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Effects of moderate exercise and a multiple vitamin and mineral complex on the arterial wall

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Abstract

Background: Aerobic exercise has favorable effects on vascular structure and function. Its beneficial role may be due to a decrease in oxidative stress. The association of vitamin and mineral supplements to exercise determined contradictory effects on arterial wall and oxidative stress parameters. The aim of this experimental study was to evaluate the effect of moderate aerobic exercise, alone or in association with a vitamin and mineral complex, on aortic wall morphology and oxidant/antioxidant balance. *Materials and Methods*: Four groups, each of 10 Wistar rats, were included in the study, as follows: (I) sedentary controls, (II) group subjected to physical exercise, (III) group subjected to physical exercise and nutritional supplement, and (IV) sedentary nutritional supplemented group. Aortic wall histological examinations and serum and aortic wall oxidative stress measurements were performed in each group. *Results*: Moderate aerobic exercise induces vascular smooth muscle cell hypertrophy and transformation in a secretory phenotype. There was a trend for increase in malondialdehyde (MDA) and decrease in thiol (SH) groups in aortic tissue homogenates, together with reduction in serum MDA values and increase in SH groups, after exercise. A reduction in aortic wall lipid peroxidation was found in supplemented trained animals compared to sedentary group, while no influence on aortic structure was noted. *Conclusions*: Moderate aerobic exercise induces adaptive modifications in the arterial wall and a favorable effect on systemic oxidative response. The association of vitamin and mineral supplement did not influence significantly arterial morphology, while its effects on aortic oxidative stress suggest an increase in local antioxidant defense.

Keywords: aerobic exercise, arteries, oxidative stress.

Introduction

Physical exercise determines complex neuromuscular, cardiovascular, respiratory, metabolic and biochemical reactions in the body. The response to exercise depends on various factors, including the intensity and duration of exercise, subjects' level of training, antioxidant status and environmental conditions [1–3].

Aerobic exercise has been documented to reduce cardiovascular risk [4]. One of its favorable effects can be explained by the modulation of arterial structure and function. One of the most important properties of great arteries, which are rich in elastin, is distensibility, which enables arteries to distend in response to pressure and volume variations and to return to initial dimensions after arterial distension. Arteries have an essential role in transporting blood from heart to peripheral tissues and also to dampen blood pressure oscillations, transforming the intermittent flow, determined by left ventricular ejection, into a continuous peripheral flow. When central arteries stiffen, systolic pressure increases, leading to left ventricular hypertrophy and subsequently, to left ventricular dysfunction. Moreover, in stiff arteries, central pressure oscillations are transmitted distally inducing alterations in highly perfused organs, such as the brain and the kidneys [5, 6].

A favorable effect of aerobic exercise on endothelial function [7, 8] and arterial distensibility has been previously reported [9–11], but the precise mechanisms that govern the effects of exercise on the vascular wall are not entirely elucidated.

Vascular redox imbalance involving nicotinamide adenine dinucleotide phosphate (NADP) oxidases, xanthine oxidases and endothelial nitric oxide synthase, with the development of endothelial dysfunction, may influence the structural and functional properties of the vascular wall [12–14]. It has been demonstrated that, at biochemical level, physical exercise plays a paradoxical role on redox homeostasis. Excessive intense physical exercise is a pro-oxidant factor, which determines an overproduction of reactive oxygen and nitrogen species (RONS), responsible for oxidative injury in various organs and tissues, including muscles, heart, liver and lungs, as well as in the blood compartment [15]. In contrast, moderate-intensity physical exercise, repeated aerobic exercise, low-intensity training and detraining have an antioxidant role, decreasing RONS and stimulating antioxidant enzymes, as a result of the antioxidant defense overregulation and redox exchange balancing [16–19].

Low RONS production and effective antioxidant defense have favorable effects on cellular signaling pathways, regarding differentiation and proliferation, as well as cell migration and apoptosis [20]. Malon-dialdehyde (MDA) is a product of lipid peroxidation and a marker of oxidative stress. It is considered a non-classical risk factor for atherosclerosis with cytotoxic

and proinflammatory effects on vascular endothelium and an adhesion molecule synthesis inducer [21, 22]. An important antioxidant system is represented by thiol (SH)-based antioxidants, which act in both intracellular and extracellular compartments [22, 23].

