ISSN 2304-3415, Russian Open Medical Journal
2024. Volume 13. Issue 3 (September). Article CID e0304
DOI: 10.15275/rusomj.2024.0304

Original article

Threonine in different phenotypes of chronic heart failure with preserved ejection fraction

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Received 2 February 2024, Revised 15 April 2024, Accepted 1 July 2024

Abstract: *Background* — Chronic heart failure with preserved ejection fraction (CHFpEF) develops as a result of many diseases that lead to significant metabolic disorders. Given the heterogeneity of this group of patients, therapeutic options for this syndrome are extremely limited. In this regard, it seems promising to study the metabolomic profile in patients with CHFpEF to identify biomarkers, examine their roles in the pathogenesis of the syndrome, and search for potential targets for targeted therapy.
Objective — The study aimed at testing the correlation between the threonine level and the features of the clinical course of CHFpEF.
Methods — The study included a total of 154 patients: 82 with CHFpEF, 45 with hypertension and/or coronary artery disease (comparison group), and 27 healthy volunteers (control group). Threonine levels were assessed using high-performance liquid chromatography-mass spectrometry.
Results — The threonine concentration was significantly reduced in patients with CHFpEF (1) vs. comparison group (2) and control group (3): $p<0.001$; $p_{1-3}<0.001$; $p_{2-3}=0.037$). A reduction in the threonine level was characteristic for patients with diabetes mellitus vs. patients without it ($p=0.029$).
Conclusion — Given the importance of threonine in energy metabolism and significant changes in its level in various pathophysiological processes, it should be considered as an additional diagnostic and prognostic criterion for CHFpEF. Additional studies are needed to better understand the role of threonine in the pathophysiology of cardiovascular diseases.

Keywords: threonine, metabolomics, arterial hypertension, chronic heart failure, diabetes mellitus.

Cite as Petruhnova MF, Krivova AV, Zektser VYu, Korobkova EO, Edniev TN, Ageev AA, Shestakova KM, Moskaleva NE, Appolonova SA, Belenkov YuN, Kozhevnikova MV. Threonine in different phenotypes of chronic heart failure with preserved ejection fraction. *Russian Open Medical Journal* 2024; 13: e0304.

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Introduction

Chronic heart failure with preserved ejection fraction (CHFpEF) is among the vital priorities in contemporary cardiology. The prevalence of this syndrome is steadily increasing: as many as half of patients with chronic heart failure are also characterized by a preserved ejection fraction [1-2]. Patients with CHFpEF are polymorbid due to the fact that the development of this phenotype is caused by such diseases as hypertension (HTN), diabetes mellitus (DM) and obesity; dyslipidemia also plays an important role in the pathogenesis of this syndrome [3-4]. The heterogeneity of the patient population poses significant challenges for the development of treatment methods, and the effectiveness of individual medicines may vary in subgroups of patients with CHFpEF. The possibility of identifying therapeutic approaches based on phenotype is discussed. The combination of CHFpEF and type 2 DM is identified as a separate phenotype [5-7].

Pathophysiological processes in carbohydrate metabolism disorders affect all levels of metabolic processes and lead to various changes in metabolism [8]. Therefore, amino acids involved in energy metabolism are considered as potential biomarkers. It has been established that a number of diseases are accompanied by changes in their concentrations, which may have diagnostic value. For example, in a study by H. Xu et al., a link was found between elevated levels of branched-chain amino acids (valine, leucine) and the risk of developing acute cerebrovascular accidents [9]. Currently, the contribution of other amino acids to the development of cardiovascular diseases (CVD) is being actively studied [10].

One of the essential amino acids in human body is threonine. It is involved in many biological processes. E.g., glycine and serine, which are the most important components of collagen and elastin, are synthesized on the basis of this amino acid. Threonine is also required for the normal functioning of the immune system, since it is involved in the formation of immunoglobulins. This amino acid plays a key role in neurotransmission. It is a glycogenic amino acid that improves energy metabolism in muscle tissue. Due to the fact that threonine is involved in purine metabolism, excess concentration of this metabolite leads to an increase in the concentration of uric acid as one of the products of the final metabolism of purine compounds. Given the importance of threonine in metabolic processes, scientific studies have been

