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ORIGINAL RESEARCH

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Investigation of the effects of chronic L-arginine supplementation on paraoxonase-1 activity in high-cholesterol diet-induced hypercholesterolemia model in rats

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ABSTRACT

Background: Hypercholesterolemia is a cardinal driver of atherosclerotic cardiovascular disease (ASCVD), one of the major global mortality causes. Associated oxidative stress and lipid peroxidation perturbes HDL-associated paraoxonase-1 (PON1) activity. This study investigates the effects of chronic L-arginine supplementation—a nitric oxide precursor—on PON1 activity, lipid profiles, and oxidative stress in hypercholesterolemic rats.

Methods: Subjects were randomly selected and divided into four equal groups (n=36): control (C), L-arginine (LA), hypercholesterolemia (HC), and hypercholesterolemia + L-arginine (HC+LA). After 8-weeks study period, serum lipid levels, PON1, and malondialdehyde (MDA) levels were measured, and the atherogenic index (ATI) was calculated. Atherosclerotic lesions were evaluated histopathologically.

Results: PON1, MDA, and lipid levels, as well as ATI and the PON1/HDL ratio, were increased in the HC group. These variables were significantly lower in the HC+LA group than in the HC group. Moreover, a significant increase in TG, VLDL, and PON1 levels was observed in the LA group compared with the C group. A strong negative correlation was found between PON1 and MDA levels.

Conclusions: Chronic L-arginine supplementation was effective in reducing ATI, LDL, and oxidative stress, and in alleviating structural vascular changes in high cholesterol diet-induced hypercholesterolemia model rats.

Keywords: atherosclerosis, hypercholesterolaemia, L-arginine, malondialdehyde, paraoxonase

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INTRODUCTION

Atherosclerotic cardiovascular diseases (ASCVD), including stroke, remain a leading cause of morbidity and mortality worldwide [1]. Atherosclerosis is a central pathology of cardiovascular diseases, accompanied by dysfunction of various cell types, particularly endothelial cells [2]. ASCVD is characterized by chronic inflammation, primarily triggered by lipid retention in the subendothelial space. The subendothelial accumulation of low-density lipoprotein cholesterol (LDL-c) launches monocyte/macrophage migration into the tunica intima of the vessel wall [3]. Subsequently, these accumulated lipids were taken up by macrophages via scavenger receptors, resulting in the formation of lipid-laden macrophages, known as foam cells. Foam cells are a major source of reactive oxygen species (ROS) in atherosclerotic lesions [4, 5]. While LDL-C remains a major causal contributor to atherosclerosis, accumulating evidence demonstrates additional pathophysiological roles for triglycerides (TG) and very low-density lipoprotein cholesterol (VLDL-C) in ASCVD. [6-8]. Besides, high-density lipoprotein cholesterol (HDL-c) is a protective factor that attenuates the progression of atherosclerosis and is related to reduced ASCVD risk, partly through its antioxidant and anti-inflammatory activities, including inhibition of LDL oxidative modification [9]. Oxidized LDL-c (Ox-LDL) is a key inflammatory component in atherosclerotic lesions. Antioxidant activity of HDL-c, which accounts for its preclusive role in Ox-LDL formation, is based on paraoxonase-1 enzyme (PON1; EC 3.1.8.1) [10, 11].

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There are three different forms of the paraoxonase genes (PON1, PON2, and PON3) in humans, located near each other on the long arm of chromosome 7. PON1, a 43-45 kDa glycoprotein esterase expressed in various tissues, is synthesized mainly in the liver and transferred to the HDL molecule in circulation. The majority of serum PON1 activity is found in HDL-c, while the remainder is found in chylomicrons and VLDL-c, but not in LDL-c. Apo A1 in HDL-c stabilizes PON1 and stimulates lactonase activity [12-14]. PON1 hydrolyzes lipid peroxides from LDL-c and prevents LDL oxidation. PON1 also prevents HDL oxidation, which enables HDL-c to maintain its antiatherogenic function in reverse cholesterol transport. According to the literature, PON1 displays its protective effect in the early and late stages of atherogenesis, in a dose-dependent manner [15, 16].

L-arginine is a semi-essential and basic amino acid that acts in various roles as both a substrate and precursor molecule, with its breakdown by arginase in the urea cycle, for the synthesis of nitric oxide (NO) through nitric oxide synthase. L-arginine bears distinct cellular functions, such as radical scavenging, antioxidant, and antiapoptotic activities. A growing body of knowledge suggests that oral L-arginine treatment exhibits a preventive role in ASCVD, but its effect on PON1 activity is yet unknown [17-19].

Reactive oxygen and nitrogen species (ROS/RNS) are produced in both pathological and physiological reactions in eukaryotic cells. In health, they are removed by enzymatic or non-enzymatic antioxidant mechanisms, and during deterioration of the organism's redox balance, ROS/RNSs accumulate and cause oxidative damage, accompanied by the attack of reactive molecules on biomolecules such as lipids, proteins, DNA, and carbohydrates [20] of lipid peroxides (malondialdehyde, MDA), associated with the oxidation of membrane lipids -end oxidation product of polyunsaturated fatty acids [21, 22].

METHODS

Animals and experimental design

Thirty-six adult (6-7 months old) male Wistar albino rats, which were obtained from the Experimental Animal Center of Trakya University, were used in the study. Subjects were randomly selected in equal numbers and divided into four groups: control group (C), L-arginine group (LA), cholesterol group (HC), and cholesterol+L-arginine group (HC+LA). Experimental groups were housed separately in order to avoid dietary cross-exposure. The rats, group-housed (3 per cage), were kept at 25±2 °C and 50±15% relative humidity with a 12 h/12 h light-dark cycle. This experimental research was approved by the local animal ethical committee of Trakya University (TUHDYEK-2010/019). All experimental procedures on animals were conducted in accordance with high ethical standards and the Guide for the Care and Use of Laboratory Animals [23, 24].