Supplementation with natural and synthetic nutritional and non-nutritional antioxidants may have favorable effects on antioxidant defense [24]. Vitamin supplements are widely used to increase exercise capacity or muscle strength [25]. Nutrients with antioxidant and anti-inflammatory effects may improve vascular properties [26]. However, the results of studies investigating the role of vitamin supplementation in trained subjects are conflicting. In rats, the association of antioxidants with exercise augmented the cardiac and metabolic beneficial effects of exercise [27], while in humans the administration of antioxidants determined contradictory results [28].

The aim of our experimental study was to analyze the effect of moderate exercise and multiple vitamin and mineral complex supplements on aortic wall morphology of old rats as well as on redox homeostasis in the serum and aortic wall homogenates. We have also evaluated the relationship between oxidative stress parameters and vascular wall modifications.

Materials and Methods

The research took place in the Experimental Research Laboratory, Department of Physiology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, between May–July 2016.

Animal groups

The subjects of this study were male Wistar rats aged 4–5 months, with a medium weight of 220±10 g, from the Animal Facility of the same University.

The subjects were assigned to four groups (n=10 animals/group):

- Group I – sedentary control group receiving lactose as placebo;
- Group II – group subjected to physical exercise;
- Group III – group subjected to physical exercise and supplemented with Daily Formula;
- Group IV – sedentary group, supplemented with Daily Formula.

Daily Formula (Universal Nutrition, New Brunswick, NY, USA) is a complex of vitamins and minerals containing vitamin A (5000 IU), vitamin C (60 mg), vitamin D (400 IU), vitamin E (30 IU), vitamin K (25 mcg), thiamine (1.5 mg), riboflavin (1.7 mg), niacin (30 mg), vitamin B6 (2 mg), folic acid (200 mcg), vitamin B12 (6 mcg), biotin (15 mcg), pantothenic acid (10 mg), calcium (170 mg), phosphorus (125 mg), iodine (25 mcg), magnesium (40 mg), zinc (5 mg), selenium (3 mcg), copper (2 mg), manganese (1 mg), chromium (2 mcg), per each tablet. The multivitamin and mineral complex was administered by oropharyngeal gavage in a dose of 0.1 mg/150 g animal, calculated in relation to the daily dose recommended for humans. This compound is commonly used in athletes for the improvement of their sport performance. The mentioned amount was administered to each animal daily, for 14 days. Lactose was used as a negative control due to its inert effects, and was administered by oropharyngeal gavage, at the same moments of the day and in equivalent doses.

The research was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, according to the requirements of the Declaration of Helsinki, Amsterdam Protocol, and the Directive 86/609/EEC.

Physical training

The trained groups were exposed to forced swimming exercise [29], 15 minutes/day, for 14 days. At the end of the experiment, the animals were euthanized, and blood samples and aortic biopsies were taken.

Pathological examinations

Histopathological examinations were performed at the Department of Veterinary Pathology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca.

Biological material was represented by tissue samples from ascending aortas, which were examined by conventional histological methods. Qualitative examination included the evaluation of the aortic wall structures: intima, media and adventitia.

The collected aortic samples were fixed in 10% neutral buffered formalin solution and processed using the paraffin technique. The paraffin blocks were cut at a thickness of 4 μm with a Leica RM 2125 RT microtome, after which the sections were displayed on routine histological slides and stained by the Hematoxylin–Eosin (HE), Periodic Acid–Schiff (PAS) and Verhoeff–Van Gieson (VVG) methods. HE staining is the basic staining method used in histology/histopathology, while the other two methods are special staining techniques: PAS staining allows evidencing polysaccharides (such as glycogen), glycoproteins, glycolipids or mucin, and VVG staining allows identifying elastic fibers. The preparations were examined with an Olympus BX 51 microscope; images were taken with an Olympus UC 30 digital camera and processed using the Olympus Stream Basic image acquisition and processing software.

Immunohistochemical (IHC) study was realized using antibodies against alpha-smooth muscle actin (α-SMA) (clone αsm-1, Leica Bond™, Leica Biosystems, Newcastle, UK). Immunohistochemistry analysis was assessed using a Leica Bond-Max™ Immunohistochemistry system.

A morphometric examination was also performed based on HE staining. Quantitative measurements consisted of determining aortic wall thickness and the diameter of smooth muscle cells (SMCs) in the structure of the aortic media, in μm, at three different points for each section.

