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CHANGES OF PLASMA FREE AMINO ACIDS IN METABOLIC SYNDROME

Alma Nurtazina[1], https://orcid.org/0000-0002-9737-4003

Ivan Voitsekhovskiy[2], https://orcid.org/0000-0001-6920-2776

Maxat Toishimanov[3], https://orcid.org/0000-0002-6070-4574

Daulet Dautov[4], https://orcid.org/0000-0003-0838-6983

[1] NJSC «Semey medical university», Semey, Republic of Kazakhstan;
[2] Al Farabi Kazakh National University, Almaty, Republic of Kazakhstan;
[3] The Kazakhstan-Japan Innovation Center KazNARU, Almaty, Republic of Kazakhstan;
[4] NJSC "Asfendiyarov Kazakh National Medical University", Almaty, Republic of Kazakhstan.

Abstract

Background: Studies have been going on for a while now to investigate the link between plasma free amino acids (PFAA) and metabolic syndrome, and the results so far have been quite promising. However, these analyses aren't commonly used for early clinical diagnosis of metabolic syndrome, partly because mass spectrometry can be pretty expensive. That's why we decided to look into and characterize the PFAA profile of blood plasma in metabolic syndrome patients using a more affordable alternative to MS\MS or GC-MS methods: high performance liquid chromatography (HPLC).

Objective: How can we describe the changes in the levels of free amino acids found in the bloodstream of individuals diagnosed with metabolic syndrome using high-performance liquid chromatography (HPLC)?

Materials and methods: We sampled thirty patients who met the diagnostic criteria for metabolic syndrome from their plasma for PFAA determination using HPLC. Additionally, we took plasma samples from fifteen confirmed healthy individuals and analyzed them using HPLC. We measured the concentrations in the samples and analyzed the relationship between the profiles of free amino acids in the plasma.

Results and discussion: The results show a significant difference in plasma concentrations of alanine, cysteine, tyrosine, valine, cystine and isoleucine between the two groups. The HPLC method, when utilized under specific conditions, can be employed for the quantitative measurement of amino acids in the blood.

Keywords: *metabolic syndrome, amino acids, BCAAs, AAAs, HPLC.*

Резюме

ИЗМЕНЕНИЕ УРОВНЯ СВОБОДНЫХ АМИНОКИСЛОТ ПЛАЗМЫ КРОВИ ПРИ МЕТАБОЛИЧЕСКОМ СИНДРОМЕ

Алма Нуртазина[1], https://orcid.org/0000-0002-9737-4003

Иван Войцеховский[2], https://orcid.org/0000-0001-6920-2776

Максат Тойшиманов[3], https://orcid.org/0000-0002-6070-4574

Даулет Даутов[4], https://orcid.org/0000-0003-0838-6983

[1] НАО «Медицинский университет Семей», г. Семей, Республика Казакстан;
[2] Казахский национальный университет им. Аль-Фараби, г. Алматы, Республика Казахстан;
[3] Казахстанско-Японский инновационный центр КазНАИУ, г. Алматы, Республика Казахстан;
[4] НАО «Казахский Национальный медицинский университет им. С.Ж. Асфендиярова», г. Алматы, Республика Казахстан.

Актуальность: исследования связи свободных аминокислот плазмы (PFAA) с метаболическим синдромом продолжаются уже давно и дали хорошие текущие результаты. Однако эти анализы не используются для ранней клинической диагностики метаболического синдрома, в том числе из-за относительно высокой стоимости масс-спектроскопии. Поэтому мы стремились изучить и охарактеризовать профиль PFAA плазмы крови у пациентов с метаболическим синдромом с использованием метода высокоэффективной жидкостной хроматографии (ВЭЖХ) как более дешевой альтернативы методам МС/МС (тандемной масс спектрометрии) или ГХ-МС (газовой хроматографии масс спектрометрии).

Цель: определить колебания уровня свободных аминокислот в плазме крови больных метаболическим синдромом методом ВЭЖХ.

Материалы и методы. У тридцати пациентов, соответствовавших критериям метаболического синдрома, были взяты образцы плазмы для определения PFAA с помощью ВЭЖХ. Также образцы плазмы проанализированы с помощью ВЭЖХ у пятнадцати здоровых. Был применен простой рандомный отбор участников исследования. Измеряли концентрации в образцах, а также взаимосвязь между профилями свободных аминокислот в плазме.