Rats in the control group were fed with standard chow and water ad libitum. At the same time, the diet-induced hypercholesterolemia model was conducted using standard chow supplemented with 3% cholesterol for the concerned groups over 8 weeks [25-27]. L-arginine was administered through daily supplementation of 3% L-arginine in the drinking water [28].

Sample preparation and biochemical analysis

All rats were sacrificed by decapitation under ketamine/xylazine anesthesia after the 8-week experimental period. Blood samples were collected into the serum tube without additives, and were immediately transferred to the biochemistry laboratory in order to prepare the sample. Serum samples were separated by centrifugation at 2000xg for 10 minutes, Hettich ROTOFIX 32A centrifuge (Andreas Hettich GmbH, Tuttlingen, Germany), and subsequently aliquoted into labeled 1.5 mL polypropylene micro-centrifuge tubes. Aliquots were stored at -80 °C until the day of biochemical analysis and were prevented from undergoing freeze-thaw cycles. The arcus and abdominal aorta tissues were fixed in 10% formalin (neutral buffered solution) for further histopathological examinations.

Serum MDA, as a lipid peroxidation product, was determined using a colorimetric thiobarbituric acid-reactive-substances (TBARS) assay. Instrumental analysis was conducted in the Medical Biochemistry Research Laboratory of Trakya University [29]. All other biochemical analyses were performed in the Central Laboratory of Trakya University Hospital using a Siemens autoanalyzer (ADVIA® 1800 Chemistry System, Siemens, Germany). Serum PON1 activity was determined by using a commercial automated colorimetric paraoxonase assay kit (Rel Assay, Mega Tıp, Gaziantep, Turkey) [30]. Serum TChol, HDL-c, and TG levels were analyzed using an automated enzymatic assay. LDL-c, levels were estimated using the Friedewald formula [31]. Additionally, VLDL-c was estimated as 20% of serum triglyceride according to this formula. All biochemical analyses were performed in triplicate.

Calculations and definitions

Total cholesterol/HDL-c ratio, atherogenic coefficient (AC), and atherogenic index (ATI) were calculated using the following equations [32, 33].

$AC = (TChol\text{-}HDL\text{-}c) / HDL\text{-}c$

$ATI = log_{10}(TG / HDL\text{-}c).$

Histopathological Examination

The arcus and abdominal aorta tissues were fixed in 10% formalin for 48 hours and prepared for paraffin embedding. Thereafter, 5 µm sections were taken from formalin-fixed paraffin-embedded samples and stained with Haematoxylin, Eosin, and examined under a light microscope to determine structural abnormalities [34].

Statistical Analysis

Data analysis was performed using Statistica 7.0 software package (StatSoft, Tulsa, OK, US), SPSS version 22.0 (SPSS, Chicago, Illinois, USA), and GraphPad Prism version 9.0 (GraphPad Software, San Diego, California, US). Continuous variables in each group were tested for normality using the Kolmogorov-Smirnov test; parametric variables were analysed using one-way ANOVA to determine the significance of differences between groups. Spearman's Rank correlation analysis was performed and presented as a correlation table to reveal the direction, degree, and importance of the relationship between variables. A binomial logistic regression analysis was conducted in the HC group to identify the dependent factor associated with atherogenic vascular changes. Descriptive statistics for each group were given as mean ± standard deviation (SD). Statistical significance level was set at $p<0.05$.

The study workflow is shown in Figure 1.

