DE GRUYTER OPEN

Short Communication

DOI: 10.1515/rrlm-2017-0024

Administration of valine, leucine, and isoleucine improved plasma cholesterol and mitigated the preatherosclerotic lesions in rats fed with hypercholesterolemic diet

Simona Ifrim[1], Cornelia Amalinei[2], Elena Cojocaru[3], Mioara Calipsoana Matei[4*]

1. Department of Preventive Medicine and Interdisciplinarity, "Grigore T. Popa" University of Medicine and Pharmacy - Iasi
2. Department of Morphofunctional Sciences I, "Grigore T. Popa" University of Medicine and Pharmacy - Iasi; Departent of Histopathology, Institute of Legal Medicine, Iasi
3. Department of Morphofunctional Sciences I, "Grigore T. Popa" University of Medicine and Pharmacy - Iasi
4. Department of Preventive Medicine and Interdisciplinarity, Grigore T. Popa University of Medicine and Pharmacy - Iasi; Iasi Clinical Epidemiology Research and Training Centre; Department of Oncogenetics

Abstract

Hypercholesterolemia has a major contribution to the occurrence and progression of atherosclerotic lesions. Recent studies report the involvement of branched-chain amino acids in cholesterol methabolism. The aim of this research was to evaluate the role of valine, leucine and isoleucine on the occurrence and progression of atherosclerosis in rats receiving hypercholesterolic diet. Material and methods: 50 male Wistar rats distributed into five groups with the following type of diets: group I (control) received standard diet; group II - cholesterol; group III - cholesterol and valine; group IV - cholesterol and leucine; group V - cholesterol and isoleucine. The experimental study was conducted over a period of 2 months. The animals were evaluated for the serum levels of total cholesterol at the beginning of the experiment, after 1 month and after 2 months. The collected tissue fragments of heart and aorta were prepared for the examination by optical microscopy in order to identify the atherosclerotic changes. Results: The most increased values of serum cholesterol were recorded in rats from group II (p=0.001), for the second and third evaluation. The histological examination showed early histopathological lesions on the vascular intima for the groups treated with cholesterol, valine, leucine, and isoleucine. These early changes (the occurrence of some superficial endothelial erosions, adhesion of erythrocytes and platelets) were correlated with the degree of the arterial wall damage, of the leukocytes adhesion to the arterial intima, and the discontinuities of the internal elastic lamina. Conclusion: The comparative study of the effects of the three essential amino acids revealed that valine induced a faster response than leucine and isoleucine on the improvement of biochemical parameters, but there were no significant differences between the three amino acids in terms of their protective ability, demonstrated by the histopathological lesion assessment.

***Corresponding author**: Mioara Calipsoana Matei, Universitatea de Medicina și Farmacie Grigore T. Popa - Iasi, Romania. E-mail: mat_calipso@yahoo.com

Keywords: valine, leucine, isoleucine, hypercholesterolemia, atherosclerosis
Received: 24th April 2017; Accepted: 12th June 2017; Published: 14th December 2017

Introduction

Among cardiovascular diseases, atherosclerosis is the *world's leading cause of death* [1]. Atherosclerosis is a multifactorial disease in which hype-/dyslipidemia and oxidative stress have major contributions to the initiation and progression of the lesions [2-5]. The branched-chain amino acids (BCAA) are diet-related essential amino acids. Valine, leucine, and isoleucine were included in this category. Recent epidemiological studies reported the involvement of BCAA in lipolysis, lipogenesis, and cholesterol methabolism [6]. Previous studies have shown that valine and leucine increase the serum level of HDL-C, proving an important contribution to the regression of atherosclerosis [7, 8]. These two BCAA were also related to a decreased LDL-C serum level with potential role as lipid-lowering agents [8, 9].

The aim of this research was to evaluate the role of valine, leucine, and isoleucine in the occurrence and progression of atherosclerosis in rats receiving hypercholesterolemic diet.

Material and methods

Animals and diets. Fifty male Wistar rats with weights of 250-300 g (provided by the Cantacuzino Institute from Bucharest) were used in this study. The rats were distributed into five groups, 10 rats for each group, with the following type of diets: group I (control) received standard diet; group II (C) received 0.4g/kgc/day cholesterol; group III (C + V) received 0.4g/kgc/day cholesterol and 62.5mg/kgc/day valine amino acid; group IV (C + L) received 0.4g kgc/day cholesterol and 69.985mg/kgc/day leucine amino acid; group V (C + iL) received 0.4g/kgc/day cholesterol and 69.985mg/kgc/day isoleucine amino acid. The cholesterol-rich diet was prepared by adding to the normal diet (1%) 0.15% (p/p) of cholesterol Sigma®C 8503 diluted in ethyl ether P.A. In order to stabilise the ethyl ether, 10 ppm of butil hidroxi-tolueno-BHT were used. The cholic acid Sigma®C 1254 (0.25%) diluted in absolute methanol Anhidro P.A. was added in order to facilitate the absorption of cholesterol. The amino acids were administered in the drinking water.