Результаты и обсуждение. Результаты выявили статистически значимую разницу в концентрациях аланина, цистеина, тирозина, валина, цистина и изолейцина в плазме между двумя группами.

Отклонения от средних значений концентраций аминокислот могут быть индикаторами метаболических процессов, связанных с развитием МС. Метод ВЭЖХ при определенных условиях может применяться для количественного определения аминокислот в крови.

Ключевые слова: *метаболический синдром, аминокислоты, BCAA, AAA, ВЭЖХ.*

Түйіндеме

МЕТАБОЛИЗМДІК СИНДРОМ ЖАҒДАЙЫНДАҒЫ ҚАН ПЛАЗМАСЫ БОС АМИН ҚЫШҚЫЛДАРЫ ДЕҢГЕЙЛЕРІНІҢ ӨЗГЕРІСТЕРІ

Алма Нуртазина[1], https://orcid.org/0000-0002-9737-4003

Иван Войцеховский[2], https://orcid.org/0000-0001-6920-2776

Максат Тойшиманов[3], https://orcid.org/0000-0002-6070-4574

Даулет Даутов[4], https://orcid.org/0000-0003-0838-6983

[1] «Семей Медицина Университеті» КеАҚ, Семей қ., Қазақстан Республикасы;
[2] «Әл-Фараби атындағы Қазақ Ұлттық университет» КЕАҚ, Алматы қ., Қазақстан Республикасы;
[3] ҚазҰАЗУ Қазақстан-Жапон инновациялық орталығы, Алматы қ., Қазақстан Республикасы;
[4] «С.Ж. Асфендияров атындағы Қазақ ұлттық медицина университеті», Алматы қ., Қазақстан Республикасы.

Өзектілігі: қан плазмасы бос аминқышқылдарының (PFAA) метаболизм синдромымен байланысын зерттеу ұзақ уақыт бойы жақсы нәтижелермен жалғасуда. Алайда, бұл талдаулар метаболизм синдромының ерте клиникалық диагностикасы үшін пайдаланылмайды, сонымен қатар масс-спектроскопияның салыстырмалы түрде жоғары құнына байланысты. Сондықтан, біз МС/МС немесе ГХ-МС арзан балама ретінде жоғары нәтижелі сұйық хроматографияны (HPLC) пайдалана отырып, метаболизм синдромы бар науқастарда плазмалық PFAA профилін зерттеуге және сипаттауға тырыстық.

Мақсаты: метаболизм синдромы бар науқастардың қан плазмасындағы бос аминқышқылдары деңгейінің ауытқуын HPLC әдісімен анықтау.

Материалдар мен тәсілдер. Метаболизм синдромының диагностикалық критерийлеріне сәйкес келетін расталған 30 пациентте ВЭЖХ әдісімен PFAA анықтау үшін плазма үлгілері алынды. Сонымен қатар, он бес сау адамнан плазма үлгілері алынды және ВЭЖХ арқылы талданды. Үлгілердегі концентрациясы, сондай-ақ плазмадағы бос аминқышқылдарының профильдері арасындағы байланыс өлшенді.

Нәтижелер мен талқылау. Нәтижелер екі топ арасындағы аланин, цистеин, тирозин, валин, цистин және изолейциннің плазмадағы концентрацияларында айтарлықтай айырмашылықты көрсетеді.

Амин қышқылы концентрациясының орташа мәндерінен ауытқулар МС дамуымен байланысты метаболизм процестерінің көрсеткіштері болуы мүмкін. ВЭЖХ әдісі белгілі бір жағдайларда қандағы амин қышқылдарын сандық анықтау үшін қолданылуы мүмкін.

Түйінді сөздер: *метаболизм синдромы, аминқышқылдары, BCAA, AAA, ВЭЖХ.*

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Background

With advances in combatting communicable diseases, noncommunicable diseases (NCDs) have emerged as the leading cause of morbidity and mortality worldwide. The World Health Organization (WHO) defines metabolic syndrome (MetS) as a pathological condition characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia. [1]. According to the 2015 Global Obesity Survey, obesity rates have doubled in 73 countries since 1980 and increased in most other countries, with 604 million adults and 108 million children in 195 countries affected by obesity. [2]. MetS clusters risk factors that precede the onset of type 2 diabetes (T2D) and cardiovascular disease (CVD), metabolic associated fatty liver disease (MAFLD) [3,4].

