ORIGINAL ARTICLE

Effect of L-Arginine on Lipid Metabolism and Morphology of Cardiomyocytes of Animals with Experimental Atherosclerosis in High-Altitude Conditions

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Abstract

Background: The Kyrgyz Republic, with its high-altitude regions, poses unique health challenges due to extreme geoclimatic factors. Cardiovascular disease (CVD) is prevalent in the region, and dyslipidemia, a significant risk factor for atherosclerosis and CVD, is associated with elevated serum cholesterol levels. This study investigates the effects of high-altitude environments on human health and the potential therapeutic benefits of L-arginine in regulating carbohydrate and lipid metabolism. **Materials and Methods:** Thirty rabbits were divided into five groups: Control (low-altitude), high-altitude (3 days), atherosclerosis model, atherosclerosis with preventive L-arginine and cholesterol treatment, and atherosclerosis with L-arginine treatment. Atherosclerosis was induced by oral cholesterol administration (500 mg/kg/day) for 60 days. L-arginine (170 mg/kg/day) was administered for 30 days for treatment and prevention. Lipid metabolism indicators (high-density lipoprotein, low-density lipoproteins, triglycerides, and total cholesterol [TC]) were measured using a biochemical autoanalyzer. Histological examination of excised plaques and myocardial morphology was performed. **Results:** Results showed a significant reduction in TC levels in the high-altitude group compared to the control. The atherosclerosis model group exhibited a tenfold increase in TC, which remained unchanged with preventive L-arginine and cholesterol treatment. However, L-arginine treatment alone decreased TC levels by approximately 65%, although still twice as high as the control. **Conclusion:** The findings suggest that L-arginine may have potential therapeutic benefits in regulating lipid metabolism and improving cardiomyocyte morphology in rabbits with induced atherosclerosis under high-altitude conditions.

Key words: Atherosclerosis, cardiomyocytes, high-altitude conditions, l-arginine, lipid metabolism

INTRODUCTION

Approximately 10% of the global population resides in mountainous regions, which encompass 20% of the land surface. In the Kyrgyz Republic, a nation characterized by 94% mountainous terrain, half of the area exceeds an altitude of 1500 m above sea level, with 41% comprising harsh highlands ascending over 3000 m. These regions are perpetually covered with ice and snow, with glaciers occupying approximately 4% of the country's territory.[1]

In recent years, industrial activities such as mining, geological industry, and infrastructure development have seen significant

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growth in the mountainous regions of the Kyrgyz Republic. The extreme geoclimatic factors in these areas pose potential risks to human health, prompting heightened concern in scientific and medical communities. Cardiovascular disease (CVD), the primary cause of morbidity and mortality among non-communicable diseases, is also prevalent in the Kyrgyz Republic.[2] Therefore, it is imperative to investigate the effects of high-altitude environments on human health and develop preventive measures to preserve it.

Dyslipidemia, a significant risk factor for atherosclerosis and CVD, correlates with elevated serum cholesterol levels and increased incidence and mortality rates of CVD. Elevated levels of total cholesterol (TC) and low-density lipoproteins (LDL), as well as decreased levels of high-density lipoproteins in the blood plasma, are associated with a higher risk of coronary heart disease, acute cerebrovascular accident, all-cause mortality, and mortality from CVD.[3]

Atherosclerosis is a multifactorial disease influenced by primary cardiovascular risk factors, including hypertension, hyperlipidemia, nicotine addiction, obesity, and diabetes mellitus, as well as interacting genetic and inflammatory factors. The dysregulation of the inflammatory response is associated with these risk factors. Atherosclerosis manifests as a chronic inflammatory condition affecting both small and large capillary vessels, its severity varying based on predisposing factors.[4]

Atherosclerosis initiates with endothelial dysfunction, leading to the retention of modified LDL and foam cell formation.[5,6] This process entails monocyte involvement and activation of inflammatory signaling pathways. This results in the accumulation of lipids within cells and the extracellular milieu, leading to the formation of fat strips, the hallmark of atherosclerosis.[7,8]

L-arginine (2-Amino-5-guanidinovaleric acid-Arg) is a prevalent amino acid found in many food sources. It plays a pivotal role in the synthesis of various compounds that regulate diverse body functions, including nitric oxide (NO) – a molecule that modulates carbohydrate and lipid metabolism. Recent research indicates potential therapeutic benefits of L-arginine in treating metabolic disorders, regulating blood pressure, and ameliorating the symptoms of Type 2 diabetes. However, the precise mechanisms of these therapeutic effects remain unclear.