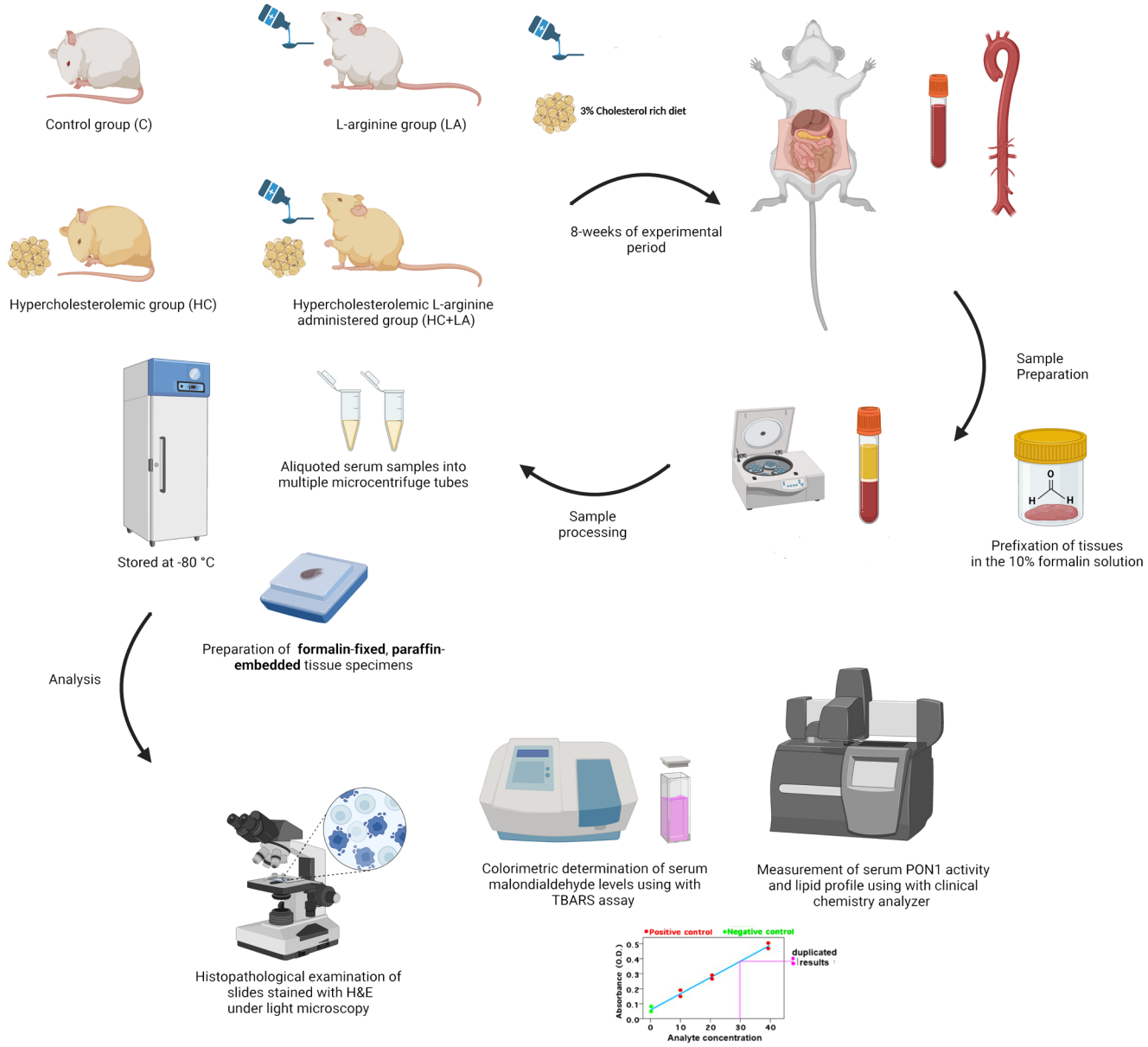

Figure 1. Overview of the study workflow, including sample collection, processing, and data analysis

In this experimental study, the objective was to investigate the metabolic effects of chronic L-arginine supplementation on serum paraoxonase-1 (PON-1) enzyme activity, lipid profiles, and early atherosclerotic vascular changes in diet-induced hypercholesterolemic rats.

RESULTS

Descriptive statistics for the study for each group are presented in Table 1, which shows that the diet-induced hypercholesterolemia model was successfully conducted. Both the initial and final weights of subjects were similar across groups according to the repeated-measures ANOVA ($F(3,32)=2.29$, $p=0.097$, η^2 $p=0.18$).

Serum TChol levels were found to be similar between C, LA and HC+LA groups; conversely, TChol levels of the subjects in the HC group were statistically higher than those of subjects in the other groups (Figure 2a). HDL-c levels were found to be similar between groups (Figure 2e). LDL-c levels in the HC group were found to be statistically higher than those of the other groups. Moreover, in the HC+LA group, LDL-c levels were found to be similar to the C and LA groups (Figure 2b).

While TG and VLDL levels in the HC group were found to be similar to those in the LA group, higher than those in the subjects in the C and HC+LA groups (Figure 2c-d).

AC, one of the indices of atherogenic risk, was found to be highest in the HC group. While LA and C groups were similar, HC and HC+LA groups were statistically different (Figure 2f). ATI, an alternate index for atherogenic risk, was found to be similar among the entire groups (Figure 2g).

NonHDL-c levels, as a more reliable atherogenic cholesterol in rodents, were found to be statistically significantly higher in the HC group according to the one-way ANOVA (Figure 2h). No significant differences in nonHDL-c were observed among the remaining three groups.

A forward stepwise binary logistic regression analysis was performed in the HC group to identify predictors of histopathologically confirmed atherosclerotic vascular alteration. Serum MDA emerged as an independent predictor. The regression model was

$$y = 2.08 + 4.59 \times MDA(\mu M)$$

where y is defined as log (p/1-p) and represents the probability of vascular alteration. Each 1 µM increase in MDA was associated with a significant increase in the odds of vascular pathology ($OR=e^{4.59}$).

Binary logistic regression analysis was performed with histologically confirmed atherosclerotic change (present/absent) as the dependent variable and experimental group as the independent variable (reference: control group) - Table 2. Compared with controls, the HC group demonstrated significantly increased odds of vascular alteration, whereas the LA group showed reduced odds. The HC+LA group was found to be similar to controls according to this analysis ($p=0.118$). Results are shown in Table 2.

Table 1. Descriptive statistics of the variables for each study group

	Control group (n=9)		LA group (n=9)		HC group (n=9)		HC+LA group (n=9)	
Variables (Unit)	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**
PON1 (U/L)	8.55±2.35	5.17-12.08	11.14±1.36	9.17-12.62	15.16±2.65	10.98-18.88	11.95±1.28	10.25-14.25
PON/HDL (U/mg)	5.47±1.50	3.30-7.90	6.59±0.72	5.30-7.40	9.24±2.63	5.80-14.20	7.74±0.69	7.10-9.10
MDA (nmol/mL)	3.00±0.69	1.85-4.30	3.16±0.45	2.33-3.63	5.06±0.32	4.52-5.67	4.34±0.31	3.94-4.74
AC	2.36±0.42	1.65-3.14	2.27±0.29	1.88-2.71	3.19±0.57	2.41-4.26	2.50±0.31	2.09-2.91
ATI	0.66±0.05	0.56-0.69	0.71±0.09	0.58-0.83	0.72±0.07	0.61-0.84	0.64±0.09	0.53-0.78
TG (mg/dL)	71.0±6.1	62.0-83.0	88.4±14.5	64.0-107.0	88.0±11.2	74.0-107.0	67.8±13.7	51.0-89.0
TChol (mg/dL)	52.2±4.2	46.0-60.0	55.3±7.0	46.0-65.0	70.2±9.0	58.0-81.0	53.1±5.4	47.0-61.0
HDL-c (mg/dL)	15.6±1.7	13.8-19.6	16.9±1.7	15.1-20.1	16.8±2.2	13.3-20.1	15.1±0.6	14.2-16.2
LDL-c (mg/dL)	22.3±4.1	17.5-31.3	20.6±5.9	9.9-28.2	35.7±9.2	22.5-45.9	24.3±5.4	17.1-34.1
VLDL-c (mg/dL)	14.2±1.2	12.4-16.6	17.6±2.9	12.8-21.4	17.6±2.2	14.8-21.4	13.5±2.7	10.2-17.8
IW (kg)	0.334±0.024	0.302-0.387	0.331±0.025	0.296-0.362	0.357±0.026	0.309-0.395	0.355±0.020	0.326-0.392
FW (kg)	0.370±0.019	0.343-0.404	0.366±0.035	0.320-0.416	0.388±0.023	0.353-0.418	0.409±0.034	0.375-0.460

