Nutrition, Metabolism & Cardiovascular Diseases (2017) **27**, 1021–1030

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Amino acid profile and metabolic syndrome in a male Mediterranean population: A cross-sectional study

A. Ntzouvani [a], T. Nomikos [a], D. Panagiotakos [a], E. Fragopoulou [a], C. Pitsavos [b], A. McCann [c], P.M. Ueland [d,e], S. Antonopoulou [a,*]

[a] Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens 17671, Greece
[b] First Cardiology Clinic, School of Medicine, National and Kapodistrian University of Athens, Athens 11527, Greece
[c] Bevital AS, Bergen 5020, Norway
[d] Department of Clinical Science, University of Bergen, Bergen 5020, Norway
[e] Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen 5020, Norway

Received 29 March 2017; received in revised form 8 July 2017; accepted 17 July 2017
Handling editor: Dr. A. Siani
Available online 25 July 2017

KEYWORDS
Metabolic syndrome;
Amino acids;
Mass spectrometry

Abstract *Background and aims:* The metabolic syndrome (MetS) refers to a cluster of clinically relevant factors that increases the risk of cardiovascular diseases and all-cause mortality. Circulating levels of several amino acids and metabolites related to one-carbon metabolism have been associated with cardiometabolic risk factors and MetS. We aimed to identify the amino acid profile that is significantly associated with MetS among an all male Mediterranean population.
Methods and results: One hundred middle-aged men (54.6 ± 8.9 years) participated in a cross-sectional study carried out during 2011–2012. The International Diabetes Federation (IDF) criteria were used to define MetS. Fasting plasma levels of 20 common amino acids and 15 metabolites related to amino acid and one-carbon metabolism were measured using gas chromatography (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Principal components analysis was applied. Fifty-six participants fulfilled the IDF criteria for defining MetS. Five factors were extracted from the 35 measured metabolites. The branched-chain amino acids/aromatic amino acids (BCAA/AAA) related pattern and the glutamine/glycine/serine/asparagine (Gln/Gly/Ser/Asn) related pattern were significantly associated with MetS (odds ratio, 95% confidence interval; 6.41, 2.43–16.91, and 0.47, 0.23–0.96, respectively) after adjustment for age, current smoking status, physical activity level and medical treatment for hypertension, dyslipidaemia, type 2 diabetes mellitus. Further adjustment for liver function markers (i.e. glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and γ-glutamyltransferase), and plasma adiponectin levels did not significantly affect the associations.
Conclusion: The BCAA/AAA pattern was positively associated, while the Gln/Gly/Ser/Asn pattern was inversely associated with established cardiometabolic risk factors and MetS. Plasma adiponectin levels or markers of liver function did not significantly affect these associations.

Abbreviations: AAA, aromatic amino acids; ADMA, asymmetric dimethylarginine; Asn, asparagine; BMI, body mass index; BCAAs, branched-chain amino acids; CVD, cardiovascular disease; DBP, diastolic blood pressure; Gln, glutamine; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GT, y-glutamyltransferase; Gly, glycine; His, histidine; IDF, International Diabetes Federation; MET, metabolic equivalent; MetS, metabolic syndrome; Orn, ornithine; PCA, principal components analysis; Pro, proline; SDMA, symmetric dimethylarginine; SBP, systolic blood pressure; Ser, serine; T2D, type 2 diabetes mellitus; TMAO, trimethylamine N-oxide.

* Corresponding author. Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Eleftheriou Venizelou 70, Athens 17671, Greece.
E-mail address: antonop@hua.gr (S. Antonopoulou).

http://dx.doi.org/10.1016/j.numecd.2017.07.006

Introduction

The metabolic syndrome (MetS) refers to a cluster of clinically relevant factors that increases the risk of cardiovascular diseases (CVD), type 2 diabetes mellitus (T2D) and all-cause mortality [1]. These factors include widely accepted indices of obesity, metabolic risk factors (i.e. elevated triglycerides, low high-density lipoprotein cholesterol levels and elevated glucose) and blood pressure homeostasis [2]. The two most widely used definitions of MetS [2,3] focus on the presence of abdominal obesity, dyslipidemia, elevated blood pressure and impaired glycaemic control and are used to identify individuals at risk of developing T2D and atherosclerotic cardiovascular disease who can benefit from lifestyle modification.

Metabolite profiling is the identification and quantification of a selected number of predefined low molecular weight compounds, generally related to (a) specific metabolic pathway(s), in specific tissues or compartments [4]. Metabolite profiling has been used to investigate differences between lean and obese subjects with or without MetS [5,6], as well as between subjects with or without cardiometabolic risk factors independent of body mass index (BMI) [7,8]. Predicting the development of MetS, dyslipidaemia, hypertension, and T2D has also utilized metabolite profiling methods [9].

A number of large prospective [10–12] and cross-sectional studies [13] have focused on a limited number of amino acids and metabolites involved in one-carbon metabolism when investigating cardiometabolic disease risk and development. Plasma homocysteine levels have been found to increase with increasing number of MetS components [10] and to associate with presence of MetS in cross-sectional analyses [10,12], as well as to predict CVD incidence in prospective analyses [10,11]. Plasma homocysteine and choline have been found to be positively correlated with waist circumference, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP), triglyceride levels and insulin resistance, and negatively with HDL-cholesterol levels [11–13]. In contrast betaine has been shown to be positively associated with HDL-cholesterol and inversely related to BMI, body fat, waist circumference, SBP and DBP, serum triglycerides and non-HDL cholesterol [13]. Dimethylglycine, creatine, proline and alanine have shown a similar association pattern with MetS components as that observed for homocysteine and choline, whereas concentrations of serine, asparagine, glutamine and glycine appear to be inversely associated with MetS components in cross-sectional analyses of large prospective cohort studies [8,9,14]. Glycine and glutamine have also been inversely associated with development of T2D [15].

Accumulating evidence from American, Northern European and Japanese populations links the branched-chain amino acids (BCAAs), and the aromatic amino acids (AAAs), especially phenylalanine and tyrosine, to cardiometabolic risk. Research has demonstrated that the BCAAs and AAAs are positively correlated with BMI, waist circumference, visceral fat, SBP, DBP, fasting blood glucose, insulin and triglyceride levels and insulin resistance, but inversely with HDL-cholesterol in cross-sectional analyses of large prospective cohort studies [8,9,16]. These amino acids have also been observed to be positively associated with development of insulin resistance [16], T2D [9,15] and hypertriglyceridemia [17]. The associations between BCAAs, AAAs and cardiometabolic disorders are thought to be underpinned by obesity and insulin resistance [18,19]. Attention has also been paid to serum tryptophan levels, and tryptophan metabolites along the kynurenine pathway. However, evidence from studies investigating cross-sectionally tryptophan, kynurenine-to-tryptophan ratio (KTR) as well as kynurenine pathway metabolites is conflicting [14,20,21].