conducted to investigate its role in the development and progression of CVD. However, controversial results were obtained. In their study, L.F. Ferreira-Divino et al. revealed that higher threonine levels were associated with a lower risk of developing any cardiovascular events [11]. An Iranian study tested the relationship between the metabolomic profile and the 10-year risk of primary atherosclerotic CVD. The study established that higher threonine levels were associated with a lower risk of developing atherosclerosis over 10 years [12]. Some studies have also examined the relationship between threonine levels and lipid spectrum parameters. For instance, a study conducted in Japan discovered that plasma threonine levels were higher in people without dyslipidemia than in people with dyslipidemia [13]. Similar results were obtained by other researchers, viz.: a negative correlation was noted between threonine concentration and triglyceride (TG) levels; threonine was also associated with a reduced risk of developing the atherogenic lipid triad [14-15]. However, the results of an Italian study demonstrated the opposite: the researchers found that individuals with high levels of high-density lipoproteins (HDL) in blood plasma were characterized by low threonine level, while patients with high TG level had elevated threonine concentration [16]. Also, an increased level of threonine after six months of observation was observed in patients who had suffered acute coronary syndrome, compared with the control group [17].

Given the relationship between the progression of atherosclerosis and type 2 DM, studies were conducted to examine changes in the levels of metabolites in this cohort of patients. According to X. Liu et al., in patients with DM and coronary artery disease (CAD) as one of the causes of heart failure, threonine metabolism was impaired: the authors observed a decrease in its concentration [18]. According to other Chinese researchers, low concentrations of amino acids (including threonine) were also associated with an increased risk of HTN [19]. In addition, a study by W.L. du Toit showed that the threonine level negatively correlated with central (aortic) systolic blood pressure and pulse wave velocity [20]. It should be noted that HTN is one of the most important diseases, the progression of which leads to the development of CHFpEF.

Thus, threonine metabolism undergoes significant changes in diseases that potentially lead to the development of CHFpEF. Given the ambiguity of the results of previous studies, the objective of this study was to test the presence of a correlation between the level of threonine and the features of the clinical course of CHFpEF.

Material and Methods

This single-stage observational study was approved by the Ethics Committee of Sechenov University and conducted in accordance with the Declaration of Helsinki. The study participants were informed about the study goal and methodology, and written informed consent was obtained from each prospective study subject before enrollment.

Patient selection

The inclusion criterion for the main group was the presence of CHFpEF diagnosed in accordance with existing clinical guidelines [21]. The comparison group included patients with HTN and CAD according to the current Russian guidelines [22, 23]. The study excluded patients with cardiomyopathy, congenital and acquired heart defects, acute myocarditis or pericarditis, stroke within the previous six months, acute or chronic renal failure, severe liver dysfunction, bronchial asthma, chronic obstructive pulmonary disease, exacerbation of gastrointestinal diseases, malignant neoplasms, thyroid disorders and autoimmune diseases. In the control group, we did not enroll patients with any CVD or disease from the list of exclusion criteria.

Our study included 154 participants distributed among Group 1 (control group with 27 healthy volunteers without signs of CVD), Group 2 (comparison group with 45 patients with HTN and/or CAD), and Group 3 (main group with 82 patients with CHFpEF).

Methods of laboratory and instrumental research

The concentration of the N-terminal fragment of the precursor of brain natriuretic peptide (NT-proBNP) in the blood serum was measured using the NT-proBNP enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp., USA). Echocardiographic parameters were assessed using two-dimensional echocardiography in M-mode and B-mode, pulsed-wave and continuous-wave Doppler ultrasound in the supine position using a Vivid 7 Dimension/Vivid 7 PRO echocardiograph version 6.0.x (GE, Germany).

Collection of biological material and sample preparation

All study participants underwent additional venous blood sampling first thing in the morning, strictly on an empty stomach. Blood samples were placed in test tubes containing the ethylenediaminetetraacetic acid dipotassium salt dihydrate. The samples were then centrifuged at 2,000 rpm for 20 min, and the resulting blood plasma was stored at -80 °C until metabolomic analysis. A total of 84 metabolites belonging to amino acid classes were identified via targeted metabolomic profiling using a Waters Acquity UPLC high-performance liquid chromatography system coupled with a high-resolution mass spectrometer (TSQ, Xevo TQ-S micro, Waters, USA) including acylcarnitines, metabolites of nitrogen and tryptophan metabolism, neurotransmitters. However, solely the essential amino acid *threonine* was assessed in this study.