While MetS symptoms such as obesity, hypertension, and T2D can vary among ethnic groups and between urban and rural populations, current scales and criteria used to diagnose MetS are based on traditional lipid profile indicators such as triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C). However, these lipid biomarkers are not sufficiently accurate for measuring dyslipidemia. [4].

Individual amino acids' quantitative representation in the blood can reveal significant shifts in crucial metabolic processes. Branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine are essential amino acids, and elevated BCAA levels predict the development of insulin resistance. [5]. These data suggest that high BCAA concentrations could negatively impact circulating blood cells, contributing to the pro-inflammatory and oxidative status observed in many pathophysiological conditions. [6]. Elevated serum BCAAs and tyrosine, phenylalanine and tryptophan - the three aromatic amino acids (AAAs) - are not only associated with insulin resistance (IR), but also closely related with lipid metabolism. Recent animal research corroborates this notion, revealing that oral BCAA administration increased lipogenic gene expression and triacylglycerol synthesis in the liver. [7].

2. Materials and methods

2.1 Ethics statement

The study protocol was approved by the Local Ethical Commission of Semey Medical University. Written informed consent were obtained from all study participants.

2.2 Study subjects

Forty-five participants were randomly recruited from Almaty city and its surroundings. Thirty subjects with MetS (24 women and 6 men) were randomly selected at one of outpatient clinics in Almaty city [8].

Participants in the control group (nine women and six men) were those who did not met the criteria of MetS.

2.3 Blood samples

Venous blood samples for biochemical analyzes were taken in standardized fasting conditions in the mornings of 9 between 7 am and 10 am. Blood was collected in EDTA K3 vacutainer tubes, plasma was separated out from whole blood and stored at -70°C within 4 hours of collection until analyzed. The tubes were kept there until (within 2 weeks to 2 months) the desired analysis for plasma amino acids.

2.4 Sample Preparation

Plasma samples (300 µl) thawed were prepared by adding volume 200 µl acetonitrile and volume 100 µl mix solution phenyl isothiocyanate (PITC:propanol, 1:9, vol/vol). Plasma proteins were precipitated by the addition of PITC. After protein precipitation, the samples were shaken and centrifuged at 14000×g for 10 min at a temperature of 4 ◦C. After the samples were centrifuged, 300 µl of the supernatant was taken into vials for HPLC analysis.

2.5. Chromatographic system

The HPLC system consisted of a Shimadzu LC-20AD Prominence HPLC Pump dual piston Column oven CTO-30A and SPD-20A / 20AV Offering dual-wavelength mode UV-VIS Detector. The HPLC separation of the derivatized amino acids required two mobile phases. Mobile phase A consists of 99% HPLC grade acetonitrile and 1% acetic acid, mobile phase B consists of 99,9% HPLC grade water, 0,1% acetic acid and 0,1 *mol* sodium acetate. All buffers were filtered through a 0.2-µm filter and degassed.

The chromatographic separation was performed using a Shimadzu Prominence LC-20 system (Shimadzu, Japan) equipped with a UV detector (SPD-20A) and a fluorescent detector (RF-10AXL). The HPLC system was equipped with a binary pump (LC-20AD), an autosampler (SIL-20AC), a degasser (DGU-20A5) and a column oven (CTO-20A) controlled by LCSolution.

Samples were separated on a Thermo Hypersil GOLD C18 HPLC column (150 mm × 4 mm, 5 µm).

The UV detection was performed at 254nm. The flow rate of the mobile phase was 0,8 ml/min. The total HPLC run time for the separation of the derivatized amino acids in a single sample or standard is 43 min.

The method of amino acid measurement in body fluids and plants was previously developed at the Food and environmental safety laboratory of the Kazakhstan-Japan Innovation Center KazNARU.

