FREE AMINO ACIDS IN THE BLOOD SERUM OF PATIENTS WITH ISCHEMIC HEART DISEASE

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S u m m a r y. For many decades coronary heart disease has been the most commonly diagnosed cardiovascular pathology in highly developed countries. According to the World Health Organization (WHO), the number of deaths from coronary heart disease will have increased from 7.1 million in 2002 to 11.1 million in 2020.

The essence of coronary heart disease (CHD) is a progressive myocardial ischemia, which leads to hypoxia and necrosis, and is associated with atherosclerosis in the coronary arteries. In the pathogenesis of CHD the key role is attributed to atherosclerotic plaques developing in the blood vessels narrowing their lumen. One of the most important pathological processes basal to the development of atherosclerosis is endothelial dysfunction.

The aim of the study was to determine the level of selected amino acids in patients with ischemic heart disease.

With age the amino acid pro□le is changed. On day 0 the level of amino acids was statistically signi□cantly higher in the majority of patients under the age of 65 years. However, the concentration of amino acids noted on days 1, 3, and 7 was statistically signi□cantly increased in the group aged 65 and older.

K e y w o r d s: ischemic heart disease, amino acid pro□le

INTRODUCTION

Cardiovascular diseases are the main cause of mortality and account for nearly 50% of all deaths. Major cardiovascular diseases include ischemic heart disease and cerebrovascular disease, which are the cause of 35% of all deaths in Europe. However, there are signi□cant differences between countries in terms of indicators such as age, gender and the distribution of determinants. Many risk factors have been identi□ed so far, the presence of which is conducive to the re-occurrence of coronary heart disease, and causes the progression of the already existing symptoms. The factors, whose relation to cardiovascular disease has been fully proven include hypertension, smoking, dyslipidemia, obesity, low physical activity, and dietary factors. Much attention is paid to the identi□cation of genetic, hemostatic, and psychosocial factors affecting the occurrence of coronary heart disease. Epidemiological studies indicate that the risk of developing or dying from a cardiovascular disease in the population is largely dependent on the prevalence of major risk factors. Their coexistence, thus the aggregation of risks, signi□cantly increases the likelihood of cardiovascular disease. From the point of view of health and survival of the population, preventive measures are of utmost importance. They should be aimed at the prophylaxis of cardiovascular diseases in those individuals who suffer from the disease. In the case of people with diagnosed disease, the goal is to prevent the progression of symptoms and complications that may set in.

Coronary heart disease is a clinical syndrome caused by an imbalance between the amount of coronary blood flow, and the requirement of the myocardial cells for oxygen and energy compounds supplied to the heart muscle [4].

In the pathogenesis of CHD the key importance is attributed to atherosclerotic plaques developing in the blood vessels narrowing their lumen. One the most important pathological processes

underlying the development of atherosclerosis is endothelial dysfunction, which results from high level of low density lipoproteins (LDL), increased shear stress, hypertension, diabetes mellitus, hyperhomocysteinemia, hypoxia, free radicals, infections, or mechanical damage [13]. Properly functioning endothelium is the source of a number of mediators which modulate the contraction and relaxation of smooth muscles, adhesion and aggregation of platelets, and leukocyte migration. They maintain adequate vasodilatation (diastole) and inhibit smooth muscle proliferation. In the case of endothelium damage, the outer membrane of the vessel becomes permeable to the plasma lipoproteins and the endothelial cells properties change from anti-adhesive to pro-adhesive [1].

The heart is supplied with blood by its own blood vessels that wrap around it like a 'crown' or 'wreath', and therefore the arteries and veins of the heart are called coronary arteries. There are two main coronary arteries which branch off directly from the aorta, i.e. the right coronary artery (RCA) and the left coronary artery (LCA) [10].

Under physiological conditions, the demand of the heart muscle for oxygen is met by the mechanisms of autoregulation of the coronary circulation, where the supply of oxygen is the product of coronary blood flow and oxygen diffusion to the myocardial cells. There are four main factors determining the demand of the heart muscle for oxygen: heart rate, myocardial contractility, the pressure in the left ventricle, the left ventricular volume, which change under the influence of e.g. physical or emotional stress.