The weight of the rats after 2 months of cholesterol-rich diet was around 12% higher. The animals included in the study were not evaluated for the existence of other metabolic diseases.

The experimental study was conducted over a period of 2 months (60 days). After the use of 75 mg/kgc intraperitoneal ketamine for anesthesia, blood samples were collected from the retro-orbitary plexus of each animal, in order to evaluate the serum levels of total cholesterol, at the beginning of the experiment (R0), after 1 month (R1), and after 2 months (R2). The current research was carried out using the instructions provided by the "*Guide for the Care and Use of laboratory animals*" [10]. The study complied with the national and international ethical regulation [11-13].

Serum cholesterol analysis. Serum total cholesterol levels were measured with reagents from Diagnosticum Zrt (Budapest, Hungary) [14].

Histopathological analysis. The collected samples (tissue fragments of heart and aorta) were prepared for the examination by optical microscopy using paraffin embedding and sectioning method. Two techniques were used for the staining of the sections: hematoxylin and eosin (HE) and Goldner-Szekely trichrome stains. The histological slides were analysed in order to identify the atherosclerotic lesions.

Statistical analysis. The collected information was introduced in a database which was analysed with Microsoft Excel statistical functions and Statistical Package for Social Sciences (SPSS) version 12 software. The measured data were represented as mean (M), standard deviation (SD), coefficient of variation (CV), and confidence interval (CI). One-way ANOVA was used to compare means between groups; $p<0.05$ was considered as statistically significant.

Results

The evolution of total serum cholesterol

At the begining of the study (R0), in the control group, the values of serum total cholesterol ranged between 33.1 and 41.2 mg/dl, with a mean of 36.9 mg/dl, followed by a slight increase in the average level over the next period to 37.7 mg/dl (for R1) and 38.2 mg/dl for R2 (p=0.399).

The level of total cholesterol was investigated three times for each group: at the beginning of the experiment (R0), after one month (R1), and after two months (R2). In all 5 groups (control, C, C+V, C+L, C+iL), the most homogeneous set of values was registered at the end of the experiment (R2): CV = 4.0% - control group; CV = 4.8% - cholesterol group; CV = 3.3% - cholesterol and valine group; CV = 3.9% - cholesterol and leucine group; CV = 4.1% - cholesterol and isoleucine group .

Among the rats from the second group (C), the level of the total cholesterol showed variable values in the range between 29.3 and 41.2 mg/dl (with a mean of 36.9 mg/dl). The trend was ascending after the initial moment of the evaluation with significant increased values: a mean of 50.3 mg/dl for the second measurement (R1) (ranged from 42.2 to 54.3 mg/dl) and 76.9 mg/dl for the final moment of the study (R2) with a variation between 70.8 and 80.4 mg/dl (p=0.001).

For the next group (C+V), at the begining of the research (R0), the total cholesterol value ranged from 35.1 to 38.5 mg/dl (mean of 37.0 mg/dl), followed by an increase at a mean of 40.9 mg/dl (range from 37.2 to 42.6 mg/dl) for the next evaluation (R1), and 44.3 mg/dl (range from 42.3 to 46.1 mg/dl) for the final moment of the experiment (R2) (p=0.001).

At the initial moment (R0), the fourth group (C+L) registred a variation of the total cholesterol between 33.1 mg/dl and 40.1 mg/dl (with a mean of 36.55 mg/dl). Variations of the total cholesterol level were noted until the end of the study. The mean values were 46.2 mg/dl after 1 month (R1) (with a minimum of 40.4 mg/dl and a maximum of 49.1 mg/dl) and 49.7 mg/dl after 2 months (R2) (with a minimum of 45.5 mg/dl and a maximum of 52.7 mg/dl) (p=0.001).

For the last group (C+iL), the first evaluation of the total cholesterol levels (R0) revealed values from 33.3 to 40.1 mg/dl, the mean being 36.8 mg/dl. After the initial measurement, important increases of the mean level of the total cholesterol were observed as follows: 46.7 mg/dl after one month (R1) (range between 40.4 and 50.1 mg/dl) and 49.9 mg/dl after two months (R2) (range between 45.5 and 52.2 mg/dl) (p=0.001).

The valine-induced decrease in cholesterol level was statistically significant compared with the other two amino acids (p=0.002).