2.6 Calculations and statistics

The two groups being compared in the analysis had an equal number of observations and exhibited a significant difference overall. To determine whether the means of the two sets of amino acid data were statistically distinct, the Mann-Whitney test for two independent samples was employed. The agglomerative hierarchical clustering technique of Hierarchical Clustering Ward Method was utilized to identify the best pair of clusters to merge at each step, based on an optimal value of an objective function. The calculations were conducted using IBM SPSS version 23.0 and JMP Statistical Discovery LLC.'s Windows version 70 software (www.jmp.com).

3. Results and discussion

Figure 1 depicts the successful separation of 18 amino acids (aspartic acid, glutamic acid, serine, asparagine, histidine, arginine, threonine, alanine, proline, cysteine, tyrosine, valine, methionine, cystine, isoleucine, leucine, phenylalanine, and lysine) using a chromatographic method on a plasma sample. The chromatographic separation was found to be of good quality. The results show significant difference between the two groups in plasma concentrations of alanine, cysteine, tyrosine, valine, cystine and isoleucine. (Table 1).

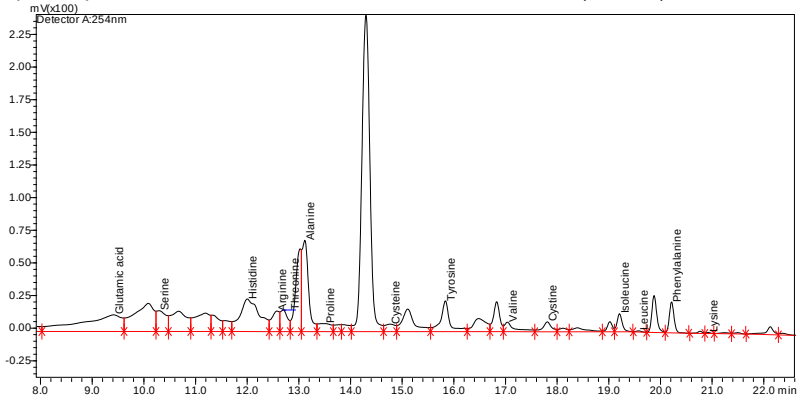


Figure 1. Chromatographic separation of a plasma sample.

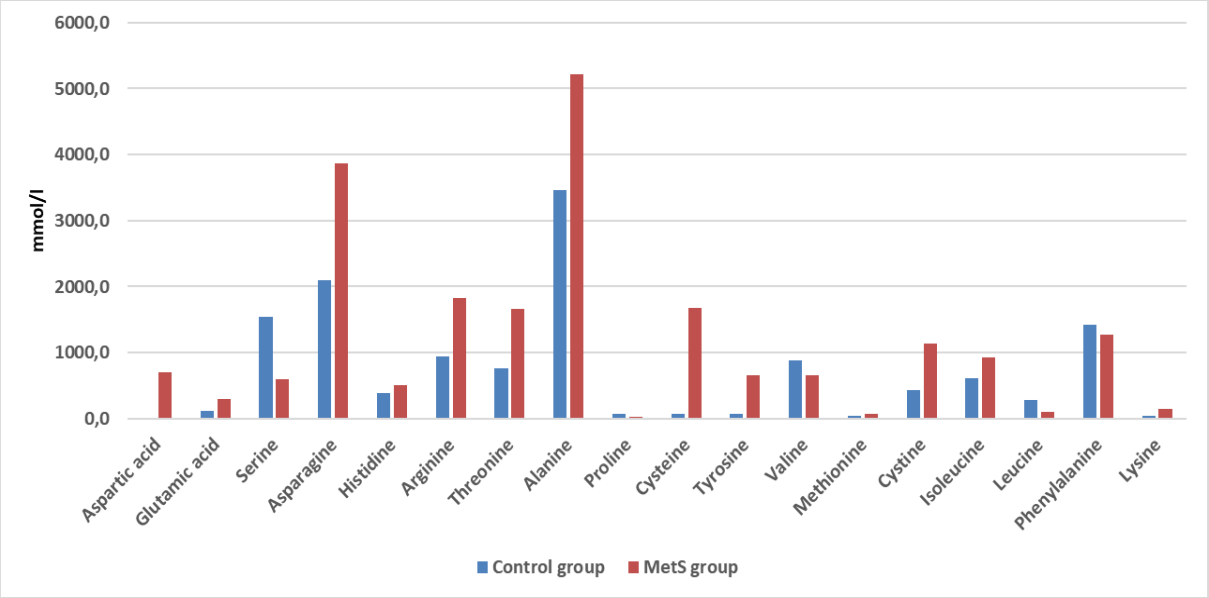


Figure 2. Plasma concentrations of identified amino acids.