Clinically, there are two basic mechanisms of ischemia manifested by anginal pain. In the first mechanism of ischemia, if coronary artery is significantly narrowed, increased myocardial oxygen demand cannot be offset by increased coronary flow, as it happens in normal conditions. In pathological cases, there is an imbalance between increased demand, decreased supply, or it may not increase proportionally to the growing demand. When the heart muscle is supplied by the narrowed vessel, that leads to ischemia. In 90% cases oxygen deficiency resulting from ischemia due to atherosclerosis of the coronary arteries accounts for chest pain. The situation is different when the patient is at rest, e.g. sleeps at night. During the night rest, the myocardial demand for oxygen is not reduced, however, if there is coronary artery spasm significantly narrowing its lumen, the supply of oxygen dramatically decreases. This leads to ischemia of the myocardium being supplied by the contracted artery. Such a mechanism of ischemia is characteristic of vasospastic angina (Prinzmetal angina) [5, 10].

The classic symptom of ischemia is coronary pain. On history taking special attention is paid to identify coronary pain. Patient is asked about the severity and nature of pain, whether it is crushing, oppressive, expanding, or burning. Patients often describe pain as a feeling of tightness, choking, burning in the esophagus, or travelling. It is important to ask about the duration of pain, if it occurred with exercise, and pain location and radiation. The coronary pain is usually located retrosternally or slightly to the left of the sternum, radiating to the left shoulder and left hand. Pain only in the interscapular area or elbow joint is considered an atypical location. Another important element in the assessment of coronary pain is its subsidence. Pain associated with transient ischemia subsides after a few minutes when the effort has stopped, and disappears immediately or after a few minutes following nitroglycerin administration. With the progression of disease, coronary pain may also occur at rest, or at night waking the patient from sleep.

The course of coronary heart disease includes a few stages, i.e. the period of stable coronary artery disease, the period of unstable angina, acute coronary syndrome (ST-segment elevation and non-ST), and sudden cardiac death. Stable coronary artery disease is defined as a clinical condition wherein the conventional coronary pain or equivalents thereof are present for at least 2-3 months with a similar frequency, severity and duration. In the majority of patients with atherosclerosis ischemia occurs during exercise and causes pain. The American Heart Association / European Society of Cardiology (AHA / ESC) and the Polish Cardiac Society (PCS) recommend classification of angina according to the Canadian Cardiovascular Society (CCS) (Table 1).

Table 1. Classification of cardio-vascular diseases according to CCS [10]

Stage I	Ordinary life activity does not cause pain. Anginal pain occurs at the large, violent or extended efforts.
Stage II	Small reduction of the usual activity, pain during fast walking, walking more than 200m, while climbing the stairs above one floor, after meals, in the morning and during the cold, wind or nervousness.
Stage III	Significant activity limitation, angina pain while walking distance of less than 200 m and below one floor level.
Stage IV	Inability to perform a little exercise without pain, angina at rest.

Unstable angina pectoris and myocardial infarction include forms of ischemic disease of acute dynamic changes, and are burdened with high mortality as they often manifest themselves as sudden cardiac death. Classification of acute coronary syndromes is based on the following criteria: presence of typical coronary pain (in all forms), biochemical markers of necrosis (typical of heart attack), and characteristic ECG changes [5].

Amino acids (AAs) are the smallest elements of the structure of peptides and proteins of all living organisms. In all the tissues and body fluids of every living organism there is also a pool of free amino acids, which in addition to creating peptides and proteins, perform many important biological functions. Free amino acids are involved in the synthesis of lipids and their derivatives. Amino acids or their derivatives are neurotransmitters (glutamic acid, aspartic acid, glycine), hormones and neurohormones (tyrosine derivatives - adreanline, norepinephrine, thyroxine, triiodo-thyronine, or derivatives of tryptophan - serotonin, melatonin). The carbon skeleton of amino acids derives from intermediate metabolites of major metabolic pathways in the body (glycolysis, Krebs cycle, the pentose-phosphate pathway). Amino acids are the precursors of nitrogenous bases, hemoglobin, biogenic amines, creatine, collagen, elastin, and many other molecules. All protein amino acids are essential for proper functioning of the body, and disturbances of enzymes necessary for the metabolism of amino acids give rise to so-called 'metabolic blocks'. In states of disease exacerbations, when nitrogen balance is impaired, free amino acids are subjected to significant disturbances, crucial to human health [15].

AIM OF THE STUDY

The aim of the study was to evaluate concentrations of free amino acids in the examined patients with coronary heart disease.

MATERIALS AND METHODS

Materials

Clinical material for the study was collected from the blood of patients with CHD. Blood was taken from the vein in the arm four times: on admission to hospital (day 0), on the first day of hospitalization, and on the third and seventh day.

