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Impact of L-Citrulline on Lipid Profile and Inflammatory Markers in Mice with Diet-Induced Atherosclerosis

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Abstract

Background: Lipid profile is the primary indicator of atherosclerosis disorder. Several strategies and therapeutic agents are targeted to reduce the risk of atherosclerosis by manipulating the lipid profile and inflammatory mediators. This study aimed to investigate the effect of citrulline on atherosclerosis and inflammation.

Methods and Results: Eighteen adult male mice were assigned randomly for the following groups (six mice for each group): the control group, which received a standard chow diet; the induced group, which received a chow diet rich with 5% cholesterol; the L-citrulline-treated group, which received a chow diet rich with cholesterol, 5% together with 100 mg/kg of citrulline. The study lasted for ten weeks; the mice monitoring continued during and after completing the study course. After ten weeks of experiments, the blood sample was collected from the animals and centrifuged at 1500rpm for 15 minutes to obtain the serum. The ELISA technique was used to measure the level of TNF-α and ET-1. TC, HDL-C, LDL-C, VLDL-C, and TG were evaluated using the colorimetric enzymatic method and the Friedewald equation.

The lipid profile components and inflammatory markers were similar at the baseline in the study groups. After ten weeks, the group of mice that received a cholesterol-rich diet showed a significant increase in all lipid profile components, excluding HDL-C, and the level of the inflammatory markers (ET-1 and TNF-α) compared with control mice. In the citrulline-treated group, the blood levels of TC, VLDL-C, LDL-C, and TNF-α were lowered insignificantly compared to the cholesterol-rich diet group. At the same time, in the citrulline-treated group, the levels of TG and ET-1 decreased significantly, and the HDL-C level increased compared to the cholesterol-rich diet group.

Conclusion: A 10-week intake of L-citrulline against the background of a cholesterol-rich diet reduces the blood TG level and proinflammatory marker (ET-1) in experimental mice. L-citrulline use may have clinical benefits in the treatment of atherosclerosis in humans.(**International Journal of Biomedicine. 2025;15(1):205-209.**)

Keywords: lipid profile • inflammatory markers • L-citrulline

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Abbreviations

CRP, C-reactive protein; **ET-1**, endothelin-1; **eNOS**, endothelial nitric oxide synthase; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **NO**, nitric oxide; **ox-LDL**, oxidized low-density lipoprotein; **SMC**, smooth muscle cells; **TC**, total cholesterol; **TG**, triglycerides; **VLDL-C**, very low-density lipoprotein cholesterol.

Introduction

Atherosclerosis is considered the main cause of cardiovascular disorders and the leading cause of death in high-risk individuals.[1]

Studies have demonstrated that elevated levels of TC, VLDL-C, LDL-C, and TG, as well as a decreased HDL-C, are directly associated with risk for atherosclerotic events.[2] In the pathogenesis of atherosclerosis, the great diversity in the cellular mechanisms leads to many structural transformations and accumulation of inflammatory mediators, eventually ending with plaque formation.[3] The place and size of plaque deposition are important factors in determining the fate of atherosclerosis; for example, if atherosclerotic plaque forms

in the coronary artery, the clinical presentation ranges from asymptomatic to acute coronary disease and cardiac death,[4] while cerebral plaque leads to with ischemic stroke.[5]

Lipid profile measurements on serum samples could give the clinician the initial picture of the risk for atherosclerosis and cardiovascular disease.[6] Moreover, many lipid-lowering medication regimens depend on monitoring and assessing the level of lipid profile components.[7]

Inflammatory mediators have an important role in the pathophysiology of atherosclerosis. Cytokines like TNF-α and IL-6 are normally involved in recruiting immune cells to regulate the body's response to infection or injury. In inflammatory diseases, their levels become abnormally high, leading to excessive inflammation; in addition, many studies indicate that TNF-α specifically can contribute to the development of atherosclerosis.[8]

ET-1 is a vasoactive peptide involved in inflammation. Its synthesis is linked to TNF-α and interleukins. In addition to its action on immune cells, ET-1 also has a mitogenic effect on atherosclerotic lesions.[9]

Citrulline is a non-essential amino acid that acts as a precursor to NO by being converted back into L-arginine, which is then oxidized by the eNOS to produce NO, a potent vasodilator agent. Many studies showed the potential hypotensive effect of citrulline and L-arginine and their beneficial role in reducing the risk of cardiovascular disorder, which is usually associated with underlying endothelial dysfunction.[10-12]

Materials and Methods

Adult domestic male mice weighing from 30g to 35g used in the study were obtained from the College of Science, Kufa University. Before the experimental work, mice were acclimated for 2 weeks with free access to water and food, and the investigation was performed according to the Guide for the Care and Use of Laboratory Animals.

Experimental animals were assigned randomly for the following groups (six mice for each group): the control group, which received a normal chow diet; the induced group, which received a chow diet rich with 5% cholesterol; the L-citrulline-treated group, which received a chow diet rich with cholesterol, 5% together with 100 mg/kg of citrulline. Both chemicals were obtained from Sigma Chemical Company.