Statistical analyses

After analyzing the metabolomic profile, correlations between clinical, structural and functional characteristics of the heart and threonine levels were examined. Statistical data processing was performed using Statistica 10.0 (StatSoft, Inc., USA) and StatTech v. 3.0.5 (Stattech LLC, Russia) software. Quantitative variables were assessed for compliance with normal distribution using the Shapiro-Wilk test (for fewer than 50 study subjects) or the Kolmogorov-Smirnov test (for more than 50 study subjects). In case of non-normal distribution of a quantitative indicator, the data were described using the median (Me) and the lower and upper quartiles (Q1 – Q3). Comparison of three or more groups by a quantitative variable the distribution of which differed from normal was performed using the Kruskal-Wallis test. Post hoc comparisons were performed using the Dunn's test with Holm adjustment. Pearson's chi-squared test was employed to compare categorical variables between three independent groups.

Table 1. Demographic and clinical characteristics of study participants

Characteristic	Group 1 (n=27)	Group 2 (n=45)	Group 3 (n=82)	p
	n (%) or Me [Q1-Q3]			
Gender (male)	9 (33.3)	24 (53.3)	31 (37.8)	0.150
Age, years	27 [26-37]	66 [56-72]	70 [64-74]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}=0.03
BMI, kg/m^2	21.6 [20.3-24.0]	29.3 [25.7-31.8]	32.4 [27.6-36.0]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}=0.02
Obesity	1 (3.7)	21 (46.7)	49 (59.8)	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
Smoking	4 (14.8)	16 (35.6)	12 (14.6)	0.015* p_{2-3}=0.02
Grade of hypertension 1 2 3	–	2 (4.4) 9 (20.0) 33 (73.3)	0 9 (11.0) 73 (89.0)	p_{2-3}=0.046*
GI or type 2 DM	0	21 (46.7)	43 (52.4)	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
Dyslipidemia	12 (44.4)	43 (95.6)	76 (92.7)	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
Atherosclerosis of BCA	0	23 (51.1)	17 (20.7)	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
Atrial fibrillation	0	12 (26.7)	42 (51.2)	<0.001* p_{1-2}=0.007 p_{1-3}<0.001 p_{2-3}=0.007
Stroke	0	4 (8.9)	17 (20.7)	0.013* p_{1-3}=0.030
PICS	0	10 (22.2)	27 (32.9)	0.002* p_{1-2}=0.02 p_{1-3}=0.002
CRF	0	3 degree – 10 (22.2) 4 degree – 2 (4.4)	3 degree – 47 (57.3) 4 degree – 1 (1.2)	<0.001* p_{1-2}=0.002 p_{1-3}<0.001 p_{2-3}=0.002

Group 1, control group (healthy volunteers); Group 2, comparison group (26 patients with HTN and 19 patients with CAD); Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45); BMI, body mass index; GI, glucose intolerance; DM, diabetes mellitus; BCA, brachiocephalic arteries; PICS, post-intensive care syndrome; CRF, chronic renal failure.

Results

Demographic, clinical, laboratory and instrumental characteristics of the groups

In our study, all three groups were similar in terms of gender, but differed in age and body mass index (BMI), viz.: healthy volunteers of the control group were significantly younger than patients with CVD and had normal body weight (*Table 1*). The CHFpEF group was mainly represented by elderly patients with dyslipidemia, 50% of the cohort had obesity, type 2 DM, atrial fibrillation and chronic renal failure (*Table 1*).

All patients with heart failure complained of shortness of breath during exercise or at rest, which significantly distinguished the main group from patients in the comparison group (53.3%, p<0.001: p_{1-3}<0.001; p_{2-3}<0.001), as well as from the control group, in which there were no complaints. Complaints of heart arrhythmia were observed in 43.9% of patients with heart failure and in 57.8% of patients with HTN or CAD (p_{2-3}>0.05). Swelling or pitting edema of the lower limbs were detected in over 50% of subjects in the main group and in 11.1% of patients in the comparison group (p_{2-3}<0.001).

In 78% of cases, stage 2a was diagnosed in patients with CHFpEF, and stage 2b was diagnosed in the remaining 22% of patients. The distribution by functional classes *sensu* NYHA was as follows: Class II, 35 (42.7%); Class III, 29 (35.4%); Class IV, 18 (22%).

The median NT-proBNP level in patients with CHFpEF was 970 [328-2722] pg/ml. The presence of signs of congestion in the pulmonary circulation confirmed by chest X-ray was diagnosed in 25 patients with CHFpEF (31.6%), which reliably distinguished the main group from patients with HTN or CAD (p_{2-3}=0.001).

The laboratory and instrumental characteristics of the patients are presented in *Tables 2* and 3. At the time of inclusion in the study, patients with CVD received pharmacotherapy in accordance with the regimen presented in *Table 4*. It should be noted that at the time of blood sampling for metabolomic profiling, two-thirds of patients with CHFpEF regularly took diuretics.