Blood was taken fasting in the morning. The sample of 5 ml of blood was collected into a tube and left for clot formation at 4°C. Then the blood was centrifuged for 15 minutes at 3,000xg to separate the serum. Centrifuged serum was deproteinized with 6% sulfosalicylic acid.

Characteristics of study groups

The examined group consisted of 60 patients: 32 men (53.33%) and 28 women (46.67%), aged 53 to 79 years, hospitalized for worsened coronary heart disease in The Świętokrzyskie Cardiology Center in Kielce (Table 2). The examined patients were divided into two groups: group 1 – patients under 65yrs, and group 2 – patients 65 years old and older (Table 3).

Table 2. Characteristics of the examined patients.

Gender	Number	%	Number of people <65yrs	%	Number of people ≥65yrs	%
Men	32	53.33%	17	53.12%	15	53.57%
Women	28	46.67%	15	46.88%	13	46.43%
Total	60	100.00%	32	100.00%	28	100.00%

Table 3. Age of the examined patients.

Gender	M	SD	Min	Max
Men	60.25	10.12	53	79
Women	67.43	1.99	64	70
Total	63.6	8.25	53	79

Determination of amino acids concentration

The concentration of free amino acids was determined in the supernatant by ion exchange chromatography on automated amino acid analyzer AAA 400 (Ingos Prague, The Czech Republic).

Amino acids were separated in a single column system 3mm x 200mm, filled with resin ion exchanger OSTION LG FA (Ingos Prague, The Czech Republic). Amino acids were separated using five lithium citrate buffers at pH 2.9; 3.1; 3.35; 4.05; 4.90. The eluted amino acids were transferred into the tephlone-coated capillary and reacted with the incoming ninhydrin forming colored compounds. Amino acids were identified by retention time compared to the standards. Acidic and alkaline amino acids were separated at 38-39°C, and neutral amino acids at 59-60°C. Amino acid serum concentrations were expressed in micromoles per $1cm^3$ ($\mu mol/cm^3$).

Statistical analysis

The results were statistically analyzed and presented as tabular and graphical illustrations. The variables characterizing the examined population were expressed as arithmetic means, minimum and

maximum values reflecting the variety of features, and standard deviation. Differentiating features between speci□c categories were calculated using statistical tests. Differences between variables were determined by non-paired Student's t-test; $p < 0.05$ was assumed statistically signi□cant [9].

RESULTS

Maintaining homeostasis of the internal environment is essential for proper functioning of a living organism. Normal levels of plasma amino acids depend on the balance between amino acids supplied and used by the system. Coronary heart disease is a clinical condition characterized by constant progression of atherosclerosis in the coronary arteries, which in turn leads to peripheral circulatory disorders. Amino acids play an important role in maintaining the normal function of the vascular endothelium in the heart and circulatory system.

A comparison of the mean concentrations of each AA in the groups of patients, minimum and maximum range, and standard deviation is shown in Table 4.

T a b l e 4. Concentrations of free amino acids in the blood serum of patients with unstable coronary artery.