Despite the increasing interest in amino acids metabolism in the context of cardiometabolic health, few studies have evaluated an array of amino acids and related metabolites in a general population. To our knowledge, this is the first study to comprehensively evaluate the amino acid profile of men with MetS in a Mediterranean population. Therefore, the aim of the present study was to evaluate the association of circulating levels of amino acids and metabolites related to amino acid and one-carbon metabolism with MetS and its components and attempt to identify candidate biomarkers for the risk of cardiovascular diseases.

Methods

Study sample

One hundred middle-aged men (mean ± standard deviation: 54.6 ± 8.9 years) were randomly selected from a previously published cross-sectional study performed during 2011–2012 in Athens, Attica [22]. In brief, the study population consisted of individuals aged >30 years from the general population. Participants responded to an invitation to health evaluation advertised at the participants' workplace. Participants diagnosed with cardiovascular disease, high-grade chronic inflammatory disease (e.g. rheumatoid arthritis, inflammatory bowel disease, atopic dermatitis, and asthma), viral infections, cold or flu, acute respiratory infection, renal disease, type 1 diabetes mellitus, dental problems or any type of surgery the month preceding the study were excluded from the study.

Ethical approval

All procedures performed in the present study were in accordance with the ethical standards of the Bioethics Committee of Harokopio University, Athens, and with the 1964 Helsinki Declaration and its later amendments. All participants provided written informed consent.

Lifestyle evaluation

Current smokers were defined as those who were smoking at least one cigarette per day during the past year or had recently stopped smoking (within the last 12 months);

remaining participants were defined as non-smokers [22]. Physical activity level was evaluated with the International Physical Activity Questionnaire (IPAQ), modified and adapted for the Greek population [23]. Physical activity level was classified as low if total physical activity was less than 600 metabolic equivalent (MET)-minutes per week and moderate if total physical activity was ≥600 MET-minutes per week [23].

Clinical evaluation

Resting blood pressure was measured twice on the right arm with an electronic monitor device. All participants rested for a minimum of 30 min before measurement, which was performed in sitting position. Diagnosis of and current medication treatment for hypertension, hypercholesterolemia, and T2D were recorded in a self-administered questionnaire. Waist and hip circumferences (in centimeters, cm), as well as height (in cm) and body mass (in kilograms, kg) were measured using standard procedures [22]. Waist-to-hip ratio was calculated as waist circumference divided by hip circumference. BMI was calculated as body mass divided by height (in meters squared, m^2).

Sample collection and biochemical analyses

Venous blood samples were collected between 08:00 and 10:00, following 12 h overnight fast. Following standard blood processing, serum and plasma aliquots were stored at −80 °C until use [22]. Serum concentrations of total cholesterol, HDL-cholesterol, triglycerides, glucose and liver enzymes (i.e. glutamic oxaloacetic transaminase or GOT, glutamic pyruvic transaminase or GPT, and γ-glutamyltransferase or γ-GT) were measured on a COBAS 8000/ROCHE analyzer at BIOMED S.A. (accreditation standard ELOT EN ISO 15189, Hellenic Accreditation System − E.SY.D.). The intra-assay coefficient of variation (CV%) was 1−2% for lipid indices (total cholesterol, HDL-cholesterol and triglycerides), 2% for glucose and 1−2% for liver enzymes (GOT, GPT, and γ-GT). Inter-assay CV% was 1−2% for lipid indices, 1% for glucose and 1−3% for liver enzymes. LDL-cholesterol was estimated with the Friedewald equation: (total cholesterol) − (HDL-cholesterol) − (triglycerides/2.2) [24]. Serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and glucose are expressed in mmol/L, serum concentrations of GOT, GPT, and γ-GT are expressed in U/L.

Serum insulin concentration (mU/L) was measured on a TOSOH AIA-600 II automated enzyme immunoassay analyser using a two-site immune-enzymometric assay (ST AIA-PACK IRI). Both intra- and inter-assay CVs were <3%. Adiponectin concentration (μg/mL) was measured in plasma EDTA samples using a commercially available ELISA method (Quantikine® ELISA, R&D Systems Europe Ltd., Abingdon, U.K.) with an assay range of 3.9−250 ng/mL. Both intra- and inter-assay CVs were <7%.

Amino acids and metabolites related to amino acid and one-carbon metabolism were measured in EDTA plasma using gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [25,26] at BEVITAL AS (www.bevital.no). Within- and between-day CVs for analytes measured by GC-MS/MS were 1−5% and 1−4%, respectively. Within- and between-day CVs for analytes measured by LC-MS/MS were 1−9% and 2−12%, respectively.

Definition of metabolic syndrome

The MetS was defined by the IDF criteria (2005) [3], which included abdominal obesity (waist circumference ≥ 94 cm for men of Europid origin) or BMI ≥ 30 kg/m^2 and any two of the following risk factors: elevated triglycerides (≥1.7 mmol/L), reduced HDL-cholesterol (<1.03 mmol/L for men), elevated blood pressure (SBP ≥ 130 mmHg or DBP ≥ 85 mmHg), elevated fasting glucose (≥5.6 mmol/L) or T2D. Participants receiving pharmacotherapy for elevated triglycerides, reduced HDL-cholesterol, elevated blood pressure or hyperglycemia were considered as meeting the aforementioned criteria.

Statistical analysis

Categorical variables are expressed as frequencies (n (%)), and continuous variables as median and percentiles (25th, 75th percentiles). Differences on continuous measurements between participants with and without MetS were evaluated using the non-parametric Mann−Whitney U test, due to skewed distributions. Mean differences in plasma metabolites between participants with and without MetS were calculated with t test. Metabolite variables had a rightly skewed distribution and values were logarithmically transformed (natural logarithm, ln) prior to t test. The association of MetS with categorical variables was evaluated using the Fischer's exact test. Normality of the distribution of continuous variables was tested with the Kolmogorov−Smirnov test and P-P plots.

Factor analysis, using the principal components method (PCA) was used to investigate potential patterns in plasma concentrations of amino acid and metabolites related to amino acid and one-carbon metabolism. Prior to PCA all variables were logarithmically transformed (natural logarithm, ln) due to rightly skewed distribution. The varimax rotation method was performed to produce interpretable components. Components with an eigenvalue ≥1.0 were extracted. The scree plot of the extracted components was also used to determine the optimal number of components to retain for further analyses. Items with a factor loading ≥|0.4| were considered as composing a given factor. Factor scores were calculated using the regression method and used in subsequent analyses investigating correlations between extracted factors and MetS components, adiponectin and liver enzymes.