Table 2. Laboratory and instrumental characteristics of study participants

Parameter	Group 1 (n=27)	Group 2 (n=45)	Group 3 (n=82)	p
	n (%) or Me [Q1-Q3]			
SBP, mm Hg	120 [112-120]	140 [125-150]	130 [120-145]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
DBP, mm Hg	75 [70-80]	80 [80-90]	80 [70-90]	<0.001* p_{1-2}<0.001 p_{1-3}=0.03 p_{2-3}=0.008
PAP, mm Hg	40 [40-46]	50 [40-60]	50 [40-60]	<0.001* p_{1-2}=0.003 p_{1-3}=0.002
Blood plasma glucose, mmol/l	4.9 [4.7-5.3]	5.7 [5.2-6.6]	5.9 [5.2-7.2]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
Glycated hemoglobin, %	–	7 [7-10]	6 [6-6]	0.03*
Total cholesterol, mmol/l	5.2 [4.8-5.8]	5.0 [4.2-5.7]	4.7 [3.5-5.9]	0.06
LDL, mmol/l	3.0 [2.7-3.6]	3.2 [2.6-3.7]	3.0 [1.9-4.6]	0.5
Triglycerides, mmol/l	0.9 [0.7-1.2]	1.3 [1.0-2.1]	1.5 [1.1-2.1]	0.001* p_{1-2}=0.004 p_{1-3}=0.001
HDL, mmol/l	1.6 [1.2-1.8]	1.3 [1.0-1.6]	1.2 [0.9-2.0]	<0.001* p_{1-2}=0.03 p_{1-3}<0.001
VLDL, mmol/l	0.5 [0.3-0.6]	0.8 [0.5-1.4]	0.9 [0.6-1.0]	0.01* p_{1-2}=0.04 p_{1-3}=0.010
Uric acid, µmol/l	261 [223-358]	304 [258-378]	395 [297-454]	<0.001* p_{1-3}<0.001 p_{2-3}=0.004
Creatinine, µmol/l	87.5 [79.8-102.0]	95.0 [81.5-106.0]	94.5 [83.9-111.5]	0.2

Group 1, control group (healthy volunteers); Group 2, comparison group (26 patients with HTN and 19 patients with CAD); Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45); SBP systolic blood pressure; DBP, diastolic blood pressure; PAP, pulmonary arterial pressure; LDL, low-density lipoproteins; HDL, high-density lipoproteins; VLDL, very-low-density lipoproteins.

Table 3. Echocardiographic parameters of study participants

Parameter	Group 1 (n=27)	Group 2 (n=45)	Group 3 (n=82)	p
	n (%) or Me [Q1-Q3]			
EDD, mm	45 [42-47]	48 [46-50]	48 [46-52]	<0.001* p_{1-2}=0.001 p_{1-3}<0.001
IVST, mm	8 [7-8]	11 [10-12]	12 [11-14]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}<0.001
LVPWth, mm	8 [7-8]	11 [10-12]	12 [11-12]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}=0.007
RWT, mm	0.36 [0.33-0.38]	0.45 [0.41-0.49]	0.48 [0.43-0.51]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}=0.03
LVMI, g/m^2	59.4 [55.3-68.3]	94.5 [78.0-107.1]	113.0 [98.8-132.0]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}<0.001
EF, %	63 [61-66]	58 [57-62]	56 [55-60]	<0.001* p_{1-2}=0.002 p_{1-3}<0.001 p_{2-3}=0.003
E/A	1.61 [1.45-1.78]	1.07 [0.73-1.31]	1.06 [0.74-1.10]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001
E/e’	5 ± 1 (5-6)	7 ± 2 (6-9)	10 ± 4 (7-13)	<0.001* p_{1-2}=0.03 p_{1-3}=0.01
LAV, ml	39 [29-42]	52 [45-70]	69 [59-85]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}<0.001
LAVI, ml/m^2	27 [23-31]	35 [29-39]	42 [35-51]	<0.001* p_{1-2}=0.002 p_{1-3}<0.001 p_{2-3}<0.001
RA-volume, ml	33 [29-38]	44 [38-57]	52 [42-68]	<0.001* p_{1-2}<0.001 p_{1-3}<0.001 p_{2-3}=0.02
PASP, mm Hg	24 [20-26]	25 [23-29]	29 [25-43]	<0.001* p_{1-3}=0.003 p_{2-3}=0.003

Group 1, control group (healthy volunteers); Group 2, comparison group (26 patients with HTN and 19 patients with CAD); Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45); EDD, end-diastolic dimension; IVST, interventricular septum thickness; LVPWth, left ventricular posterior wall thickness; RWT, relative wall thickness of the left ventricle; LVMI, left ventricular mass index; EF, ejection fraction; E/A, the ratio of the early (E) to late (A) ventricular filling velocities in the left ventricle; E/e’, the ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity; LAV, left atrial volume; LAVI, left atrial volume index; RA-volume, right atrial volume; PASP, pulmonary arterial systolic pressure.

