

Effect of L-arginine on myocardial vascular atherosclerosis in rabbits under high mountain environment

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Abstract. The study assessed blood lipid metabolism indicators and myocardial vascular morphology in rabbits with atherosclerosis under long-term adaptation under high mountain environment against the background of L-arginine therapy and prophylaxis. The experiment was conducted on 30 rabbits divided into 5 groups. Using biochemical analyzers, changes in the concentrations of total cholesterol, triglycerides, low- and high-density lipoproteins were determined before and after atherosclerosis simulation; additionally, the effect of L-arginine on the dynamics of the lipid spectrum indicators under long-term adaptation to high mountain environment was assessed. The results were processed using SPSS 16.0 at a statistical significance level of $p < 0.05$. Administration of L-arginine in rabbits with high cholesterol rate under long-term adaptation under high mountain environment resulted in a significant decrease in its content related to the initial increased level. Triglyceride level decreased after L-arginine therapy under adaptation to high mountain environment; however, an increase in the level of this lipid type was observed in animals receiving cholesterol simultaneously with L-arginine (prophylaxis). The therapy and prophylaxis with L-arginine resulted in a decrease in LDL level in animals with atherosclerosis under long-term adaptation to high mountain environment that may indicate the potential efficacy of the drug in the treatment of this condition.

1 Introduction

Currently, about 10% of the world population lives in high mountain regions, which occupy 20% of the land surface. Approximately 94% of the territory of Kyrgyzstan is covered by mountains and about half of the highlands are beyond 1,500 m above sea level; 41% of the mountainous territory is in the harsh highlands going beyond 3,000 m [1].

It is believed that people endure the comparable altitudes differently in various mountainous regions and, conversely, the comparable body functional shifts can be

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observed at various altitudes. These assertions are explained by at least two reasons: firstly, the specific impact of different mountainous regions due to their own geographical features distinguished by specific factors and factor combinations in the natural environment, and, secondly, wide individual differences in the tolerability to the environment conditions [2].

The most significant adaptive reactions that contribute to an enhancement in oxygen transport to tissues during the development of acute oxygen deficiency are an increase in the minute blood volume (MBV) as well as an acceleration of the blood flow and its redistribution that results in an augment of the blood supply to organs that are highly sensitive to hypoxia, primarily the brain, and organs in a state of hyperfunction including the heart and lungs.

Since the effects of unfavorable factors in the highlands most affect the cardiovascular system, of particular concern is the steady increase in mortality from coronary heart disease and its serious complication that is myocardial infarction stipulated by atherosclerosis as the main cause [3].

Atherosclerosis is a chronic disease characterized by the accumulation of cholesterol in the blood vessel walls in the form of cholesterol plaques and related to a lipid metabolism disorder [4]. Every year, more than 17.5 million people in the world die from cardiovascular pathology; in Kyrgyzstan this rate reaches more than 18 thousand people [5]. The key factor in the development of atherosclerosis is an increase in the level of the plasma lipoproteins rich in cholesterol including low-density lipoproteins (LDL) [6-8]. Many studies have shown that oxidized LDL particles contribute to the atherosclerosis progression [8, 9]. Arterial endothelial dysfunction causes modification of ApoB-containing LDL and triggers monocyte infiltration into the arterial wall. Due to endothelial dysfunction, LDL particles can be deposited in the arterial wall and retained by the extracellular matrix [10]. LDL particles accumulate and bind to intimal proteoglycans producing aggregate formation. Further they can penetrate into smooth muscle cells via LDL receptors [11]. Activation of vascular endothelial cells also results in the expression of cell adhesion molecules such as VCAM-1 and ICAM-1, which facilitate the binding and attachment of immune cells, specifically monocytes, to the arterial wall [12].

We selected L-arginine, a semi-essential amino acid and nitric oxide donor as the investigational agent. NO is known to be an unorthodox messenger molecule, which plays an important role in many biological functions, especially in the blood circulatory system; it is involved in the regulation of vasodilation, the inhibition of the platelet aggregation and adhesion, the retardation of leukocyte adhesion and vascular inflammation, as well as controlling vascular insufficiency and smooth muscle proliferation [13]. Consequently, L-arginine is critical for maintaining endothelial function and preventing cardiovascular diseases [14]. Notably, L-arginine supplementation reduces endothelial dysfunction induced by insulin resistance (IR) in patients with type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD) [15]. Yan J et al. demonstrated that this supplement can increase NO bioavailability through inducing higher expression and phosphorylation of eNOS [16]. Moreover, L-arginine has been shown to inhibit inflammatory activation and regulate redox homeostasis in aortic valve interstitial cells by suppressing procalcific differentiation [17]. In this regard, L-arginine supplementation has attracted much attention in terms to the prevention and treatment of cardiovascular diseases such as hypertension, atherosclerosis, coronary heart disease, heart failure and peripheral arterial disease, although its clinical efficacy is still controversial. From the standpoint of evidence-based medicine, the appropriateness of using L-arginine for the treatment of atherosclerosis of the cardiovascular system is underexplored. All of the forgoing determined the relevance of conducting the scientific investigation in this direction.