		Patients < 65yrs								**Patients ≥65yrs**							
		Day 0		**Day 1**		**Day 3**		**Day 7**		**Day 0**		**Day 1**		**Day 3**		**Day 7**	
		M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
alkaline	**LYS**	0.320	0.091	0.169	0.001	0.196	0.056	0.225	0.006	0.190	0.035	0.195	0.010	0.176	0.025	0.231	0.033
	HIS	0.089	0.006	0.069	0.011	0.068	0.019	0.075	0.015	0.068	0.010	0.076	0.003	0.070	0.008	0.082	0.014
	ARG	0.123	0.030	0.066	0.001	0.051	0.017	0.045	0.020	0.084	0.030	0.066	0.001	0.078	0.013	0.092	0.011
acid	**ASP**	0.095	0.030	0.031	0.014	0.036	0.009	0.039	0.002	0.041	0.002	0.044	0.007	0.046	0.005	0.050	0.010
	GLU	0.385	0.019	0.438	0.061	0.171	0.078	0.152	0.011	0.169	0.041	0.232	0.038	0.227	0.084	0.256	0.117
sulphur	**MET**	0.019	0.008	0.008	0.001	0.012	0.006	0.017	0.006	0.018	0.014	0.017	0.001	0.020	0.006	0.029	0.009
aromatic	**TYR**	0.134	0.062	0.041	0.009	0.059	0.031	0.083	0.008	0.080	0.020	0.061	0.010	0.073	0.022	0.100	0.020
	PHE	0.106	0.035	0.046	0.006	0.060	0.026	0.070	0.001	0.062	0.013	0.060	0.004	0.080	0.015	0.131	0.038
branched chain	**VAL**	0.250	0.038	0.210	0.034	0.260	0.086	0.293	0.012	0.295	0.025	0.260	0.021	0.257	0.013	0.303	0.025
	ILE	0.084	0.030	0.067	0.010	0.078	0.035	0.097	0.004	0.100	0.021	0.086	0.011	0.082	0.010	0.102	0.010
	LEU	0.166	0.052	0.133	0.004	0.152	0.050	0.176	0.011	0.132	0.049	0.154	0.003	0.162	0.025	0.194	0.042
non-protein	**TAU**	0.199	0.095	0.111	0.065	0.062	0.033	0.087	0.037	0.106	0.089	0.110	0.076	0.127	0.077	0.104	0.063
	ORN	0.126	0.035	0.067	0.022	0.069	0.021	0.084	0.011	0.100	0.009	0.104	0.003	0.090	0.013	0.114	0.027
	CIT	0.011	0.009	0.015	0.014	0.019	0.011	0.017	0.012	0.027	0.018	0.020	0.010	0.014	0.009	0.019	0.006
small protein	**SER**	0.170	0.032	0.076	0.057	0.102	0.022	0.093	0.019	0.112	0.030	0.129	0.032	0.125	0.029	0.135	0.037
	GLY	0.447	0.148	0.183	0.018	0.179	0.034	0.185	0.020	0.213	0.040	0.228	0.036	0.230	0.096	0.245	0.057
	ALA	0.322	0.045	0.321	0.137	0.342	0.139	0.480	0.029	0.387	0.095	0.423	0.081	0.387	0.114	0.473	0.112
	THR	0.271	0.097	0.110	0.013	0.133	0.030	0.134	0.022	0.153	0.058	0.148	0.029	0.145	0.029	0.183	0.045

In the age group <65 yrs amino acid concentrations on day 0 and 1 were higher than in the patients ≥65 yrs (Table 4). In the patients with CHD ≥65 yrs serum free amino acid concentrations presented different values throughout the observation period, i.e. from day 0 to day 7. The concentrations of sulfur amino acids were low throughout the study period in both age groups.

Tables 5-8 present the results of Student's t-test comparing serum free AA concentrations between the groups.

T a b l e 5. Amino acid concentrations in the blood serum of patients with CHD (μmol/cm^3) on day 0.

		Patients < 65yrs		**Patients ≥65yrs**		**T**	**p**
		M	**SD**	**M**	**SD**		
alkaline	**LYS**	0.3198	0.091	0.1902	0.035	28.364	**0.000**
	HIS	0.0890	0.006	0.0684	0.010	27.964	**0.000**
	ARG	0.1230	0.030	0.0836	0.030	10.303	**0.006**
acid	**ASP**	0.0948	0.030	0.0414	0.002	58.704	**0.000**
	GLU	0.3853	0.019	0.1688	0.041	189.780	**0.000**
sulphur	**MET**	0.0188	0.008	0.0181	0.014	0.014	0.908
aromatic	**TYR**	0.1335	0.062	0.0800	0.020	11.339	**0.003**
	PHE	0.1060	0.035	0.0621	0.013	21.888	**0.000**
branched chain	**VAL**	0.2503	0.038	0.2951	0.025	12.708	**0.002**
	ILE	0.0838	0.030	0.1000	0.021	2.593	0.120
	LEU	0.1660	0.052	0.1319	0.049	2.607	0.119
non-protein	**TAU**	0.1985	0.095	0.1061	0.089	5.756	**0.025**
	ORN	0.1260	0.035	0.1002	0.009	8.809	**0.007**
	CIT	0.0110	0.009	0.0267	0.018	4.989	**0.038**
small protein	**SER**	0.1695	0.032	0.1123	0.030	19.240	**0.000**
	GLY	0.4470	0.148	0.2132	0.040	40.258	**0.000**
	ALA	0.3215	0.045	0.3872	0.095	3.454	0.075
	THR	0.2705	0.097	0.1532	0.058	14.731	**0.001**

On day 0, the concentrations of free AAs: LYS, HIS, ARG, ASP, GLU, TYR, PHE, TAU, ORN, SER, GLY, THR were signi□cantly lower in the patients aged 65yrs and older. On the same day serum concentrations of VAL, CIT were signi□cantly higher in the patients aged 65 and older. No statistically signi□cant differences were observed for the concentrations of ILE, LEU, and ALA (Table 5, Figure 1).

Table 6. Amino acid concentrations in the blood serum of patients with CHD (μmol/cm^3) on day 1.