The most increased values were recorded in rats from group C (cholesterol) (p=0.001), for the second evaluation (R1) and for the final moment of the experiment (R2). The differences between the mean levels of the total cholesterol among the studied groups did not show a statistical significance (p=0.993) in the initial measurement (R0) (Table 1).

The highest individual values of total cholesterol were recorded for the second group, which received only cholesterol (Figure 1).

The histopathological study

The aim of the histopathological study was to explore the lesions initiation related to the ath-

Table 1. Statistical indicators of total cholesterol values compared by study groups

Group	N	Mean (mg/dl)	StandardDeviation (mg/dL)	CV (%)	95% CI for mean (mg/dl)		Total cholesterol (mg/dl)		p value (FANOVA test)
					Min	Max	Min	Max	
R0									
Control group	10	36.9	2.2		35.4	38.6	33.1	41.3	
Group C	10	36.9	3.4		34.4	39.3	29.3	41.2	
Group C+V	10	37.0	0.9		36.3	37.7	35.1	38.5	0.993
Group C+L	10	36.6	2.4		34.83	38.3	33.1	40.1	
Group C+iL	10	36.8	2.3		35.1	38.5	33.3	40.1	
R1									
Control group	10	37.7	2.0		36.3	39.1	34.4	40.1	
Group C	10	50.3	3.5		47.8	52.8	42.2	54.3	
Group C+V	10	40.9	1.6		39.9	42.1	37.2	42.6	0.001
Group C+L	10	46.2	2.5		44.5	47.9	40.4	49.1	
Group C+iL	10	46.7	2.8		44.7	48.7	40.4	50.1	
R2									
Control group	10	38.2	1.8		36.9	39.5	35.9	40.9	
Group C	10	76.9	3.1		74.7	79.2	70.9	80.4	
Group C+V	10	44.3	1.5		43.2	45.3	42.2	46.1	0.001
Group C+L	10	49.7	1.9		48.3	51.1	45.5	52.7	
Group C+iL	10	49.9	2.1		48.4	51.3	45.5	52.2	

erosclerotic process and the evaluation of the correlation between pathological changes associated with the hypercholesterolemic diet and the administration of some essential amino acids (valine, leucine, and isoleucine).

In this current experimental research, aorta and coronary artery walls showed the following features: superficial erosions of the endothelium lining the vessel lumen, variable adhesion of erythrocytes, leukocytes, and platelets to the arterial wall correlated with the degree of arterial wall damage, occurrence of rare foamy macrophages on the arterial intima, and mild thickening of the arterial wall (Table 2).

For the control group (including rodents that received a standard diet) the histopathological examination showed a normal feature of the arterial wall using both HE and Goldner-Szekely trichrome stains. The intima of the analysed arterial wall fragments revealed a delicate simple squamous epithelium separated from the internal elastic lamina by a loose subendothelial connective tissue containing several fibroblasts, smooth muscle cells, and thin collagen fibers. Multiple concentrical layers of muscle fibers have been noticed in the tunica media of coronary arteries. The tunica adventitia was relatively thin and contained fibroblasts, longitudinal bundles of collagen fibers, and a loose network of elastic fibers.