Of interest is the statistically significant reduction in the threonine concentration in CHFpEF group patients with the presence of type 2 DM, pulmonary vascular congestion diagnosed by chest X-ray, and also in patients taking diuretics and mineralocorticoid receptor antagonists (MRA) (*Table 5*).

Assessment of the threonine level in the study population

In our study, we compared the threonine levels between the study groups (*Figure 1*). In patients with CHFpEF, the threonine concentration was 97.6 [74.7-115.2] μM; in comparison group patients, it was108.8 [92.0-121.0] μM; finally, in healthy volunteers, it was 130.9 [100.8-142.3] μM. The threonine level was significantly lower in patients with CHFpEF (p<0.001: p_{1-3}<0.001; p_{2-3}=0.037).

As a result of constructing a prognostic model (dependence of threonine concentration on the factors such as gender, age, BMI, presence of DM and pulmonary congestion based on chest X-ray data, intake of diuretics and MRA) using the linear regression method (*Table 6*), the resulting regression model is characterized by the correlation coefficient rxy=0.531, which corresponds to a noticeable close relationship according to the Chaddock scale. The model is statistically significant (p=0.002). It established a relationship between the threonine concentration and the presence of type 2 DM (B=-18.219; standard error [SE]=6.661; t=-2.735; p=0.008) and with MRA intake (B=-14.798; SE=5.929; t=-2.496; p=0.015). A negative correlation between the threonine level and BMI (r=-0.244, p=0.027) was also revealed in our study; however, this relationship is weak on the Chaddock scale, and given that the main group is represented by patients with signs of congestion, it is not possible to reliably establish the cause of the increase in BMI. We also revealed a moderate inverse relationship between the threonine concentration and the glucose level (r=-0.338, p=0.002) (*Figure 2*). In addition, weak relationships were detected between threonine and TG levels (r=-0.244, p=0.038), as well as between threonine and the uric acid levels (r=-0.267, p=0.021).

Discussion

Threonine plays a key role in metabolic processes; hence, its concentration can vary widely depending on the types of pathophysiological mechanisms. Based on the results of our study, the hypothesis about the relationship between the level of this amino acid and CHFpEF has been proven: we discovered that in patients suffering from CHFpEF, the level of threonine was significantly lower than in patients of the control group and the comparison group. Moreover, we demonstrated a relationship between threonine and type 2 DM in patients with CHFpEF.

Table 4. Initial therapy for patients with CVD

Pharmaceutical drug class	Group 2 (n=27)	Group 3 (n=45)	p
	n (%)		
ACEI	27 (60)	44 (53.7)	0.491
ARA	15 (33.3)	38 (46.3)	0.302
BAB	29 (64.4)	67 (81.7)	0.030*
CCB	19 (42.2)	36 (43.9)	0.855
MRA	4 (8.9)	48 (58.5)	<0.001*
Diuretics	22 (48.9)	53 (64.6)	0.084
Statins	31 (68.9)	53 (64.6)	0.628
Hypoglycemic medications	12 (26.7)	22 (26.8)	0.984
Class 3 antiarrhythmics	4 (8.9)	6 (7.3)	0.742
NOAC	13 (28.9)	51 (62.2)	<0.001*
Antiplatelet agents	20 (44.4)	35 (42.7)	0.848

Group 2, comparison group (26 patients with HTN and 19 patients with CAD); Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45); ACEI, angiotensin-converting enzyme inhibitors; ARA, angiotensin receptor antagonists; BAB, beta-adrenergic-blocking drugs; CCB, calcium channel blockers; MRA, mineralocorticoid receptor antagonists; NOAC, new oral anticoagulants.