Biochemical determinations

The oxidant/antioxidant balance was assessed by measuring the levels of MDA as a marker of lipid peroxidation, using the fluorescence method [30], and SH antioxidants as a marker of the endogenous antioxidant system, using Hu's method [31]. Biochemical oxidative stress parameters were evaluated in serum and ascending aortic wall homogenates. The values of serum parameters were expressed in nmol/mL, and those of aortic wall homogenates in nmol/mL protein. Biochemical deter-

minations were performed in the Laboratory for the Study of Oxidative Stress, Department of Physiology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

Statistical analysis

Descriptive statistic elements were calculated, data being presented using centrality, location and distribution indicators. Normal distribution was tested with the Shapiro–Wilk test and variance, with the *F*-test. For the analysis of three or more samples, the analysis of variance (ANOVA) test or the non-parametric Kruskal–Wallis test was used. For the analysis of two samples, the Student's *t*-test or the non-parametric Mann–Whitney *U*-test was employed. The significance threshold for the tests utilized was α=0.05 (5%), 0.01 (1%) or 0.001. The Pearson's correlation coefficient (r) was used to detect the correlation between two continuous quantitative variables with normal (uniform) distribution, and the Spearman's rank correlation coefficient (ρ) was used in the case of variables with non-uniform distribution. Correlation coefficients were analyzed using Colton's rule. Statistical analysis was performed with the Excel application (Microsoft Office 2010) and the StatsDirect *v*.2.7.2 software. Results were graphically represented with the Excel application (Microsoft Office 2010).

Results

Histopathological analysis of the aortic wall

For the evaluation of morphological changes in the aortic wall, the assessment of the polysaccharides' presence and the distribution of elastic fibers, sections stained with HE, PAS and VVG were analyzed.

In control animals, no significant morphological changes were observed (Figure 1, A–C). The vascular wall structures described were: the intima, which comprises the endothelial cell layer, the subendothelial layer and the internal elastic limiting membrane, the media, formed by fusiform SMCs, elastic fibers and collagen, and the adventitia, composed of connective tissue, predominantly consisting of collagen fibers and rare elastic fibers, with a circular and longitudinal arrangement (Figure 1A).

PAS staining showed the presence of a large amount of PAS-positive material (glycogen, glycoproteins) in the cytoplasm of SMCs in the aortic media (Figure 1B). The number and distribution of elastic fibers were normal (Figure 1C).

The sections from the aorta of animals subjected to physical exercise (Figure 2, A–C) showed marked hypertrophy of SMCs in the aortic media (Figure 2A) and a significant reduction of the amount of PAS-positive material in the cytoplasm of these cells (Figure 2B). Thus, the shape of SMCs was different from that observed in the control group. Rhomboid cells were predominant compared to fusiform cells. In addition to changes in the amount of PAS-positive material, alterations of its distribution in the vascular media were observed, with a higher concentration in the superficial layers. VVG staining demonstrated the presence of thicker elastic fibers arranged between smooth muscle cell layers (Figure 2C), without any morphological changes in the vascular intima and adventitia.

In animals subjected to physical exercise and supplemented with vitamins and minerals (Figure 3, A–C), SMCs in the aortic media were hypertrophied (Figure 3A) and the amount of intracytoplasmic PAS-positive material was increased compared to group II (Figure 3B). Rhomboid SMCs were also predominant compared to fusiform cells, observed for group I. Regarding elastic fibers, their number was higher compared to group II, while their thickness and distribution were similar (Figure 3C).

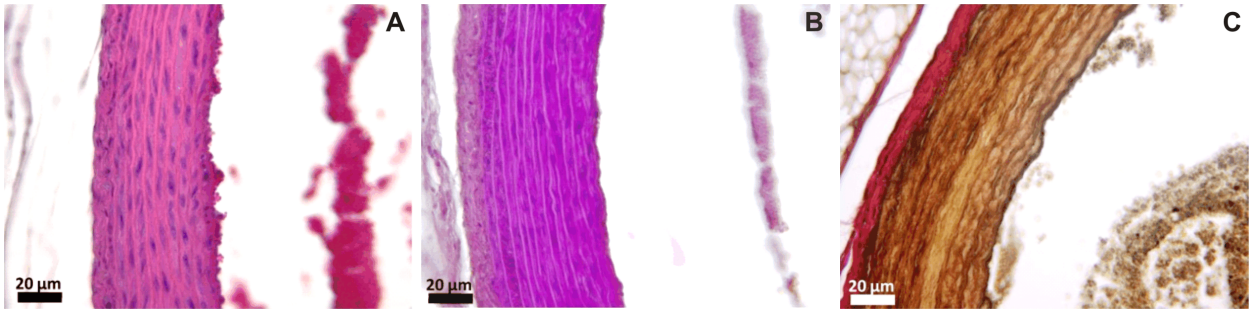

Figure 1 – ***Cross-sections of the ascending aorta of animals from the control group, showing normal morphology (×400): HE staining (A), PAS staining (B), Verhoeff–Van Gieson staining (C). HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff.***