Correlations between PCA factors and MetS components (i.e. HDL-cholesterol, triglycerides, glucose, insulin, SBP, DBP, BMI, waist circumference, waist-to-hip ratio), adiponectin, GOT, GPT and γ-GT were evaluated after

adjustment for age. All five factors, as well as HDL-cholesterol, triglycerides, SBP, DBP, BMI, waist circumference, waist-to-hip ratio, adiponectin, GOT and GPT had normal distribution and were used in correlations as continuous variables. In contrast, glucose, insulin and γ-GT had a rightly skewed distribution and values were logarithmically transformed (natural logarithm, ln) prior to analysis. Correlations are reported as coefficients of linear correlation (Pearson's r) and level of significance (p).

Logistic regression was applied with MetS as the dependent variable and factors as independent variables with adjustments for age, current smoking status, physical activity level, and medical treatment for hypertension, dyslipidaemia and type 2 diabetes mellitus (Model 1). We adjusted for age, current smoking status, physical activity level and medical treatment for hypertension, dyslipidaemia and type 2 diabetes mellitus in order to take into account unexpected discrepancies in the distribution of these variables between MetS groups, due to the observational design of the study, and because of previously shown associations with MetS components, amino acids and metabolites related to amino acid and one-carbon metabolism [8,9,13,16,20,27]. Potential mediation by GOT, GPT, γ-GT or adiponectin on the association between the extracted factors and MetS, was examined by adding these variables to the basic Model 1. Results are presented as odds ratio (OR) and 95% confidence interval (95% CI).

SPSS version 21 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All statistical tests were two-tailed, with a nominal level of p-value < 0.05 considered as significant. We employed a Bonferroni-corrected p-value threshold of 0.0014 which was calculated as the nominal p-value threshold (i.e. 0.05) divided by the number of metabolites analysed (i.e. 35 metabolites) in order to account for multiple comparisons.

Results

Characteristics of participants with MetS and without MetS

Lifestyle, clinical and biochemical characteristics of the participants with MetS and those without MetS are presented in Table 1. Using the IDF criteria for defining MetS, fifty-six participants were considering as having the MetS. Participants with MetS did not differ significantly from those without MetS with respect to age, serum concentration of total cholesterol, LDL-cholesterol, GOT and GPT, as well as current smoking status, physical activity level and medical treatment for dyslipidaemia or type 2 diabetes mellitus. Serum concentrations of triglycerides, glucose, insulin and γ-GT, SBP, DBP, BMI, waist circumference and waist-to-hip circumference ratio were

Table 1 [a]Characteristics of participants with MetS (IDF criteria) and without MetS.

	Without MetS (n = 44)	MetS (n = 56)	p*,**
Age (years)	53.5 (47.3, 57.0)	57.5 (47.3, 63.8)	0.068
Current smoking (yes)	12 (27.9)	22 (40)	0.285
Physical activity level (moderate)	28 (63.6)	29 (51.8)	0.309
MetS components:			
Total cholesterol (mmol/L)	5.3 (4.9, 5.9)	5.1 (4.7, 5.9)	0.264
HDL-cholesterol (mmol/L)	1.3 (1.1, 1.5)	1.0 (0.9, 1.2)	<0.001
LDL-cholesterol (mmol/L)	3.5 (3.0, 3.9)	3.2 (2.6, 3.9)	0.252
Triglycerides (mmol/L)	1.1 (0.8, 1.4)	1.9 (1.4, 2.5)	<0.001
Glucose (mmol/L)	5.1 (4.8, 5.4)	5.5 (5.0, 6.1)	0.003
Insulin (mU/L)	6.4 (5.0, 9.3)	11.8 (8.7, 16.7)	<0.001
SBP (mm Hg)	123.5 (116.0, 131.0)	134.3 (125.5, 138.0)	<0.001
DBP (mm Hg)	80.0 (71.0, 86.0)	85.0 (79.2, 90.0)	0.035
Waist circumference (cm)	90.5 (87.0, 93.0)	105.0 (100.0, 112.4)	<0.001
Waist-to-hip ratio	0.92 (0.88, 0.95)	1.0 (0.95, 1.06)	<0.001
BMI (kg/m^2)	24.9 (24.1, 26.5)	29.3 (27.1, 32.4)	<0.001
Medical treatment for:			
Hypertension (yes)	3 (6.8)	17 (30.4)	0.005
Dyslipidaemia (yes)	7 (15.9)	14 (25.0)	0.327
Type 2 diabetes mellitus (yes)	0	4 (7.1)	0.128
GOT (U/L)	19.59 (16.71, 21.91)	18.67 (14.19, 21.16)	0.271
GPT (U/L)	17.22 (11.99, 23.35)	19.00 (11.76, 28.13)	0.588
γ-GT (U/L)	21.54 (17.51, 30.66)	28.48 (21.54, 39.88)	0.021
Adiponectin (μg/mL)	6.50 (4.55, 9.53)	4.71 (3.38, 7.28)	0.006

*Significant differences between participants with and without MetS with respect to continuous variables were evaluated using the non-parametric Mann–Whitney U test.

**The association of MetS with categorical variables was evaluated using the Fischer's exact test.

[a]Continuous variables (i.e. age, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, insulin, SBP, DBP, waist circumference, waist-to-hip ratio, BMI, GOT, GPT, γ-GT and adiponectin) are expressed as median (25th, 75th percentiles); Categorical variables (i.e. current smoking, physical activity level, and medical treatment for hypertension, dyslipidaemia and type 2 diabetes mellitus) are expressed as absolute and relative frequencies, n (%); Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GT, γ-glutamyltfansferase; MetS, metabolic syndrome; SBP, systolic blood pressure.

significantly higher among participants with MetS compared to participants without MetS. Conversely, serum concentration of HDL-cholesterol and plasma concentration of adiponectin were significantly lower among participants with MetS compared to participants without MetS. Participants with MetS differ significantly from those without MetS with respect to medical treatment for hypertension.

MetS and metabolite profile

Differences between participants with MetS and those without MetS with respect to plasma concentrations of amino acids and metabolites related to amino acid and one-carbon metabolism are reported in Supplementary Table 1. Mean differences of logarithmically transformed plasma metabolites between participants with MetS and those without MetS are reported in Fig. 1. Participants with MetS had significantly higher plasma concentrations of alanine, aspartic acid, glutamic acid, the BCAAs (isoleucine, leucine and valine), the AAAs (phenylalanine, tryptophan and tyrosine), kynurenine, total cysteine and TMAO. After applying the Bonferroni rule for multiple comparisons, statistically significant differences were observed only for alanine, glutamic acid, kynurenine, and valine between participants with MetS and those without MetS.

