mean BMI of the studied population was 34 ± 4 kg/m^2 with a waist circumference of 110 ± 8 cm. According to International Diabetes Federation (IDF) criteria [35], 8.6% of participants suffered from diabetes mellitus.

An overview of levels of individual dietary amino acids as well as other dietary characteristics and physical activity by means of MET is given in Supplemental Table 1.

A principal component analysis (PCA) was applied to explore patterns of the distributions of dietary amino acids. Thus, we identified a principal component which explains 79.7% of the variation and was integrated by all the dietary amino acids (ALA ARG ASP CYS GLU GLY HIS ILE LEU LYS MET PHE PRO SER THR TRP TYR VAL) (Supplemental Table 2). After performing analyses between tertiles of this principal component and hepatic status and glucose metabolism-related variables, we found significant positive associations with ferritin, liver fat mass and TyG index (Supplemental Table 3).

Subjects were classified according to the medians of the specific dietary amino acids. The mean intake of dietary amino acids was AAA: 1538 mg/day; BCAA: 3480 mg/day and SAA: 700 mg/day. These quantities of amino acids correspond to an average intake of 112 g of protein per day. Participants with higher amino acid consumption showed higher glucose, insulin, HbA1c and HOMA-IR values; however, no significant differences were observed between low (below median) and high (above median) dietary amino acid intakes except for SAA consumption. Participants above the dietary SAA median had significantly higher HbA1c and HOMA-IR values than subjects below the median. The TyG index was significantly higher in participants above the dietary AAA and BCAA median (Table 1).

The analysis of the hepatic health-related variables showed that participants above the dietary amino acid median registered significantly higher liver fat content compared to those participants below the median. Liver iron concentration was significantly higher only in participants consuming greater quantities of AAA. Ferritin levels as well as the hepatic volume were significantly higher in participants with greater dietary AAA and BCAA consumption. No relevant differences were observed in transaminase

Table 1 Glucose metabolism markers and hepatic status related variables of the study participants according to the median of specific amino acid consumption

n = 112	All	AAA (mg/day)		BCAA (mg/day)		SAA (mg/day)	
		< 1240	≥ 1240	< 2905	≥ 2905	< 649	≥ 649
Age (years)	50.8 (9)	51.8 (10)	50.4 (8)	51.6 (10)	50.7 (9)	51.0 (10)	51.3 (9)
Sex (men/women)	65/47	32/25	33/22	30/26	35/21	39/27	26/20
BMI (kg/m^2)	33.7 (4)	33.6 (4)	33.9 (4)	33.5 (3)	34.0 (4)	33.3 (3)	34.4 (4)
Waist Circumference (cm)	109.8 (8)	108.7 (10)	111.0 (9)	108.2 (10)	111.4 (10)	108.5 (9)	111.8 (10)
Glucose metabolism variables							
Glucose (mg/d)	109 (32)	103.7 (19)	114.4 (40)	103.8 (19)	114.2 (40)	103.2 (17)	117.3 (43)
Insulin (mU/L)	18.3 (11)	16.6 (9)	20.0 (12)	16.7 (9)	19.9 (12)	16.9 (9)	20.2 (12)
HbA1c (%)	5.9 (1)	5.8 (0.9)	6.0 (1)	5.8 (0.9)	6.0 (1)	5.8 (0.9)	6.1 (1)*
HOMA-IR	5.2 (5)	4.3 (3)	6.1 (6)	4.4 (3)	6.1 (6)	4.3 (3)	6.5 (7)*
TyG index	8.8 (0.6)	8.6 (0.4)	8.9 (0.7)*	8.6 (0.4)	8.9 (0.7)*	8.7 (0.5)	8.9 (0.7)
Liver status variables							
ALT (IU/L)	33.2 (18)	31.6 (18)	34.8 (17)	30.6 (17)	35.8 (18)*	31.5 (17)	35.6 (18)
AST (IU/L)	24.5 (10)	24.4 (10)	24.5 (9)	23.9 (10)	25.1 (9)	24.0 (10)	25.1 (10)
GGT (IU/L)	37.6 (25)	37.6 (26)	37.5 (25)	37.3 (27)	37.8 (24)	38.9 (28)	35.6 (20)
Ferritin (ng/mL)	150.1 (130)	119.4 (101)	182.0 (148)*	112.6 (90)	188.2 (152)*	131.7 (100)	176.5 (161)
Liver fat mass (%)	11.7 (8)	10.0 (7)	13.4 (9)*	9.3 (7)	14.1 (9)**	9.7 (7)	14.5 (9)**
Liver iron (%)	26.9 (4)	25.8 (4)	27.9 (4)*	26.5 (3)	27.2 (5)	27.0 (3)	26.6 (5)
Hepatic volume (mL)	1813 (530)	1719 (479)	1906 (565)*	1709 (474)	1913 (565)*	1736 (457)	1922 (606)

Values are represented as mean (SD)

AAA aromatic amino acids; *BCAA* branched-chain amino acids; *SAA* sulfur amino acids; *HbA1c* glycosylated hemoglobin; *HOMA-IR* Homeostatic Model Assessment of Insulin Resistance; *TyG index* triglyceride–glucose index; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *GGT* gamma-glutamyl transferase

*indicates p values < 0.05, **indicates p values < 0.01

concentration among participants above and below the specific amino acid dietary medians (Table 1).

A sub-analysis concerning the gender was performed to evaluate the effect of sex in the link between dietary amino acids and variables of interest. Remarkably, stronger associations of dietary amino acids with both hepatic and glucose status were found in men. In women, some of the significant differences disappeared although the same trends were maintained when analyzing liver and glucose metabolism-related variables.