Table 5. Analysis of the threonine concentration in patients with CHFpEF depending on the presence of signs of pulmonary congestion, type 2 diabetes mellitus or intake of diuretics

Parameter	Category	Group 3		
		M±SD	95% CI	p
Signs of pulmonary vascular congestion based on chest X-ray	No pulmonary congestion	102±28	94-109	0.039*
	Pulmonary congestion	88±24	78-98	
Intake of diuretics	No diuretics taken	106±28	95-117	0.041*
	Taking diuretics	93±26	86-100	
MRA	No MRA intake	105±31	95-116	0.027*
	MRA intake	92±23	85-99	
IGT and/or type 2 DM	No IGT or type 2 DM (subgroup 1)	106±27	97-115	0.004*
	IGT (subgroup 2)	102±26	87-117	p_{1-3}=0.003
	Type 2 DM (subgroup 3)	84±24	75-93	

Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45); MRA, mineralocorticoid receptor antagonists; IGT, impaired glucose tolerance; DM, diabetes mellitus.

Table 6. Analysis of threonine levels by a number of indicators

	B	*Standard deviation*	*t*	*p*
Intercept	114.359	34.519	3.313	0.001*
Gender	4.771	6.132	0.778	0.439
Age, years	0.198	0.383	0.519	0.605
BMI, kg/m²	-0.431	0.491	-0.878	0.383
Pulmonary circulation based on chest X-Ray	-6.559	6.307	-1.040	0.302
IGT	2.522	8.022	0.314	0.754
Type 2DM	-18.219	6.661	-2.735	0.008*
Taking diuretics	-4.344	7.033	-0.618	0.539
Taking MRA	-14.798	5.929	-2.496	0.015*

BMI, body mass index; IGT, impaired glucose tolerance; DM, diabetes mellitus; MRA, mineralocorticoid receptor antagonists.

Such data can be explained by the pathophysiological changes investigated in experimental studies. For example, C.M. Ross-Inta et al. examined the effect of a diet with a reduced threonine content on changes in energy metabolism [24]. An anorexigenic effect of this diet was noted, specifically: laboratory animals consumed less food, which was probably a protective mechanism to prevent the aggravation of amino acid imbalance.

It was also established that threonine deficiency promoted the progression of mitochondrial uncoupling in the liver and NADP-dependent substrates (malate, pyruvate), which resulted in increased use of FAD-dependent substrates (such as succinate). These metabolic shifts favored the use of fatty acids as an energy source for ATP synthesis. However, lipid catabolism by ß-oxidation of fatty acids produces less ATP and is energetically less favorable. In the context of CVD pathophysiology, a decrease in intracellular ATP content can lead to the progression of HTN (as one of the most common etiologic factors in the development of CHFpEF) due to increased activation of the sympathetic nervous system. In addition, mitochondrial dysfunction is accompanied by intense formation of reactive oxygen species, which leads to disruption of NO-dependent vascular relaxation, decreased cGMP formation and, as a consequence, a decrease in the cardioprotective effect of protein kinase G. This contributes to the development of endothelial dysfunction, which subsequently leads to remodeling of the vascular wall, including the coronary arteries. This process plays an important role in the progression of diastolic myocardial dysfunction [19, 25].

