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State of endothelial function, lipid spectrum and features of coronary vessels structure of rats with obesity and insulin resistance under iodine deficiency conditions

T.V. Todoriv[1], M.M. Bagriy[2], N.M. Voronych-Semchenko[1]

[1]Ivano-Frankivsk National Medical University, Ukraine,
[2]Institute of the Pathology and Cytology, Clinik of Medical School of Brandenburg, Neuruppin, Germany; e-mail: taniastrokosh@gmail.com

The aim of the study was to investigate the changes of endothelin-1 content, blood lipid spectrum parameters, structural features of coronary vessels of rats with insulin resistance and obesity under conditions of adequate iodine supply and iodine deficiency. For the modeling of insulin resistance, rats were kept on high-fructose, obesity – high-calorie, iodine deficiency – iodine deficiency diets. It was found that the development of insulin resistance, obesity and iodine deficiency was accompanied by an increase of endothelin-1 level in 2.41, 2.31 times and at 80.17% in blood serum, relative to the data in intact animals. Insulin resistance and obesity under conditions of limited iodine supply leads to the significant changes in endothelial dysfunction (increase in the level of endothelin-1 in 3.02 and 2.50 times relative to control and at 67.38 and 39.40% – relative to mono iodine deficiency) and dyslipidemia (increase in the atherogenic factor at 48.08% – 4.20 times relative to isolated insulin resistance, obesity and iodine deficiency). Such changes were consistent with the structural violations. In insulin-resistant animals focal unevenness of the outer and inner contours, their uneven thickness, areas of homogeneous enlightenment were observed under the conditions of iodine deficiency in the arterioles and minor arteries of the myocardium. In obese animals under the conditions of iodine deficiency, the vessels of the microcirculatory bed were dilated and overflown with erythrocytes. Endotheliocytes with nuclei elongated along the wall, in some places there is a swelling of the cytoplasm of endotheliocytes. In the wall of minor arteries there are transparent vacuoles, areas of homogeneous eosinophilia, which are caused by the accumulation of glycoproteins. Thus, the development of insulin resistance and obesity in iodine deficiency is accompanied by more significant changes in endothelial function and an increase in proatherogenic fractions in the blood lipid spectrum, as evidenced by changes in the structural organization of myocardial vessels than with proper iodine supply.
Key words: endothelin-1; blood lipid spectrum; obesity; insulin resistance; iodine deficiency; cardiovascular system.

INTRODUCTION

The problem of cardiovascular diseases is a priority for the population's health in the global structure of morbidity, especially for industrialized countries. Cardiovascular pathology, according to statistical studies, ranks first among the causes of death, disability, and increased spending on public health in Ukraine [1]. According to the list of tasks approved by the World Health Organization (WHO) in 2013, it is planned to reduce non-infective diseases by 25% by 2025 [2]. Early diagnosis and prevention of cardiovascular pathologies will help to achieve this goal, which will help improve the quality of life, preserve the health and economic development of each country.

According to the results of numerous experimental and clinical studies in recent years, the dominant place in the pathogenesis of the development of cardiovascular system

lesions belongs to the violation of endothelial dysfunction [3]. Among them, there is the formation of pro-inflammatory, prothrombotic, and vasoconstrictor substances on the one hand and the imbalance of vasodilating, anti-inflammatory, antiatherogenic, antiproliferative factors on the other hand [4]. Endothelin-1 polypeptide is one of the most important regulators of the functional state of vascular endothelium, which determines the vasoactive effect. Low concentrations of the peptide lead to vascular relaxation associated with the disorder of endothelin receptors type B as a result of the release of vasoactive factors from the endothelium (nitric oxide, prostacyclin and natriuretic peptide of the atria). High levels of endothelin-1 mediate the vasoconstrictor effect due to the stimulation of type A receptors on the surface of vascular smooth muscle, which promotes the activation of G-proteins and phospholipase C, followed by an increase in calcium concentration in vascular smooth cells and the development of vasoconstriction [5]. The main activators of endothelin-1 secretion are hypoxia, ischemia, inflammation, growth of proatherogenic fractions of lipids in blood serum [6].

According to epidemiological studies of the last decade, there has been a steady tendency towards an increase of cases of overweight, diabetes mellitus (especially insulin-resistant) and thyroid pathology, which are considered to be the diseases of civilization that may increase the risk of cardiovascular disease [1, 2]. It's important, that non-insulin-dependent diabetes and obesity are associated with a more complex course, more severe prognosis, and higher mortality in patients with COVID-19. Vascular endothelial dysfunction is one of the main reasons for initiating the development of the cardiovascular system damage and its progression, in particular, in coronavirus disease. In this regard, the study of vascular endothelial function in obesity, insulin resistance, and hypothyroidism remain relevant.

The aim of the study: to study changes in endothelin-1 content, blood lipid spectrum parameters, structural peculiarities of myocardial vessels of rats with obesity, and insulin resistance under the conditions of adequate iodine supply and iodine deficiency.

METHODS

The studies were performed using non-linear mature male rats weighing 150-180 g, randomized by the method of convenience sampling. Animals were divided into the following groups: group 1st – control (intact animals, n = 15), group 2nd – animals with insulin resistance under the conditions of proper iodine supply (n = 15), group 3rd – obese animals under the conditions of proper iodine provision (n = 15), group 4th – animals with hypothyroid dysfunction against the background of iodine deficiency (n = 15), group 5th – insulin-resistant animals under the conditions of iodine deficiency, and group 6th – animals with obesity under the conditions of iodine deficiency (n = 15).