The relationship between amino acid intake and liver and glucose metabolism-related variables was further explored after adjusting for age, BMI and gender. AAA consumption was positively correlated with liver fat, ferritin concentration (Fig. 1) and TyG index (Fig. 2). Also, positive associations of BCAA consumption with liver fat, hepatic iron, ferritin (Fig. 1) and TyG (Fig. 2) were observed. When analyzing SAA intake, positive correlations were found with liver fat (Fig. 1) and HbA1c (Fig. 2).

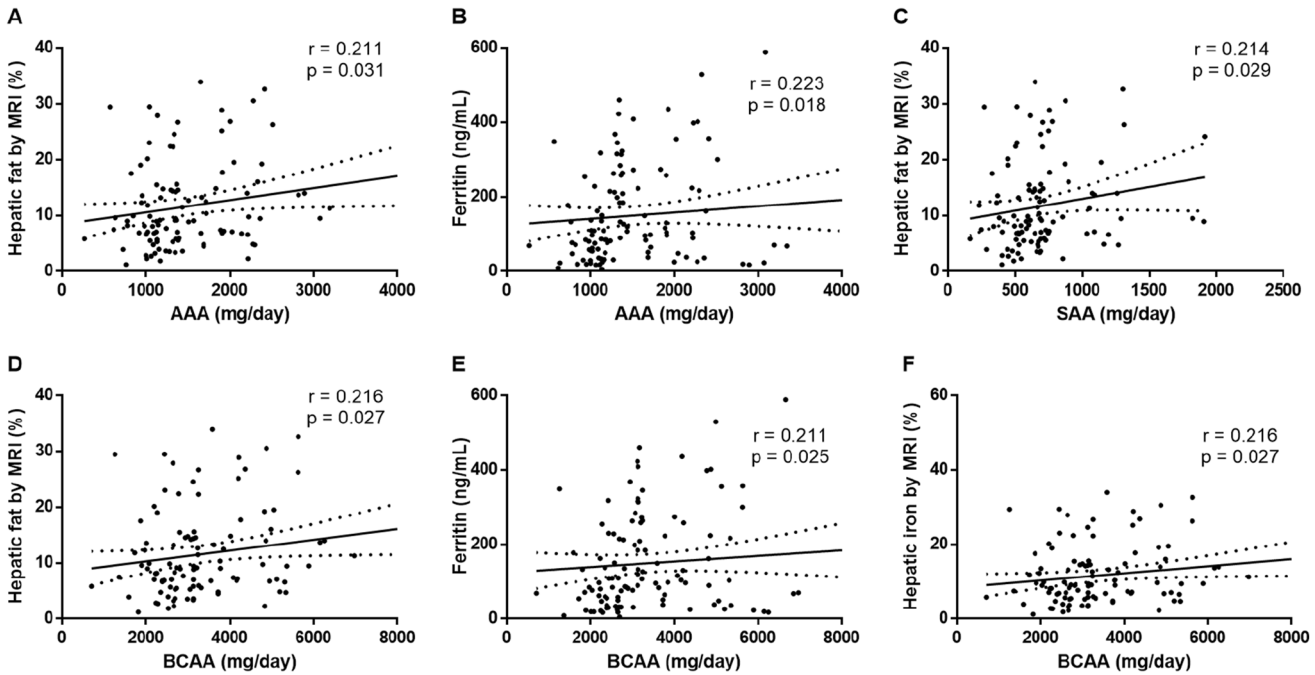

Fig. 1 Correlation analysis between dietary intake of amino acids and liver status variables adjusted by age, sex and BMI. **a** Correlation between AAA consumption and liver fat by MRI; **b** correlation between AAA consumption and serum ferritin; **c** correlation between SAA consumption and liver fat by MRI; **d** correlation between BCAA intake and liver fat by MRI; **e** correlation between BCAA consumption and serum ferritin; **f** correlation between BCAA consumption and hepatic iron by MRI. *AAA* aromatic amino acids; *BCAA* branched-chain amino acids; *SAA* sulfur amino acids; *MRI* magnetic resonance imaging

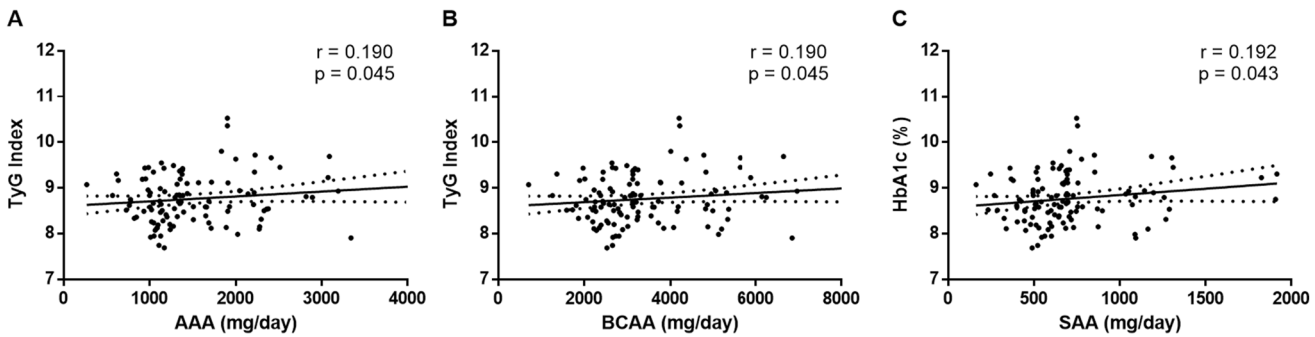

Fig. 2 Correlation analysis between dietary intake of amino acids and glucose metabolism variables adjusted by age, sex and BMI. **a** Correlation between AAA consumption and TyG index; **b** correlation between BCAA intake and TyG index; **c** correlation between SAA consumption and HbA1c. *AAA* aromatic amino acids; *BCAA* branched-chain amino acids; *SAA* sulfur amino acids; *TyG index* triglyceride–glucose index; *HbA1c* glycated hemoglobin

