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Effect of diet-induced hypercholesterolemia on metabolic processes in the heart, liver, and pancreas in rats

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Abstract

Aim. To analyze the biochemical changes in the cells of the heart muscle, liver and pancreas, as well as to establish their pathogenetic significance in diet-induced experimental hypercholesterolemia.

Methods. The study was conducted on 65 outbred male rats. During the experiment, the animals were divided into groups: the first (control, n=30) — animals that were kept on a general vivarium diet; the second (experimental, n=35) — animals with diet-induced hypercholesterolemia for three months by keeping on a special diet. At the end of the experiment, the concentrations of pyruvic acid, lactate, reduced glutathione, the activity of glutathione reductase, and glutathione peroxidase were determined in the tissues by using biochemical methods. The Student's t-test was used for the experimental data of the samples after normality testing.

Results. The analysis of energy metabolism indicators in animals with hypercholesterolemia relative to the control group revealed a lower level of pyruvic acid in the heart muscle (0.29±0.03 mmol/mg protein; $p \leq 0.05$) and liver (0.25±0.02 mmol/mg protein; $p > 0.001$). A significantly higher lactate level was recorded in all tissues, most pronounced in the liver (6.73±0.6 mmol/mg protein; $p \leq 0.001$). The results obtained indicate the predominance of the anaerobic glycolysis in the tissues and the accumulation of incomplete-oxidation products. The study of the key glutathione-linked enzymes in animals with hypercholesterolemia relative to the control showed a lower activity of glutathione reductase in the pancreas — 0.52±0.05 mmol/mg protein/min ($p \leq 0.001$), as well as its higher activity in the liver — 0.297±0.03 mmol/mg protein/min ($p \leq 0.001$) and heart — 13.58±1.4 mmol/mg protein/min ($p > 0.001$). The activity of glutathione peroxidase and reduced glutathione in all organs remained practically unchanged, or the differences were insignificant. This trend indicates a violation of the antioxidant defense system and oxidative stress.

Conclusion. Changes in the metabolic link of adaptive-compensatory responses in the cells of the heart muscle, liver, and, most pronounced in the pancreas, indicate the role of the pancreas as a "target organ" in the pathogenesis of diet-induced hypercholesterolemia.

Keywords: hypercholesterolemia, heart muscle, liver, pancreas.

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Background. Hypercholesterolemia is one of the triggering mechanisms for the development of atherosclerosis and pathology of the cardiovascular system that remains unelucidated [1, 2]. Nevertheless, impaired cholesterol homeostasis can be regarded as a stress factor leading to an imbalance of regulatory influences on the major systems of the body and altering the adaptive potential of the body [3].

Therefore, it should be assumed that changes in key metabolic processes in the tissue of the heart, liver, and pancreas in hypercholesterolemia lead to an impairment of adaptive-compensatory mechanisms and cause structural and functional damage.

The degree of damage to cellular structures depends on the organism's reactivity and has its own characteristics in various organs. To date, the effect of hypercholesterolemia on metabolic interorgan relationships remains unknown [4, 5], which is required in the assessment of the pathogenetic significance of organ-specific changes needed in the development of complex drug correction schemes and the testing of new dietary products.

The existing data on the mechanisms of cholesterol homeostasis impairment are largely controversial due to the ambiguity of methodological approaches and research design [6–9], as well as the involvement of experimental models since hypercholesterolemia is achieved by exogenous administration of cholesterol [10, 11]. The alimentary origin of hypercholesterolemia and the related nature of the rearrangements of metabolic processes in the blood, organs, and tissues remain unclear.

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Useful information can be obtained by creating adequate models of hypercholesterolemia using the alimentary factor. In this study, we developed and used a model of essential hypercholesterolemia based on the preparation of a high-fat diet composed of semolina, cane sugar, butter, and unsalted lard, which was characterized by a change in the lipid status and the formation of dyslipidemia, fatty infiltration, disruption of the structure of the vascular wall and cellular elements of organs, such as the swelling of the walls of arterioles and hyalinosis of the walls of individual vessels [12].

The nodal metabolites of carbohydrate-energy metabolism and the glutathione link of the antioxidant system play a key role in the control of cellular metabolism. The stable activity of key enzymes of the glutathione link is the basis for reliable antioxidant protection, which determines the adaptive capabilities of the body [13].