		Patients < 65yrs		Patients ≥65yrs		T	P
		M	SD	M	SD		
alkaline	LYS	0.1693	0.001	0.1947	0.010	38.315	**0.000**
	HIS	0.0690	0.011	0.0757	0.003	3.958	0.064
	ARG	0.0660	0.001	0.0662	0.001	0.245	0.630
acid	ASP	0.0310	0.014	0.0440	0.007	11.128	**0.004**
	GLU	0.4383	0.061	0.2323	0.038	79.006	**0.000**
sulphur	MET	0.0077	0.001	0.0170	0.001	190.061	**0.000**
aromatic	TYR	0.0407	0.009	0.0610	0.010	16.947	**0.001**
	PHE	0.0460	0.006	0.0600	0.004	30.745	**0.000**
branched chain	VAL	0.2097	0.034	0.2603	0.021	15.552	**0.001**
	ILE	0.0667	0.010	0.0863	0.011	13.571	**0.002**
	LEU	0.1333	0.004	0.1540	0.003	150.745	**0.000**
non-protein	TAU	0.1110	0.065	0.1103	0.076	0.000	0.986
	ORN	0.0670	0.022	0.1037	0.003	34.658	**0.000**
	CIT	0.0153	0.014	0.0200	0.010	0.627	0.440
small protein	SER	0.0763	0.057	0.1293	0.032	6.561	**0.021**
	GLY	0.1827	0.018	0.2277	0.036	8.228	**0.011**
	ALA	0.3213	0.137	0.4230	0.081	3.979	0.063
	THR	0.1097	0.013	0.1483	0.029	9.396	**0.007**

On the □rst day, the concentrations of free AA levels: LYS, ASP, MET, TYR, PHE, VAL, ILE, LEU, ORN, SER, GLY, THR were signi□cantly higher in the patients aged 65 and older. Serum GLU concentrations were signi□cantly lower in that group of patients. No statistically signi□cant differences were noted only for alkaline HIS, ARG, and TAU, CIT and ALA (Table 6, Figure 1).

Table 7. Amino acid concentrations in the blood serum of patients with CHD (μmol/cm^3) on day 3.