Abbreviations: LA- L-arginine, HC- hypercholesterolemia, HC+LA- hypercholesterolemia+L-arginine, AC- atherosclerotic coefficient, ATI- atherogenic index, IW- initial weight, FW- final weight.

Table 2. Comparison of the atherosclerotic scores (AS) between experimental groups

Chi-Square Test	**Histological changes associated with atherosclerosis**		**Result**
	No changes	**Changes present**	
Control group (C)	5	4	$X^2 = 13.143$ df = 3 $p = 0.004$
L-arginine group (LA)	8	1	
Hypercholesterolemia group (HC)	1	8	
HC + LA group	2	7	
Binary logistic regression analysis	***p* value**	**β coefficient**	**Odds ratio (OR)**
Control group (C)	0.739		
L-arginine group (LA)	0.05	β = -2,079	0.125
Hypercholesterolemia group (HC)	0.05	β = 2,079	8.000
HC + LA group	0.118		

Atherosclerotic scores were obtained from histopathological examination of the aorta specimens of each group. Groups are different from each other in terms of atherosclerotic scores according to the Chi-square test.

Serum PON1 enzyme activity in the HC group was found to be significantly higher according to the one-way ANOVA test. Additionally, PON1 activity in the LA group was found to be statistically significantly higher than that in the C group. PON1 activities were found to be similar in LA and HC+LA groups (Figure 3a). While PON/HDL ratio in C and LA; HC and HC+LA groups were similar to each other, in the HC group, it was higher than C and LA groups (Figure 3b).

Notwithstanding that serum MDA levels in the HC group were found to be the highest, C and LA groups were similar in their levels. Moreover, serum MDA levels in HC+LA groups were found to be higher than in C and LA groups (Figure 3c).

Spearman's rank correlation analysis was performed to evaluate the associations among lipid parameters, oxidative stress markers, and atherosclerotic indices across the experimental groups (Table 3).

In the C group, HDL-c showed a significant negative correlation with the MDA (ρ=–0.789), whereas LDL-c demonstrated strong positive associations with TChol (ρ=0.778) and ATI (ρ=0.917). In addition, TChol was moderately correlated with TG (ρ=0.715).

In the LA group, LDL-c displayed strong positive correlations with TChol (ρ=0.862) and ATI (ρ=0.778). The AC was strongly correlated with final body weight (ρ=0.837). Fur-