This study aimed to analyze the biochemical changes in the cells of the heart muscle, liver, and pancreas and establish their pathogenetic significance in experimental hypercholesterolemia induced by an alimentary factor.

Materials and methods. The study was performed on 65 outbred male rats aged 12 months with a body weight of 300 ± 50 g from September to November 2020 in the Department of General and Clinical Biochemistry No. 1 of the Rostov State Medical University of the Ministry of Health of Russia.

The experimental study using animals was agreed and approved by the local ethics committee of the Rostov State Medical University of the Ministry of Health of Russia (protocol No. 17/16 of 10/20/2016).

During the experiment, the animals were divided into two groups. Group 1 consisted of animals (control group, $n = 30$) which received the general diet of the vivarium, whereas Group 2 (experimental group, $n = 35$) included rats with essential hypercholesterolemia induced by feeding with a special diet for 3 months (semolina, cane sugar, and butter in a ratio of 1:2:2 once daily and each animal was individually given 50 g of white unsalted lard), and when the target cholesterol level of 3.83 ± 0.31 mmol/L was achieved, they were taken to the experiment (control 2.2 ± 0.2 mmol/L) [12].

At the end of the experiment, the animals were decapitated under ether anesthesia.

With a temperature regime of 4°C, the rat heart, liver, and pancreas were extracted, and a homogenate was prepared in a 1:9 ratio with a cooled isotonic sodium chloride solution, centrifuged at 3000 rpm to precipitate large cell debris.

The concentration of substrates of carbohydrate-energy metabolism, the level of reduced glutathione, and the activity of glutathione enzymes were determined spectrophotometrically using SF U-2900. The concentration of pyruvic acid was determined according to V.S. Kamyshnikov (2009) [14], the level of lactate was determined according to the reaction with parazone, as described by L.A. Danilova (2003) [15], the concentration of reduced glutathione was determined according to the method proposed by George L. Ellman (1959) [16], the activity of glutathione peroxidase was determined according to the method described by L.A. Danilova (2003) [15], and the activity of glutathione reductase was determined according to the method proposed by L.B. Yusupova (1989) [17].

The concentration of total protein was calculated using the Lowry method (1951) [18].

Statistica 10.0 and Microsoft Office Excel Worksheet were used to statistically evaluate the experimental results. After testing the normality of distribution, the significance of the differences in the considered indicators of the compared groups was determined by the value of the Student's *t*-test. Statistical significance was set at $p \leq 0.05$.

Results and discussion. The study results obtained are presented in Table 1.

Upon analyzing the parameters of energy metabolism in animals with essential hypercholesterolemia relative to the control group, the level of pyruvic acid in the tissue of the heart muscle and in the liver decreased by 25.5% ($p \leq 0.05$) and 35.9% ($p < 0.001$), respectively, although no significant change was observed in the pancreas. Moreover, lactate levels significantly increased in all tissues by 72.18% in the heart muscle ($p < 0.001$), 511.82% in the liver ($p < 0.001$), and 95.24 % in the pancreas ($p < 0.001$).

Evaluation of the results obtained revealed predominance in the tissues of the anaerobic pathway of glycolysis and the accumulation of under-oxidized products. An increase in lactate levels indicates a change in the interaction between organs (heart, liver, and pancreas), which disrupts the Cori cycle, gluconeogenesis reactions, hemostasis processes, and other metabolic changes [19].

The balance of the level of reduced and oxidized glutathione in cells is controlled by the ratio of glutathione reductase to glutathione peroxidase enzymes [13]. The analysis of the key enzymes in the pancreas in rats with hypercholesterolemia relative to the control group revealed a decrease in the activity of glutathione reductase by 59.38% ($p > 0.001$) and reduced glutathione by 28.31% ($p > 0.05$), although no significant increase in the activity of glutathione peroxidase was observed [23.58% ($p > 0.05$)], which indicates the dysfunction of the antioxidant defense system and formation of oxidative stress.

Table 1. Biochemical changes in parameters in the heart muscle, liver, and pancreas of rats with essential hypercholesterolemia (M ± m, *p*).