The study was aimed to the investigation of the lipid metabolism indicators in the blood serum and the morphology of myocardial vessels in rabbits with simulated atherosclerosis

against the background of L-arginine therapy during long-term adaptation under high mountain environment.

2 Materials and methods

The work was carried out at the high-mountain scientific base on the Tuya-Ashu pass (3200 m above sea level) and at the Interdisciplinary Educational and Scientific Center for Biomedical Research of the Kyrgyz State Medical Academy named after I.K. Akhunbaev. The investigations were confirmed by a certificate from the Bioethics Committee, in particular by the extract from protocol No. 3 dated 04/28/2021 of the KSMA named after I.K. Akhunbaev.

The experiments were conducted on laboratory rabbits of both sexes aged 8-12 months weighing 3.5-4.5 kg. A total of 30 laboratory rabbits were used, which were divided into 5 groups of 6 animals each. Group 1 included the intact animals under low mountain relief conditions. Group 2 included the intact animals on the 30th day of their adaptation under high mountain environment. Group 3 consisted of the animals with simulated atherosclerosis (simulated according to the Anichkov method by oral administration of cholesterol at a dose of 500 mg / kg of body weight once a day). Group 4 included the animals that were administered cholesterol simultaneously with L-arginine for 30 days (prophylaxis). Group 5 consisted of animals with simulated atherosclerosis treated with L-arginine.

During the investigation, L-arginine was used in the form of capsules under the trade name "Vazoton", produced by the company "Altayvitaminy" (Russia).

Before and after simulation of atherosclerosis, as well as after the administration of L-arginine, the following lipid metabolism indicators were studied: high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), and total cholesterol (TC) with a desktop biochemical autoanalyzer "RESPONS 920" (DiaSysDiagnosticSystemsGmbH, Germany).

Statistical processing of the obtained data was performed using the SPSS 16.0 software. The data were presented as the mean value \pm the average statistical error (Independent Samples T-Test). The level of statistical significance was $p < 0.05$.

Additionally, a histological study of the removed plaques was conducted to assess vessel state. For investigation of the morphology of the rabbit myocardium, microscope slides with 5–6-micron thick histological sections were made and stained with hematoxylin and eosin. A comparative assessment of the histological picture was carried out before and after atherosclerosis simulation, as well as after the course of therapy and prophylaxis with L-arginine.

3 Results and discussion

Examination of animals on the 30th day of adaptation under high mountain environment showed that the level of total cholesterol in intact animals decreased to 1.24 ± 0.04 mmol/l at $p < 0.001$ in comparison with the indicator in rabbits under low mountain conditions, which amounted to 3.96 ± 0.07 mmol/l. In animals with simulated vascular atherosclerosis, compared to animals of the intact group under high mountain environment, the level of TC increased to 5.67 ± 0.2 mmol/l ($p < 0.001$). In rabbits, which received cholesterol simultaneously with L-arginine for 30 days, the cholesterol level increased to 20.1 ± 0.3 mmol/l at $p \leq 0.001$. After 30 days of L-arginine administration, the level of this indicator decreased to 4.63 ± 0.1 / l ($p < 0.004$) but still remained almost 4 times higher than in intact animals after 30th day adaptation under high mountain environment (Fig. 1).