Animals of the control (1st) group were on a standard diet. In order to simulate insulin resistance (2nd and 5th groups), the animals received a 10% solution of fructose instead of drinking water for eight weeks [7]. To model obesity (3rd and 6th groups) rats were kept on a high-calorie diet [8]. Rats of groups 4th-6 th were on an iodine deficiency diet for two months [9]. Carbohydrate metabolism markers (insulin content, serum glucose concentration and glycosylated hemoglobin – HbA1c in whole blood) were determined of experimental rats, followed by calculation of the HOMA-IR index. Control over the reproduction of alimentary obesity was carried out by weighing the animals, measuring the nasal-anal length and calculating the body mass index (BMI). To assess the thyroid status of animals, the content of free triiodothyronine (FT_3) and free thyroxine (FT_4), thyroid-stimulating hormone (TSH) in the blood serum was studied by using the kit enzyme-linked immunosorbent assay "ELISA" (Elabscience, USA), followed by calculation of

FT_3/FT_4, TSH/FT_4 indices. The iodine supply of rats was assessed according to the concentration of iodine in the daily portions of urine, which was collected by the method of metabolic cells [10].

The functional state of the endothelium was examined according to the level of endothelin-1 in the blood serum by enzyme-linked immunosorbent assay using reagents "Biocompare" (Austria).

In blood serum, the levels of total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides (TG) were determined and also the atherogenic index (AI) was calculated. The content of TC, LDL, HDL, TG was determined using standard sets of LLC SPE "Filicit-Diagnostics" (Dnipropetrovsk, Ukraine). AI was calculated according to the formula: AI = (TC – HDL) : HDL.

For general histological examination and special histological examinations, the heart was fixed in a 10% solution of neutral formalin (pH = 7.0). In the next step, the pieces of the heart were placed in an ascending battery of alcohols for dehydration, chloroform, a mixture of chloroform-paraffin (1:1), paraffin (at a temperature of 37°C). After paraffin preparation, pieces of the heart were poured into paraffin. The production of serial paraffin sections with a thickness of 4-6 μm was performed using a sledge microtome. Histologic cuts of the heart were stained with hematoxylin and eosin, according to Shabadash (identification of glycogen), PAS-staining was performed (verification of glycoproteins) [11].

Conditions of keeping and manipulation of animals during the study, removal of rats from the experiment met the requirements of Ukrainian legislation (Law of Ukraine No. 3447-IV "On protection of animals from cruel treatment", 2006), Order of the Ministry of Health of Ukraine No. 281 dated 01.11.2000 "On measures to further improvement of the organizational standards for the use of experimental animals" and the principles of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986).

Statistical analysis of digital data was performed using the computer program Exel of Microsoft Office 365 ProPlus. For each of the samples it was checked whether the distribution of the studied indicator was normal, using the Kolmogorov-Smirnov and Lilliefors criteria. Taking into account that the obtained data corresponded to Gauss law, the results are represented by the interval M ± m. The significance of differences was assessed by the Student's t-test, the correlation – by Pearson index r. The difference in parameters was considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

As a result of the study performed in animals of the control (1st) group, the indices of thyroid homeostasis corresponded to the reference data for intact animals. In insulin resistance (2nd group) a decrease in FT_3 and FT_4 at 18.99 ($P < 0.05$) and 13.06% ($P < 0.05$) relative to control data was observed. In obesity 3rd group in animals, there was found an increase in the content of TSH in the blood serum at 23.08% ($P < 0.05$) relative to the values in intact animals. In animals of groups 4th-6th, changes in thyroid homeostasis were the most significant and reflected the development of hypothyroid dysfunction (Table 1). In particular, in isolated iodine deficiency (4th group) there was a decrease in FT_3 and FT_4 at 35.11 and 37.02% ($P < 0.001$) against the background of an increase in TSH at 53.85% ($P < 0.001$), FT_3/FT_4 – at 18.18% ($P < 0.05$) and TSH/FT_4 – in 2.2 times ($P < 0.001$) relative to control. In rats of the 5th and 6th groups, changes in thyroid status were more pronounced. Thus, there were found a decrease in FT_3 and FT_4 at 64.13 ($P < 0.001$) and at 58.28% ($P < 0.001$) and at 28.27 ($P < 0.001$) and at 23.92% ($P < 0.01$), an increase in TSH at 30.77 ($P < 0.01$) and at 61.53% ($P < 0.001$), TSH/FT_4 – twice ($P < 0.05$) and in 2.4 times

($P < 0.001$) relative to the data in intact animals.

The most pronounced changes in carbohydrate homeostasis were observed in animals of the groups 2nd and 5th (Table 2). Thus, in rats of the 2nd group there was found an increase in blood serum insulin level at 20.85% ($P < 0.001$), glucose – at 43.06% ($P < 0.01$), HbA1c – at 91.92% ($P < 0.001$) and HOMA-IR index – at 94.02% ($P < 0.001$) relative to control values. In animals of the 5th group there was an increase in the concentration of insulin – at 54.16% ($P < 0.001$), glucose – at 64.58% ($P < 0.01$), HbA1c – in 2.05 times ($P < 0.001$) and HOMA-IR index – in 2.54 times ($P < 0.001$) relative to data in intact animals. In obesity there was found an increase in the concentration of insulin – at 20.85% ($P < 0.01$), glucose – at 33.79% ($P < 0.01$), HbA1c – at 35.65% ($P < 0.05$) and HOMA-IR index – at 64.14% ($P < 0.01$) relative to control data. Carbohydrate metabolism in rats on an iodine deficiency diet did not differ significantly from the control group.