Quantile regression models were set up with ferritin, liver fat and hepatic iron content as dependent variables and dietary variables such as AAA, BCAA and SAA as independent factors (Table 2). Both minimally adjusted (Model 1: age and sex) and multiple adjusted [Model 2: age, sex, body mass index, energy intake (kcal/day) and physical activity (METs-min/week); Model 3: age, sex, physical activity (METs-min/week), protein intake (%), carbohydrate intake (%) and fat intake (%); Model 4: age, sex, physical activity (METs-min/week) and protein intake (%); Model 5: age, sex, physical activity (METs-min/week), fruits (g/day) and vegetables (g/day)] models showed that all types of amino acids were significantly associated with the hepatic fat content. AAA and BCAA consumption was also positively associated with liver iron concentration. Regarding ferritin levels, a positive association was found with BCAA.

An ancillary analysis was carried after excluding diabetic individuals. A total of ten diabetics were excluded. The statistical significances were maintained in the quantile regression models when analyzing the association between hepatic status parameters (ferritin, liver fat and iron content) and dietary amino acids (AAA, BCAA and SAA) without diabetic participants (data not shown).

Additionally, dietary amino acids were not significantly associated with glucose metabolism variables in the quantile regression analyses except for SAA, which were positively associated with HbA1c (Table 3).

Complementarily, a sensitivity analysis was carried out adjusting the intake of specific amino acids for energy, using the residual method. Although the association of amino acid variables with ferritin and liver iron disappeared when energy-adjusted AAA, BCAA and SAA were analysed, the significance remains when exploring liver fat by MRI, which is the most important outcome (Supplemental Table 4). Concerning energy-adjusted amino acids and glucose metabolism variables, we did not find statistically significant differences, as in the case of any energy amino acid adjustment (Supplemental Table 3).

Discussion

The present study aimed to investigate the association between dietary amino acids and both hepatic and glucose metabolism-related variables in adults affected by overweight/obesity and NAFLD. Dietary amino acids showed a relevant association with liver fat accumulation and in some cases with hepatic iron content, suggesting a potential role in the pathogenesis of the disease. The association observed between dietary amino acids and glucose metabolism-related variables was weak since the significance disappeared in the multivariate adjusted model.

Diet has a direct impact on the development of obesity and related metabolic disorders like NAFLD and IR [36, 37]. Protein quality, including the intake of specific dietary amino acids, has an important role in the promotion of optimal health status although available studies concerning amino acid requirements in the population are inconsistent [19, 38]. However, recent research has evidenced that a dietary amino acid pattern increased in BCAA, derived from a variety of food sources (both animal and vegetable proteins), are strongly associated with obesity, IR, T2D, NAFLD and

Table 2 Quantile regression models with ferritin, MRI Liver fat mass and MRI Liver iron as the dependent variables and different amino acid consumption (AAA, BCAA and SAA) as independent variables

($n=112$)	Model 1		Model 2		Model 3		Model 4		Model 5	
	β	p	β	p	β	p	β	p	β	p
Ferritin										
AAA	45.73	0.092	44.79	0.134	51.07	0.074	51.36	0.065	42.58	0.115
BCAA	45.73	0.090	51.90	0.088	66.11	0.025	51.36	0.067	53.39	0.079
SAA	21.55	0.448	24.81	0.443	42.60	0.177	33.87	0.264	16.05	0.596
MRI liver fat mass										
AAA	3.62	0.021	3.09	0.119	3.62	0.031	3.11	0.065	3.98	0.020
BCAA	5.05	0.001	6.16	0.001	4.76	0.002	4.37	0.006	4.36	0.006
SAA	5.60	0.003	6.12	0.001	4.82	0.008	4.55	0.016	4.97	0.007
MRI liver iron										
AAA	1.78	0.010	2.17	0.003	1.69	0.011	1.64	0.013	1.62	0.009
BCAA	0.94	0.204	2.00	0.006	1.35	0.037	1.35	0.045	1.30	0.058
SAA	0.80	0.290	1.44	0.064	0.80	0.239	0.89	0.230	1.16	0.122

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, energy intake (kcal/day) and physical activity (METs-min/week). Model 3: adjusted for age, sex, physical activity (METs-min/week), protein intake (%), carbohydrate intake (%) and fat intake (%). Model 4: adjusted for age, sex, physical activity (METs-min/week) and protein intake (%). Model 5: adjusted for age, sex, physical activity (METs-min/week), fruits (g/day) and vegetables (g/day). *AAA* aromatic amino acids; *BCAA* branched-chain amino acids; *SAA* sulfur amino acids; *MRI* magnetic resonance imaging

Table 3 Quantile regression models with HbA1c, HOMA-IR and TyG index as the dependent variables and different amino acid consumption (AAA, BCAA and SAA) as independent variables