The literature suggests potential health benefits associated with L-arginine. However, certain studies have found that excessive consumption of L-arginine may exacerbate pre-existing health conditions or increase the risk of certain diseases. The precise mechanisms underlying the role of L-arginine in the regulation of carbohydrate and lipid metabolism remain unclear and are currently under investigation.[9]

At high altitudes, the cardiovascular system is subjected to adverse external factors, and L-arginine is commonly used as a vasodilator for CVDs in the Kyrgyz Republic. Therefore, investigating its effects on lipid metabolism and cardiomyocyte morphology in animals with induced atherosclerosis is warranted.

This study analyzed blood serum lipid metabolism and cardiomyocyte morphology in rabbits before and after inducing atherosclerosis, with and without L-arginine therapy, during short-term high-altitude acclimatization.

MATERIALS AND METHODS

In this study, 30 rabbits (15 males, 15 females, 8–12 months old, 3.5–4.5 kg) were studied at a high-altitude scientific base on the Tuya-Ashu Pass (3200 m) and the Interdisciplinary Educational and Scientific Center for Biomedical Research at the Kyrgyz State Medical Academy.

Blood was collected from all rabbits through venipuncture of the auricular vena cava. The study included five groups: Control rabbits at low altitude, intact rabbits at high altitude for 3 days (group 1), rabbits with simulated atherosclerosis (group 2), rabbits with atherosclerosis receiving L-arginine and cholesterol as preventive treatment (group 3), and rabbits with atherosclerosis treated with L-arginine (group 4).

Experimental atherosclerosis was induced in rabbits by administering 500 mg/kg of cholesterol orally daily for 60 days, using a reversible dietary model of hypercholesterolemia, similar to the initial therapeutic steps for patients. L-arginine, under the brand name Vasoton (Altayvitamin, Biysk, Russia), was administered at 170 mg/kg daily for 30 days to treat and prevent atherosclerosis. Lipid metabolism indicators, including high-density lipoprotein (HDL), LDL, triglycerides (TG), and TC, were measured using a biochemical autoanalyzer (respons®920, DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

Histological examination involved analysis of the excised plaques. Micropreparations with a thickness of 5–6 μm were prepared and stained with hematoxylin and eosin to study the myocardial morphology of the rabbits. A comparative evaluation was conducted before and after inducing atherosclerosis as well as after treatment and prevention with L-arginine.

Data were statistically processed using Statistical Packages for the Social Sciences 16.0 and are presented as mean ± standard deviation (independent samples *t*-test). Statistical significance was defined as $P < 0.05$.

RESULTS

On the 3rd day of high-altitude adaptation, TC levels of Group 1 in the control group dropped from 3.97 ± 0.07 mmoL/L to

2.33 ± 0.3 mmoL/L ($P < 0.001$). TC levels of Group 2 surged nearly tenfold to 24.4 ± 0.9 mmoL/L ($P < 0.001$). In Group 3, with 30 days of cholesterol and L-arginine treatment, TC levels remained stable at 24.1 ± 0.5 mmoL/L ($P \leq 0.7$). Conversely, after 30 days of L-arginine in Group 4, TC levels fell to 8.46 ± 0.5 mmoL/L ($P < 0.001$), still about twice the levels of the control group [Figure 1].

Figure 2 indicates that TG levels in the blood serum of group 1 significantly dropped from 1.38 ± 0.1 to 0.86 ± 0.06 mmoL/L ($P < 0.01$) compared to the control group. Group 3 exhibited an increase in TG levels, rising from 0.86 ± 0.06 to 2.73 ± 0.1 mmoL/L ($P < 0.001$) relative to the control group. Compared to Group 2, TG levels in Group 3 significantly decreased from 2.73 ± 0.1 to 1.57 ± 0.05 mmoL/L ($P < 0.01$). Group 4 also showed a notable reduction in TG levels from 2.73 ± 0.1 to 1.2 ± 0.05 mmoL/L compared to group 2 ($P \leq 0.001$).

On the 3rd day of adaptation to high altitudes, the LDL level in the blood serum of Group 1 decreased from 2.4 ± 0.1 to 1.4 ± 0.2 mmoL/L, compared to the control group ($P < 0.002$) [Figure 3]. In contrast, Group 2 showed a significant increase in LDL levels, which rose to 22.5 ± 0.9 mmoL/L and reached levels that were more than 16 times higher than those in Group 2 ($P \leq 0.001$).