The histopathological examination of the tissue fragments of

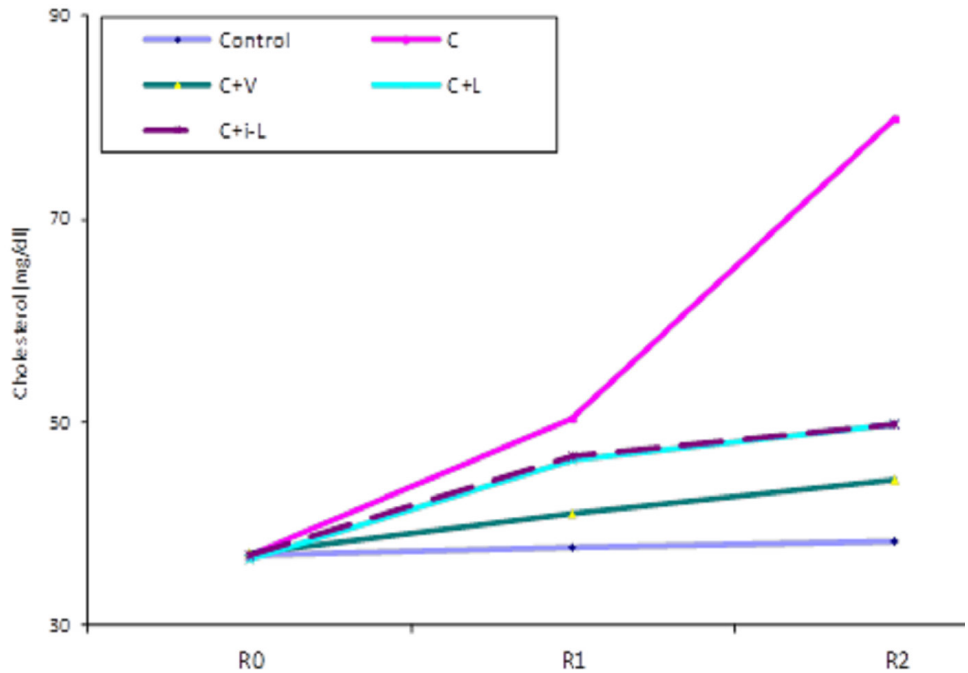

Fig.1. The trend of total serum cholesterol values for experimental groups

the rodents that received hypercholesterolemic diet revealed some changes in the arterial wall structure that affected the endothelium and the subendothelial space. Optical microscopy examination showed specific features of endothelium characteristic for the prelesional stage of atherosclerosis, revealing a degree of endothelial dysfunction, such as adhesions of erythrocytes and platelets, associated with endothelial discontinuity (table 2). Leukocyte margination and rare macrophage occurrence in the subendothelial tissue were also observed. The endothelial damage may have resulted from LDL cholesterol endocytosis, followed by phagocytosis by macrophages that were consequently transformed into foam cells, in the subendothelial space (Figure 2a).

The above described changes were reduced within the groups which received valine, leucine, and isoleucine, respectively, comparing with the group receiving cholesterol, demonstrating the protective role of the three amino acids associated with hypercholesterolemic diet.

Thus, reduced erythrocyte and platelet adhesion to both types of arterial walls (aorta and coronaries) were noticed within the third group

Table 2. The assessed histopathological changes related to early atherosclerotic process by study groups

Group	Adhesions of leucocytes	Adhesions of erythrocytes and platelets	Superficial erosions of the endothelium
CONTROL	-	-	-
C	++	++	++
C+V	+	+	-
C+L	+	+	+/-
C+iL	+	+	+/-