($n=112$)	Model 1		Model 2		Model 3		Model 4		Model 5	
	β	p	β	p	β	p	β	p	β	p
HbA1c (%)										
AAA	0.08	0.275	0.09	0.315	0.05	0.553	0.09	0.266	0.06	0.386
BCAA	0.10	0.223	0.12	0.199	0.06	0.528	0.09	0.295	0.11	0.185
SAA	0.13	0.124	0.18	0.049	0.22	0.021	0.26	0.004	0.22	0.012
HOMA-IR										
AAA	0.89	0.194	1.22	0.059	0.98	0.067	0.87	0.140	0.80	0.160
BCAA	0.80	0.195	0.85	0.227	0.97	0.084	0.88	0.126	0.78	0.204
SAA	0.85	0.200	0.88	0.207	0.94	0.081	1.01	0.122	0.72	0.232
TyG index										
AAA	0.16	0.150	0.18	0.176	0.23	0.054	0.18	0.153	0.17	0.161
BCAA	0.18	0.144	0.18	0.162	0.23	0.059	0.20	0.113	0.13	0.341
SAA	0.16	0.169	0.16	0.220	0.11	0.442	0.16	0.275	0.07	0.631

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, energy intake (kcal/day) and physical activity (METs-min/week). Model 3: adjusted for age, sex, physical activity (METs-min/week), protein intake (%), carbohydrate intake (%) and fat intake (%). Model 4: adjusted for age, sex, physical activity (METs-min/week) and protein intake (%). Model 5: adjusted for age, sex, physical activity (METs-min/week), fruits (g/day) and vegetables (g/day). Abbreviations: AAA: aromatic amino acids; BCAA: branched-chain amino acids; SAA: sulfur amino acids; MRI: magnetic resonance imaging

liver injury [39, 40], even though BCAA supplementation in elderly and athletes is often related to positive effects on muscle growth and energy expenditure [41, 42]. In this context, Zhang et al. reported that BCAA supplementation attenuates weight gain induced by increased adipose lipolysis in mice following a high-fat diet. At the same time, however, BCAA intake seems to cause liver injury thought increased adipose lipolysis, resulting in hyperlipidemia, IR, and hepatic lipotoxicity [39]. In this way, BCAA activate AMPKα2 and stimulate lipolysis in the adipocyte, increasing plasma free fatty acids (FFA), which results in hepatic FFA accumulation. In the liver, BCAA activate mTOR and inhibit FFA to TG conversion and autophagy, intensifying FFA lipotoxicity [39]. At this point, we hypothesized that dietary AAA, BCAA and SAA could have a direct effect on liver fat accumulation acting possibly via oxidative stress or inflammatory processes. Indeed, inflammation and oxidative stress are major factors implicated in the evolution of NAFLD; liver steatosis accompanied by hepatic inflammation (NASH) as well as IR [11, 12].

On the contrary, some researchers reported opposite results regarding the effect of BCAA on liver health. In this context, Beppu et al. found that BCAA supplementation improved functional liver regeneration in individuals undergoing portal vein embolization and subsequent hepatectomy [43]. In line with these results, Mattick et al. studied the importance of BCAA supplementation in the link between liver and muscle, and its relevance to critical illness [44]. Interestingly, Honda et al. suggested that BCAA can alleviate hepatic steatosis and liver injury associated with NASH [45]. Regarding studies in animal models, Takegoshi also reported beneficial effects of BCAA, inducing an antifibrotic effect, preventing apoptosis in hepatocytes, and decreasing the incidence of hepatocellular carcinoma in a nonalcoholic steatohepatitis mouse model [46]. Additionally, BCAA supplementation ameliorated liver fibrosis and suppress tumor growth in a rat model of hepatocellular carcinoma with liver cirrhosis [47]. All these studies reported beneficial effects of BCAA evidencing inconsistencies in the literature. At this point, it is important to highlight that dietary BCAA consumption/supplementation seems to have differential impact depending on the stage of NAFLD. Circulating amino acids appear significantly higher in the early stages of NAFLD/NASH, but the level rapidly decreases in cirrhosis [48]. However, it is not determined whether this is due to increased liver protein catabolism, impaired muscle, obesity, and/or increased IR or impaired tissue metabolism [21]. Thus, BCAA intake could be detrimental in the early stage of NAFLD and simultaneously, beneficial in cirrhosis, although further investigations should be conducted to identify the impact of BCAA on overall hepatic status.

In the present study, significant associations were also observed between amino acid consumption and glucose metabolism variables of the participants. However, these results should be interpreted with caution, as most of the differences are not significant after adjusting for potential confounders in the quantile regression models. In this context, the intake of this subgroup of amino acids does not appear to have a great impact on glucose homeostasis, being other variables that have the most impact on glucose metabolism. In the literature, most observational studies show that dietary BCAA are associated with higher risk of T2D [49].

Nonetheless, several clinical trials suggest beneficial effects of short-term use of BCAA supplementation on IR condition on specific populations, although with a small sample size [49, 50]. Further investigation is needed as there is limited research analyzing the association between specific dietary amino acids and glucose homeostasis.

Dietary amino acids also influenced iron metabolism. Higher hepatic iron accumulation was found with increased dietary amino acid consumption and individuals with higher AAA and BCAA intake exhibited differences in serum ferritin concentrations. Published data have linked increased serum ferritin levels to impaired glucose homeostasis (T2D and IR) [51] as well as liver metabolism disruption [52]. Ferritin is not only a serum marker of total body iron stores, but also it is an acute phase protein elevated in inflammation, acting as a pro-inflammatory cytokine inducing liver damage [53, 54]. On the other hand, increased hepatic iron content often coexists with IR, T2D and NAFLD [55, 56]. The association of specific dietary amino acid consumption with greater liver iron content and plasma ferritin levels in adults with obesity and NAFLD may provide biochemical insights towards metabolic pathways linking dietary amino acids, hepatic status and glucose metabolism.