Table 1.

Differences of amino acids plasma concentrations between patients with MetS and apparently healthy subjects.

	Asp	His	Arg	Thr	Ala	Pro	Cys	Tyr	Val	Cyst	Ile	leu	Phe	Lys
Total N	28	38	44	44	47	28	44	46	47	47	47	47	47	47
Mann-Whitney U	55.00	170.00	225.00	200.00	148.00	60.00	48.000	70.00	403.00	78.00	135.000	256.00	294.00	158.00
Test Statistic	55.00	170.00	225.00	200.00	148.00	60.00	48.000	70.00	403.00	78.00	135.500	256.00	294.000	158.00
Standard Error	18.84	30.16	38.87	38.87	43.81	21.76	40.389	41.88	43.81	43.81	43.818	43.81	43.81	43.81
Exact Sig. (2-sided test)	.348	.334	.54	.96	.036	.085	.000	.0001	.0001	.0001	.017	.071	.21	.061

New research indicates that alterations in plasma amino acid levels, specifically branched chain amino acids and aromatic amino acids, are linked to insulin resistance, visceral obesity, and the likelihood of developing cardiovascular disease and diabetes in the future. The Plasma Free Amino Acid (PFAA) index, which is linked to visceral fat obesity, has shown a relatively strong correlation with various variables [9]. Several studies have also demonstrated that the plasma concentrations of certain amino acids, including isoleucine, glutamic acid, aspartic acid, alanine, histidine, methionine, and asparagine, were significantly higher in individuals with MetS [10].

Furthermore, an increase in the levels of Branched-Chain Amino Acids (BCAAs) and specific Aromatic Amino Acids (AAAs), such as alanine, in the plasma, is associated with insulin resistance (IR) in Type 2 Diabetes (T2D) [11].

Elevated levels of alanine were found in patients with MAFLD, and elevated plasma concentrations of asparagine and alanine were found in patients diagnosed with MAFLD and T2D. Obese patients were found to have elevated levels of alanine [12], and diabetic patients have lower plasma concentrations of serine than non-diabetic individuals, as supported by other studies [13].

People characterized as late chronotype have elevated type 2 diabetes and cardiovascular disease risk compared to early chronotype. Plasma tricarboxylic acid cycle (TCA) intermediates - some AA (proline, isoleucine) were lower in early chronotype, other AA (threonine, histidine, arginine) were higher compared with late chronotype [14].