The study lasted for ten weeks; the mice monitoring continued during and after completing the study course. The blood sample was collected from the animals (the mice underwent anesthetic procedures using a combination of ketamine and xylazine injected intraperitonially to collect the blood). Blood samples were centrifuged at 1500 rpm for 15 minutes to obtain the serum, and then the ELISA technique was used to measure the level of TNF-α and ET-1. TC, HDL-C, LDL-C, VLDL-C, and TG were evaluated using the colorimetric enzymatic method and the Friedewald equation.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Data are shown as mean+SEM. The Mann-Whitney (U Test) was used to compare the differences between groups. A P-value of <0.05 was considered statistically significant.

Results

The lipid profile components and inflammatory markers were similar at the baseline in the study groups. After ten weeks, the group of mice that received a cholesterol-rich diet showed a significant increase in all lipid profile components, excluding HDL-C, and the level of the inflammatory markers (ET-1 and TNF-α) compared with control mice (Table 1, Figures 1-3). In the citrulline-treated group, the blood levels of TC, VLDL-C, LDL-C, and TNF-α were lowered insignificantly compared to the cholesterol-rich diet group. At the same time, in the citrulline-treated group, the levels of TG and ET-1 decreased significantly, and the HDL-C level increased compared to the cholesterol-rich diet group (Table 2, Figures 1-3).

Table 1.

Serum lipid profile parameters (mg/dL) and inflammatory markers (pg/mL) in the control and cholesterol-rich diet groups.

Group	TC	TG	VLDL-C	LDL-C	HDL-C	ET-1	TNF-α
Control group (n=6)	95.5±2.7	72.8±1.9	14.5±0.3	50.7±3.1	10.5±0.5	20.7±0.6	144.2±19.6
Cholesterol-rich diet group (n=6)	796.3±43.3	138.6±17.6	24.1±2.3	731.0±47.1	15.6±1.9	32.0±1.4	189.4±16.0
P-value	<0.001	0.005	0.002	<0.001	0.009	0.001	0.001

Table 2.

Serum lipid profile parameters (mg/dl) and inflammatory markers (pg/ml) in the cholesterol-rich diet and L-citrulline-treated groups.

Group	TC	TG	VLDL-C	LDL-C	HDL-C	ET-1	TNF- α
Cholesterol-rich diet group (n=6)	796.3±43.3	138.6±17.6	24.1±5.3	731.0±47.1	15.6±1.9	32.0±1.4	189.4±16.0
L-citrulline-treated group (n=6)	883.3±31.3	99.5±5.4	20.7±1.4	819.4±8.2	26.4±3.7	21.4±2.3	177.3±13.1
P-value	0.083	0.032	0.220	0.091	<0.001	0.001	0.200

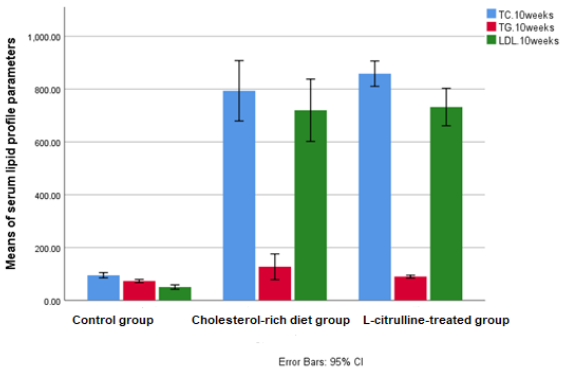

Fig. 1. *Serum levels (mg/dL) of TC, TG and LDL-C in the study groups.*

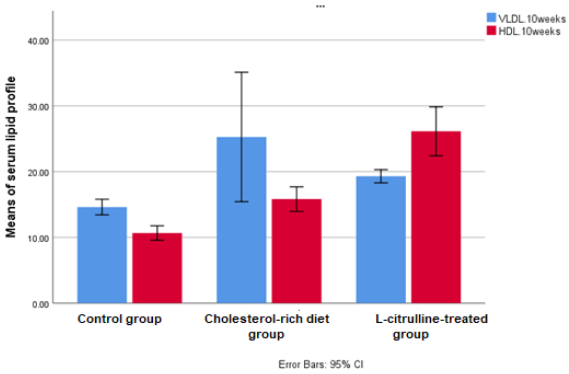

Fig. 2. *Serum levels (mg/dL) of HDL-C and VLDL-C in the study groups.*

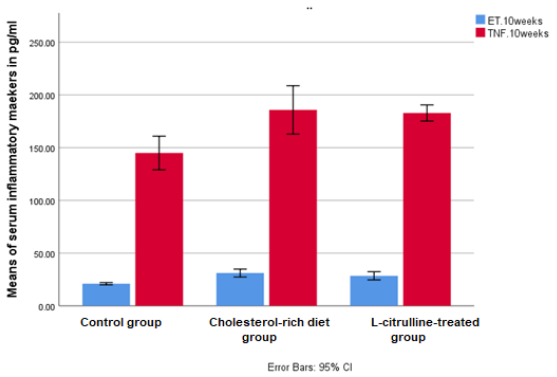

Fig. 3. *Serum levels (pg/dL) of ET-1 and TNF-α in the study groups.*

Discussion

This study, like several others, has demonstrated the role of a cholesterol-rich diet in increasing atherogenic components of the blood lipid profile and inflammatory markers associated with an increased risk of atherosclerotic plaque formation and damage. Systemic inflammatory responses are considered to play a major role in destabilizing atherosclerotic plaques.[13] Obesity and a high-fat diet are associated with chronic inflammation in human adipose tissues and arteries.[14] Oxidative modification of LDL-C is an essential mechanism that increases its inflammatory potential.