Increased plasma amino acid levels are associated with NAFLD, increased risk of IR and T2D [57, 58], and also probably with metabolic syndrome and overweight/obesity [59]. On the other hand, published data suggest that plasma amino acids seem to be weakly associated with dietary amino acids [60]. Recent studies have shown that plasma amino acids are not only important as substrates for various metabolic pathways but also for the nutrient-sensitive signaling pathway that acts synergistically with insulin, mTOR, or epigenomic regulation [61]. Herman et al. exhibited downregulated expression of mitochondrial BCAA-degrading enzymes in the white adipose tissue (WAT) in obesity and T2D, suggesting that adipose tissue contributes to the regulation of circulating BCAA levels [62]. Also, another study conducted by Yoneshiro et al. showed that brown adipose tissue (BAT) acts like a metabolic filter for circulating BCAA levels and protects against obesity and IR whereas impaired BAT activity in obese and diabetic states reduces systemic BCAA clearance [63]. Moreover, reduced expression of hepatic BCAA enzymes has been found in fatty liver [21]. Accumulating evidence points at mitochondrial dysfunction as the main factor in the pathophysiology and progression of NAFLD [64]. In this context, it has been suggested that reduced mitochondrial BCAA oxidation and subsequent intracellular accumulation of BCAA leads to activation of mTORC1 [64]. mTORC1 is an essential factor in the insulin-regulated pathway, being responsible for insulin receptor substrate-1 (IRS-1) phosphorylation and consequently, inhibition of insulin signaling [57]. Also, some studies indicated that some free amino acids, especially BCAA, modulate the size and heterogeneity of lipid droplets in hepatocytes in individuals with NAFLD/NASH. Indeed, activation of mTORC1 also stimulates hepatic lipogenesis and IR induces heterogeneity of lipid droplets in liver [65].

On the other hand, several studies have found increased AAA in liver disease [66]. Phenylalanine is irreversibly converted to tyrosine mainly in the liver where tyrosine is further metabolized. Elevated tyrosine concentrations are often detected in individuals affected by NAFLD, possibly because of impairment in hepatic metabolism of this AAA [21]. Plasma concentrations of AAA were also found increased with higher severity of liver diseases [21]. These results are not surprising because the liver is the site of protein and AAA metabolism. Also, early data showed a close relationship between SAA and fatty liver status [22]. In fact, methionine has a lipotropic effect which may be mediated by sulfane sulfur whereas the hepatosteatogenic effect of cystine may be related to the removal of sulfane sulfur by cysteine catabolites [22].

This investigation was not devoid of limitations. First, the sample size is relatively low, but the results are plausible. Second, given the cross-sectional nature of the study, causality could not be identified. Thirdly, dietary intake was evaluated using self-reported information of the participants, which might have affected results that depended on such evaluation. Fourthly, there is a lack of reliable data on AAA, BCAA and SAA values for some foods, which may have led to inaccurate values. Fifthly, further studies are needed to verify the impact of the dietary amino acids on plasma amino acid concentrations as well as is relation to NAFLD and IR condition. Moreover, interventional strategies focused on dietary amino acids may be contemplated regarding the metabolic status and stage of NAFLD in individuals with liver damage and IR. On the other hand, some strengths can also be mentioned. The selection of participants was carefully carried out. Participants have been well characterized and an appropriate methodology was used to evaluate liver status (ultrasonography and MRI). To our knowledge, little has been investigated regarding the association of specific dietary amino acids in populations with NAFLD and IR risk profile. The current findings are of great importance in the management of NAFLD and IR. Further research concerning this issue will allow a better understanding of the mechanisms involved in NAFLD, IR and dietary amino acids.

Conclusion

These findings suggest that the consumption of specific dietary amino acids might negatively impact liver status, suggesting a potential role in the pathogenesis of NAFLD and, to a lesser extent also in glucose metabolism, in subjects

with overweight/obesity and NAFLD. A healthy dietary pattern promoting a balanced dietary amino acid composition might be considered for the management of NAFLD. Further investigations should be performed to better understand the molecular interactions underlying this disease.

Acknowledgements The authors are very grateful to all the participants of the study. The authors wish to express their gratitude to the Government of Navarra, Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBERobn), and Fundació La Marató de TV3 for the financial support. Cristina Galarregui appreciates the predoctoral grant received from Congelados de Navarra, Government of Navarra, and Ministerio de Educación, Cultura y Deporte. Thanks are given to all the staff (www.clinicaltrials.gov; NCT03183193) for their contribution to FLiO project. Helen Hermana M. Hermsdorff and Josefina Bressan are CNPq Research Productivity fellows.

Author contributions CG: conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, roles/writing—original draft; writing—review and editing. IC: conceptualization and methodology. BAM-A: conceptualization, data curation, and methodology. JIM: conceptualization and methodology. ME: conceptualization and methodology. ABB: conceptualization and ethodology. José IH: conceptualization and methodology. VlO: conceptualization and methodology. MR-C: conceptualization and methodology. HHMH: conceptualization and methodology. JB: conceptualization and methodology. JAT: conceptualization, formal analysis, funding acquisition, methodology, and project administration. JAM: conceptualization, cata curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, roles/writing—original draft, and writing—review and editing. MAZ: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, roles/writing—original draft, writing—review and editing. IA: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, roles/writing—original draft, writing—review and editing.

Funding This work was supported by the Health Department of the Government of Navarra [61/2015], CIBERobn (Physiopathology of Obesity and Nutrition) [CB12/03/3002] and Fundació La Marató de TV3 [201630.10]. Cristina Galarregui was partially supported by fellowships from Congelados de Navarra, Government of Navarra, and Ministerio de Educación, Cultura y Deporte [FPU17/06330].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The present study has been approved by the Research Ethics Committee of the University of Navarra on 24 April 2015 (ref. 54/2015).and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent All participants of the present study gave their informed consent prior to their inclusion in the study.

Availability of data and material All data and materials support their published claims and comply with field standards.