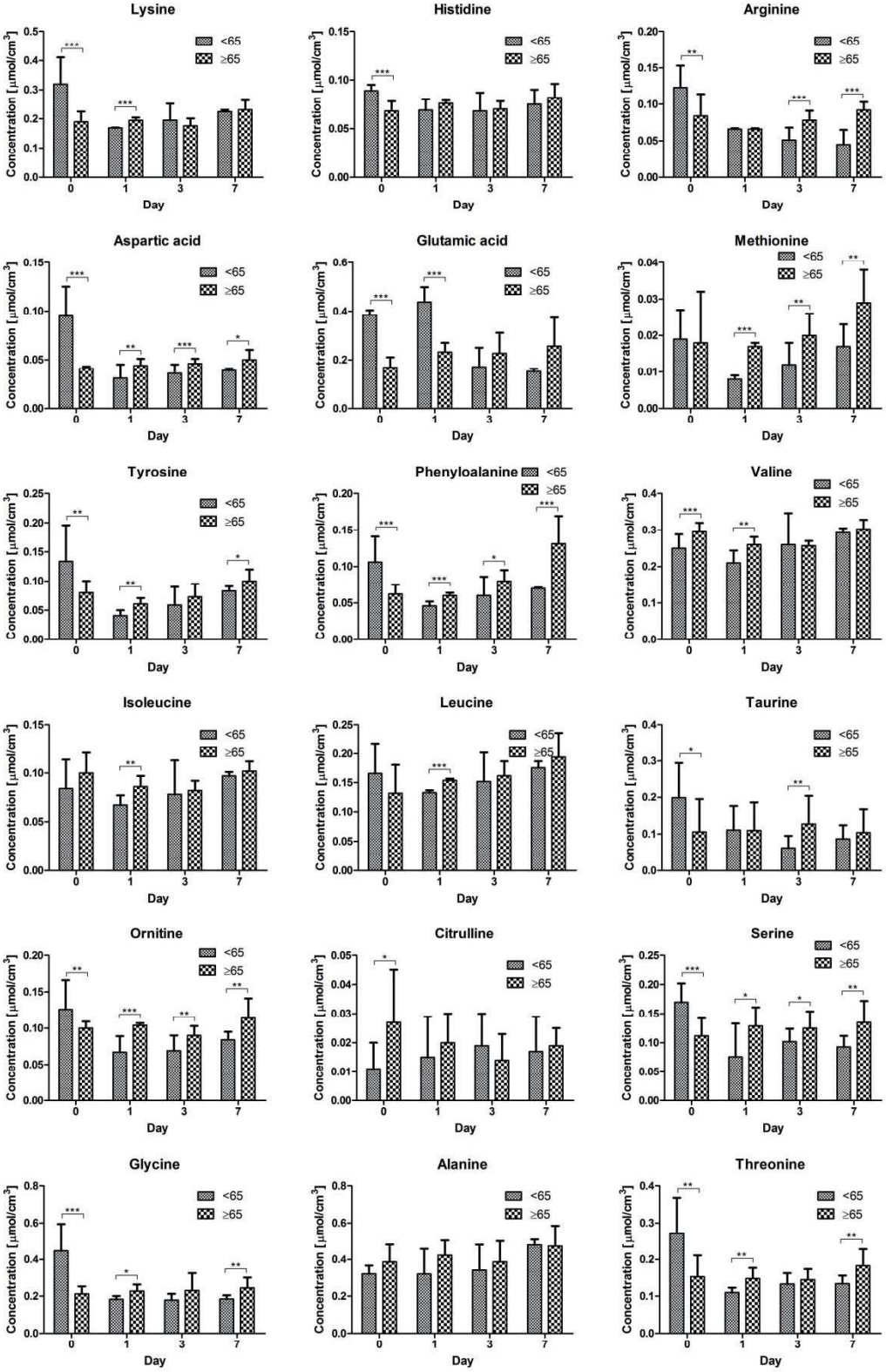
		Patients < 65yrs		Patients ≥65yrs		T	P
		M	SD	M	SD		
alkaline	LYS	0.1958	0.056	0.1763	0.025	1.720	0.200
	HIS	0.0678	0.019	0.0696	0.008	0.122	0.729
	ARG	0.0510	0.017	0.0782	0.013	25.196	**0.000**
acid	ASP	0.0362	0.009	0.0463	0.005	16.568	**0.000**
	GLU	0.1713	0.078	0.2268	0.084	3.306	0.080
sulfur	MET	0.0122	0.006	0.0204	0.006	13.896	**0.001**
aromatic	TYR	0.0592	0.031	0.0732	0.022	2.123	0.156
	PHE	0.0600	0.026	0.0802	0.015	7.420	**0.011**
branched chain	VAL	0.2598	0.086	0.2570	0.013	0.019	0.890
	ILE	0.0775	0.035	0.0824	0.010	0.333	0.568
	LEU	0.1515	0.050	0.1616	0.025	0.537	0.470
non-protein	TAU	0.0617	0.033	0.1274	0.077	7.797	**0.009**
	ORN	0.0685	0.021	0.0899	0.013	11.459	**0.002**
	CIT	0.0187	0.011	0.0137	0.009	1.478	0.237
small protein	SER	0.1017	0.022	0.1251	0.029	5.570	**0.025**
	GLY	0.1787	0.034	0.2297	0.096	3.094	0.089
	ALA	0.3422	0.139	0.3868	0.114	0.930	0.343
	THR	0.1327	0.030	0.1451	0.029	1.287	0.266

In the patients with CHD aged 65yrs and older the concentrations of free AAs on the 3rd day were signi□cantly higher in the case of ARG, ASP, MET, PHE, TAU, ORN, and SER (Table 7, Figure 1). No statistically signi□cant differences were observed in the case of other AAs.

Table 8. Amino acid concentrations in the blood serum of patients with CHD (μmol/cm^3) on day 7.

		Patients < 65yrs		Patients ≥65yrs		T	P
		M	SD	M	SD		
alkaline	LYS	0.2248	0.006	0.2311	0.033	0.283	0.599
	HIS	0.0750	0.015	0.0822	0.014	1.438	0.242
	ARG	0.0450	0.020	0.0916	0.011	101.574	**0.000**
acid	ASP	0.0393	0.002	0.0497	0.010	7.682	**0.011**
	GLU	0.1520	0.107	0.2564	0.117	4.679	0.420
sulfur	MET	0.0170	0.006	0.0290	0.009	12.659	**0.002**
aromatic	TYR	0.0828	0.008	0.1001	0.020	5.609	**0.026**
	PHE	0.0695	0.001	0.1313	0.038	20.373	**0.000**
branched chain	VAL	0.2933	0.012	0.3029	0.025	1.067	0.312
	ILE	0.0970	0.004	0.1024	0.010	2.035	0.167
	LEU	0.1760	0.011	0.1940	0.042	1.366	0.254
non-protein	TAU	0.0873	0.037	0.1042	0.063	0.492	0.490
	ORN	0.0843	0.011	0.1140	0.027	8.821	**0.007**
	CIT	0.0168	0.012	0.0185	0.006	0.211	0.651
small protein	SER	0.0933	0.019	0.1347	0.037	8.733	**0.007**
	GLY	0.1848	0.020	0.2451	0.057	8.262	**0.008**
	ALA	0.4798	0.029	0.4734	0.112	0.024	0.878
	THR	0.1340	0.022	0.1826	0.045	8.163	**0.009**