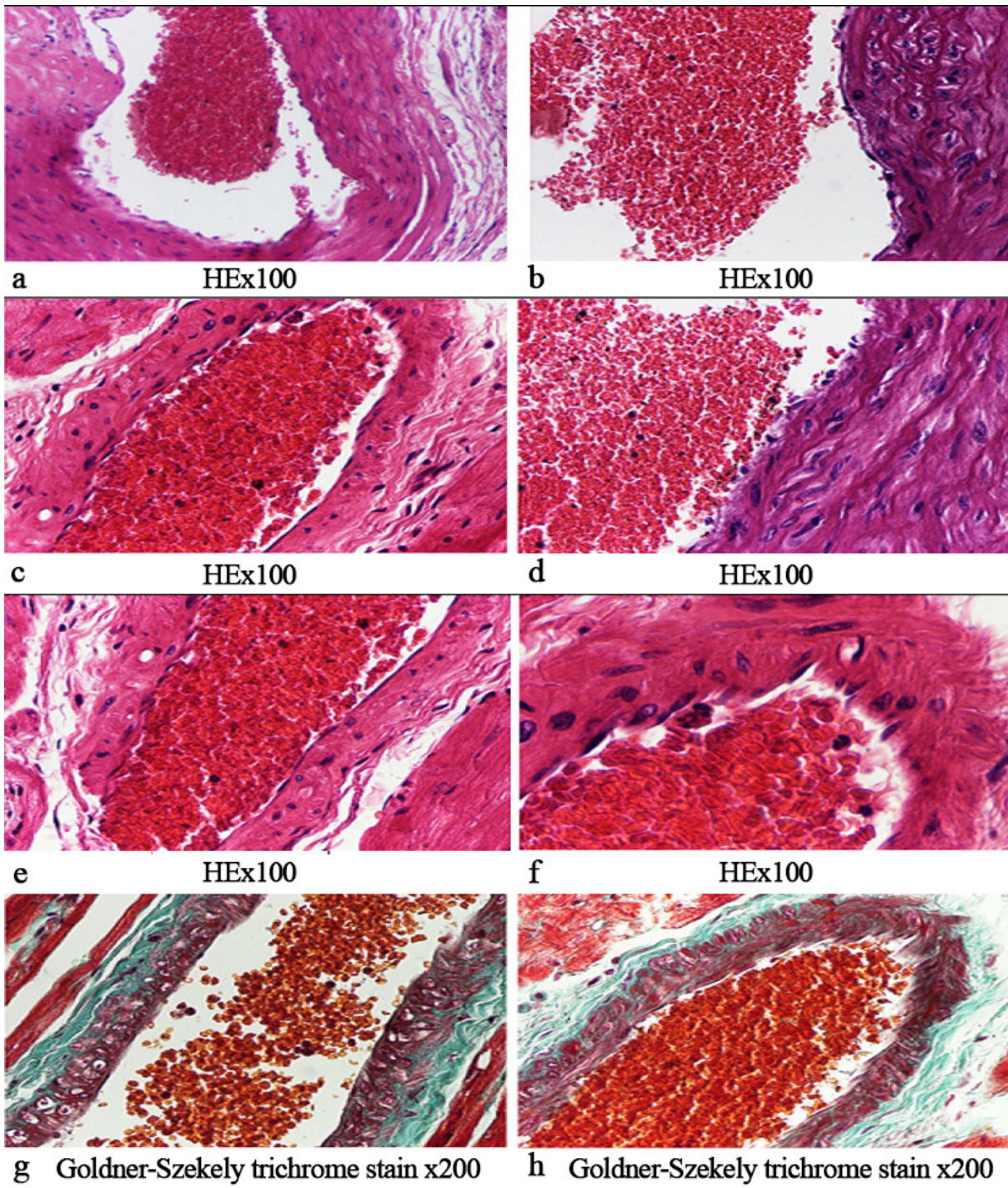

Fig. 2a. Vascular wall with early atherosclerotic changes in group II (Hypercholesterolemic rats): adhesions of erythrocytes and platelets associated with endothelial discontinuity; leukocyte margination and rare macrophages at the level of the subendothelial tissue (HE stain - a, c, e x100; b, d x 200; f x400; Goldner-Szekely trichrome stain - g, h x200)

(rodents receiving a diet rich in cholesterol associated with valine) (Table 2). The internal elastic lamina preserved its continuity, and the elastic layers of the coronary wall structure showed a structure within normal histological limits in HE and Goldner-Szekely trichrome stains (Figure 2b).

The evolution of changes in the rats from the fourth group (the animals receiving a cholesterol diet associated with leucine) revealed a similar feature with that observed in animals from the third group (cholesterol and valine diet). Microscopic examination showed reduced erythrocyte, leukocyte, and platelet adhesion to the endothelium, preserving the disposition of the elastic layers within the structure of coronary tunica media.

The fifth group (rodents which received a high cholesterol diet associated with isoleucine) exhibited similar morphological features when compared with groups III and IV, as follows: presence of some arterial lumens, sometimes with irregular endothelium, and with a slight adhesion of erythrocytes, and platelets due to intimal discontinuity.

Discussions

Cardiovascular disease is the main cause of death in the United States and globally. An insight into the major risk factors of cardiovascular disease identifies hypercholesterolemia, as one of its major consequences is atherosclerosis, involving many genetic and environmental factors which are difficult to control [15]. Furthermore, oxidative stress contributes to the pathogenesis of hypercholesterolemic atherosclerosis. Anandhi et al. conducted an experimental study on atherosclerosis, using male Wistar rats which were fed with an atherogenic diet, for 45 days. The study results indicate significantly higher average levels of serum lipid profile parameters (total cholesterol, triglycerides, and low-density lipoprotein cholesterol) [16], the fact being also reported in our study, regarding the level of total cholesterol.

Of the 8 branched-chain amino acids, leucine, isoleucine, and valine are considered the essential amino acids. Xiao et al. found that a diet deficient in amino acids used for a period of 7 days quickly reduces abdominal fat mass in mice [17]. Recent scientific evidence from studies conducted on animals and humans have reported a link between levels of branched-chain essential amino acids and obesity [18]. Moreover, recent research suggests that leucine is involved in systemic cholesterol metabolism. Thus, Zhao et al. investigated the effects of leucine supplementation on the development of atherosclerosis in mice. The mice were fed with a diet supplemented with leucine (1.5% w/v), in drinking water, for 8 weeks. Leucine supplementation resulted in a 57.6% reduction in aortic atherosclerotic lesions in mice, a 41.2% decrease in serum levels of LDL cholesterol, and an increase by 40.2% in serum levels of HDL-cholesterol [4]. Unlike Zhao's study that found no change in the level of total cholesterol after leucine supplementation, the current research revealed that the level of total cholesterol was significantly lower in the rats from the group receiving a diet supplemented with leucine. In an analogous manner, the study developed by our research team noted a further lower level of total cholesterol in the group receiving valine.