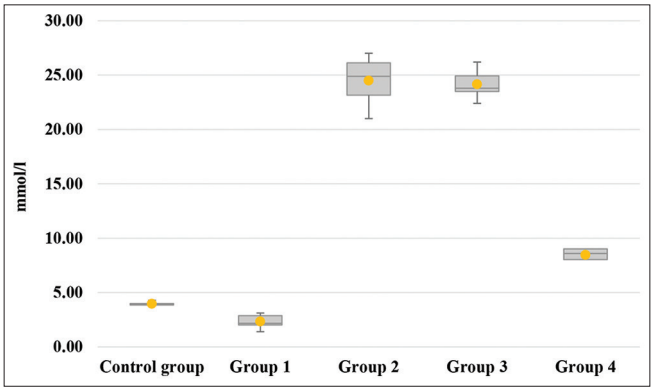

Figure 1: The level of total cholesterol in the blood serum of rabbits in different groups

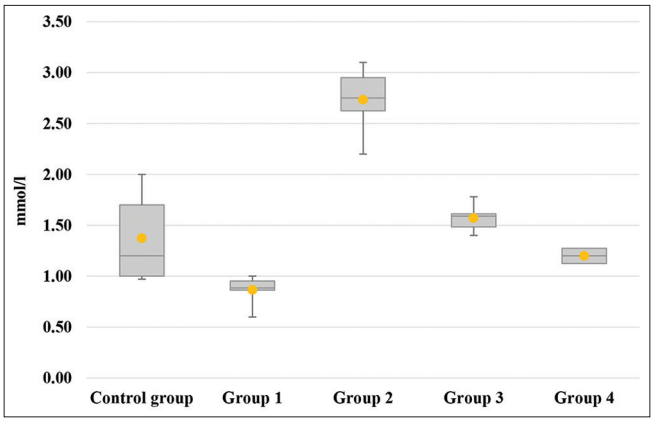

Figure 2: The level of triglycerides in the blood serum of rabbits in different groups

Group 3 showed a decrease in LDL levels from 22.5 ± 0.9 to 21.6 ± 0.5 mmoL/L ($P < 0.3$). Group 4, on the other hand, experienced a significant decrease in LDL levels to 7.4 ± 0.9 mmoL/L ($P \leq 0.01$).

On the 3rd day of high-altitude adaptation, Group 1's LDL level decreased from 0.98 ± 0.03 to 0.97 ± 0.07 mmoL/L, similar to the control group ($P \leq 0.8$). In Group 3, HDL levels dropped from 0.97 ± 0.07 to 0.96 ± 0.03 mmoL/L following cholesterol administration ($P \leq 0.9$). However, HDL levels in Group 3 significantly rose to 1.08 ± 0.01 mmoL/L after L-arginine administration ($P \leq 0.01$). Conversely, Group 4 experienced a significant HDL increase from 0.96 ± 0.03 to 1.18 ± 0.06 mmoL/L, a statistically significant difference compared to group 2 ($P \leq 0.01$) [Figure 4].

Morphological assessment of rabbit hearts at low altitudes revealed a typically structured myocardium with closely juxtaposed myocytes showing cytoplasmic vascularization or granularity. Homogenization was observed in some of the myocytes. Vessel structures varied, being either muscular-elastic or muscular, with thickening and structural alterations, such as vessel wall dystrophy and small decay areas [Figure 5a].

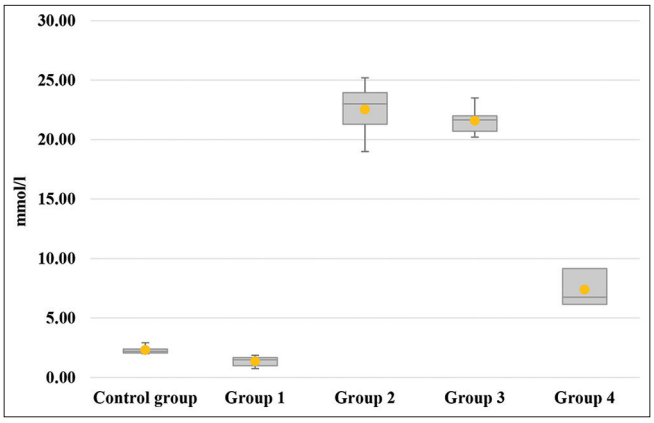

Figure 3: The levels of low-density lipoproteins in the blood serum of rabbits in different groups