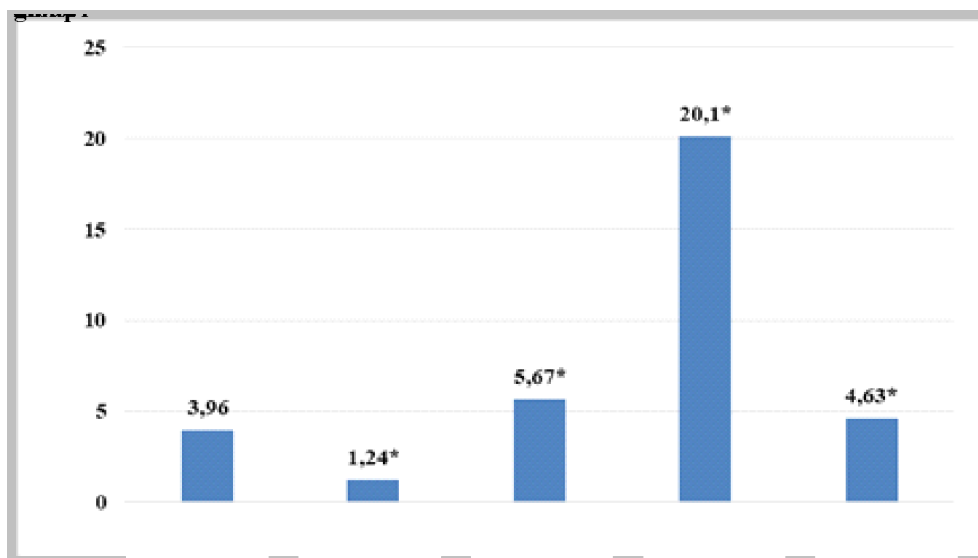


Fig. 1. The level of TC in the blood serum of the control and experimental rabbits under high mountain environment

In Fig. 1 – group 1 – the intact animals under low mountain conditions; Group 2 – the intact animals on the 30th day of the adaptation under high mountain environment; Group 3 – the animals with simulated atherosclerosis; Group 4 – the animals, which were administered cholesterol simultaneously with L-arginine for 30 days (prophylaxis); Group 5 – the animals with simulated atherosclerosis treated with L-arginine. Note: * – $p < 0.05$ when comparing group 2 with group 1; group 3 with group 2; group 4 with group 3; group 5 with group 3.

Figure 2 shows that the blood serum TG level in intact animals on the 30th day of adaptation under high mountain environment significantly decreased to 0.4 ± 0.04 mmol/l ($p < 0.002$) in reference to the indicator in the intact animals under low mountain conditions, which amounted to 1.38 ± 0.1 mmol/l. Determining TG in blood serum of rabbits in 60 days after the administration of cholesterol attested a significant increase in the TG concentration to 1.52 ± 0.07 mmol/l ($p \leq 0.001$). In animals, which received cholesterol simultaneously with L-arginine, we revealed a significant increase in the TG level to 1.73 ± 0.05 mmol/l ($p < 0.001$) in comparison to the indicator in the group of animals with simulated atherosclerosis, which was 1.52 ± 0.07 mmol/l. Examination of rabbits that received L-arginine for 30 days also showed a decrease in the TG level to 1.0 ± 0.03 mmol/l in comparison to animals of the 3rd group with the indicator of 1.52 ± 0.07 mmol/l ($p \leq 0.001$).

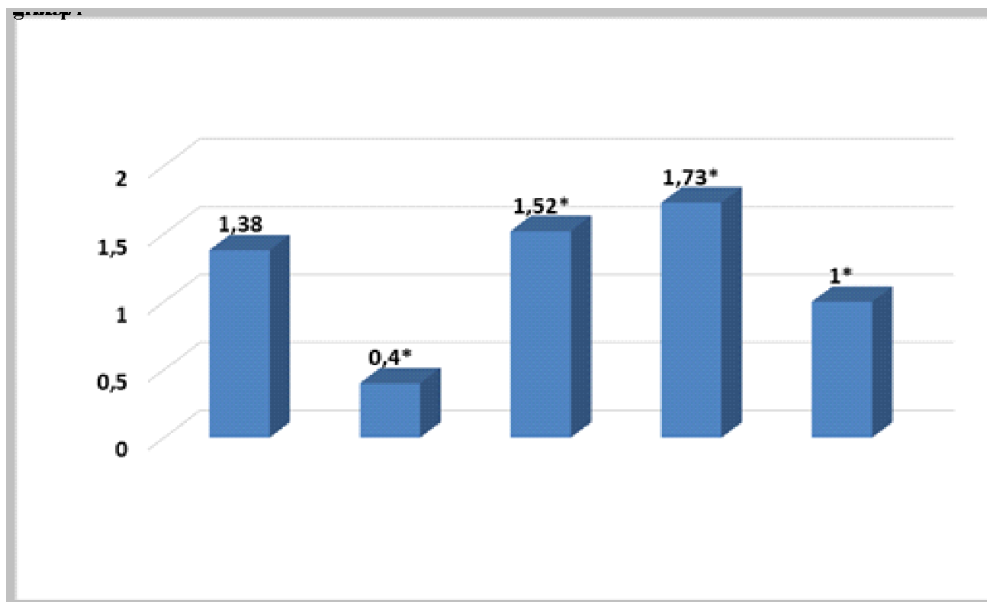


Fig. 2. The level of TG in the blood serum of the control and experimental rabbits under high mountain environment