Table 2 describes the results obtained from factor analysis for the whole cohort. Initially, twelve factors with an eigenvalue ≥ 1 were extracted, however, five factors were retained for further analyses based on scree plot; *Factor 1*, the dominant factor explaining 23% of the variance, comprised mainly all BCAAs and AAAs, glutamic and aspartic acid, alanine, lysine and methionine; *Factor 2* comprised glutamine, glycine, serine, asparagine, threonine, ornithine, lysine, histidine and proline; *Factor 3* comprised total homocysteine, cystathionine, creatinine, trimethyllysine, methylmalonic acid, proline and kynurenine; *Factor 4* comprised betaine, choline, SDMA, dimethylglycine and creatinine; *Factor 5* comprised arginine, TMAO, ornithine and methionine.

Correlations between PCA factors and MetS components, adiponectin and liver enzymes

Factors derived from the PCA were correlated with MetS components after adjustment for age (Fig. 2). Factor 1 was negatively associated with HDL-cholesterol ($r = -0.435$, $p \leq 0.001$), and positively with triglycerides ($r = 0.416$, $p \leq 0.001$), glucose ($r = 0.222$, $p < 0.05$), insulin ($r = 0.451$, $p \leq 0.001$), BMI ($r = 0.389$, $p \leq 0.001$) and waist circumference ($r = 0.426$, $p \leq 0.001$). In contrast, Factor 2 was positively associated with HDL-cholesterol ($r = 0.289$, $p \leq 0.005$), and negatively with insulin ($r = -0.271$, $p < 0.05$), SBP ($r = -0.296$, $p \leq 0.005$) and BMI ($r = -0.219$, $p < 0.05$). Factor 3 was positively associated with waist-to-hip ratio ($r = 0.203$, $p < 0.05$), and Factor 4 was negatively associated with glucose ($r = -0.225$, $p < 0.05$). Factor 5 was not associated with any MetS component.

Correlations between PCA factors and adiponectin, as well as liver enzymes (i.e. GOT, GPT and γ-GT), after adjustment for age, were also investigated (Fig. 2). Factor 1 was negatively associated with adiponectin ($r = -0.384$, $p \leq 0.001$), and positively with γ-GT ($r = 0.453$, $p \leq 0.001$). Factor 4 was positively associated with

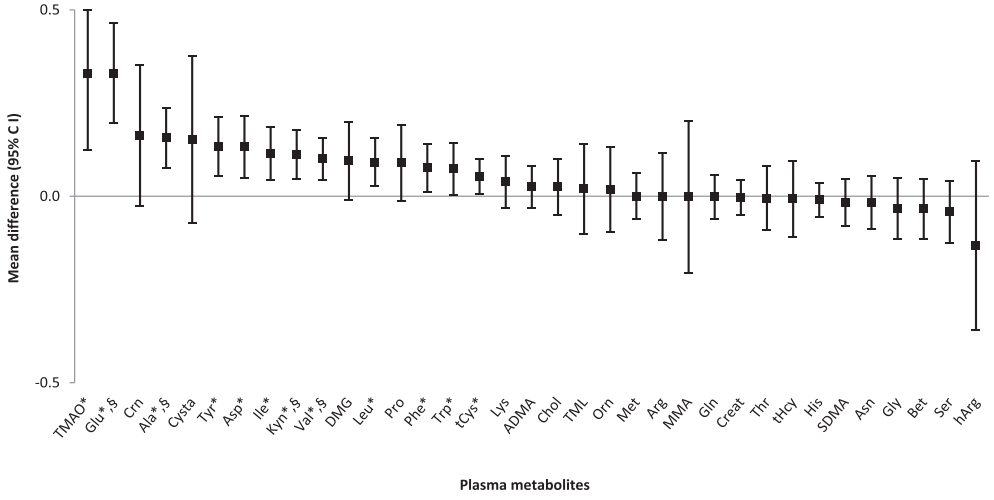

Figure 1 Mean differences between participants with MetS (IDF criteria) and without MetS with respect to logarithmically transformed metabolite concentrations. Plot shows mean difference and 95% confidence interval (CI) of the mean difference (vertical axis) for each plasma metabolite (horizontal axis). Mean difference is marked with a rectangular (■), and 95% confidence interval (CI) of the mean difference is marked with low and high lines. Metabolite variables have a rightly skewed distribution and values are logarithmically transformed (natural logarithm, ln) prior to t test for comparison of normally distributed variables between participants with MetS and participants without MetS. Significant differences in plasma metabolites are marked with (*) for a p-value lower than the nominal threshold (i.e. p-value <0.05), and (§) for a p-value lower than the Bonferroni-adjusted threshold (i.e. p-value <0.0014). Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; ADMA, asymmetric dimethylarginine; Bet, betaine; Chol, choline; Crn, creatine; Creat, creatinine; Cysta, cystathionine; DMG, dimethylglycine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; hArg, homoarginine; Ile, isoleucine; Kyn, kynurenine; Leu, leucine; Lys, lysine; Met, methionine; MMA, methylmalonic acid; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; SDMA, symmetric dimethylarginine; Thr, threonine; tCys, total cysteine; tHcy, total homocysteine; TMAO, trimethylamine N-oxide; TML, trimethyllysine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

Table 2 [a]Results from factor analysis (using the PC method) exploring patterns of plasma amino acids and metabolites related to amino acid and one-carbon metabolism.