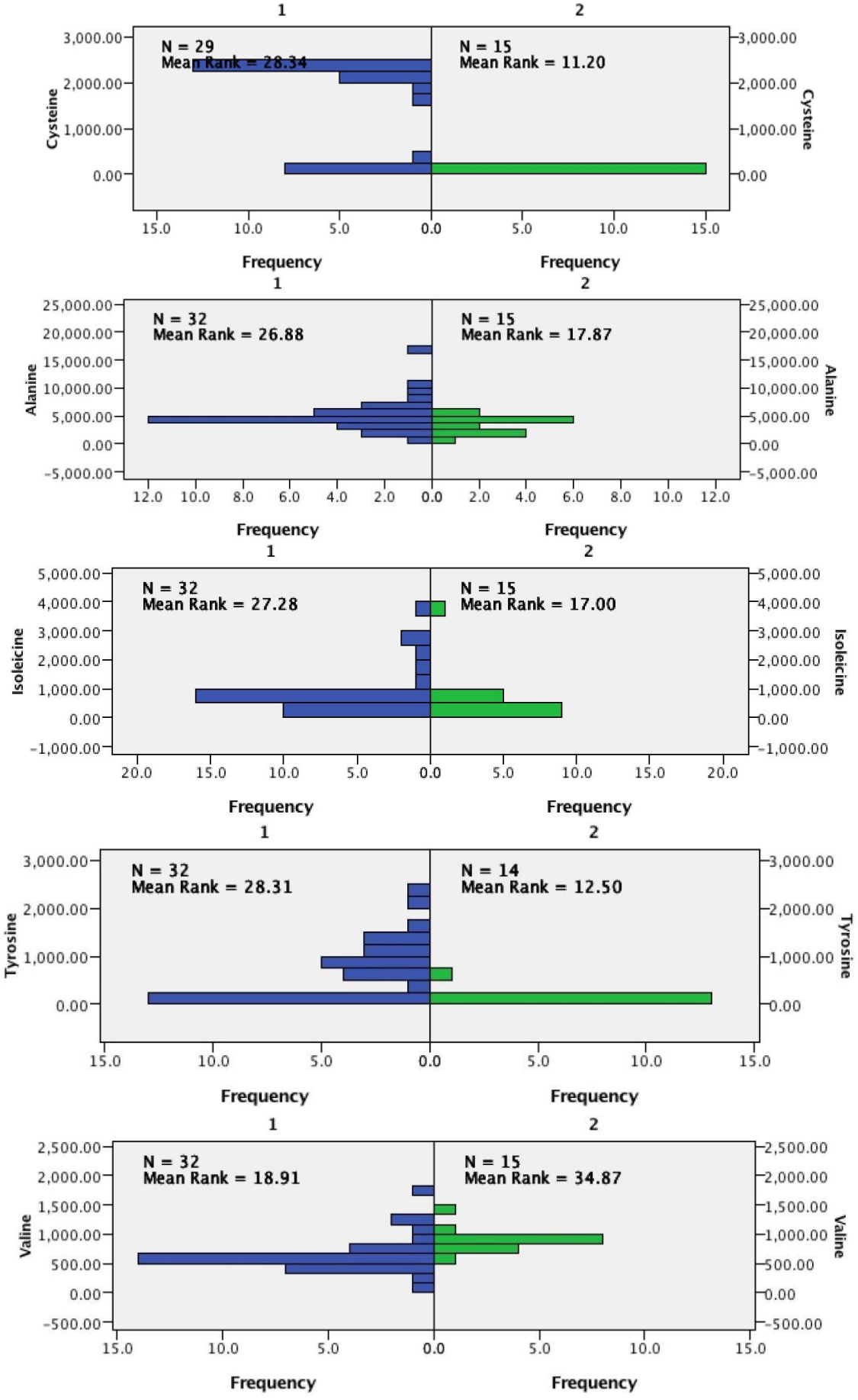
Significant disturbances in amino acid metabolism, the tricarboxylic acid cycle, and glycerol and phospholipid metabolism may affect overall glucose homeostasis in T2D x [15]. Valine, leucine and isoleucine degradation, and tryptophan metabolism) were associated with the presence of metabolic syndrome [16].

BCAAs are found to be closely associated with Insulin resistance. Reduction in leucine/isoleucine along with glycerol during oral glucose tolerance test represents the strongest predictor of insulin sensitivity and the decrease of plasma BCAA levels is blunted in insulin-resistant subjects during the oral glucose tolerance test [17].

In summary, elevated plasma concentrations amino acids are associated with general changes in the metabolic background and many processes that regulate amino acid metabolism. Deviations from the mean values of amino acid concentrations can be indicators of metabolic processes associated with the development of MetS. BCAAs and AAAs of the 19 amino acids showed greatest association with obesity and lipid variables.

Further large-scale studies are needed to clarify the rationale of our suggestion as well as addressing cost-effective analysis of HPLC in the measurement of plasma free amino acids.

Fig. 3 Difference in values of amino acids between patients with MetS (1) and apparently healthy subjects (2).



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Declaration of conflicting interests:

The authors declare that there is no conflict of interest in the present study.

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Author contribution statement:

All authors have contributed equally.

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Контактная информация:

Войцеховский Иван Владимирович – докторант PhD, Казахский национальный университет им. Аль-Фараби, Факультет Биологии и Биотехнологии, г. Алматы, Республика Казахстан

Почтовый индекс: 050032, г. Алматы, Медеуский район, мкрн. Алатау,31

Email: ivan.voitsekhovskiy@gmail.com

Телефон: 8 700 262 69 53