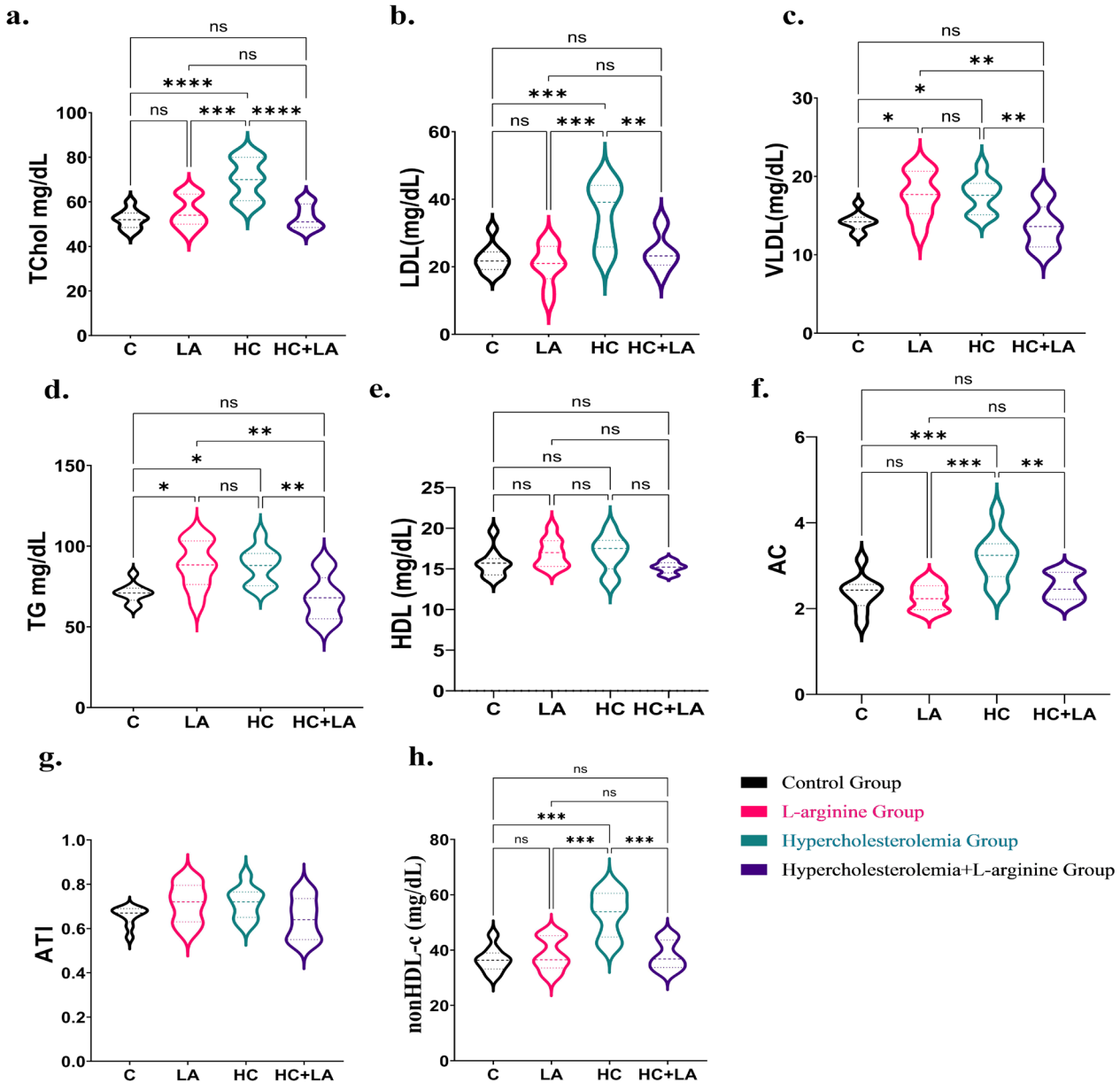

Figure 2. Comparison of serum lipid profiles and cardiovascular risk indices among the study groups

Each group consisted of nine rats: C - control group, LA - L-arginine group, HC - hypercholesterolemia group, HC+LA - hypercholesterolemia + L-arginine group. Abbreviations: AC - atherosclerotic coefficient, ATI - atherogenic index, HDL - high-density lipoprotein (cholesterol), LDL - low-density lipoprotein (cholesterol), non-HDL-c - non-HDL cholesterol, TChol - total cholesterol, TG - triglycerides, VLDL - very low density lipoprotein (cholesterol).

thermore, PON1 exhibited robust positive correlations with both final weight (ρ=0.753) and initial weight (ρ=0.811).

In the HC group, the total atherosclerotic score (AS) correlated positively with MDA (ρ=0.690), paraoxonase-1 (PON1; ρ=0.773), and PON1/HDL ratio (ρ=0.755). LDL-c was positively correlated with both total cholesterol (ρ=0.916) and ATI (ρ=0.720), while negatively correlated with AC (ρ= –0.717). AC itself showed an inverse association with HDL-c (ρ=–.717) but positive correlations with MDA (ρ=0.720) and TG (ρ= –0.697).

In the combined HC+LA group, LDL-c correlated positively with total cholesterol (ρ=0.828), ATI (ρ=0.667), and AC (ρ=0.992). It is important to be cautious when assessing ATI values since ATI is mathematically derived from serum TG and HDL-c concentrations. Therefore, ATI is inherently dependent on variations in these parameters. PON1 enzyme activity demonstrated a strong positive correlation with AC (ρ=0.966). Similarly, total cholesterol was strongly associated with AC (ρ=0.887). Final body weight correlated positively with both AC (ρ=0.800) and PON1 enzyme activity (ρ=0.783), whereas it exhibited a strong negative correlation with total AS (ρ=–0.777).

Overall, these findings indicate that while hypercholesterolemia induced strong positive associations among LDL-c, total cholesterol, and atherogenic indices, L-arginine supplementation partially modified these interactions, as evidenced by significant correlations involving PON1 and body weight parameters.

Light microscopic examination of hematoxylin and eosin-stained aortic tissue sections revealed normal vascular endothelium and vascular structures in both C and LA groups (Figure 4a-4b). Moreover, light microscopic findings in the HC group confirmed precursor atherosclerotic lesions such as endothelial disorganization and hyaline/mucoid degeneration limited to the endothelial and subendothelial layers of the tunica intima. However, atherosclerotic plaque formation and light microscopic changes involving vascular smooth muscle cells were not assessed, except for the increased thickness of the tunica media relative to the C and LA groups (Figure 4c). Apart from that, chronic L-arginine administration ameliorated hypercholesterolemia-induced changes in aorta tissue specimens of the subjects (Figure 4d).

DISCUSSION

In this experimental study using a diet-induced hypercholesterolemia model, we investigated the effects of chronic oral L-arginine administration for 8 weeks on adult male Wistar albino rats, focusing. The initial and final weights of the subjects, serum PON1 activity, serum lipoprotein, and MDA levels were measured. Additionally, the AC and ATI indices were calculated uniformly across all groups to facilitate internal comparisons with the intention of evaluating atherosclerotic risk. On the other hand, these indices are originally validated in human populations, and their direct extrapolation to assess rodent atherosclerotic risk should be interpreted cautiously. In this preclinical research, AC and ATI were used as relative markers of lipid distribution rather than definitive predictors of atherosclerotic burden. Histopathological examination of aortic samples was performed under the light microscope to evaluate atherosclerotic lesions by staining the slides with H&E.

In our study, the chronic L-arginine administered hypercholesterolemic group (HC+LA), it was found that the serum TChol, LDL-c, TG, VLDL-c levels, and the AC index were lower compared to the group where only hypercholesterolemia was modeled (HC), while the ATI ratio was similar between the groups. An experimental study conducted in rabbits, which involved subcutaneous administration of acute L-arginine at a dose of 10 mg/kg on days 1, 2, and 8, studied the effects of nitric oxide on plasma lipid profiles and was shown to increase plasma HDL-c levels on day 2 and decrease the AC index (35). Similarly, in an experimental study where a type 2 diabetes mellitus model was applied to adult male Wistar rats and the metabolic effects of chronic L-arginine supplementation were examined, L-arginine administration decreased serum TChol, TG, HDL-c, and LDL-c levels [36]. According to another experimental study, chronic parenteral L-arginine and L-norvaline (argininase inhibitor agent, also a non-proteogenic α amino acid) administration to rats fed a high-fat diet diminished TChol, LDL-c, and MDA levels [37].