	Factor				
	1	2	3	4	5
Leucine	**0.829**	0.221	−0.080	0.113	−0.185
Isoleucine	**0.807**	0.098	0.147	0.095	−0.120
Valine	**0.785**	0.171	0.027	−0.024	−0.306
Tyrosine	**0.710**	0.234	0.198	−0.085	0.183
Tryptophan	**0.707**	0.393	−0.063	−0.056	−0.009
Phenylalanine	**0.701**	0.354	0.168	0.149	0.167
Glutamic acid	**0.663**	−0.262	−0.115	−0.136	0.030
Aspartic acid	**0.653**	0.107	0.092	0.060	0.155
Alanine	**0.615**	0.265	0.288	−0.167	0.068
Lysine	**0.539**	**0.454**	−0.240	0.223	0.035
Methionine	**0.461**	0.317	0.138	0.023	**0.459**
Creatine	0.286	−0.002	−0.086	−0.081	−0.270
Glutamine	0.119	**0.754**	0.147	−0.018	−0.006
Glycine	−0.004	**0.734**	−0.044	−0.257	0.119
Serine	0.244	**0.712**	−0.189	0.015	0.038
Asparagine	0.361	**0.677**	−0.039	0.094	0.185
Threonine	0.345	**0.660**	0.162	−0.063	0.058
Ornithine	0.107	**0.594**	0.107	0.290	**−0.503**
Histidine	0.240	**0.453**	−0.173	0.177	0.279
Total homocysteine	−0.067	−0.066	**0.719**	0.051	0.082
Cystathionine	0.193	−0.162	**0.553**	−0.055	−0.040
Creatinine	−0.137	0.014	**0.508**	**0.447**	−0.030
Trimethyllysine	0.077	−0.012	**0.479**	0.199	−0.251
Methylmalonic acid	0.046	0.075	**0.452**	0.096	0.210
Proline	0.262	**0.413**	**0.445**	−0.360	−0.228
Kynurenine	0.316	0.211	**0.410**	0.227	−0.259
ADMA	−0.036	0.162	0.244	0.130	−0.122
Betaine	−0.061	0.009	−0.026	**0.663**	0.027
Choline	0.116	0.040	0.082	**0.644**	−0.244
SDMA	−0.225	0.001	0.294	**0.577**	0.216
Dimethylglycine	0.267	−0.046	0.341	**0.562**	0.112
Total cysteine	0.080	−0.168	0.216	0.264	0.165
Arginine	0.026	0.035	0.142	−0.002	**0.636**
TMAO	−0.014	−0.091	0.198	0.134	**−0.589**
Homoarginine	−0.017	0.078	−0.055	0.192	0.365
% of variance	22.857	9.288	7.160	6.007	5.052

[a]Extraction Method: Principal components analysis (PCA) with varimax rotation; Values are logarithmically transformed (ln) prior to analysis because of rightly skewed distribution; Items with a loading $\geq|0.4|$ were reported as composing a given factor (bold font type); Abbreviations: ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; TMAO, trimethylamine N-oxide.

adiponectin ($r = 0.246$, $p < 0.05$), and Factor 5 was positively associated with GOT ($r = 0.282$, $p \leq 0.005$). Factors 2 or 3, were not significantly correlated with adiponectin, GOT, GPT or γ-GT.

Association between amino acid patterns and MetS

Factors derived from the PCA representing patterns in plasma concentrations of amino acids and metabolites related to amino acid and one-carbon metabolism were used in logistic regression analysis in order to investigate whether those patterns were significantly associated with MetS. The results obtained from logistic regression analysis are presented in Table 3, Model 1. After adjustment for age, current smoking status, physical activity level, and medical treatment for hypertension, dyslipidaemia and type 2 diabetes mellitus, Factor 1 (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine, glutamic and aspartic acid, alanine, lysine and methionine) was positively associated with MetS (OR, 95% CI; 6.41, 2.43−16.91), whereas Factor 2 (glutamine, glycine, serine, asparagine, threonine, ornithine, lysine, histidine and proline) was inversely associated with MetS (OR, 95% CI; 0.47, 0.23−0.96). None of the other factors (Factors 3−5) were significantly associated with MetS in the multivariate adjusted Model 1 (Table 3).

Mediation analysis of GOT, GPT, γ-GT or adiponectin

The results obtained from logistic regression analysis examining the potential mediating effect of GOT, GPT, γ-GT or adiponectin on the association between emergent amino acid and related metabolite factors and MetS, are presented in Table 3 (Models 2−5). None of the potential mediators significantly altered the association between the extracted factors and the MetS, nor was GOT, GPT, γ-GT or adiponectin significantly associated with MetS, following various adjustments (Table 3, Models 2−5). Factor 3 (total homocysteine, cystathionine, creatinine, trimethyllysine, methylmalonic acid, proline and kynurenine) was found to be positively associated with MetS (OR, 95% CI; 1.96, 1.03−3.72) after adjustment for adiponectin (Table 3, Model 5).

Discussion

The present study revealed two distinct amino acids patterns significantly correlated but in opposite directions with MetS components in men. The one, with unfavourable effect, includes elevated BCAA/AAA, glutamic acid, aspartic acid, and alanine levels, while the other one mainly comprises reduced levels of glutamine, glycine, serine, asparagine, and threonine. In addition, only the BCAA/AAA related amino acid pattern was also significantly correlated with adiponectin and γ-GT both strongly linked to the development of adverse cardiometabolic outcomes. The results clearly demonstrate that there is a strong relationship between specific amino acids and MetS also in male Mediterranean population.

Association between amino acid patterns and MetS

The amino acid pattern which included the BCAAs (leucine, isoleucine, valine), the AAAs (tyrosine, tryptophan, phenylalanine), glutamic acid, aspartic acid, alanine, lysine and methionine, denoted “Factor 1”, was the dominant factor (accounting for 23% of the variance in PCA). All the amino acids composing Factor 1, with the exception of lysine and methionine, were significantly higher among participants with MetS. The amino acids that had the highest loadings in Factor 1 were the BCAAs and the AAAs, and for that reason this factor will be referred to as BCAA/AAA related amino acid pattern