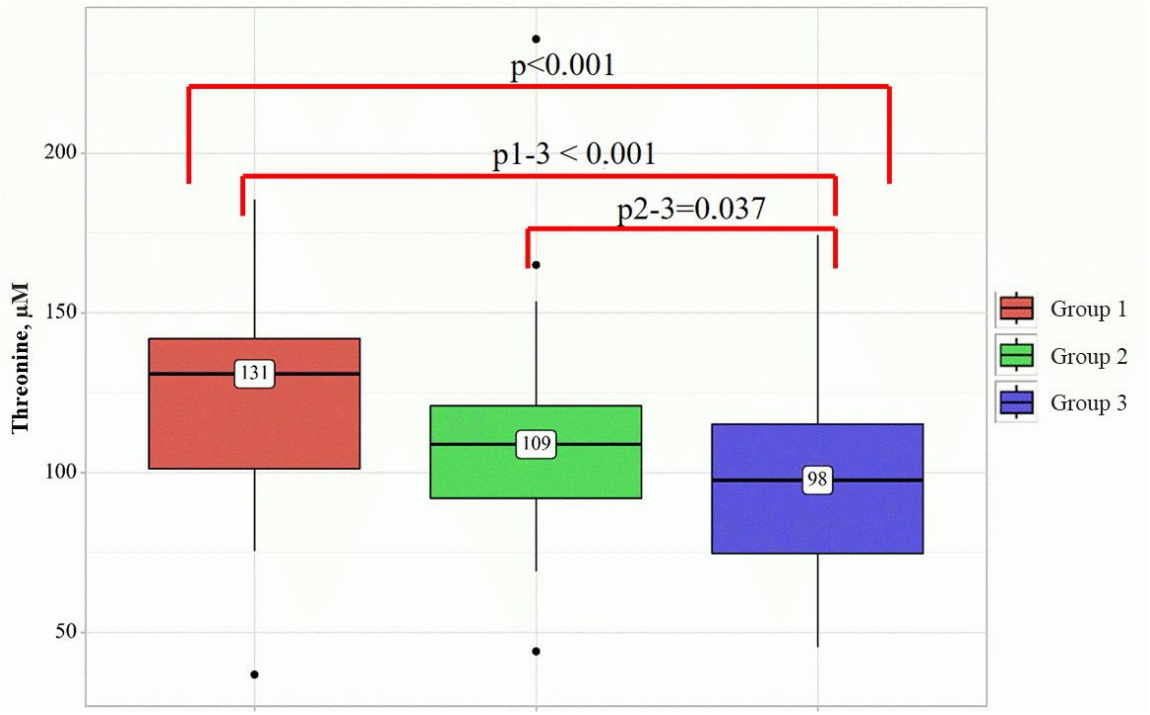

Figure 1. Threonine concentration in study groups.

Group 1, control group (healthy volunteers); Group 2, comparison group (26 patients with HTN and 19 patients with CAD); Group 3, main group (82 patients with CHFpEF: CHFpEF+HTN, n=37; CHFpEF+CAD, n=45).

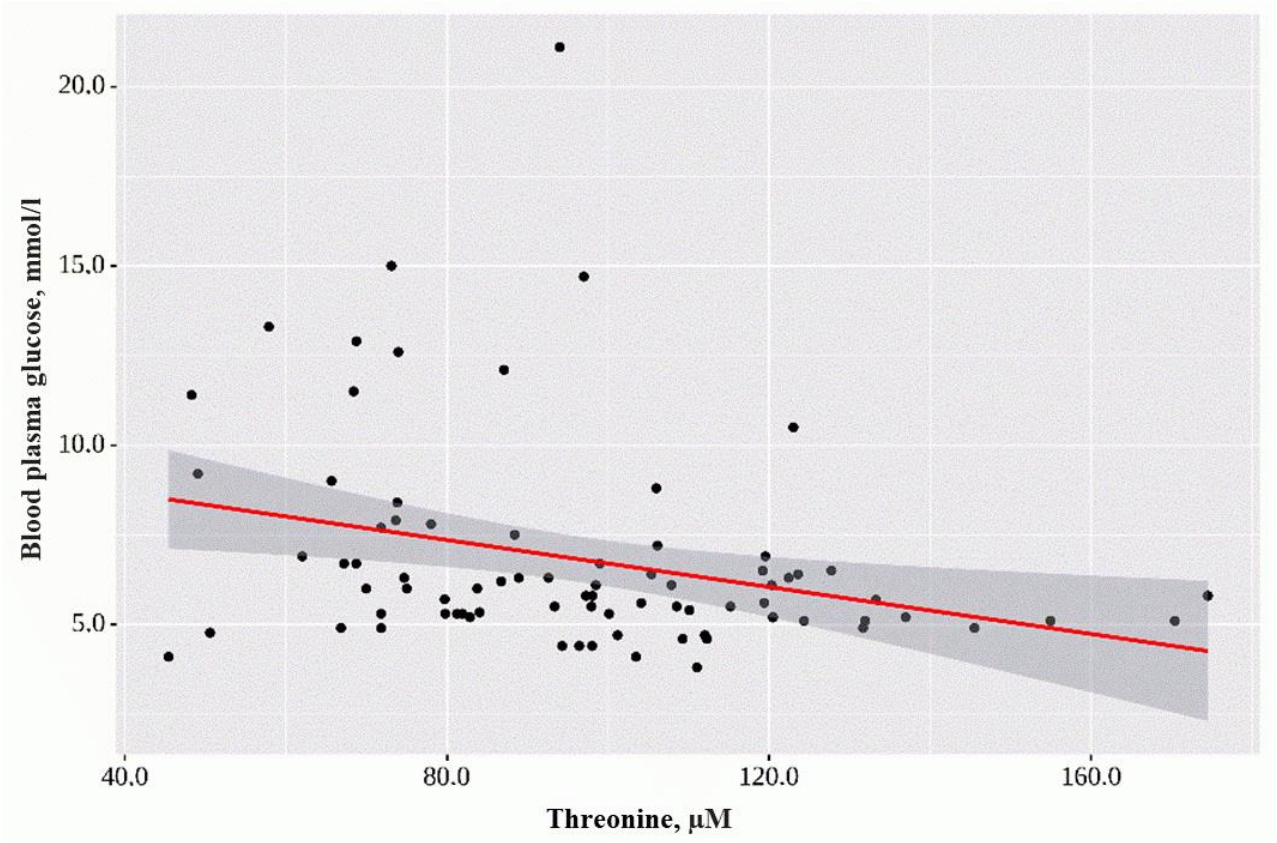

Figure 2. Correlation of threonine concentration with glucose level.