Moreover, L-arginine influx into RBCs and nitric oxide synthase activity were found to be statistically significantly reduced in C57BL/6 mice fed a high-fat diet for 10 weeks. According to the concerned study, serum TChol and TG levels were found to be statistically significantly increased compared to the control group, while MDA levels remained unchanged [38]. According to a clinical study, in which the effects of chronic oral L-arginine supplementation on fasting plasma glucose (FPG), HbA1C, nitric oxide, and total antioxidant status (TAS) were investigated in diabetic patients with atherosclerotic peripheral vascular disease, both NO concentrations and TAS levels increased [39]. In a single-blinded, placebo-controlled clinical trial, chronic oral L-arginine administration in obese patients statistically significantly diminished TC, TG, LDL-c, and MDA levels in a dose-dependent manner, while HDL-c levels were elevated in the group administered with high-dose L-arginine compared to baseline [40].

Preclinical and clinical studies in scientific literature show that L-arginine supplementation increases NO levels in peripheral blood [19, 41, 42]. Moreover, the current literature indicates a strong association between

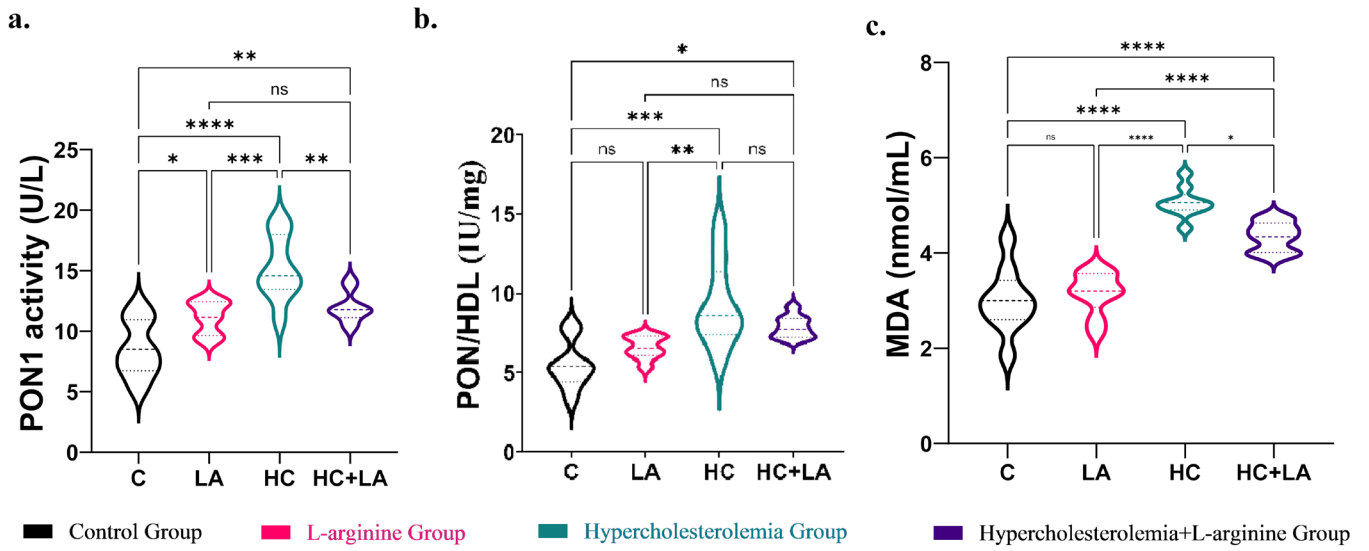

Figure 3. Group comparison of serum MDA levels, PON1 activity, and its derived ratio PON1/HDL

Each group consisted of nine rats: C - control group, LA - L-arginine group, HC - hypercholesterolemia group, HC+LA - hypercholesterolemia + L-arginine group. Abbreviations: HDL - high-density lipoprotein (cholesterol), MDA - malondialdehyde, PON1 - paraoxonase-1 enzyme.