The designations in the figure correspond to the description given in Fig. 1. Note: * – $p < 0.05$ when comparing group 2 with group 1; group 3 with group 2; group 4 with group 3; group 5 with group 3.

On the 30th day of adaptation under the mountain climate, the LDL level in the blood serum of the intact animals decreased to 1.01 ± 0.07 mmol/l ($p < 0.001$) in comparison to the intact animals under low mountain conditions with the indicator of 2.4 ± 0.1 mmol/l (Fig. 3). In animals with simulated atherosclerosis, the amount of this lipid type in the blood serum increased to 18.5 ± 0.2 mmol/l ($p \leq 0.001$) in contrast to the animals of the 2nd group. After the administration of cholesterol simultaneously with L-arginine, the animals of the experimental group showed a decrease in the LDL level to 3.59 ± 0.1 mmol/l at $p < 0.001$ in reference to 18.5 ± 0.2 mmol/l in the animals with simulated atherosclerosis. And in the animals of the 5th group, received L-arginine therapy, the LDL level significantly decreased to 2.19 ± 0.1 mmol/l ($p \leq 0.001$).

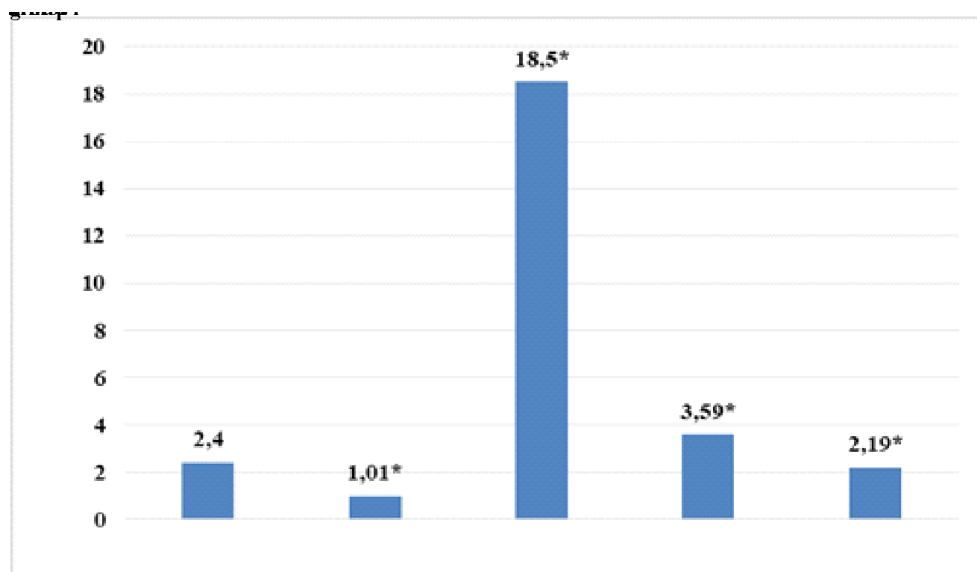


Fig. 3. LDL level in the blood serum of the control and experimental rabbits under high mountain environment

The designations in the figure correspond to the description given in Fig. 1. Note: * – $p < 0.05$ when comparing group 2 with group 1; group 3 with group 2; group 4 with group 3; group 5 with group 3.

On the 30th day of adaptation under high mountain environment, the quantitative level of HDL in the blood serum of intact animals significantly decreased to 0.73 ± 0.02 mmol/l in reference to the control value of 0.98 ± 0.03 mmol/l in rabbits under low mountain conditions at $p \leq 0.001$. The HDL level after cholesterol administration significantly increased to 1.19 ± 0.01 mmol/l ($p \leq 0.001$) relative to the second group with the indicator of 0.73 ± 0.02 mmol/l. In animals of the 4th group, the level of this lipoprotein slightly increased to 1.26 ± 0.03 mmol/l, with $p \leq 0.1$ relative to the indicators in the group with simulated atherosclerosis (1.19 ± 0.01 mmol/l). Examination of rabbits with atherosclerosis, which received the investigational drug, revealed a significant decrease in the HDL level to 0.91 ± 0.03 mmol/l at $p \leq 0.001$ in comparison to animals of the 3rd group (Fig. 4).