Lipid metabolism largely depends on the level of threonine. Therefore, in the course of our research, we studied the relationship between the level of threonine and lipid spectrum parameters. As a result, an inverse correlation was established between the levels of TG and threonine. In the study by C.M. Ross-Inta et al. [24], it was also proven that threonine deficiency contributes to disruption of lipid metabolism, leading to excessive accumulation of TG in liver tissue. One of the mechanisms of these changes is the effect of threonine on gene expression, specifically, increased expression of genes encoding fatty acid synthesis, while simultaneously suppressing genes associated with TG transport and fatty acid oxidation. In the experimental study by Chinese researchers [26], a link was proven between threonine deficiency in the diet and impaired fatty acid oxidation. The important role of threonine in the regulation of lipid metabolism was also proven in the study by F.-H. Wang et al [14], the results of which revealed an inverse relationship between threonine concentration and TG level. In patients with higher threonine values in blood plasma, the risk of developing atherogenic lipid triad was significantly reduced. Thus, the results of previous studies are consistent with the results highlighted in this article.

We revealed a negative correlation between a reduction in threonine concentration and an increase in BMI. This relationship can probably be considered in the context of changes in lipid metabolism: for instance, an increase in threonine content in the diet of experimental animals leads to a decrease in body weight and normalization of such parameters as serum leptin and adiponectin [27]. These changes are associated with a change in the expression of thermogenin (uncoupling protein 1). In obesity, a decrease in the level of this protein was observed, which contributed to lipogenesis. The introduction of foods high in threonine into the diet contributed to lipolysis and normalization of body weight. Also, threonine indirectly affects lipid metabolism by regulating the signaling pathway of peroxisome proliferator-activated receptors (PRARs). This group of nuclear receptors is expressed in many tissues. One type of receptors (PPARγ) regulates adipocyte differentiation and, in addition, affects glucose homeostasis and insulin sensitivity via increasing the expression of glucose transporter type 4 (GLUT4) [28]. PPARγ is a therapeutic target for one of the groups of oral hypoglycemic agents (thiazolidinediones). The hypothesis about the relationship between the level of plasma glycemia and the threonine concentration was confirmed by the results of this study: a negative correlation was detected between the level of glycemia in patients and the concentration of threonine. In addition, the level of plasma threonine in patients with DM who participated in this study was significantly lower than in patients without it, which accentuates the important role of the level of this amino acid in the pathogenesis of carbohydrate metabolism disorders and the development of insulin resistance. Similar results were obtained in the publication by Chinese researchers [29].

The results of our study revealed an inverse relationship between the level of threonine and the concentration of uric acid. Threonine is one of the main carbon sources in the synthesis of purine bases and thymidine; uric acid is the end product of purine base metabolism [30]. In this regard, it is assumed that the concentration of threonine is directly dependent on the level of uric acid. However, because the established inverse correlation is weak, it is not statistically significant.

Conclusion

Our study demonstrated a statistically significant reduction in threonine concentration in patients with CHFpEF vs. those with HTN and CAD, and vs. healthy volunteers. At the same time, the presence of type 2 DM and MRA intake were significant factors influencing the decrease in the biomarker concentration in patients with CHFpEF, which was confirmed by the presence of a moderate inverse correlation of threonine with glucose levels. These findings suggest that changes in threonine concentration are characteristic of the CHFpEF metabolic phenotype. However, additional studies are needed to clarify the association of threonine with different CHFpEF phenotypes.

One way or another, the results of our study imply that it is possible to correct metabolic disorders in patients with CVD and DM by adding threonine to the patient's diet in order to improve metabolic processes. However, this issue requires further study to identify the most characteristic changes in threonine in specific pathologies and determine the reliability of the relationship between these events.

Conflict of interest

The authors declare no competing interests.

Funding

The study was conducted with financial support of the Innovative Science School at Sechenov University within the framework of the *Priority 2030* program.

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