The experiment there was determined an absolute increase in BMI in animals of all groups at 30.43 – 71.74% (most significantly in rats of group 3rd – at 52.17%, $P < 0.01$ and group 6th – at 71.74%, $P < 0.01$).

In animals, under conditions of insulin resistance (2nd group), there were observed an increase (in 2.41 times) in blood serum level of endothelin-1 ($P < 0.01$), TC – at 33.06% ($P < 0.05$), TG – in 2,04 times ($P < 0.01$) and LDL – at 53.85% ($P < 0.05$) against the background of a decrease in HDL – at 50.96% ($P < 0.01$) relative to the data in the control group (Table 3). Under such conditions, the AI increased in 5.32 times

Table 1. Indexes of thyroid status in rats with insulin resistance, obesity, iodine deficiency and their combination (M ± m; n = 15)

Groups of animals	FT_3, pmol/l	FT_4, pmol/l	TSH, mIU/l	FT_3/FT_4,	ТТГ/FT_4,	Iodine in urine,mcg/l
1st control group (intact animals)	6.58 ± 0.30	28.93 ± 1.04	0.13 ± 0.01	0.22 ± 0.01	0.005 ± 0.001	103.24 ± 4.01
2nd experimental group (animals with insulin resistance)	5.33 ± 0.37 $P_{1-2} < 0.05$	25.15 ± 1.24 $P_{1-2} < 0.05$	0.10 ± 0.02	0.20 ± 0.01	0.004 ± 0.001	94.63 ± 3.31
3rd experimental group (animals with obesity)	6.96 ± 0.47 $P_{2-3} < 0.05$	29.33 ± 1.62	0.16 ± 0.009 $P_{1-3} < 0.05$ $P_{2-3} < 0.05$	0.24 ± 0.07 $P_{2-3} < 0.05$	0.007 ± 0.001	92.62 ± 2.98 $P_{1-3} < 0.05$
4th experimental group (iodine deficiency animals)	4.27 ± 0.33 $P_{1-4} < 0.001$ $P_{2-4} < 0.05$ $P_{3-4} < 0.05$	18.22 ± 1.74 $P_{1-4} < 0.001$ $P_{2-4} < 0.01$ $P_{3-4} < 0.001$	0.20 ± 0.01 $P_{1-4} < 0.001$ $P_{2-4} < 0.001$ $P_{3-4} < 0.01$	0.26 ± 0.01 $P_{1-4} < 0.05$ $P_{2-4} < 0.001$	0.011 ± 0.001 $P_{1-4} < 0.001$ $P_{2-4} < 0.001$ $P_{3-4} < 0.05$	52.65 ± 6.58 $P_{1-4} < 0.001$ $P_{2-4} < 0.001$ $P_{3-4} < 0.001$
5th experimental group (insulin-resistant animals under conditions of iodine deficiency)	2.36 ± 0.38 $P_{1-5} < 0.001$ $P_{2-5} < 0.001$ $P_{3-5} < 0.001$ $P_{4-5} < 0.01$	13.17 ± 2.29 $P_{1-5} < 0.001$ $P_{2-5} < 0.001$ $P_{3-5} < 0.001$	0.17 ± 0.009 $P_{1-5} < 0.01$ $P_{2-5} < 0.01$ $P_{4-5} < 0.01$	0.18 ± 0.01 $P_{1-5} < 0.05$ $P_{3-5} < 0.001$ $P_{4-5} < 0.001$	0.010 ± 0.002 $P_{1-5} < 0.05$ $P_{2-5} < 0.05$	61.68 ± 6.45 $P_{1-5} < 0.001$ $P_{2-5} < 0.001$ $P_{3-5} < 0.001$
6th experimental group (animals with obesity under conditions of iodine deficiency)	4.72 ± 0.30 $P_{1-6} < 0.001$ $P_{5-6} < 0.001$	22.01 ± 1.85 $P_{1-6} < 0.01$ $P_{3-6} < 0.01$ $P_{5-6} < 0.01$	0.21 ± 0.02 $P_{1-6} < 0.01$ $P_{2-6} < 0.01$ $P_{3-6} < 0.05$	0.25 ± 0.01 $P_{2-5} < 0.01$ $P_{5-6} < 0.001$	0.012 ± 0.75 $P_{1-6} < 0.01$ $P_{2-6} < 0.01$ $P_{3-6} < 0.05$	65.20 ± 5.62 $P_{1-6} < 0.001$ $P_{2-6} < 0.001$ $P_{3-6} < 0.001$

Note: Here and in the following Tables P – a reliable difference between the indexes experimental groups

($P < 0.01$) relative to the basic data. It is known that insulin resistance causes changes in the activity of lipoprotein lipase and hepatic triglyceride lipase and stimulates the synthesis of TG from fructose in the liver, which reduces the synthesis of phospholipids, causes the accumulation of LDL and deficiency of HDL [12].