Cojocaru et al. showed in previous studies that the combination of amino acids like valine and leucine in human diets can produce the regression of atherosclerosis or other clinical entities associated with hyperlipidemia [7, 9]. Miasoedov et al. conducted an experimental study regarding the administration of essential amino acids leucine, glycine, and proline to rats and showed that this diet may reduce the risk of vascular blood clots, and thus may prevent the occurrence of atherosclerotic plaques in the arterial wall [19].

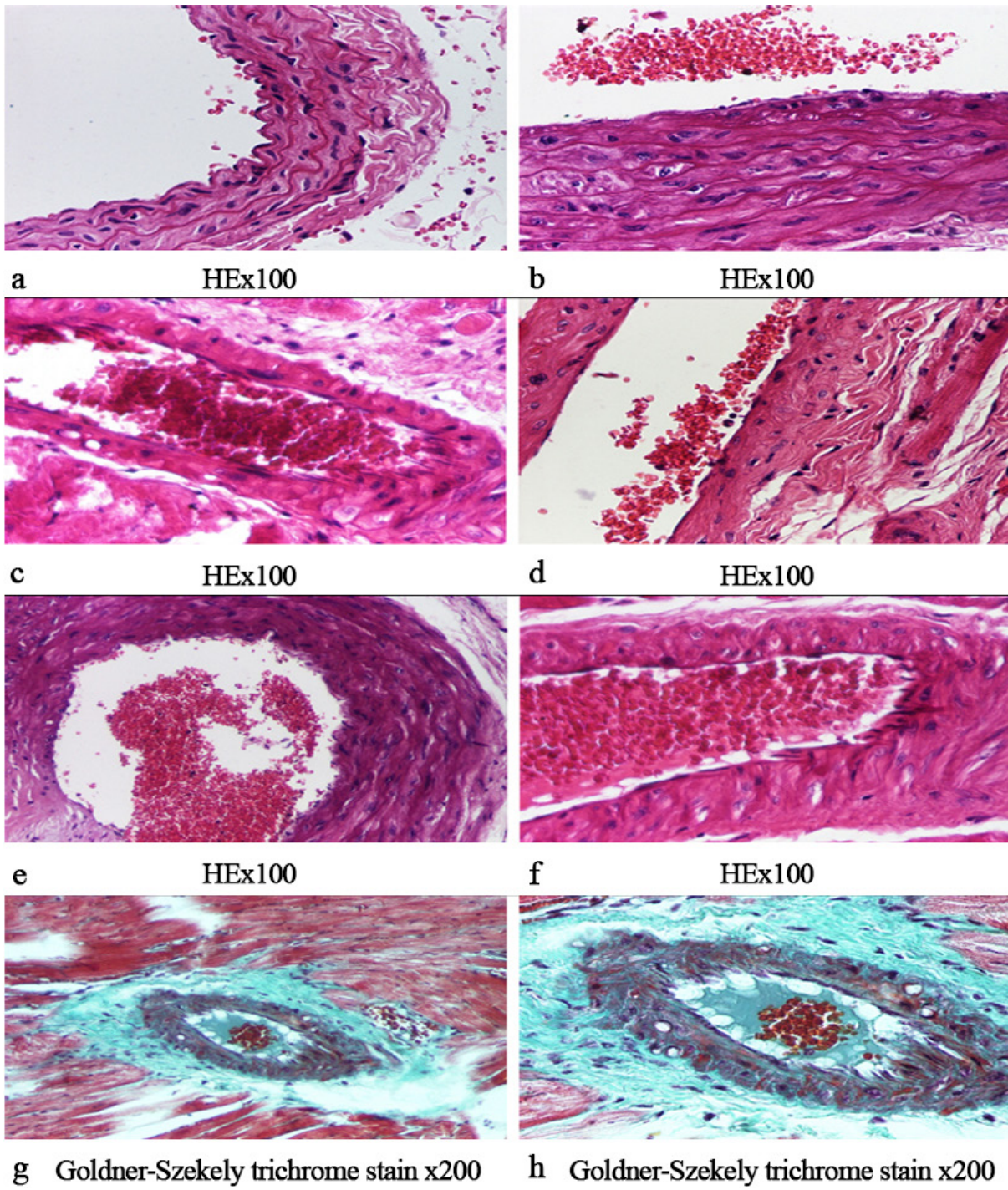

Fig. 2b. Vascular wall with early atherosclerotic changes – group III (hypercholesterolemia+Valine): reduced erythrocytes and platelets adhesion to the arterial walls; internal elastic lamina and elastic layers of the coronary wall within normal histological limits (HE stain - a, b, c, d, e, f x200; Goldner-Szekely trichrome stain – g x100, h x200)

An important characteristic is that atherosclerotic plaques developed in mice show common features with human atherosclerotic plaques [20], facilitating the translation of results to the human body.