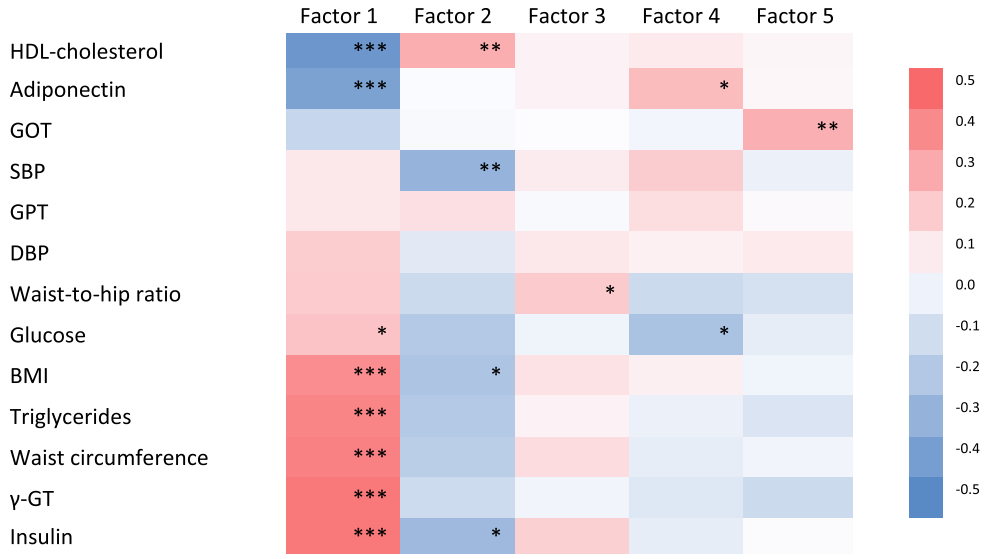

Figure 2 Correlations between PCA factors and MetS components, adiponectin and liver enzymes. Pearson's correlation coefficients were calculated after adjustment for age. Glucose, γ-GT and insulin have a rightly skewed distribution and values are logarithmically transformed (natural logarithm, ln) prior to analysis. Significant correlations are marked with asterisk(s): (*) for $p < 0.05$, (**) for $p \leq 0.005$, (***) for $p \leq 0.001$. Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GT, γ-glutamyltransferase; SBP, systolic blood pressure; Factor 1 (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine, glutamic and aspartic acid, alanine, lysine and methionine); Factor 2 (glutamine, glycine, serine, asparagine, threonine, ornithine, lysine, histidine and proline); Factor 3 (total homocysteine, cystathionine, creatinine, trimethyllysine, methylmalonic acid, proline and kynurenine); Factor 4 (betaine, choline, SDMA, dimethylglycine and creatinine); Factor 5 (arginine, TMAO, ornithine and methionine).

hereafter. The BCAA/AAA related pattern was associated with established components of the MetS, related to dyslipidaemia, impaired glycaemic control, and increased body mass. As mentioned in the introduction, the amino acids composing the BCAA/AAA related amino acid pattern in the present study have been previously associated with cardiometabolic risk factors in cross-sectional analyses [5,8,14,28]. Our results are in accordance with previous cross-sectional investigations, which also used principal component analysis to identify emergent amino acid profiles/patterns [5,27,29]. An amino acid profile characterized by elevated BCAAs, methionine, Glx (glutamate/glutamine), the aromatic amino acids phenylalanine and tyrosine, as well as C3 and C5 acylcarnitines, has been significantly associated with obesity status and insulin resistance [5]. Similar BCAA/AAA related amino acid patterns have been inversely associated with insulin sensitivity [29], positively associated with circulating levels of triglycerides and insulin, BMI, insulin resistance and SBP, but negatively associated with HDL-cholesterol [27]. Yamakado and colleagues (2015) followed an approach for calculation of a plasma free amino acid (PFAA) index, which is different from the PCA method, but also found that the indices which included BCAAs and AAAs, were positively associated with visceral fat and circulating levels of insulin in cross-sectional analyses [9].

The amino acid pattern which included glutamine, glycine, serine, asparagine, threonine, ornithine, lysine, histidine and proline, denoted "Factor 2", accounted for 9% of the variance in PCA. None of the amino acids composing Factor 2 were significantly different between participants with and without MetS. The first four amino acids composing Factor 2 in the present study, i.e. glutamine (Gln), glycine (Gly), serine (Ser) and asparagine (Asn), have previously been found to be consistently associated with MetS components related to decreased cardiometabolic risk in cross-sectional analyses. Specifically, Gln has been found to be inversely associated with fasting glucose, triglycerides, insulin, SBP, DBP [8] and insulin resistance [18], and positively related to HDL-cholesterol [8]. Gly levels have been found to be lower among obese subjects compared to subjects with normal weight [5], and Gly has been inversely associated with BMI, waist circumference [14] and insulin resistance [28]. Ser and Asn have been found to be inversely associated with insulin resistance [28]. In contrast with Gln, Gly, Ser and Asn, previous studies have shown that ornithine (Orn), histidine (His) and proline (Pro) are positively associated with an adverse cardiometabolic risk profile in cross-sectional analyses. Even though His, Orn, and Pro have not been found to be significantly different between obese subjects and subjects with normal weight [5], both Pro and Orn have been positively associated with BMI and waist circumference [14]. In addition, Pro and His have been positively associated with insulin resistance [28]. In our study, His, Pro and Orn did not differ significantly between participants with and without MetS, and all three amino acids were found to contribute to Factor 2. As a whole, we found that Factor 2 was associated with cardiometabolic profile typically related to decreased risk, i.e. high HDL-cholesterol levels, low insulin levels and systolic blood pressure, as well as decreased body mass. These associations could be driven by the fact that the amino acids with the strongest loadings in Factor 2 are Gln, Gly, Ser and Asn, whereas Pro, His

Table 3 [a]Results from logistic regression models that evaluated the association between amino acid patterns and presence of MetS.

	Model 1	Model 2	Model 3	Model 4	Model 5
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Factor 1	6.41 (2.43–16.91)	6.45 (2.42–17.22)	6.35 (2.40–16.77)	6.17 (2.00–19.00)	5.13 (1.90–13.85)
Factor 2	0.47 (0.23–0.96)	0.47 (0.23–0.96)	0.46 (0.22–0.95)	0.47 (0.23–0.96)	0.45 (0.22–0.92)
Factor 3	1.83 (0.98–3.41)	1.83 (0.98–3.41)	1.81 (0.97–3.38)	1.81 (0.97–3.38)	1.96 (1.03–3.72)
Factor 4	0.66 (0.36–1.21)	0.66 (0.36–1.22)	0.66 (0.36–1.21)	0.66 (0.35–1.25)	0.72 (0.39–1.35)
Factor 5	0.69 (0.38–1.26)	0.69 (0.36–1.32)	0.69 (0.37–1.26)	0.69 (0.38–1.26)	0.73 (0.39–1.34)
GOT (U/L)		1.00 (0.91–1.11)			
GPT (U/L)			1.00 (0.97–1.05)		
γ-GT (ln)				0.95 (0.25–3.68)	
Adiponectin (μg/mL)					0.88 (0.71–1.09)

[a]Factor 1 (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine, glutamic and aspartic acid, alanine, lysine and methionine); Factor 2 (glutamine, glycine, serine, asparagine, threonine, ornithine, lysine, histidine and proline); Factor 3 (total homocysteine, cystathionine, creatinine, trimethyllysine, methylmalonic acid, proline and kynurenine); Factor 4 (betaine, choline, SDMA, dimethylglycine and creatinine); Factor 5 (arginine, TMAO, ornithine and methionine); Age, Factor 1, Factor 2, Factor 3, Factor 4, Factor 5, GOT, GPT and adiponectin are continuous with normal distribution; γ-GT has a rightly skewed distribution and values are logarithmically transformed (natural logarithm, ln) prior to analysis; Model 1: adjustment for age, current smoking status (yes vs. no), physical activity level (moderate vs. low), medical treatment for hypertension (yes vs. no), dyslipidaemia (yes vs. no), type 2 diabetes mellitus (yes vs. no), plus Factors 1–5; Model 2: Model 1 plus GOT (U/L); Model 3: Model 1 plus GPT (U/L); Model 4: Model 1 plus γ-GT (ln); Model 5: Model 1 plus adiponectin (μg/mL); Results are presented as odds ratio (OR) and 95% confidence interval (95% CI).

and Orn had the lowest loadings. For that reason, Factor 2 will be referred to as Gln/Gly/Ser/Asn related amino acid pattern hereafter.