Morphological examination of the vessels of the myocardium of insulin-resistant rats in part of the capillaries revealed endothelial cells with hypercellularity. The nuclei of such endothelial cells are irregularly shaped, slightly hyperchromic, in places layered on top of each other. Freely located erythrocytes are noted perivascularly around a part of capillaries. In arterioles and minor arteries like capillaries, endothelial hypercellularity is noted. The wall of part of such vessels is unevenly homogeneous, with areas of enlightenment (Fig. 1A). This causes unevenness of its thickness and inner and outer surfaces. Endothelial hypercellularity and uneven thickening of the vessel wall are accompanied by a narrowing of their lumen.

In obese rats, unidirectional but more significant changes in the studied parameters were observed. Thus, in the blood serum of animals of the 3th experimental group there was found an increase in the content of endothelin-1 – in 2.31 times ($P < 0.001$), TC – at 51.62% ($P < 0.01$), TG – in 2.67 times ($P < 0.001$), LDL – at 76.92% ($P < 0.001$) and AI – in 9.38 times ($P < 0.01$) against the background of a decrease in the content of HDL at 64.42% ($P < 0.001$) (Table 3). In obesity, some lipoproteins accumulate in adipocytes, which inhibits the increased synthesis of cholesterol in the liver. At the same time, the activity of LDL receptors

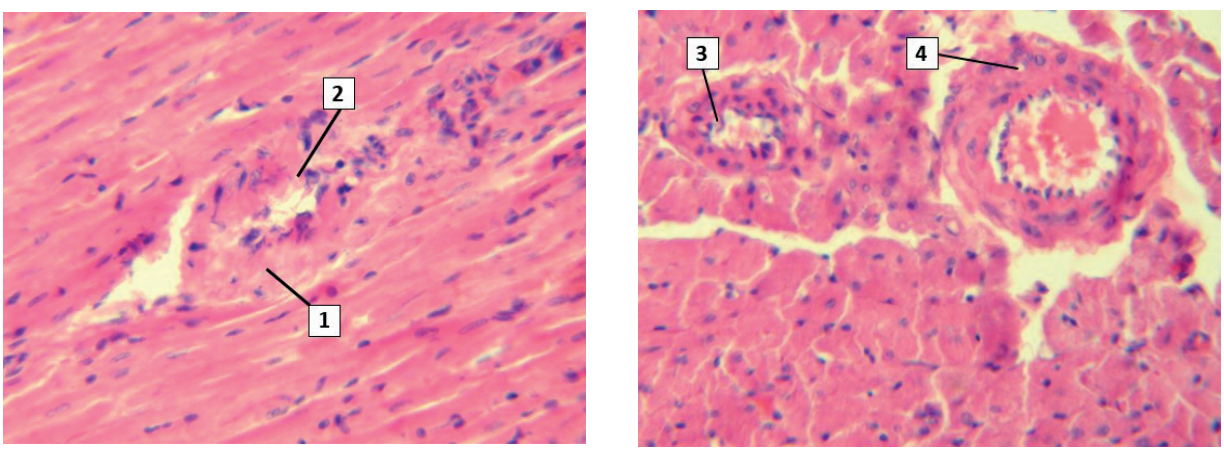
Table 2. Indexes of carbohydrate metabolism in rats with insulin resistance, obesity, iodine deficiency and their combination (M ± m; n = 15)

Groups of animals	Insulin, mkOD/l	Glucose, mmol/l	Glycosylated Hb, μmol of fructose/g Hb	HOMA-IR index
1st control group (intact animals)	13.09 ± 0.53	4.32 ± 0.40	3.59 ± 0.42	2.51 ± 0.28
2nd experimental group (animals with insulin resistance)	17.71 ± 0.52 $P_{1-2} < 0.001$	6.18 ± 0.41 $P_{1-2} < 0.01$	6.89 ± 0.47 $P_{1-2} < 0.001$	4.87 ± 0.35 $P_{1-2} < 0.001$
3rd experimental group (animals with obesity)	15.82 ± 0.72 $P_{1-3} < 0.01$ $P_{2-3} < 0.05$	5.78±0.28 $P_{1-3} < 0.01$	4.87 ± 0.39 $P_{1-3} < 0.05$ $P_{2-3} < 0.01$	4.12 ± 0.37 $P_{1-3} < 0.01$
4th experimental group (iodine deficiency animals)	13.41 ± 0.75 $P_{2-4} < 0.001$ $P_{3-4} < 0.05$	4.28 ± 0.44 $P_{2-4} < 0.01$ $P_{3-4} < 0.05$	4.23 ± 0.92 $P_{2-4} < 0.05$	2.55 ± 0.29 $P_{2-4} < 0.001$ $P_{3-4} < 0.01$
5th experimental group (insulin-resistant animals under conditions of iodine deficiency)	20.18 ± 1.05 $P_{1-5} < 0.001$ $P_{3-5} < 0.01$ $P_{4-5} < 0.001$	7.11 ± 0.30 $P_{1-5} < 0.001$ $P_{3-5} < 0.01$ $P_{4-5} < 0.001$	7.36 ± 0.82 $P_{1-5} < 0 .001$ $P_{3-5} < 0.05$ $P_{4-5} < 0.05$	6.37 ± 0.53 $P_{1-5} < 0.001$ $P_{2-5} < 0.05$ $P_{3-5} < 0.01$ $P_{4-5} < 0.001$
6th experimental group (animals with obesity under conditions of iodine deficiency)	16.01 ± 0.81 $P_{1-6} < 0.01$ $P_{4-6} < 0.05$ $P_{5-6} < 0.01$	6.72 ± 0.36 $P_{1-6} < 0.001$ $P_{4-6} < 0.001$	5.12 ± 0.56 $P_{1-6} < 0.001$ $P_{2-6} < 0.05$ $P_{5-6} < 0.05$	4.79 ± 0.25 $P_{1-6} < 0.001$ $P_{4-6} < 0.001$ $P_{5-6} < 0.01$