Although the effect of hypercholesterolemia in the atherosclerotic process is well-known, a limited number of studies regarding the effect of hypercholesterolemia without development of atherosclerotic lesions are available in literature. Studies were carried out to investigate the effects of hyperlipidemia induced by high-cholesterol diet on the rat aorta, isolated in the absence of the atherosclerotic lesion. The rats from the control group were fed with a standard diet and two other groups were fed with a high fat diet for 36 days. The blood lipid level was measured and a sample of the thoracic aorta was collected for histological study, at the end of the experiment. The results of the study conducted by Garjani et al. revealed that high-cholesterol diet significantly increased both the serum levels of total cholesterol and LDL-cholesterol ($p<0.001$). Increased serum cholesterol level was associated with a significant reduction of the endothelial vasodilation in the thoracic aorta, although the histopathological study revealed no atherosclerotic lesion. The study concluded that high cholesterol level was associated with endothelial dysfunction despite the absence of aortic atherosclerotic lesions [21]. The situation is similar to that observed in the current study where the atherosclerotic plaques were not developed because the duration of the experiment was 60 days, not long enough to cause severe damage. However, we found biochemical level changes in lipid metabolism that characterise prelesional atherosclerosis stages. Also, the histopathological study indicated some features that precede the consequent development of atherosclerotic lesions.

Although LDL and HDL values are more specific targets in the management of hyper/dislipidemias, we did not evaluate these markers, so this could be a limitation of our current research.

The biochemical study conducted by our team reveals, by comparing the different sets of lipid metabolism parameters according to the experiment design, that the nonpolar type of essential amino acids like valine, leucine, and isoleucine have a direct effect in reducing the total cholesterol plasma levels. Consequently, the vascular endothelium is protected with an important result in diminishing the risk of endothelial dysfunction. The comparison between the three essential amino acids demonstrates that valine induces a faster response than leucine and isoleucine, regarding the improvement of biochemical parameters, but no differences between the three amino acids are found in terms of their protective ability by histopathological lesion assessment, very probably due to short term evaluation.

The research which was carried out by our team found that the occurrence of early changes in the vascular intima that involves the endothelium, the subendothelial tissue, and the tunica media (the occurrence of some superficial endothelial erosions, of a discrete, medium, or severe adhesion of erythrocytes and platelets) was correlated with the degree of the arterial wall damage, of leukocyte adhesion to the arterial intima, and of the discontinuities of the internal elastic lamina.

These microscopic aspects of endothelial dysfunction can be explained by the oxidative theory of atherogenesis according to which the origin of this process is located in the endothelial cells. As a consequence, any type of aggression exerted on the endothelium has a major significance, contributing to the initiation of the lesion. The excess of reactive oxygen species affects endothelial cells and the subendothelial extracellular matrix which leads to adhesion of formed elements of blood [22].

Conclusions

The final conclusions of the study support the fact that the hypercholesterolemic diet accelerated the occurrence of biochemical and morphological changes specific to atherosclerosis. Valine, leucine, and isoleucine cause a significant reduction of histopathological lesions and biochemical changes produced as a consequence of a high cholesterol diet. The histopathological examination showed that valine, leucine, and isoleucine had a beneficial contribution to the vascular wall, early histopathological lesions.

The experimental study provides material evidence that supports the protective effects of the diet supplemented with essential amino acids, opening perspectives for new product development that could be used in the prevention of atherosclerosis.

Conflicts of interest

The authors declare that they have no conflict of interest.

List of abbrevations

BCAA = Branched-Chain Amino Acids
C = cholesterol
C + V = cholesterol + valine
C + L = cholesterol + leucine
C + iL = cholesterol + isoleucine
M = mean
SD = standard deviation
CV = coefficient of variation
CI = confidence interval