On the seventh day serum amino acid concentrations of ARG, ASP, MET, TYR, PHE, ORN, SER, GLY, and THR were signi□cantly higher in the patients aged 65yrs and older. Other differences were statistically insigni□cant (Table 8, Figure 1).



F i g u r e 1. Analysis of amino acid levels in the serum of patients with coronary heart disease. Data are presented as means and standard deviation (n = 60; * - p <0.05; ** - p <0.01, *** - p <0.001 by t-Student test).

DISCUSSION

Poland is a country with a high risk of cardiovascular events. Coronary heart disease is the most common heart disease among middle-aged and older people. Clinical symptoms of the disease occur in nearly 30% of people over the age of 65yrs. In developed countries, it remains the most common cause of death in men over the age of 45yrs and women over the age of 65. In the elderly population women constitute a signi□cantly greater proportion of patients suffering from ischemic heart disease. Ischemic heart disease is often accompanied by other conditions, such as hypertension or diabetes. In the etiology of coronary heart disease bigger or smaller dynamic changes develop within the blood vessels, which results in narrowed vascular lumen and eventual closure of the vessel developing with time [8].

In the course of unstable angina, a signi□cant increase in acute phase proteins occurs [14]. In the case of impaired circulation the blood flow is insuf□cient and tissue hypoxia develops. This results in increased catabolism of proteins and altered amino acid pro□le. At the cellular level anaerobic metabolism develops, resulting in metabolic acidosis. This impairs the function of cells and can even lead to irreversible damage.

Our study compared the concentrations of free AAs in the blood serum of the patients with coronary heart disease between two age groups: the patients under 65yrs and 65yrs old and older. We found fluctuating pro□le of amino acids in the serum of the examined patients observed from the admission to hospital till day 7 of hospitalization. The concentration of free amino acids in the serum of CHD patients varied depending on the time of treatment. With age amino acid pro□le was changed. On day 0, the level of amino acids was statistically signi□cantly higher in the majority of patients under the age of 65 years. However, the concentration of amino acids noted on days 1, 3, and 7 was statistically signi□cantly increased in the group aged 65 and older.

Bertolini et al. indicated the effect of age and gender on the concentration of amino acids in the human blood [3]. Also, Armstrong who investigated a large population (100 women and 90 men) found a lower level of free amino acids in the serum of women [2]. The importance of amino acid changes in the pathogenesis of many diseases has been recognized in recent years. This is reflected in the introduction of new analytical methods to determine the concentrations of free amino acids in the body fluids.

Górski et al. presented the most important data about the use of energy substrates by the heart muscle in the course of coronary artery disease and diabetes [6]. The heart muscle has a unique ability to use different energy substrates. It may draw energy from glucose, free fatty acids, lactic acid, and ketones. Moreover the heart muscle may use certain amino acids as energy substrates, e.g. glutamine, glutamic acid, and aspartic acid and alanine.

Moreover, researchers investigated amino acid metabolism in obesity taking into account the fact that increased levels of plasma free amino acids may stimulate insulin secretion [6]. Zwaigzne et al. found that with decreasing body weight of obese children the concentrations of free amino acids in the plasma and red blood cells were changed. After patients' body weight had been reduced, AA levels were close to the values of free amino acids recorded in the children with normal body weight. The authors' □ndings indicate that there is a need to study the metabolism of amino acids in obesity. The results con□rm the existence of altered protein metabolism in obese children [17].

Iłżecka et al. investigted amino acid concentrations in the plasma of patients with atherosclerosis [7]. They found that amino acids may play an important role in the etiology of the disease. The aim of their study was to determine the concentration of amino acids in the blood serum of patients with atherosclerosis, and to assess the correlation between amino acids, type of atherosclerosis and its duration. Amino acid concentrations varied. Signi□cantly decreased levels were observed for isoleucine, leucine, tyrosine, and valine. Moreover, the clinical condition of patients with serious atherosclerosis signi□cantly affected the concentrations of amino acids, where the highest concentrations of alanine were found in terminally ill patients. However, no statistically signi□cant correlation with concomitant diseases was found. No dependence was found between the clinical symptoms of those diseases and their duration, and the concentration of amino acids [12, 16, 11].