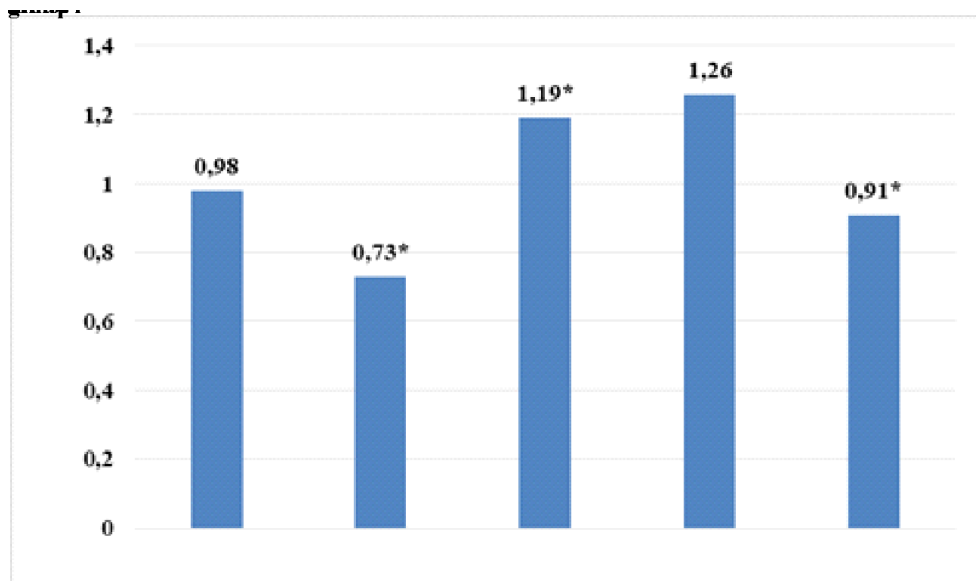


Fig. 4. Level of HDL in the blood serum of the control and experimental rabbits under high mountain environment

The designations in the figure correspond to the description given in Fig. 1. Note: * – $p < 0.05$ when comparing group 2 with group 1; group 3 with group 2; group 4 with group 3; group 5 with group 3.

Morphological examination of the heart in the intact rabbits under low mountain conditions revealed a myocardium of normal structure; myocytes were closely adjacent to each other; individual myocytes had the vascularization or granularity of the cytoplasm. Along with that, homogeneity was found in certain myocytes. Upon examination we identified different types of vessel structure, specifically, muscular-elastic and muscular ones. Both types of vessels had areas of thickening and disruption of the normal structure in these loci, which manifested by dystrophy of the vessel wall and small areas of decay (Fig. 5, a).

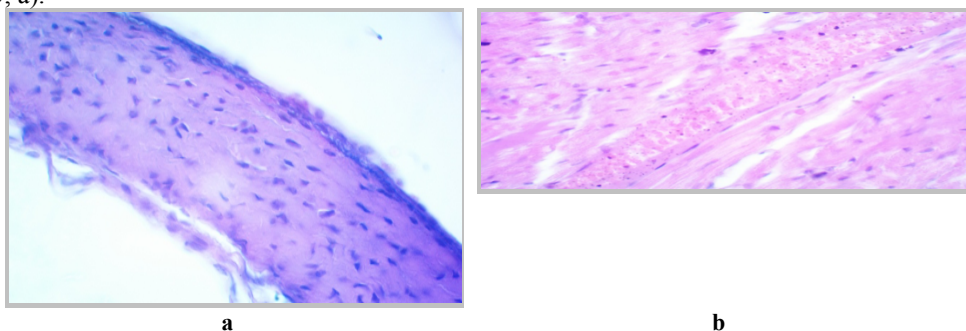


Fig. 5. Hematoxylin + eosin staining. Magnification $\times 180$. (a) The vessel wall in an intact rabbit. (b) The vessel wall in a rabbit on the 30th day of adaptation under high mountain environment.