References

1. Chalasani N, Younossi Z, Lavine JE et al (2018) The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 67:328–357
2. Ahmed A, Wong RJ, Harrison SA (2015) Nonalcoholic fatty liver disease review: diagnosis, treatment, and outcomes. Clin Gastroenterol Hepato 13:2062–2070. https://doi.org/10.1016/j.cgh.2015.07.029
3. Cantero I, Abete I, Babio N et al (2018) Dietary inflammatory index and liver status in subjects with different adiposity levels within the PREDIMED trial. Clin Nutr 37:1736–1743. https://doi.org/10.1016/j.clnu.2017.06.027
4. Bessone F, Razori MV, Roma MG (2019) Molecular pathways of nonalcoholic fatty liver disease development and progression. Cell Mol Life Sci 76:99–128
5. Byrne CD, Targher G (2015) NAFLD: a multisystem disease. J Hepatol 62:S47–S64
6. Haas JT, Francque S, Staels B (2016) Pathophysiology and mechanisms of nonalcoholic fatty liver disease. Annu Rev Physiol 78:181–205. https://doi.org/10.1146/annurev-physiol-021115-105331
7. Loomba R, Abraham M, Unalp A et al (2012) Nonalcoholic steatohepatitis clinical research network. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology 56:943–951. https://doi.org/10.1002/hep.25772
8. Tessari P, Cecchet D, Cosma A et al (2011) Insulin resistance of amino acid and protein metabolism in type 2 diabetes. Clin Nutr 30:267–372. https://doi.org/10.1016/j.clnu.2011.02.009
9. Seko Y, Yamaguchi K, Itoh Y (2018) The genetic backgrounds in nonalcoholic fatty liver disease. Clin J Gastroenterol 11:97–102
10. Marin-Alejandre BA, Abete I, Cantero I et al (2019) Association between sleep disturbances and liver status in obese subjects with nonalcoholic fatty liver disease: a comparison with healthy controls. Nutrients 11:1–16
11. Fontana L (2018) Interventions to promote cardiometabolic health and slow cardiovascular ageing. Nat Rev Cardiol 15:566–577
12. Spahis S, Delvin E, Borys JM et al (2017) Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. Antioxidants Redox Signal 26:519–541. https://doi.org/10.1089/ars.2016.6776
13. Alisi A, Carpino G, Oliveira FL et al (2017) The role of tissue macrophage-mediated inflammation on NAFLD pathogenesis and its clinical implications. Med Inflamm. https://doi.org/10.1155/2017/8162421
14. Marin-Alejandre BA, Abete I, Cantero I et al (2019) The metabolic and hepatic impact of two personalized dietary strategies in subjects with obesity and nonalcoholic fatty liver disease: the fatty liver in obesity (FLiO) randomized controlled trial. Nutrients 11:2543. https://doi.org/10.3390/nu11102543
15. Schübel R, Nonnenmacher T, Sookthai D et al (2019) Similar weight loss induces greater improvements in insulin sensitivity and liver function among individuals with NAFLD compared to individuals without NAFLD. Nutrients 11:1–12
16. Volynets V, Machann J, Küper MA et al (2013) A moderate weight reduction through dietary intervention decreases hepatic fat content in patients with non-alcoholic fatty liver disease (NAFLD): a pilot study. Eur J Nutr 52:527–535. https://doi.org/10.1007/s00394012-0355-z
17. Recaredo G, Marin-Alejandre BA, Cantero I et al (2019) Association between different animal protein sources and liver status