Our study compared the concentrations of free AAs in the blood serum of patients with coronary heart disease between two age groups: the patients under 65yrs and 65yrs and older.

CONCLUSIONS

1. Coronary heart disease causes changes in the pro□le of free amino acids.

2. Chronic and progressive nature of CHD leads to considerable disturbances in amino acid concentrations.

3. On day 0, the level of amino acids was statistically signi□cantly higher in the majority of patients under the age of 65 years. However, the concentrations of amino acids noted on days 1, 3, and 7 were statistically signi□cantly increased in the group aged 65 and older.

REFERENCES

1. Aird W.C. (2004). Endothelium as an organ system. *Crit. Care Med.* 32. p.271–279.
2. Zapolska-Downar D., Zapolska-Downar A. (2002). Miażdżyca jako przewlekła choroba zapalna. *Przeg. Lek.* 3. p.147–152 [in Polish].
3. Armstrong S. D. (1973). A study of plasma free amino acid levels. Normal values for children and adults. *Metabolism* 22. p.561-563.
4. Bertolini A. M., Santagostino A., Belgioioso G. B. (1972). The behavior of free amino acids in the plasma of the aged. *Gerontol. Clin.* 14. p.43-49.
5. Camm John A., Luscher Thomas F., Serryus Patrick W. (2006). Choroby serca i naczyń. *Podręcznik Europejskiego Towarzystwa Kardiologicznego. Poznań.* p.255-343 [in Polish].
6. Daniluk J., Jurkowska G. (2005). Zarys Chorób Wewnętrznych. *Czelej*. p.8-30.
7. Górski J., Knapp M., Musiał W. (2000). Metabolizm substratów energetycznych w mięśniu sercowym. Wpływ niedotlenienia i cukrzycy. *Czyn. Ryz.* 2 (3). p.18-23 [in Polish]..
8. Iłżecka J., Stelmasiak Z., Solski J., Wawrzycki S., Szpetnar M. (2003). Plasma amino acids concetration in amyotrophic lateral sclerosis patients. *Amino Acids*. 25. p.69-73.
9. Janion M., Wożakowska-Kapłon B., Sadowski J. (2004). Cardiac rupture in acute myocardial infarction wit ST segment elewation. *Kordiol. Pol.* 61. p.127-131 [in Polish].
10. Jędrychowski W., Penar A. (2000). Statystyczna analiza wyniku badań naukowych w medycynie i biologii. *Wyd. U J, Kraków* [in Polish].
11. Kośmicki M. (2010). Choroba niedokrwienna serca w Polsce i na świecie – nierozwiązany w pełni problem. *Kardiologia Oparta na Faktach*. 1. p.35-48 [in Polish].
12. Modi P., Suleiman S. M., Reeves B. C., Pawade A., Parry A. J., Angelini G.D., Caputo M. (2006). Changes in myocardial free amino acids during pediatric cardiac surgery: a randomised controlled trial of three cardioplegic techniques. *Eur. J. Cardiovasc. Surg.* 30. p.41-48.
13. Murray R.K., Granner D. K., Mayes P. A., Rodwell V. W. (1995). Biochemia Harpera. *PZWL, Warszawa* [in Polish].
14. Naruszewicz M., Zapolska-Downar D. (2006). Molekularne podłoże miażdżycy. *Pol. Przeg. Chir.* 7. p.821–846 [in Polish].
15. Ravel R. (1989). Clinical Laboratory medicine. Clinical application of laboratory data. *Year Book Med. Pub. Inc. London*.
16. Szpetnar M. (2014). Aminokwasy i peptydy białek. Chemia organizmów żywych. *RTN Radom*. p.96-116 [in Polish].
17. Tingberg E., Öhlin A. K. Gottsäter A., Öhlin H. (2006). Lipid peroxidatoin in not increased in heart failure patients on modern pharmacological therapy. *International journal of cardiology*. 112. p.275-281.
18. Zwaigzne-Raczyńska J., Gołębiowska M. (1990). Zachowanie się wolnych aminokwasów w osoczu i krwinkach czerwonych u dzieci z otyłością prostą pod wpływem leczenia odchudzającego. *Pediatr. Pol.* 65 (11/12). p.16-23 [in Polish].