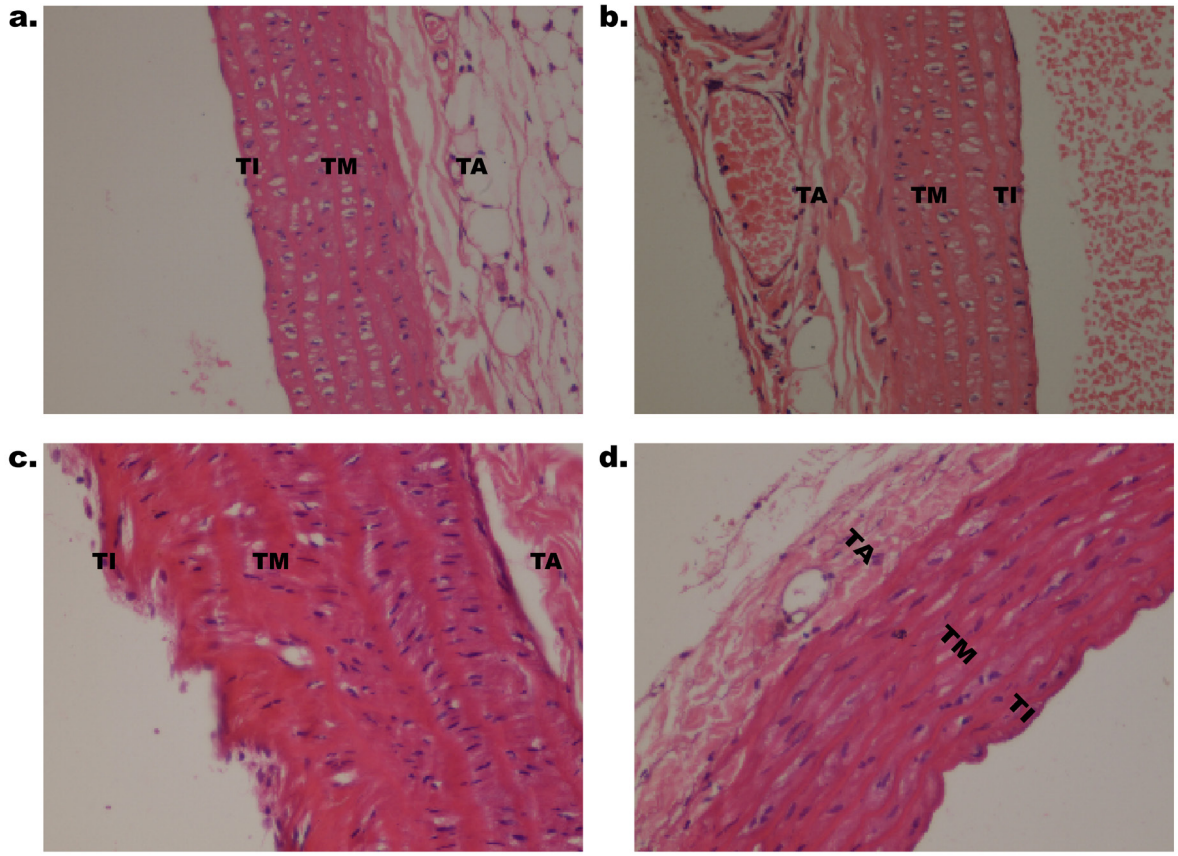

Figure 4. Histopathological examination of aorta specimens of the subjects

Light microscopic examination of hematoxylin and eosin (H&E)-stained aortic tissue sections. **(a)** Control (C) group; **(b)** L-arginine (LA) group; **(c)** Hypercholesterolemia (HC) group; **(d)** Hypercholesterolemia + L-arginine (HC+LA) group. The C and LA groups showed normal vascular endothelium and vascular structures. HC group showed precursor atherosclerotic lesions such as endothelial disorganization and hyaline/mucoid degeneration limited to the endothelial and subendothelial layers of the tunica intima, as well as increased thickness of the tunica media. In the HC+LA group, chronic L-arginine administration ameliorated hypercholesterolemia-induced histological changes Abbreviations: TA - tunica adventitia, TI - tunica intima, TM - tunica media.

Table 3. Spearman rank correlation analysis of the data for all experimental groups

Groups	Variables	Total AS	HDL-c	LDL-c	AC	MDA	PON1	PON/ HDL	TChol	ATI	TG	Weightt Diff.	Final Weight	Initial Weight
C	HDL-c									-.678				
	LDL-c								.778	.917				
	MDA						-.789							
	PON/HDL													
	TChol			.778							.715			
	ATI		-.678	.917										
	TG								.715					
	Final weight													.695
LA	LDL-c								.862	.778				
	AC										.837			
	PON1												.753	.811
	TChol			.862										
	ATI			.778										
	Final weight						.753					.870		.828
HC	Total AS									.690			.773	.755
	LDL-c				-.717				.916					
	AC			-.717							.720	-.667		
	MDA											-.828		
	TG				.720								-.697	
	Final weight										-.697			.908
HC + LA	Total AS											-.807	-.777	
	LDL-c					-.706			.828	.667				
	AC										.992			
	PON1							.966						
	TChol			.828						.887				
	Final weight	-.777										.800		.783

Each experimental group consisted of nine rats: C - Control group, LA - L-Arginine group, HC - Hypercholesterolemia group; HC+LA - hypercholesterolemia + L-arginine group. Spearman rank correlation was used for analysis and relevant correlation coefficients (ρ) are presented in the table. The strength of correlation was interpreted as follows: $0.80 \leq |\rho| \leq 1.00$ very strong; $0.60 \leq |\rho| < 0.80$ strong; $0.40 \leq |\rho| < 0.60$ moderate: $0.20 \leq |\rho| < 0.40$; weak: $0.00 < |\rho| < 0.20$ very weak. Correlation coefficients were color-coded as follows: red color refers to strong positive correlation, light red color refers to moderate positive correlation, blue color refers to strong negative correlation, light blue color refers to moderate negative correlation. Abbreviations: AC - atherogenic coefficient, ATI - atherogenic index, AS - atherosclerotic score, HDL-c - high-density lipoprotein cholesterol, MDA - malondialdehyde, PON1 - paraoxonase-1 enzyme, TChol - total cholesterol, TG - triglycerides.

serum lipid profiles and NO levels [43, 44]. Additionally, we found that the serum lipid profile of rats supplemented with chronic oral L-arginine was similar to that of the control group. Consistent with our findings, a systematic review and meta-analysis of randomized controlled trials reported that oral L-arginine supplementation had no statistically significant effect on serum lipid profile. Particularly, it was stated that L-arginine supplementation had no significant effect on serum TChol, LDL-c, or HDL-c levels, while TG levels were significantly, though slightly, reduced compared to placebo controls [45]. Researchers who conducted a clinical study in healthy individuals to investigate the metabolic effects of low- and high-dose L-arginine supplementation over one week reported that low-dose arginine reduced TChol and VLDL-c levels while increasing HDL-c levels (46). Similar to both preclinical and clinical studies in the literature, our study also demonstrates that L-arginine supplementation leads to a favourable change in the lipid profile of hypercholesterolemic individuals. In our study, HDL-c levels were found to be similar between the L-arginine-supplemented groups and other experimental groups. Furthermore, research examining the effects of L-arginine administration showed consistent findings regarding its reduction in TC, LDL-c, and MDA levels, while its effect on HDL-c levels indicated contradictory findings [35, 36, 46].