Table 3. The content of endothelin-1 and indicators of lipid metabolismin rats with insulin resistance, obesity, iodine deficiency and the conditions of their combination (M ± m; n = 15)

Groups of animals	Endothelin-1, fmol/l	TC, mmol/l	TG, mmol/l	HDL, mmol/l	LDL, mmol/l	AI, cu
1st control group (intact animals)	8.27 ± 0.41	1.24 ± 0.15	0.52 ± 0.13	1.04 ± 0.13	0.39 ± 0.05	0.39 ± 0.11
2nd experimental group (animals with insulin resistance)	19.94 ± 3.31 $P_{1-2} < 0.01$	1.65 ± 0.12 $P_{1-2} < 0.05$	1.06 ± 0.13 $P_{1-2} < 0.01$	0.51 ± 0.12 $P_{1-2} < 0.01$	0.60 ± 0.07 $P_{1-2} < 0.05$	2.04 ± 0.69 $P_{1-2} < 0.05$
3rd experimental group (animals with obesity)	19.12 ± 2.47 $P_{1-3} < 0.001$	1.88 ± 0.10 $P_{1-3} < 0.01$	1.39 ± 0.15 $P_{1-3} < 0.001$	0.37 ± 0.08 $P_{1-3} < 0.001$	0.69 ± 0.06 $P_{1-3} < 0.01$	3.66 ± 0.64 $P_{1-3} < 0.01$
4th experimental group (iodine deficiency animals)	14.90 ± 2.18 $P_{1-4} < 0.01$	1.57 ± 0.04 $P_{1-4} < 0.05$ $P_{2-4} < 0.05$	0.86 ± 0.09 $P_{1-4} < 0.05$ $P_{3-4} < 0.01$	0.68 ± 0.07 $P_{1-4} < 0.05$ $P_{3-4} < 0.05$	0.57 ± 0.06 $P_{1-4} < 0.05$	1.29 ± 0.39 $P_{1-4}<0.05$
5th experimental group (insulin-resistant animals under conditions of iodine deficiency)	24.94 ± 3.84 $P_{1-5} < 0.001$ $P_{4-5} < 0.05$	1.85 ± 0.12 $P_{1-5} < 0.01$ $P_{2-5} < 0.05$	1.21 ± 0.18 $P_{1-5}<0.01$	0.39 ± 0.06 $P_{1-5} < 0.001$ $P_{4-5} < 0.01$	0.78 ± 0.11 $P_{1-5} < 0.01$	3.76 ± 0.81 $P_{1-5} < 0.01$
6th experimental group (animals with obesity under conditions of iodine deficiency)	20.77 ± 2.78 $P_{1-6} < 0.001$ $P_{4-6} < 0.05$	2.13 ± 0.21 $P_{1-6} < 0.01$	1.62 ± 0.18 $P_{1-6} < 0.001$ $P_{2-6} < 0.05$ $P_{4-6} < 0.01$	0.31 ± 0.10 $P_{1-6} < 0.001$ $P_{4-6} < 0.01$	1.11 ± 0.11 $P_{1-6} < 0.001$ $P_{2-6} < 0.01$ $P_{3-6} < 0.01$ $P_{4-6} < 0.001$ $P_{5-6} < 0.05$	5.42 ± 1.05 $P_{1-6} < 0.001$ $P_{2-6} < 0.05$ $P_{5-6} < 0.05$

in adipose tissue increases, and the reverse transport of TC in HDL decreases. Being overweight is characterized by a decrease in tolerance to exogenous lipids. A long and high hyperlipidemic reaction to food intake in response to fat load has been established [13].



A B

Fig. 1. Myocardial arteries of the rat with insulin resistance (A) and obesity (B); 1 – plasma permeation of the artery wall of the muscular type, 2 – deformation of endothelial cells, 3 – vacuoles in the cytoplasm of endothelial cells of small arteries of the muscular type, 4 – vacuoles in the cytoplasm of smooth myocytes of the middle lining of the arteries. Staining: hematoxylin and eosin, 400×

In overweight animals in the minor arteries of the myocardium, the focal clarifications of the cytoplasm of the inner membrane cells are singly visualized due to the presence of small transparent vacuoles. This is accompanied by a slight protrusion of the cytoplasm to the lumen of the capillary. The nuclei of such endothelial cells retain an elongated character. Small-vacuolar enlightenments in a middle cover in smooth myocytes are visualized in vessels (see Fig. 1B). There is a slight growth of connective tissue fibers around single minor arteries.