in obese subjects with non-alcoholic fatty liver disease: fatty liver in obesity (FLiO) study. Nutrients 11:2359. https://doi.org/10.3390/nu11102359
18. Katsagoni CN, Papatheodoridis GV, Ioannidou P et al (2018) Improvements in clinical characteristics of patients with non-alcoholic fatty liver disease, after an intervention based on the Mediterranean lifestyle: a randomised controlled clinical trial. Br J Nutr 120:164–175
19. Katz DL, Doughty KN, Geagan K et al (2019) Perspective: the public health case for modernizing the definition of protein quality. Adv Nutr 10:755–764
20. Teymoori F, Asghari G, Mirmiran P et al (2017) Dietary amino acids and incidence of hypertension: a principle component analysis approach. Sci Rep 4:16838. https://doi.org/10.1038/s41598-017-17047-0
21. Grajeda-Iglesias C, Aviram M (2018) Specific amino acids affect cardiovascular diseases and atherogenesis via protection against macrophage foam cell formation: review article. Rambam Maimonides Med J 9:3. https://doi.org/10.5041/RMMJ.10337
22. Hanvold SE, Vinknes KJ, Bastani NE et al (2018) Plasma amino acids, adiposity, and weight change after gastric bypass surgery: are amino acids associated with weight regain? Eur J Nutr 57:2629–2637. https://doi.org/10.1007/s00394-017-1533-9
23. Gaggini M, Carli F, Rosso C et al (2018) Altered amino acid concentrations in NAFLD: Impact of obesity and insulin resistance. Hepatology 67:145–158. https://doi.org/10.1002/hep.29465
24. Toohey JI (2014) Sulfur amino acids in diet-induced fatty liver: A new perspective based on recent findings. Molecules 19:8334–8349. https://doi.org/10.3390/molecules19068334
25. Lee SS, Park SH (2014) Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol 20:7392–7402
26. Sanyal AJ, Brunt EM, Kleiner DE et al (2011) Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 54:344–353
27. Zulet MA, Bondia-Pons I, Abete I et al (2011) The reduction of the metabolic syndrome in Navarra-Spain (RESMENA S) study: a multidisciplinary strategy based on chrononutrition and nutritional education, together with dietetic and psychological control. Nutr Hosp 26:16–26
28. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
29. Acosta AM, Escalona M, Maiz A (2002) Determinación del índice de resistencia insulínica mediante HOMA en una población de la Región Metropolitana de Chile. Rev Méd Chile 130:17
30. Navarro-González D, Sánchez-Íñigo L, Pastrana-Delgado J et al (2016) Triglyceride-glucose index (TyG index) in comparison with fasting plasma glucose improved diabetes prediction in patients with normal fasting glucose: the Vascular-Metabolic CUN cohort. Prev Med (Baltim) 86:99–105. https://doi.org/10.1016/j.ypmed.2016.01.022
31. Pineda N, Sharma P, Xu Q et al (2009) Measurement of hepatic lipid: High-speed T2-corrected multiecho acquisition at 1H MR spectroscopy - a rapid and accurate technique. Radiology 252:568–576. https://doi.org/10.1148/radiol.252308208488
32. Martin-Moreno JM, Boyle P, Gorgojo L et al (1993) Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol 22:512–519
33. Fernández-Ballart JD, Piñol JL, Zazpe I et al (2010) Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 103:1808–1816
34. Souci SW, Fachmann W, Kraut H (2008) Food Composition and Nutrition Tables. Medpharm, Swedon
35. Perez-Cornago A, Lopez-Legarrea P, de la Iglesia R et al (2014) Longitudinal relationship of diet and oxidative stress with depressive symptoms in patients with metabolic síndrome after following a weight loss treatment: the RESMENA project. Clin Nutr 33:1061–1067. https://doi.org/10.1016/j.clnu.2013.11.011
36. Galarregui C, Zulet MA, Cantero I et al (2018) Interplay of glycemic index, glycemic load, and dietary antioxidant capacity with insulin resistance in subjects with a cardiometabolic risk profile. Int J Mol Sci 19:E3662. https://doi.org/10.3390/ijms19113662
37. Tricò D, Frascerra S, Baldi S et al (2019) The insulinotropic effect of a high-protein nutrient preload is mediated by the increase of plasma amino acids in type 2 diabetes. Eur J Nutr 58:2253–2261. https://doi.org/10.1007/s00394-018-1778-y
38. National Research Council (US) (1989) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances Protein and Amino Acids. Recommended Dietary Allowances, Washington DC
39. Zhang F, Zhao S, Yan W et al (2016) Branched chain amino acids cause liver injury in obese/diabetic mice by promoting adipocyte lipolysis and inhibiting hepatic autophagy. EBioMedicine 13:157–167. https://doi.org/10.1016/j.ebiom.2016.10.013
40. Isanejad M, LaCroix AZ, Thomson CA et al (2017) Branched-chain amino acid, meat intake and risk of type 2 diabetes in the Women's Health Initiative. Br J Nutr 117:1523–1530
41. Valenzuela PL, Morales JS, Emanuele E et al (2019) Supplements with purported effects on muscle mass and strength. Eur J Nutr 58:2983–3008. https://doi.org/10.1007/s00394-018-1882-z
42. Jackman SR, Witard OC, Philp A et al (2017) Branched-chain amino acid ingestion stimulates muscle myofibrillar protein synthesis following resistance exercise in humans. Front Physiol 8:390
43. Beppu T, Nitta H, Hayashi H et al (2015) Effect of branched-chain amino acid supplementation on functional liver regeneration in patients undergoing portal vein embolization and sequential hepatectomy: a randomized controlled trial. J Gastroenterol 50:1197–1205. https://doi.org/10.1007/s00535-015-1067-y
44. Mattick JSA, Kamisoglu K, Ierapetritou MG et al (2013) Branched-chain amino acid supplementation: impact on signaling and relevance to critical illness. Wiley Interdiscip Rev Syst Biol Med 5:449–460. https://doi.org/10.1002/wsbm.1219
45. Honda T, Ishigami M, Luo F et al (2016) Branched-chain amino acids alleviate hepatic steatosis and liver injury in choline-deficient high-fat diet induced NASH mice. Metabolism 69:177–187. https://doi.org/10.1016/j.metabol.2016.12.013
46. Takegoshi K, Honda M, Okada H et al (2017) Branched-chain amino acids prevent hepatic fibrosis and development of hepatocellular carcinoma in a non-alcoholic steatohepatitis mouse model. Oncotarget 8:18191–18205. https://doi.org/10.18632/oncotarget.15304
47. Cha JH, Bae SH, Kim HL et al (2013) Branched-chain amino acids ameliorate fibrosis and suppress tumor growth in a rat model of hepatocellular carcinoma with liver cirrhosis. PLoS ONE 8:e77899. https://doi.org/10.1371/journal.pone.0077899
48. Buzzetti E, Petta S, Manuguerra R et al (2019) Evaluating the association of serum ferritin and hepatic iron with disease severity in non-alcoholic fatty liver disease. Liver Int 1:1–10
49. de la OV, Zazpe I, Ruiz-Canela M, (2019) Effect of branched-chain amino acid supplementation, dietary intake and circulating levels in cardiometabolic diseases: an updated review. Curr Opin Clin Nutr Metab Care. https://doi.org/10.1097/MCO.0000000000000614
50. Ntzouvani A, Nomikos T, Panagiotakos D et al (2017) Amino acid profile and metabolic syndrome in a male Mediterranean population: a cross-sectional study. Nutr Metab Cardiovasc Dis 27:1021–1030