According to another study, which employed a similar experimental design to ours and was conducted on adult male Wistar albino rats, high cholesterol diet-fed subjects in the experimental group were supplemented with 3% oral L-arginine for 8 weeks and showed 42% lower serum PON1 activity and 152% lower ATI compared to the peer hypercholesterolemic group. Similar to the aforementioned data, serum MDA and LDL-c levels were found to be significantly lower [47]. We found that serum PON1 activity in the LA group was higher compared to the C group, while subjects in the HC group displayed the highest PON1 activity. Moreover, the PON/HDL ratio was found to be similar to that of the HC and HC+LA groups, but higher than in the C and LA groups. Whereas there is a common thought for reduced risk of atherosclerosis with elevated serum PON1 activity, current scientific literature, based on experimental research, shows contradictory findings regarding the effects of a cholesterol-rich diet on serum PON1 activity [48-50]. A part of these studies provided an explanation for the inconsistent response of PON1 activity in rats fed a high-cholesterol diet, which is caused by discrepancies in the antioxidant system [51-53]. It is possible that the increased oxidative stress and related adaptive metabolic responses associated with the hypercholesterolemic diet in the HC+LA group may have neutralized the effect of L-arginine supplementation on PON1 activity, though there are studies showing positive effects [47, 54, 55].

In this experimental study, in which diet-induced hypercholesterolemia was modelled and an atherogenic lipid profile was obtained, the AC index in the HC group was higher than in the other groups. Histopathological examinations of the arcus and abdominal aorta tissues revealed that atherosclerotic plaque formation had not yet occurred. On the other hand, in the HC group, early signs of atheroma formation, such as endothelial irregularities and hyaline/mucoid degeneration, were detected. The histological findings in the HC+LA group, being similar to those in the C group, suggest that the atherosclerotic effects of the cholesterol-rich diet were mitigated by L-arginine. Zhang et al. demonstrated in their study, which was conducted on male Sprague-Dawley rats, that chronic L-arginine supplementation over 12 weeks could prevent high-fat diet-induced atherosclerotic changes [56]. The in vitro experimental part of the aforementioned study showed that L-arginine diminished endothelial cell damage caused by oxidized-LDL.

In conformity with another study, carried out with a similar experimental design, MDA levels were correlated with high-fat diet-induced atherosclerotic vascular changes, since high-fat diet-mediated elevations in MDA were reduced by both antioxidant-rich tomato extract and the conventional lipid-lowering treatment atorvastatin [57]. Histopathological findings of our study, which are in harmony with current literature, showed that atherosclerotic vascular changes were statistically significantly more pronounced in the HC group compared to the other groups. The results indicated a positive correlation with lower serum MDA and LDL-c levels in the group administered chronic L-arginine, aligning with the histopathological vascular findings.

In another experimental study conducted on Wistar albino rats fed with a high-cholesterol diet for 6 weeks to investigate the anti-atherogenic effects of fruit extract, it was reported that the ATI and AC were higher in the experimental groups in which atherosclerosis was modelled. AC was defined as the coronary risk index in the paper. Furthermore, in that group, histopathological examination of H&E-stained aorta sections revealed lesions characterized by lipid deposition [58].

CONCLUSIONS

In normal physiological conditions, L-arginine supplementation displayed limited effects on both the serum lipid profile and MDA levels. However, chronic oral L-arginine administration, together with a high-cholesterol diet, significantly altered serum PON1 activity, contrary to expectations, as serum PON1 activity dropped in the group of rats fed a high-cholesterol diet in response to L-arginine supplementation. That phenomenon can be attributed to the high-cholesterol diet-associated eleva-

tion in oxidative stress. This may be associated with upregulation of the antioxidant defence mechanism as an adaptive response to oxidative stress induced by high cholesterol. According to our research findings, L-arginine supplementation would be beneficial for atherosclerotic vascular changes, acting as an antioxidant, in individuals with hypercholesterolemia. Nonetheless, the following limitations should be acknowledged. First, the relatively small number of animals per group may limit statistical power and increase the risk of type II error. Second, LDL-c values were estimated using the Friedewald equation, which was developed and validated in humans and may not accurately reflect lipoprotein distribution in rodents, an HDL-dominant species with distinct lipid metabolism. Experimental evidence suggests that the Friedewald formula may overestimate LDL-c in hypercholesterolemic rats [59]. Similarly, atherogenic indices such as the atherogenic coefficient (AC) and atherogenic index (ATI) were originally developed for human cardiovascular risk assessment and may not optimally represent atherogenic burden in rat models. Although these methodological considerations do not affect the internal statistical comparisons between experimental groups, they may limit extrapolation regarding absolute cardiovascular risk and translational interpretation.

ABBREVIATIONS

AC – Atherogenic Coefficient

ATI – Atherogenic Index

ApoA1 – Apolipoprotein A1

AS – Atherosclerotic score

ASVCD – Atherosclerotic cardiovascular diseases

HC – Hypercholesterolemia

HDL-c – High-density lipoprotein cholesterol

H&E – Haematoxylin and eosin stain

kDa – Kilodalton

LA – L-arginine

LDL-c – Low-density lipoprotein cholesterol

MDA – Malondialdehyde

PON – Paraoxonase

Ox-LDL – Oxidized LDL

ROS – Reactive oxygen species

RNS – Reactive nitrogen species

TBARS – Thiobarbituric acid-reactive-substances

TChol – Total cholesterol

TG – Triglyceride

VLDL – Very low-density lipoprotein

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AUTHORS' CONTRIBUTION

ODO: writing draft; analysis, data management; concept/design; data collection; data analysis; interpretation; critical revision; final approval

SU: writing draft; analysis; data management; interpretation; data collection; final approval

FH: writing draft; analysis; data visualization; interpretation; final approval

SE: writing draft; analysis; concept/design; data collection; interpretation; final approval

SA: writing draft; analysis; data management; data collection; interpretation; final approval

SO: writing draft interpretation; final approval

SSG: writing draft; analysis, critical revision

All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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