Restriction of iodine supply in animals has led to an increase of endothelin-1 in blood serum – at 80.17% ($P < 0.01$), TC – at 26.61% ($P < 0.05$), TG – at 65.38% ($P < 0.01$), LDL at 46.15% ($P < 0.01$), AI – in 3.30 times ($P < 0.05$) and to a decrease in HDL concentration at 34.61% ($P < 0.05$) (Table 3). It is known that iodine deficiency contributes to the development of hypercholesterinemia due to increased reabsorption of TC in the intestine with a decrease in the catabolism of LDL, which is due to a decrease in the content of LDL receptors in the liver and increase of its serum content. Prolonged iodine deficiency leads to a violation of the HDL structure, the change in the reverse transport of TC, which is the main antiatherogenic process [14].

In the arterioles of the myocardium of obese rats there is a weak condensation of chromatin in the nuclei of endothelial cells, in some – edema and vacuolation of the cytoplasm of endothelial cells, single macrophages and lymphocytes periarterially. Minor arteries with the phenomena of edema and vacuolation of the cytoplasm of endothelial cells, which slightly penetrates into the lumen of blood vessels. The nuclei are somewhat hyperchromic, irregularly rounded. Other endothelial cells that do not experience edematous phenomena have spindle-shaped extensions along the nucleus wall. The minor veins of the myocardium in the lumen contain a moderate number of compact erythrocytes. Endotheliocytes with clear spindle-shaped homogeneous nuclei extended along the vessel wall, a small amount of cytoplasm without signs of vacuolation, clasmatosis.

Attention is drawn to the changes in the studied parameters in insulin-resistant animals having been on the iodine deficiency diet (5^{th} group). In rats of this group, signs of endothelial dysfunction increased, reflecting a threetimes increase in endothelin-1 level ($P < 0.001$). Dyslipidemia, which we've observed in insulin resistance against the background of iodine deficiency, was characterized by an increased content of LDL (twice, $P < 0.01$), TC (at 50.00%, $P < 0.01$) and TG (in 2.33 times, ($P < 0.01$), AI – in 7.49 times ($P < 0.05$) relative to control data (see Table 3). Such changes of the lipid spectrum of blood can be caused by deficiency of hormones of a thyroid gland and insulin resistance which causes a decrease in the activity of lipoprotein lipase, which in turn increases the flow of free fatty acids into the blood from adipose tissue. The level of HDL, which has anti-atherogenic properties, in the blood serum of animals with combined pathology is reduced [12].

In animals of the group 5^{th}, the studied parameters differed significantly from the data under conditions of isolated iodine deficiency (endothelin-1 concentration increased at 67.38%, $P_{4-5} < 0.05$; AI increased in 2.91 times, $P_{4-5} < 0.05$) and insulin resistance (the content of TC – at 12.73%, $P_{2-5} < 0.05$; HDL – at 57.35%, $P_{2-5} < 0.01$ have increased), which confirms the potentiation of the negative impact on the cardiovascular system of combined endocrinopathy.

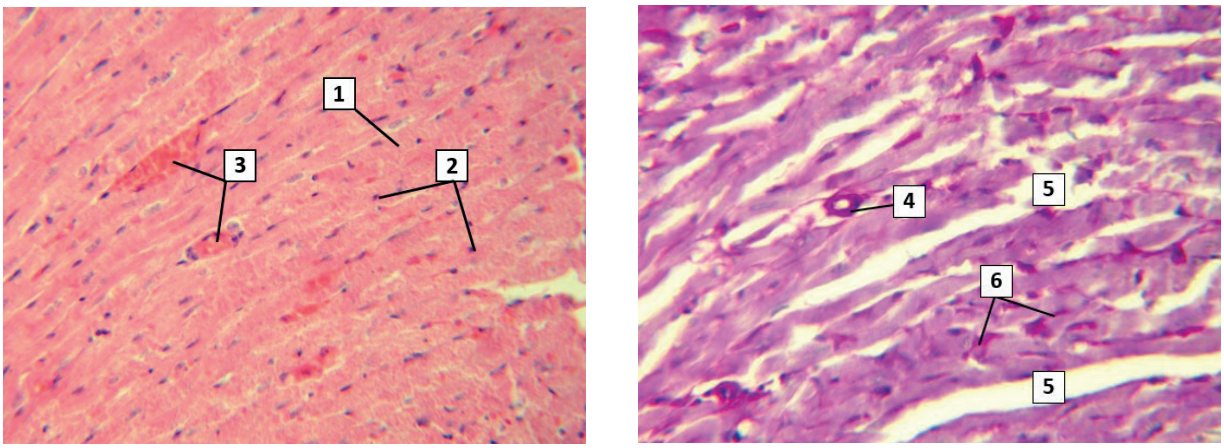
Vessels of the microcirculatory bed of the animals' myocardium of the experimental 5^{th} group are filled with erythrocytes, dilated (Fig. 2A). In the lumen of individual vessels, red blood cells are compact, the boundaries between them are not clearly defined. Erythrocytes are also visualized perivascularly. In a small number of vessels of the microcirculatory bed, along with erythrocytes, there are single leukocytes. Endothelial cells are focal with slightly vacuolated cytoplasm, nuclei are

of irregular ovoid shape, in places they are layered on top of each other. In a wall of single arterioles and minor arteries zones of the strengthened eosinophilic coloring are found, with homogenization of a wall in the given areas, hyalinosis of an arteriole of the myocardium is observed (Fig. 2B).