Histological examination of the myocardium taken out of rabbits on the 30th day of exposure under high mountain environment revealed that the vessels were randomly thickened, with the deposits of fatty tissue and the accumulation of macrophages and xanthomatous cells under intima in thickened areas. Upon examining the myocardium,

some myocytes were found to have signs of multiple hemorrhages against the background of pronounced plethora and dystrophy of cardiomyocytes (Fig. 5, b).

Histological examination of the myocardium taken out of rabbits with simulated atherosclerosis under high mountain environment revealed a number of characteristic features. Among them were the following:

- a) a plaque containing many fat cells; signs of sclerosis in a certain area related to finding many connective tissue cells
- b) signs of a later stage of sclerosis development with increased proliferation of connective tissue cells, i.e. the beginning of its next stage, liposclerosis;
- c) infiltration of the vessel wall with lipids; finding of proliferation of macrophages in the inner layer of the vessel
- d) infiltration of the vessel wall with lipids in the intima and in the inner layer of the vessel; pronounced proliferation of macrophages (Fig. 6, a).

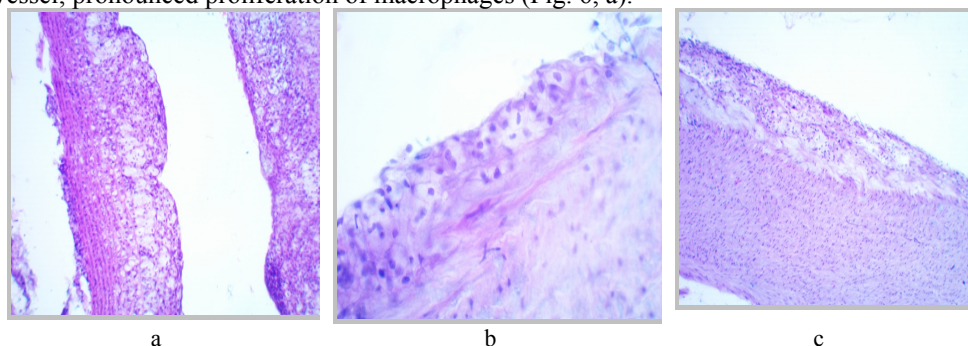


Fig. 6. Hematoxylin + eosin staining. Magnification $\times 180$. (a) The vessel wall in a rabbit with simulated atherosclerosis. (b) The vessel wall in a rabbit after prophylaxis with L-arginine. (c) The vessel wall in a rabbit with simulated atherosclerosis after treatment with L-arginine

Microscopic examination of the histological samples of the section through the left part of heart in rabbits, which received cholesterol simultaneously with L-arginine, revealed a number of important features. 1) In terms of vessel we identified a clearly marked fatty plaque with thickening of the intima; in the thickness of the plaque there was fat-protein destritis. In addition, fat and xanthomatous cells were revealed. Among these cells there were many macrophages. 2) In the subepicardial zone we identified hemorrhage into the heart muscles against the background of pronounced plethora. Intermuscular capillaries were sharply dilated. Some intramuscular vessels had thickened walls. Besides, we observed dystrophy of individual groups of cardiomyocytes (Fig. 6, b).

Microscopic examination of the cardiac muscle in animals with simulated atherosclerosis under conditions of long-term adaptation under high mountain environment, which were treated with L-arginine, reveals the following features. 1) The vessels were muscular-elastic type; the intima was thickened in a certain area and under it there were accumulations of fat and xanthomatous cells and macrophages. 2) Myocardium was of normal structure, some myocytes manifested moderate dystrophy. There were small hemorrhages in the subepicardial zone (Fig. 6, c).

4 Conclusion

Thus, the conducted experimental study of the effect of L-arginine on the indicators of the blood serum lipid spectrum in rabbits with simulated atherosclerosis showed that the investigational drug had a normalizing effect on the ratio of proatherogenic (CL, LDL) and antiatherogenic (HDL) fractions of lipids. The examination of myocardial morphology in

rabbits with simulated atherosclerosis and L-arginine therapy under high mountain environment revealed various changes in the structure of the heart and blood vessels. Animals with atherosclerosis under high mountain environment showed signs of myocardial dystrophy, vascular structure disorders, formation of fatty plaques, and proliferation of connective tissue. Upon the treatment and prophylaxis with L-arginine in rabbits with atherosclerosis under high mountain environment, specified characteristics were still observed but with moderate myocardial dystrophy. L-arginine therapy may have a beneficial effect on the structure of the heart and blood vessels at atherosclerosis under high mountain environment adaptation but further investigations may be necessary for gaining a complete understanding of this drug effects.

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