When investigating MetS as an outcome we showed that the BCAA/AAA related pattern and the Gln/Gly/Ser/Asn related pattern were the only amino acid patterns which were significantly associated with MetS, after adjustment for age, current smoking status, physical activity level, and medical treatment for hypertension, dyslipidaemia, type 2 diabetes mellitus. Previous studies have demonstrated the factor comprising BCAAs and AAAs to be cross-sectionally associated with MetS even after adjustment for age, BMI and waist circumference [27]. Factors consisting of BCAAs, AAAs, methionine, alanine, histidine, proline, glutamate and aspartate have been found to be significantly and positively associated with a metabolically unwell state (MUW), characterized by presence of two or more cardiometabolic abnormalities, and these associations remained after adjustment for BMI in cross-sectional analyses [7]. In contrast, a factor composed of glycine, serine and ornithine was associated with metabolically well (MW), compared to metabolically unwell participants (individuals with at least two cardiometabolic abnormalities) [7].

Influence of liver function and adiponectin

We found that the BCAA/AAA related amino acid pattern was negatively associated with adiponectin, but positively with γ-GT, whereas the Gln/Gly/Ser/Asn related pattern was not associated with any of these biomarkers. Previously, Cheng et al. (2015) reported elevated serum levels of BCAAs, phenylalanine and tyrosine alongside decreased adiponectin levels and elevated liver enzymes [30]. The BCAAs and tryptophan have also been previously found to be inversely associated with plasma adiponectin levels [31,32], and these amino acids have clustered together with adiponectin in hierarchical cluster analysis [31]. We have recently found that adiponectin has a high discriminative accuracy for MetS even after adjustment for, age, sex and MetS components [22]. In addition, a recent meta-analysis has shown that γ-GT level is positively and significantly associated with risk of the MetS even within normal reference levels of γ-GT [33]. On the evidence of these findings, based on cross-sectional evaluation [22,30–32] and meta-analysis of prospective cohort studies [33], we studied the potential mediating effect of liver function markers (i.e. GOT, GPT and γ-GT) and adiponectin on the association between amino acid patterns and MetS, and to our knowledge we are the first to do so. We found that despite adjusting for GOT, GPT, γ-GT and adiponectin, the significant relations between the BCAA/AAA related amino acid pattern and the Gln/Gly/Ser/Asn related pattern with MetS remained largely unchanged.

Potential mechanisms

Important considerations for the present study include the use of fasting plasma samples and acknowledgement that the amino acids, which characterize the pattern positively and significantly associated with MetS (i.e. BCAA and AAA), was dominated by essential amino acids. Circulating fasting levels of these amino acids are dependent on both the rate of appearance in the blood, which reflects proteolysis and amino acid release from tissues, mainly the skeletal muscle, and the rate of disappearance from the blood, which is partly due to increased amino acid catabolism [34]. Elevated circulating levels of the BCAAs and AAAs may be explained by obesity-related insulin resistance which has been associated with an increase in muscle protein breakdown [35]. Furthermore, decreased circulating adiponectin levels have been identified as an underlying mechanism influencing activation of proteolysis in insulin-resistant state [35]. In the case of BCAAs' catabolism, gene expression analysis of subcutaneous adipose tissue has revealed that the BCAA degradation

pathway is down-regulated in obese compared to normal weight individuals [36]. Disturbed BCAA catabolic pathway could also explain the increased glutamate and alanine levels observed in participants with MetS in the present study. Specifically, increased glutamate levels could be explained by the fact that glutamate is synthesized in the first step of BCAA catabolism [5]. We should point out that in the present study, all participants with MetS were overweight or obese and had significantly higher fasting insulin and glucose levels (indicative of reduced insulin sensitivity) and significantly lower adiponectin levels compared with the participants without MetS.

Similarly to our finding concerning the Gln/Gly/Ser/Asn related pattern, Yamakado et al. (2015) have found that glutamine, glycine, serine, and asparagine form a cluster which has been negatively correlated with fat mass and insulin resistance [9]. Previous studies have speculated that these amino acids may also be linked to cardiometabolic risk via insulin-associated mechanisms [8]. For instance, gluconeogenesis which is increased in an insulin-resistant state, can drive glucose synthesis from both glycine and serine in hepatocytes thereby reduced circulating levels of both these amino acids [9]. In summary, the possible mechanisms underlying the observations of the present study, and in particular the association between the BCAA/AAA pattern and MetS include enhanced proteolysis in skeletal muscle tissue and reduced amino acid catabolism in adipose tissue underpinned by increased body mass and presence of insulin resistance among subjects with MetS.

Limitations and strengths

This was a cross-sectional study that does not allow conclusions regarding causality. Furthermore, sample size was relatively small, which limits the generalization of the findings. This study has also strengths. Biochemical analyses and metabolite profiling were based on fasting blood samples which limits confounding from postprandial associations and in the context of MetS research, the study measured a unique panel of metabolites. In addition, results of the present study were consistent with results from previously published studies. Finally, factor analysis yielded metabolite factors that are connected both statistically and biochemically, suggesting that these were not randomly extracted factors.

Conclusions

In the present study the MetS was characterised by two distinct amino acid profiles; the first, dominated by BCAA/AAA, was positively associated, and the second, an amino acid pattern/signature dominated by Gln/Gly/Ser/Asn, was inversely associated with established cardiometabolic risk factors and the MetS. These associations were not affected by plasma adiponectin levels or levels of liver aminotransferases. Our results suggest that both amino acid profiles are indicative of MetS and may serve as potential biomarkers in identifying subjects with MetS subjects. These results motivate further research in order to study mechanistic hypotheses and evaluate potential intervention strategies for tackling cardiometabolic risk.

Funding

The present study was financially supported by the Postgraduate Program of the Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens, and was partly funded by the Hellenic Atherosclerosis Society (HAS). The funders had no role in the design and analysis of the study or in the writing of this article.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to thank the cardiologists, general practitioners, nurses and dietitians who collaborated on this project, as well as the participants for their cooperation during the study. The authors would also like to thank Gry Kvalheim for laboratory facilities, scientific advice and guidance, and Ove Årseth and Saleh Rayyan for their technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2017.07.006.