Parameter	Group 1 (control, *n* = 30)			Group 2 (hypercholesterol diet, *n* = 35)		
	H	L	P	H	L	P
Pyruvic acid, μmol/mg protein	0.4±0.038	0.39±0.04	0.39±0.04	0.29±0.03 p ≤0.05	0.25±0.02 p >0.001	0.4±0.03 p >0.05
Lactate, μmol/mg protein	2.66±0.24	1.1±0.1	3.15±0.29	4.58±0.47 p <0.001	6.73±0.6 p <0.001	6.15±0.57 p <0.001
Reduced glutathione, μmol/mg protein	295.17±30.6	496.6±49.83	49.94±5.1	378.74±38.1 p >0.05	642.2±63.8 p >0.05	17.62±1.8 p <0.001
Glutathione peroxidase, μmol/mg protein/min	21.55±2.2	46.85±4.7	16.2±1.5	29.05±3.1 p ≥0.05	45.49±4.6 p >0.05	20.02±1.9 p >0.05
Glutathione reductase, μmol/mg protein/min	8.94±0.91	0.158±0.01	1.28±0.13	13.58±1.4 p >0.001	0.297±0.03 p <0.001	0.52±0.05 p <0.001

Note: *p* — degree of significance relative to the indicators of the control group (statistical significance at $p \leq 0.05$); H — heart muscle; L — liver; P — pancreas

Relative to the control group, The activity of glutathione reductase increased by 87.97% ($p > 0.001$) in the liver of the experimental group and by 51.9% in the heart muscle ($p > 0.001$), while the activity of glutathione peroxidase and reduced glutathione in both organs was almost unaltered ($p > 0.05$). This tendency may be because myocytes have a small amount of O_2 supplied by diffusion from capillaries. The data obtained indicate the intense work of the antioxidant system aimed at restoring the pool of glutathione.

We assume that the data obtained demonstrate the peculiarities of the pathogenesis of hypercholesterolemia and the different roles of the organs being studied in the organization of the adaptive-compensatory reactions of organisms.

It should be noted that a high level of lactate, especially expressed in the liver, may be associated with the accumulation of macrophages inter alia, which produce the tumor necrosis factor in an activated state that affects lipid metabolism [20], promotes the accumulation of active forms oxygen, and stimulates lipid peroxidation [21].

The acidification of the environment due to an excessive amount of lactate and the development of hypoxia in the liver can induce non-alcoholic fatty disease, including fatty hepatosis and steatohepatitis [9].

Furthermore, the accumulation of lactate in the heart is accompanied by "local acidification," which leads to impaired microcirculation, insufficient oxygen supply, impaired systolic function, and diastole defect [21]. These processes in cardiomyocytes induce apoptosis, which triggers the caspase cascade and activates protein catabolism and leads to the accumulation of toxic products.

As a result of oxidative stress, the level of prooxidants and endotoxins increases, which enter the intestines and pancreas along with blood flow.

In the pancreas, a decrease in the pH value caused by the accumulation of lactate is filled with the activation of proteolytic enzymes, self-digestion, morphological changes leading to necrosis of cellular elements, and stromal lipomatosis [9].

Notably, the decrease in the pool of reduced glutathione is most pronounced in the pancreas, whereas the work of the glutathione link of the antioxidant system is enhanced in the heart and liver.

The sensitivity of pancreatic cells due to a lack of oxygen is known, although this also applies to the heart muscle. However, the pronounced depletion of glutathione reserves and a decrease in the intensity of its recovery in the glutathione reductase reaction indicate the special vulnerability of pancreatic cells in the impairment of cholesterol homeostasis, which makes the pancreas a target organ.

CONCLUSION

Changes in the metabolic link of adaptive reactions in the organs being studied were characterized by an increase in anaerobic processes, especially in the liver, accompanied by the depletion of glutathione reserves in the pancreas, which indicates its role as a target organ in the pathogenesis of alimentary hypercholesterolemia.

Author contributions. Z.I.M. created the study concept and design and edited the text; A.V.R. performed the literature review, collected and processed the results obtained, and wrote the text; I.A.S. performed the literature review, analyzed the results obtained, and wrote the text.

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