Today, many publications on ox-LDL can be found to provide evidence of its role in atherosclerosis.[15] ox-LDL instigates atherosclerotic events throughout the disease, from endothelium dysfunction, white blood cell activation, foam cell formation, SMC migration, and proliferation to platelet adhesion and aggregation.[16-20]

HDL, along with its major lipid-poor apolipoprotein A-I, aids in removing cholesterol from foam cells for clearance by the liver. The efflux from macrophage foam cells is the first step towards reverse cholesterol transport, lowering further inflammation and preventing atherosclerosis.[21,22]

Chronic supplementation with L-citrulline plus L-arginine has been shown to exhibit anti-atherosclerotic effects.[23,24] NO produced from L-arginine by eNOS plays an important role in regulating endothelium-dependent vasodilatation,[25-27] preventing the adhesion of blood cells and platelets along the endothelial cell layer of blood vessels,[28] and inhibiting vascular SMC proliferation.[29] NO also shows scavenging effects against oxygen radical species, including preventing ox-LDL.[30] Oral treatment with l-arginine in animals[31-33] and humans [34-36] has been studied extensively to suppress the progression of atherosclerosis or its component processes by restoring physiological levels of NO. However, relatively large doses of 5–15 g/day would be required to improve endothelial function in humans.

L-citrulline is a potent endogenous precursor of l-arginine. In a study by Schwedhelm et al.,[37] L-citrulline supplementation dose-dependently increased plasma L-arginine levels in healthy human volunteers more effectively than equivalent doses of L-arginine. Furthermore, several clinical trials demonstrated that L-citrulline supplementation functionally improved arterial stiffness[38] and decreased lipoprotein oxidation.[39]

A study by Morita et al.[23] focused on a potential strategy for promoting the L-citrulline-to-L-arginine recycling pathway—which is the principal mechanism for sustaining localized L-arginine availability for eNOS-catalyzed NO production, by simultaneous application of l-citrulline and l-arginine—and showed that L-citrulline plus L-arginine supplementation caused a more rapid increase in plasma L-arginine levels and marked enhancement of NO bioavailability, including plasma cyclic guanosine monophosphate (cGMP) concentrations, than with dosage with the single amino acids.

A study by Asgary et al.[40] aimed to assess the effects of l-citrulline supplementation on the clinical and laboratory outcomes in critically ill patients who were expected to receive mechanical ventilation for more than 72 hours. The patients in the placebo group received 10 g of microcrystalline cellulose, and the patients in the intervention group were given L-citrulline daily for 7 days. The citrulline group showed a notable reduction in fasting blood sugar (P=0.04), TC (P=0.02), LDL-C (P<0.001), and high-sensitivity CRP (P<0.001). The total duration of invasive ventilation and the mean Sequential Organ Failure Assessment (SOFA) score on Day 7 were significantly lower in the citrulline group than in the control group. Moreover, a significant increase in days alive and ventilator-free days within 28 days after admission was found in the citrulline group at the end of the trial.

These results support the effect of citrulline in our study, which showed a decrease in TC and LDL-C, but different results appear in the TG level, which was lowered significantly after 70 days of treatment with L-citrulline in our study. The notable differences in the results between these studies may be attributed to the difference in the duration of treatment and the dosage of the supplement. In several studies, oral L-citrulline ingestion has reduced serum inflammatory cytokine concentrations, such as IL-6, TNF-α, and CRP, in aged animals[41] and humans.[42] Although the exact mechanisms underlying the citrulline-mediated improvements in systemic inflammation remain unknown, it is possible, according to Breuilard et al.,[43] to dampen macrophage cytokine production.

Barkhidarian et al.[44] performed a comparative study between the patients in the intensive care unit who received citrulline supplements and those who received arginine supplements for ten days. The researchers found a significant reduction in the serum level of IL-6 only in the citrulline-treated group.

In another study, Miczke et al.[45] reported that the administration of L-arginine enhances the production of NO, which is necessary for maintaining normal endothelial function and increasing insulin sensitivity in visceral tissues; these findings were supported by Bode-Böger et al.,[46] who found that the treatment with L-arginine could be beneficial for patients with cardiovascular diseases developed from atherosclerosis and endothelial dysfunction.

In conclusion, the results of our study show that a 10-week intake of L-citrulline against the background of a cholesterol-rich diet reduces the blood TG level and proinflammatory marker (ET-1) in experimental mice. L-citrulline use may have clinical benefits in the treatment of atherosclerosis in humans.

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