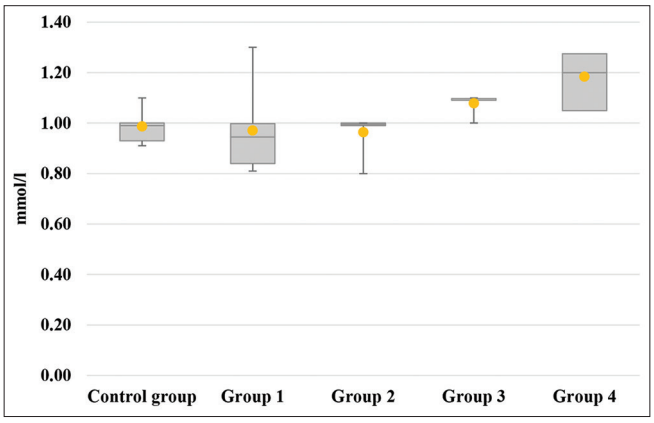

Figure 4: The levels of high-density lipoprotein in the blood serum of rabbits in different groups

Histological analysis of the myocardium from rabbits euthanized after 3 days at high altitude showed vessel thickening, fatty tissue deposition, and macrophage and xanthoma cell accumulation in the intima. Fat cell deposition extended deep into vessel walls. The myocardial structure remained normal, with myocytes and multiple hemorrhages with pronounced fullness [Figure 5b].

Histological examination of the myocardium in Group 2 showed the following findings: (1) For a significant length beneath the intima, there was an extensive accumulation of xanthoma and adipose cells that extended to a considerable depth of the vessel wall. Locally, clusters of fat-protein debris were present in the xanthoma cells, and the intima was unevenly thickened. (2) The myocardium showed moderate dystrophy in the form of myocyte cytoplasmic vacuolization and homogenization. In one of the intracardiac vessels, the intima thickened toward the wall beneath an intimate cluster of xanthoma cells. Hemorrhaging was observed in the myocardium [Figure 6a].

Microscopic examination of the myocardium from Group 3 revealed the following findings: (1) a well-defined fatty plaque accompanied by an increase in intimacy thickness. Plaques were composed of fat, protein debris, and xanthoma cells and several macrophages were present in the area. (2) Hemorrhages were pronounced within the heart muscle in the subepicardial region. The capillaries in this area were noticeably enlarged, and some intramuscular vessels displayed thickened walls. Cardiomyocytes showed signs of dystrophy [Figure 6b].

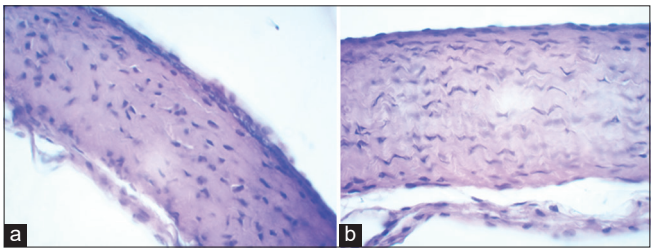

Figure 5: Microscopic images of vessel walls in intact rabbits (hematoxylin-eosin staining, ×180) (a) Control group. (b) Group 1

Microscopic examination of the myocardium from Group 4 revealed the following observations: (1) The vessel was of the muscle-elastic type with thickening in the intima in one area, accompanied by the accumulation of fat, xanthoma cells, and macrophages beneath. (2) The myocardium exhibited a normal structure, but some of the myocytes showed moderate dystrophy. Small hemorrhages were also observed in the subepicardial zone [Figure 6c].

The benefits of enhanced heart function are limited, and insufficient strain on the heart muscles can have detrimental consequences in both humans and animals. Pharmacological support at the cellular level is essential to ensure that the body's oxygen demand increases during stressful situations and improves blood flow to active organs and tissues.