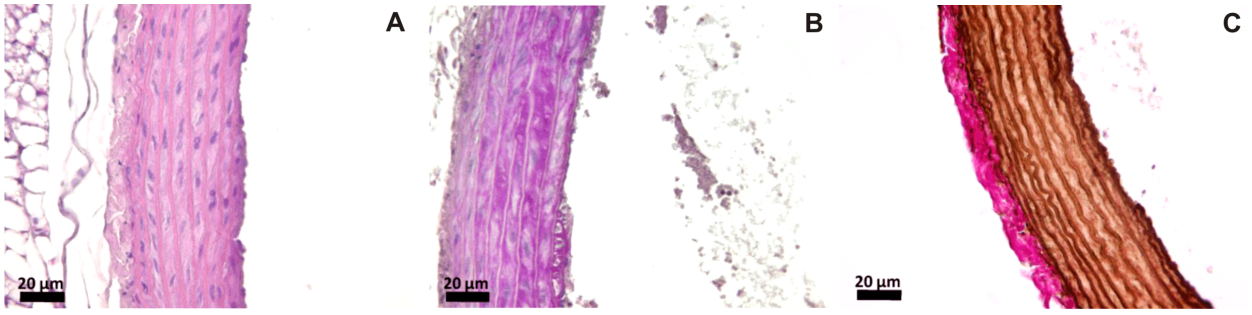

Figure 2 – ***Cross-sections of the ascending aorta of animals from group II (×400): HE staining (A), PAS staining (B), Verhoeff–Van Gieson staining (C). HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff.***

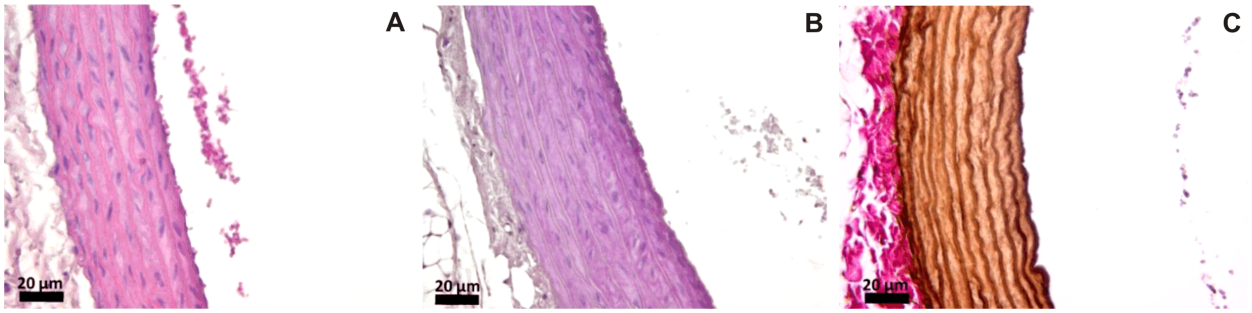

Figure 3 – ***Cross-sections of the ascending aorta of animals from group III (×400): HE staining (A), PAS staining (B), Verhoeff–Van Gieson staining (C). HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff.***

For group IV (Figure 4, A–C), including sedentary animals supplemented with vitamins and minerals, similar aspects to those described for group I were observed. The amount of PAS-positive material in the media was more reduced compared to group I. The number and distribution of elastic fibers were similar to those described for group I, which suggests that changes observed in groups II and III were mainly due to moderate exercise and not to vitamin and mineral supplementation.

IHC examination identified the α-SMA positive SMCs in the media of the aorta (Figure 5).

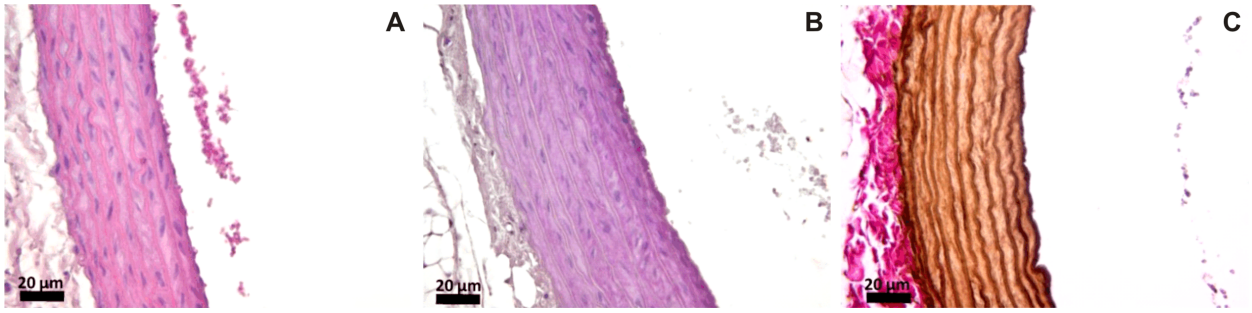

Figure 4 – ***Cross-sections of the ascending aorta of animals from group IV (×400): HE staining (A), PAS staining (B), Verhoeff–Van Gieson staining (C). HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff.***