References

1. Mc Nair E, Qureshi M, Prasad K, Pearce C. Atherosclerosis and hypercholesterolemic Age-Rage axis. Int J Angiol. 2016 Jun;25(2):110-6. DOI: 10.1055/s-0035-1570754
2. Li Q, Gu W, Ma X, Liu Y, Jiang L, Feng R, et al. Amino acid and biogenic amine profile deviations in an oral glucose tolerance test: a comparison between healthy and hyperlipidaemia individuals based on targeted metabolomics. Nutrients. 2016 Jun;8(6):379. DOI: 10.3390/nu8060379
3. Vergeer M, Holleboom AG, Kastelein JJP, Kuivenhoven JA. The HDL hypothesis: does highdensity lipoprotein protect from atherosclerosis? J Lipid Res. 2010 Aug;51(8):2058–73. DOI: 10.1194/jlr.R001610
4. Zhao Y, Dai XY, Zhou Z, Zhao GX, Wang X, Xu MJ. Leucine supplementation via drinking water reduces atherosclerotic lesions in apoE null mice. Acta Pharmacol Sin. 2016 Feb;37(2):196-203. DOI: 10.1038/aps.2015.88
5. Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. Ann N Y Acad Sci. 2012 Apr;1254:18-32. DOI: 10.1111/j.1749-6632.2012.06480.x
6. Zhang S, Zeng X, Ren M, Mao X, Qiao S. Novel metabolic and physiological function of branched chain amino acids: a review. J Anim Sci Biotechnol. 2017 Jan;8:10. DOI: 10.1186/s40104-016-0139-z
7. Cojocaru E, Zamfir C, Zamosteanu N, Trandafirescu M, Cotuţiu C. The effects of branched chain aminoacids on LDL-cholesterol in experimental animals subjected to dietary hypercholesterolemia. Rev Med Chir Soc Med Nat Iasi. 2012 Jan-Mar;116(1):200-6.
8. Yang R, Dong J, Zhao H, Li H, Guo H, et al. Association of Branched-Chain Amino Acids with Carotid Intima-Media Thickness and Coronary Artery Disease Risk Factors. PLoS ONE. 2014 Jun;9(6):e99598. DOI: 10.1371/journal.pone.0099598
9. Cojocaru E, Zamfir C, Amihăesei C, Trandafirescu M, Leon M, Mitu F. The influence of branched amino acids on LDL-cholesterol levels in a model of experimental atherosclerosis. Romanian Journal of Functional and Clinical, Macro- and Microscopical Anatomy and of Anthropology 2012;11(1):35-40.
10. *** National Research Council of the National Academies. Guide for the Care and Use of laboratory animals. 8th edition. The National Academies Press, Washington, USA, 2011.
11. *** Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

12. *** The ARRIVE Guidelines — Animal Research: Reporting In Vivo Experiments. Available at http://www.nc3rs.org.uk/page.asp?id=1357.
13. Festing MFW, Overend P, Gaines Das R, Cortina Borja M, Berdoy M. The design of animal experiments: reducing the number of animals in research through better experimental design, Laboratory Animal Handbooks Series 14. Royal Society of Medicine Press, London, 2002.
14. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of the total cholesterol serum. Clin Chem. 1973 Dec;19(12):1350-6.
15. Bennett BJ, Davis RC, Civelek M, Orozco L, Wu J, Qi H et al. Genetic architecture of atherosclerosis in mice: A Systems Genetics Analysis of Common Inbred Strains. PLoS Genet. 2015 Dec;11(12):e1005711. DOI: 10.1371/journal.pgen.1005711
16. Anandhi R, Thomas PA, Geraldine P. Evaluation of the anti-atherogenic potential of chrysin in Wistar rats. Mol Cell Biochem. 2014 Jan;385(1-2):103-13. DOI: 10.1007/s11010-013-1819-z
17. Xiao F, Du Y, Ly Z, Chen S, Zhu J, Sheng H, et al. Effects of essential amino acids on lipid metabolism in mice and humans. J Mol Endocrinol. 2016 Nov;57(4):223-231. DOI: 10.1530/JME-16-0116
18. Kitsy A, Carney S, Vivar JC, Knight MS, Pointer MA. Effects of leucine supplementation and serum withdrawal on branched-chain amino acid pathway gene and protein expression in mouse adipocytes. PLoS One. 2014 Jul;9(7):102615. DOI: 10.1371/journal.pone.0102615
19. Miasoedov NF, Shubina TA, Obergan T, Grigorieva ME, Andreeva LA, Liapina LA. Cholesterol-lowering effect of the regulatory peptide Pro-Gly-Pro-Leu. Vopr Pitan. 2013;82(5):41-5.
20. Matoba T, Sato K, Egashira K. Mouse models of plaque rupture. Curr Opin Lipidol. 2013 Oct;24(5):419-25. DOI: 10.1097/MOL.0b013e3283646e4d
21. Garjani A, Azarmiy Y, Zakheri A, Allaf Akbari N, Andalib S, Maleki-Dizaji N. Vascular Dysfunction in Short-Term Hypercholesterolemia despite the Absence of Atherosclerotic Lesions. J Cardiovasc Thorac Res. 2011 Aug;3(3):73-7.
22. De Brito FCF. Inhibition of inflammatory pathways promotes an improving effect on endothelial dysfunction: The effects of Longxuetongluo capsule in an experimental model of atherosclerosis. Atherosclerosis. 2016 Oct;255:111-2. DOI: 10.1016/j.atherosclerosis.2016.10.040