DISCUSSION

The number of *in vitro* studies on L-arginine and lipid metabolism remains insufficient to fully elucidate its mechanism of action. Researchers have predominantly focused on endothelial cells due to their pivotal role in the development of atherosclerosis. This complex process involves vascular repair and plaque formation, influenced by factors such as monocyte adhesion, LDL cholesterol penetration, and foam cell formation. Notably, not all animal studies have consistently indicated increased L-arginine levels or improved cholesterol levels.[10,11] However, some studies have indicated that L-arginine supplementation can improve lipid levels in animals.[12]

For example, L-arginine has been found to reduce cholesterol or lipoprotein levels by modulating hepatic mRNA expression, promoting lipolysis and fatty acid oxidation, and increasing plasma adiponectin levels.[13] Conversely, other studies have indicated that L-arginine increases HDL cholesterol levels.[14-16] In addition, Cooke *et al*. found that L-arginine improved endothelial function, reduced atherosclerotic plaques, and decreased atherosclerosis in male rabbits with hypercholesterolemia.[17] A study by Wang *et al*. in rabbits showed that L-arginine, when combined with an inhibitor

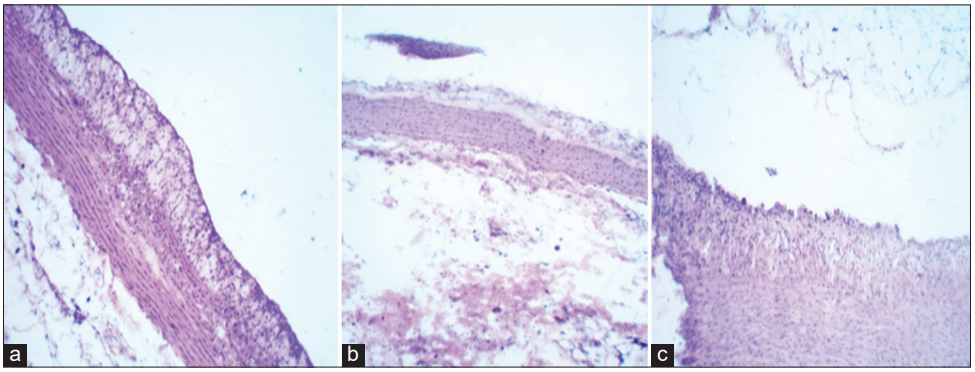

Figure 6: Microscopic images of vessel walls in intact rabbits (hematoxylin-eosin staining, ×180) (a) Group 2. (b) Group 3. (c) Group 4

of NO synthesis, led to an improvement in endothelium-dependent relaxation and restored endothelial function in hypercholesterolemia.[18]

L-arginine supplementation (2.25–2.5% in drinking water for 1–10 weeks) prevents coronary artery intima thickening, reduces monocyte and macrophage accumulation, and decreases atherosclerotic lesion formation in hypercholesterolemic rabbits.[19,20] L-arginine (2% in drinking water) or alpha-tocopherol (300 mg/day) enhances endothelium-dependent vasodilation, increases NO production, reduces oxidative stress, and slows atherosclerosis progression in these animals.[21] In hypercholesterolemic individuals, L-arginine supplementation improves endothelial function without affecting the lipid profile, showcasing its antiatherogenic properties.[22]

Suzuki *et al*. administered L-arginine (100 mg/mL) via catheter for 15 min to measure neointimal volume in stents, observing a 35% increase without changes in luminal volume.[23] Creager *et al*. found that intravenous L-arginine (10 mg/kg/min) improved endothelium-dependent vasodilation in 14 hypercholesterolemic patients.[24] Long-term L-arginine supplementation (10 g/day for 3–6 months) may enhance nitrite utilization in atherosclerotic conditions by improving carbonic anhydrase-dependent renal nitrite reabsorption. Future studies need to determine the optimal oral L-arginine dose for treating NO-related dysfunction.[25]

CONCLUSION

Findings on lipid metabolism and myocardial changes in rabbits adapting to high altitudes suggest that L-arginine in the blood serum effectively corrects lipid metabolism in these animals. Significant changes were observed in the lipid fractions of the animal models with induced atherosclerosis after 3 days of high-altitude adaptation. TC and LDL levels increased more than tenfold, while high-density lipoprotein levels decreased. The administration of L-arginine for 30 days during high-altitude adaptation delayed the progression of experimental atherosclerosis and resulted in decreased overall blood cholesterol, triglyceride, and LDL levels and increased HDL cholesterol levels. In addition, when L-arginine and cholesterol were administered simultaneously to animals under high-altitude conditions, triglyceride levels decreased, HDL levels increased, and TC and LDL levels remained unchanged, thus preventing the formation of cholesterol plaques.

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