51. Fernández-Real JM, Mcclain D, Review MM (2015) Mechanisms linking glucose homeostasis and iron metabolism toward the onset and progression of type 2 diabetes. Diabetes Care 38:2169–2176. https://doi.org/10.2337/dc14-3082
52. Britton LJ, Subramaniam VN, Crawford DHG (2016) Iron and non-alcoholic fatty liver disease. World J Gastroenterol 22:8112–8122. https://doi.org/10.3748/wjg.v22.i36.8112
53. Modares Mousavi SR, Geramizadeh B, Anushiravani A et al (2018) Correlation between serum ferritin level and histopathological disease severity in non-alcoholic fatty liver disease. Middle East J Dig Dis 10:90–95
54. Britton L, Bridle K, Reiling J et al (2018) Hepatic iron concentration correlates with insulin sensitivity in nonalcoholic fatty liver disease. Hepatol Commun 2:644–653
55. McKay A, Wilman HR, Dennis A et al (2018) Measurement of liver iron by magnetic resonance imaging in the UK Biobank population. PLoS ONE 13:1–14
56. Rousseau M, Guénard F, Garneau V et al (2019) Associations between dietary protein sources, plasma BCAA and short-chain acylcarnitine levels in adults. Nutrients 11:5. https://doi.org/10.3390/nu11010173
57. Lynch CJ, Adams SH (2014) Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 10:723–736
58. Mahbub M, Yamaguchi N, Takahashi H et al (2017) Association of plasma free amino acids with hyperuricemia in relation to diabetes mellitus, dyslipidemia, hypertension and metabolic syndrome. Sci Rep. https://doi.org/10.1038/s41598-017-17710-6
59. van den Berg EH, Flores-Guerrero JL, Gruppen EG et al (2019) Non-alcoholic fatty liver disease and risk of incident type 2 diabetes: Role of circulating branched-chain amino acids. Nutrients. https://doi.org/10.3390/nu11030705
60. Meijer AJ, Dubbelhuis PF (2004) Amino acid signalling and the integration of metabolism. Biochem Biophys Res Commun 313:397–403. https://doi.org/10.1016/j.bbrc.2003.07.012
61. Ruiz-Canela M, Guasch-Ferre M, Toledo E et al (2018) Plasma branched chain/ aromatic amino acids, enriched Mediterranean diet and risk of type 2 diabetes: case-cohort study within the PREDIMED Trial. Diabetologia 61:1560–1571
62. Herman MA, She P, Peroni OD et al (2010) Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J Biol Chem 285:11348–11356. https://doi.org/10.1074/jbc.M109.075184
63. Yoneshiro T, Wang Q, Tajima K (2019) BCAA catabolism in brown fat controls energy homeostasis through SLC25A44. Nature 572:614–619. https://doi.org/10.1038/s41586-019-1503
64. Wei Y, Rector RS, Thyfault JP et al (2008) Nonalcoholic fatty liver disease and mitochondrial dysfunction. World J Gastroenterol 14:193–199. https://doi.org/10.3748/wjg.14.193
65. Kakazu E, Sano A, Morosawa T (2019) Branched chain amino acids are associated with the heterogeneity of the area of lipid droplets in hepatocytes of patients with non-alcoholic fatty liver disease. Hepatol Res 49:860–871. https://doi.org/10.1111/hepr.13346
66. Cheng S, Wiklund P, Autio R et al (2015) Adipose tissue dysfunction and altered systemic amino acid metabolism are associated with non-alcoholic fatty liver disease. PLoS ONE. https://doi.org/10.1371/journal.pone.0138889

Affiliations

Cristina Galarregui[1] · Irene Cantero[1] · Bertha Araceli Marin-Alejandre[1] · J. Ignacio Monreal[2,3] · Mariana Elorz[2,4] · Alberto Benito-Boillos[2,4] · José Ignacio Herrero[2,5,6] · Víctor de la O[2,7] · Miguel Ruiz-Canela[2,7,8] · Helen Hermana M. Hermsdorff[9] · Josefina Bressan[9] · Josep A. Tur[8,10] · J. Alfredo Martínez[1,2,8] · M. Angeles Zulet[1,2,8] · Itziar Abete[1,2,8]

Cristina Galarregui
cgalarregui@alumni.unav.es

Irene Cantero
icgonzalez@unav.es

Bertha Araceli Marin-Alejandre
bmarin.1@alumni.unav.es

J. Ignacio Monreal
jimonreal@unav.es

Mariana Elorz
marelorz@unav.es

Alberto Benito-Boillos
albenitob@unav.es

José Ignacio Herrero
iherrero@unav.es

Víctor de la O
vdelao@unav.es

Miguel Ruiz-Canela
mcanela@unav.es

Helen Hermana M. Hermsdorff
helenhermana@ufv.br

Josefina Bressan
jbrm@ufv.br

Josep A. Tur
pep.tur@uib.es

J. Alfredo Martínez
jalfmtz@unav.es

[1] Department of Nutrition, Food Sciences and Physiology and Centre for Nutrition Research, Faculty of Pharmacy and Nutrition, University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain

[2] Navarra Institute for Health Research (IdiSNA), 31008 Pamplona, Spain

[3] Clinical Chemistry Department, Clinica Universidad de Navarra, 31008 Pamplona, Spain

[4] Department of Radiology, Clinica Universidad de Navarra, 31008 Pamplona, Spain

[5] Liver Unit, Clinica Universidad de Navarra, 31008 Pamplona, Spain

[6] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 28029 Madrid, Spain

[7] Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, 31008 Pamplona, Spain

[8] Biomedical Research Centre Network in Physiopathology of Obesity and Nutrition (CIBERobn), Instituto de Salud Carlos III, 28029 Madrid, Spain

[9] Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa 36570-900, Brazil

[10] Research Group On Community Nutrition and Oxidative Stress, University of Balearic Islands and Balearic Islands Institute for Health Research (IDISBA), 07122 Palma, Spain