Under conditions of animals' being on high-fat and iodine-deficient diets at the same time (experimental 6th group), the content of endothelin-1 in the blood serum has increased by 2.50 times ($P < 0.001$) compared to data of intact animals. Under such conditions, the indices of the lipid spectrum of the blood had the following dynamics relative to the control data: the concentration of total cholesterol has increased at 71.77% ($P < 0.01$), TG – by 3.11 times ($P < 0.001$), and LDL – by 2.85 times ($P < 0.001$), the HDL content has decreased at 70.19% ($P < 0.001$) (see Table 3). Such changes in the lipid spectrum of the blood caused an increase in AI – by 13.89 times ($P < 0.001$) relative to control.

Significant differences between the studied parameters in animals of groups 6th, 3rd and 4th were established. In particular, in the blood serum of obese rats under conditions of iodine deficiency the concentration of LDL cholesterol was higher at 60.87% ($P_{3-6} < 0.01$) compared to data in animals with isolated obesity. There was higher TG level – in 2.13 times ($P_{4-6} < 0.001$), LDL cholesterol at 94.74% ($P_{4-6} < 0.001$), but lower HDL cholesterol at 54.41% ($P_{4-6} < 0.01$) regarding data in animals with mono iodine deficiency. At the same time, the AI in rats of the 6th group was higher at 48.08% ($P_{3-6} < 0.05$) relative to the data under the conditions of obesity and in 4.20 times ($P_{4-6} < 0.01$) – under the conditions of iodine deficiency. The higher serum endothelin-1 content is noteworthy at 39.40% ($P_{4-5} < 0.05$) compared to animals with isolated iodine deprivation.

In obese rats, against the background of limited iodine supply, the vessels of the microcirculatory bed are dilated and overflown with erythrocytes, which are sometimes compactly located next to each other. Endotheliocytes are with nuclei elongated along the wall, there is a swelling of the cytoplasm of endotheliocytes. In a wall of some minor arteries there are small, and also average-sized transparent vacuoles (Fig. 3A). The presence of vacuoles of medium caliber leads to the displacement of the nucleus to the periphery of the cytoplasm of individual smooth myocytes. Along with this,



A B

Fig 2. The myocardium of rat with insulin resistance under iodine deficiency (1 – granularity of the cytoplasm of cardiomyocytes, 2 – karyopyknosis of cardiomyocyte nuclei, 3 – the plethora of the microcirculatory bed, 4 – circular equable accumulation of glycoproteins in the wall of arteriole, 5 – myocardial interstitial edema, 6 – the plethora of vessels of the microcirculatory bed vessels). Staining: hematoxylin and eosin (A), PAS (B), 400×

local enlightenments with blurred borders and irregular shapes are visualized in the wall, as well as areas of homogeneous eosinophilia, which are caused by excessive accumulation of glycoproteins in the vessel wall (Fig. 3B).

As a result of the correlation analysis between the concentration of endothelin-1 in the blood serum and AI ($r = 0.56$, $P < 0.05$), TC ($r = 0.63$, $P < 0.01$), there was established a significant, direct correlation. There was also a direct, significant correlation between the content of total cholesterol and BMI in the blood serum ($r = 0.52$, $P < 0.05$). Such data confirm the role of endothelial dysfunction among the main pathogenetic mechanisms of cardiovascular disease and a significant risk factor for thromboembolic complications (heart attack, stroke, etc.).

In general, the tendency to increase the content of endothelin-1 in the blood serum and proatherogenic fractions of the lipid profile, AI, in experimental animals that were on high-fat and high-carbohydrate, iodine-deficient diets, draws attention. Due to the fact that endothelin-1 controls vascular tone, local processes of hemostasis, cell proliferation, such an imbalance can be a trigger mechanism for changes in lipid metabolism and a predictor of atherosclerosis, as well as one of the links in vascular complications in coronavirus disease. These biochemical disorders were consistent with structural changes in myocardial vessels. It can be argued that hypothyroid dysfunction, which can develop in residents of goiter-endemic regions, will adversely affect the development of endothelial dysfunction and dyslipidemia, and iodine deficiency is a factor that potentiates comorbid structural-metabolic changes in the cardiovascular system.

CONCLUSIONS

The development of insulin resistance, obesity and iodine deficiency is accompanied by the development of endothelial dysfunction and dyslipidemia. Burdening by insulin resistance and iodine deficiency obesity is accompanied by an increase of endothelin-1 level in blood serum at 67.38% (only in the insulin resistance against the background of iodine deficiency relative to monoiodine deficiency), TC (at 12.73 – 49.19%), TG (at 52.83% – in 2.13 times), LDL (60.87% – 94.74%) and AI (at 48.08% in 4.20 times) in relation to isolated models of insulin resistance, iodine deficiency and obesity. BMI substantially affects the concentration of total cholesterol