References

[1] Franco OH, Massaro JM, Civil J, Cobain MR, O'Malley B, D'Agostino Sr RB. Trajectories of entering the metabolic syndrome: the Framingham heart study. Circulation 2009;120(20):1943–50.
[2] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120(16):1640–5.
[3] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112(17):2735–52.
[4] Dunn WB, Ellis DI. Metabolomics: current analytical platforms and methodologies. Trends Anal Chem 2005;24(4):285–94.
[5] Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9(4):311–26.
[6] Allam-Ndoul B, Guénard F, Garneau V, Cormier H, Barbier O, Pérusse L, et al. Association between metabolite profiles, metabolic syndrome and obesity status. Nutrients 2016;8(6):324.
[7] Batch BC, Shah SH, Newgard CB, Turer CB, Haynes C, Bain JR, et al. Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness. Metabolism 2013;62(7):961–9.

[8] Cheng S, Rhee EP, Larson MG, Lewis GD, McCabe EL, Shen D, et al. Metabolite profiling identifies pathways associated with metabolic risk in humans. Circulation 2012;125(18):2222–31.
[9] Yamakado M, Nagao K, Imaizumi A, Tani M, Toda A, Tanaka T, et al. Plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population. Sci Rep 2015;5:11918.
[10] Hajer GR, van der Graaf Y, Olijhoek JK, Verhaar MC, Visseren FLJ. SMART study group. Levels of homocysteine are increased in metabolic syndrome patients but are not associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome. Heart 2007;93(2):216–20.
[11] Esteghamati A, Hafezi-Nejad N, Zandieh A, Sheikhbahaei S, Ebadi M, Nakhjavani M. Homocysteine and metabolic syndrome: from clustering to additional utility in prediction of coronary heart disease. J Cardiol 2014;64(4):290–6.
[12] Wang X, Ye P, Cao R, Yang X, Xiao W, Zhang Y, et al. The association of homocysteine with metabolic syndrome in a community-dwelling population: homocysteine might be concomitant with metabolic syndrome. PLoS One 2014;9(11):e113148.
[13] Konstantinova SV, Tell GS, Vollset SE, Nygård O, Bleie Ø, Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. J Nutr 2008;138(5):914–20.
[14] Ho JE, Larson MG, Ghorbani A, Cheng S, Chen MH, Keyes M, et al. Metabolomic profiles of body mass index in the framingham heart study reveal distinct cardiometabolic phenotypes. PLoS One 2016;11(2):e0148361.
[15] Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 2016;39(5):833–46.
[16] Würtz P, Soininen P, Kangas AJ, Rönnemaa T, Lehtimäki T, Kähönen M, et al. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes Care 2013;36(3):648–55.
[17] Mook-Kanamori DO, Römisch-Margl W, Kastenmüller G, Prehn C, Petersen AK, Illig T, et al. Increased amino acids levels and the risk of developing of hypertriglyceridemia in a 7-year follow-up. J Endocrinol Investig 2014;37(4):369–74.
[18] Würtz P, Mäkinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes 2012;61(6):1372–80.
[19] Giesbertz P, Daniel H. Branched-chain amino acids as biomarkers in diabetes. Curr Opin Clin Nutr Metab Care 2016;19(1):48–54.
[20] Theofylaktopoulou D, Midttun Ø, Ulvik A, Ueland PM, Tell GS, Vollset SE, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland health study. Clin Exp Immunol 2013;173(1):121–30.
[21] Mangge H, Summers KL, Meinitzer A, Zelzer S, Almer G, Prassl R, et al. Obesity-related dysregulation of the tryptophan–kynurenine metabolism: role of age and parameters of the metabolic syndrome. Obesity (Silver Spring) 2014;22(1):195–201.
[22] Ntzouvani A, Fragopoulou E, Panagiotakos D, Pitsavos C, Antonopoulou S. Reduced circulating adiponectin levels are associated with the metabolic syndrome independently of obesity, lipid indices and serum insulin levels: a cross-sectional study. Lipids Health Dis 2016;15(1):140.
[23] Papathanasiou G, Georgoudis G, Papandreou M, Spyropoulos P, Georgakopoulos D, Kalfakakou V, et al. Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. Hell J Cardiol 2009;50(4):283–94.
[24] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.
[25] Midttun Ø, McCann A, Aarseth O, Krokeide M, Kvalheim G, Meyer K, et al. Combined measurement of 6 fat-soluble vitamins and 26 water-soluble functional vitamin markers and amino acids in 50 μL of serum or plasma by high-throughput mass spectrometry. Anal Chem 2016;88(21):10427–36.
[26] Midttun Ø, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal Bioanal Chem 2013;405(6):2009–17.
[27] Wiklund PK, Pekkala S, Autio R, Munukka E, Xu L, Saltevo J, et al. Serum metabolic profiles in overweight and obese women with and without metabolic syndrome. Diabetol Metab Syndr 2014;6(1):40.
[28] Yamada C, Kondo M, Kishimoto N, Shibata T, Nagai Y, Imanishi T, et al. Association between insulin resistance and plasma amino acid profile in non-diabetic Japanese subjects. J Diabetes Investig 2015;6(4):408–15.
[29] Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 2009;32(9):1678–83.
[30] Cheng S, Wiklund P, Autio R, Borra R, Ojanen X, Xu L, et al. Adipose tissue dysfunction and altered systemic amino acid metabolism are associated with non-alcoholic fatty liver disease. PLoS One 2015;10(10):e0138889.
[31] Nakamura H, Jinzu H, Nagao K, Noguchi Y, Shimba N, Miyano H, et al. Plasma amino acid profiles are associated with insulin, C-peptide and adiponectin levels in type 2 diabetic patients. Nutr Diabetes 2014;4:e133.
[32] Combs TP, Marliss EB, Lamarche M, Chevalier S. Adiponectin is an independent predictor of circulating branched-chain amino acids in men. Can J Diabetes 2016;40(5):S55–6.
[33] Kunutsor SK, Apekey TA, Seddoh D. Gamma glutamyltransferase and metabolic syndrome risk: a systematic review and dose–response meta-analysis. Int J Clin Pract 2015;69(1):136–44.
[34] Cynober LA. Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. Nutrition 2002;18(9):761–6.
[35] Wang X, Hu Z, Hu J, Du J, Mitch WE. Insulin resistance accelerates muscle protein degradation: activation of the ubiquitin-proteasome pathway by defects in muscle cell signaling. Endocrinology 2006;147(9):4160–8.
[36] Badoud F, Lam KP, DiBattista A, Perreault M, Zulyniak MA, Cattrysse B, et al. Serum and adipose tissue amino acid homeostasis in the metabolically healthy obese. J Proteome Res 2014;13(7):3455–66.