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ORIGINAL CONTRIBUTION

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

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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.

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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).

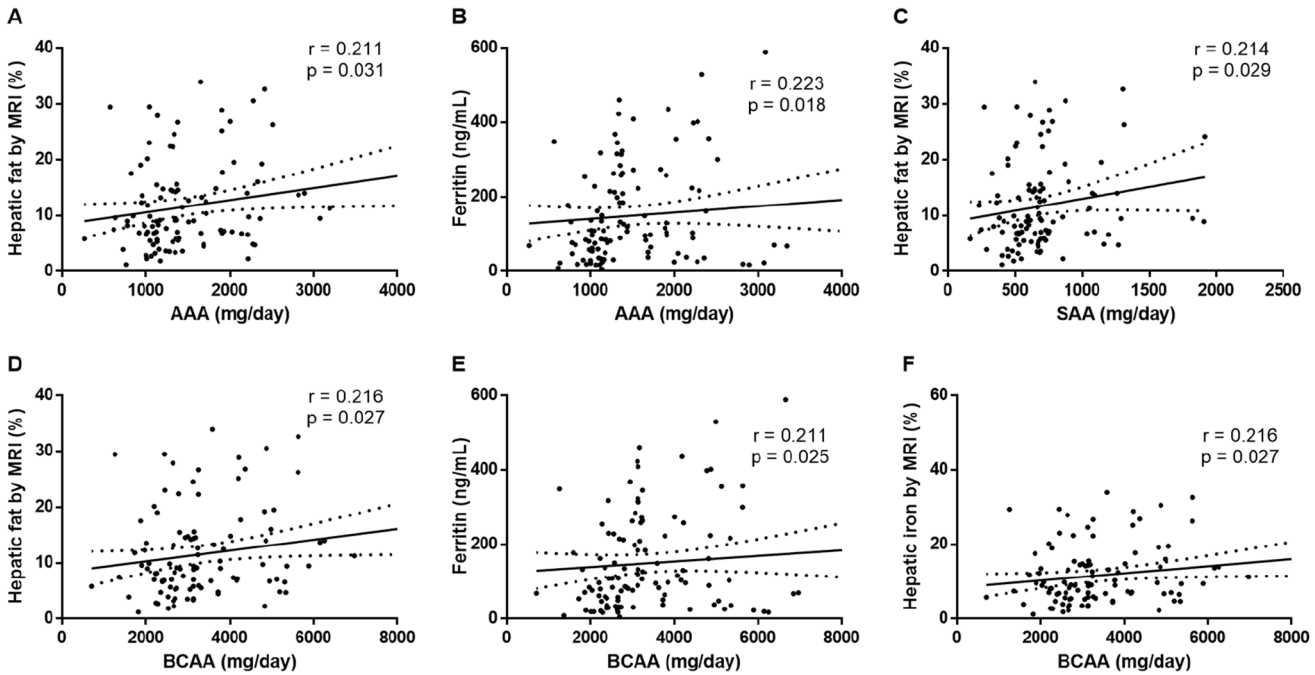

Fig. 1 Correlation analysis between dietary intake of amino acids and liver status variables adjusted by age, sex and BMI. **a** Correlation between AAA consumption and liver fat by MRI; **b** correlation between AAA consumption and serum ferritin; **c** correlation between SAA consumption and liver 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

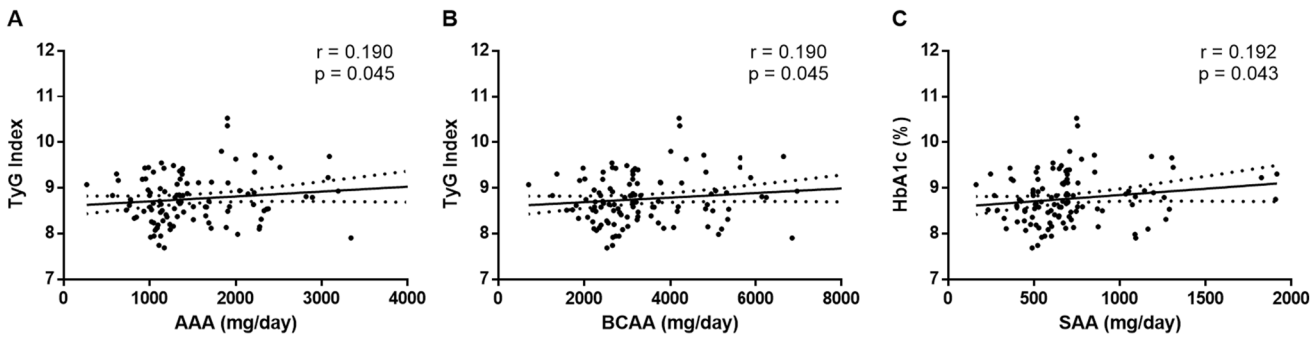

Fig. 2 Correlation analysis between dietary intake of amino acids and glucose metabolism variables adjusted by age, sex and BMI. **a** Correlation between AAA consumption and TyG index; **b** correlation between BCAA intake and TyG index; **c** correlation between SAA consumption and HbA1c. *AAA* aromatic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

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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.

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