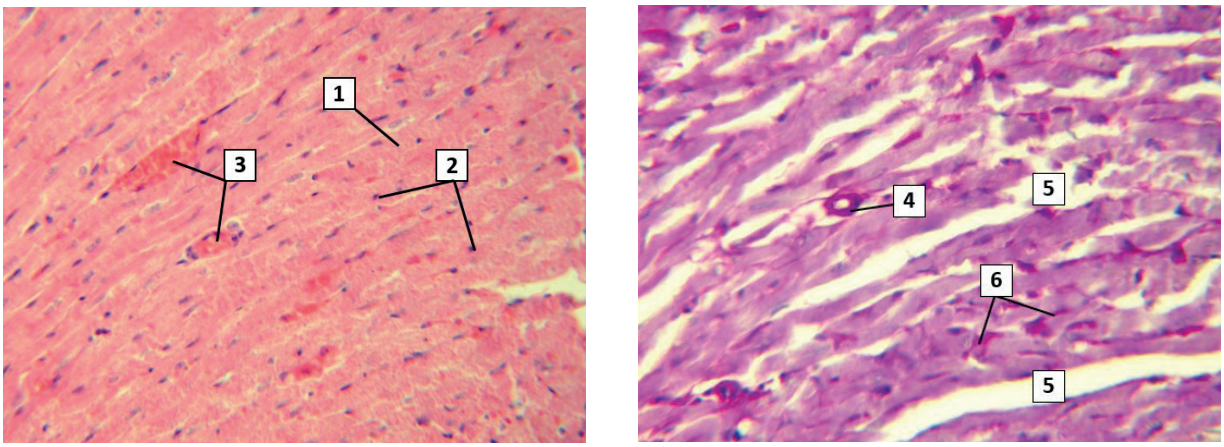


Fig. 3. The myocardium of rat with insulin resistance under iodine deficiency (1 – vacuolation of smooth myocytes of the wall of minor arteries of muscular type, 2 – local enlightenment in the middle lining of arteries, 3 – dilation of the interstitium perivascularly, 4 – dilation of the interstitium between the cardiac muscle cells; 5 – glycoproteins in the arteriole wall, 6 – edema of the myocardial interstitium). Staining: hematoxylin and eosin (A), PAS (B), 400×

in the blood serum of experimental animals (r = 0.52). As a result of correlation analysis, a direct, medium-strength correlation was found between the concentration of endothelin-1 in the serum and AI (r = 0.56), TC (r = 0.63).

Such changes were consistent with structural violations. Morphological examination of myocardial vessels in experimental rats revealed structural changes in the microcirculatory bed (in arterioles and minor arteries, like in capillaries, endothelial hypercellularity is noted, which with uneven thickening of the vessel wall causes narrowing of their lumen). The most pronounced changes in the structural organization of blood vessels were observed in combined endocrinopathies, which significantly increases the cardiovascular risks in such conditions. In general, comorbid pathologies are manifested by the increased endothelial dysfunction and proatherogenic changes in the blood lipid spectrum, which is confirmed by changes in the structural organization of myocardial vessels.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

Т.В. Тодорів[1], М.М. Багрій[2], Н.М. Воронич-Семченко[1]

СТАН ЕНДОТЕЛІАЛЬНОЇ ФУНКЦІЇ, ЛІПІДНИЙ СПЕКТР ТА ОСОБЛИВОСТІ СТРУКТУРИ КОРОНАРНИХ СУДИН ЩУРІВ З ОЖИРІННЯМ Й ІНСУЛІНОРЕЗИСТЕНТНІСТЮ ЗА УМОВ ЙОДОДЕФІЦИТУ

[1]Івано-Франківський національний медичний університет, Україна;
[2]Інститут патології та цитології медичної клініки та медичного інституту Федеральної Землі Бранденбург, Німеччина; e-mail: taniastrokosh@gmail.com

Метою роботи було дослідження зміни вмісту ендотеліну-1, показників ліпідного спектра крові, структурних особливостей коронарних судин щурів із інсулінорезистентністю й ожирінням за умов належного забезпечення йодом і йододефіциту. Для моделювання інсулінорезистентності щурів утримували на високофруктозній, ожиріння – висококалорійній, йододефіциту – йододефіцитній дієтах. Виявлено, що розвиток інсулінорезистентності, ожиріння та йододефіциту супроводжувався збільшенням у сироватці крові вмісту ендотеліну-1 у 2,41, 2,31 раза та на 80,17% відповідно щодо значень у інтактних тварин. Інсулінорезистентність та ожиріння за умов обмеженого забезпечення йодом зумовлює суттєвіші зміни ендотеліальної функції (збільшення вмісту ендотеліну-1 у 3,02 і 2,50 раза щодо контролю й на 67,38 і 39,40% – щодо значень при монойоддефіциті) і дисліпідемії (зростання коефіцієнта атерогеності на 48,08% – у 4,20 раза щодо ізольованих інсулінорезистентності, ожиріння та йододефіциту). Такі зміни узгоджувалися зі структурними порушеннями. В інсулінорезистентних тварин за умов йододефіциту в артеріолах та дрібних артеріях міокарда спостерігали осередкову нерівномірність зовнішнього та внутрішнього контурів, нерівномірну їх товщину, ділянки гомогенного просвітлення. У тварин із ожирінням за умов йододефіциту судини мікроциркуляторного русла розширені та переповнені еритроцитами. Ендотеліоцити з витягнутими вздовж стінки ядрами, місцями відзначається набряк їх цитоплазми. У стінці дрібних коронарних артерій виявляються прозорі вакуолі, ділянки гомогенної еозинофілії, які зумовлені накопиченням глікопротеїнів. Таким чином, розвиток інсулінорезистентності та ожиріння за умов йододефіциту супроводжується більш істотними змінами ендотеліальної функції та збільшенням проатерогенних фракцій у ліпідному спектрі крові, що підтверджується змінами структурної організації судин міокарда, ніж при належному забезпеченні йодом.

Ключові слова: ендотелін-1; ліпідний спектр крові; ожиріння; інсулінорезистентність; йододефіцит; серцево-судинна система.

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