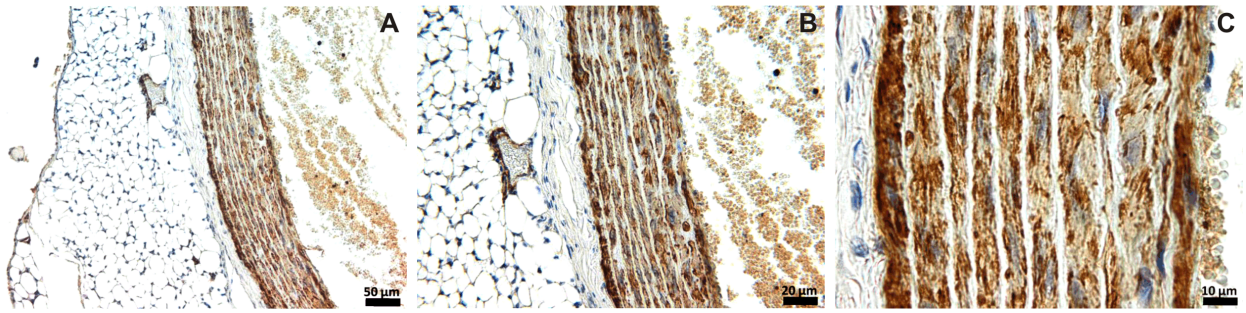

Figure 5 – ***Immunohistochemical identification of the α-SMA positive smooth muscle cells in the media of the aorta. Anti-α-SMA antibody immunomarking: (A) ×200; (B) ×400; (C) ×1000. α-SMA: Alpha-smooth muscle actin.***

Quantitative statistical analysis of morphological parameters (Table 1) showed a significant increase in the diameter of SMCs in groups subjected to physical exercise compared to control group and to sedentary group receiving vitamins and minerals.

Regarding aortic wall thickness (Table 2), no statistically significant differences were found between the groups (p>0.05).

Oxidative stress assessment in blood and aortic tissue

Serum MDA (MDAs) decreases non-significantly after exercise but, in supplemented trained animals, the increase was significant compared to controls. Thus, exercise and vitamins increase systemic oxidative stress, while exercise alone reduces MDA levels. This indicates that vitamin supplementation may increase lipid peroxidation when associated with exercise (Table 3).

The values of serum SH (SHs) groups increase significantly with exercise, but also in sedentary animals taking vitamin supplements and in trained supplemented animals, compared to controls, suggesting that both exercise and vitamins or their association may ameliorate serum antioxidant defense (Table 4).

The analysis of MDA levels from aortic homogenates (MDAaoh) revealed statistically significant differences between the sedentary group with lactose administration and the sedentary group treated with vitamin and mineral supplements, as well as between the groups subjected to exercise with and without vitamin and mineral supplementation (p<0.001). MDAaoh increased non-significantly after exercise. In animals subjected to exercise and

supplemented with vitamins, the level of MDAaoh was similar to that of controls. In sedentary animals taking supplements, MDAaoh increases significantly compared to controls (2.4×) and to exercise group taking supplements (2.9×) suggesting that sedentariness may intensify lipid peroxidation (Table 5).

SH groups measured in aortic homogenates (SHaoh) were increased in the group subjected to physical exercise and supplemented with vitamins and minerals compared to the sedentary supplemented group, suggesting a favorable aortic effect of supplement association in trained animals with increase in local antioxidant defense ($p<0.05$) (Table 6).

Table 1 – ***Comparative analysis of smooth muscle cells diameter values (measured in μm) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	8.01	0.31	7.87	0.95	6.36	9.17	*I–II–III–IV*		0.003	
II	11.71	0.8	11.03	2.42	8.83	15.61	*I–II*	0.001	*II–III*	0.03
III	9.56	0.53	8.8	1.59	8.18	12.6	*I–III*	0.003	*II–IV*	0.003
IV	8.28	0.52	8.66	1.58	5.99	10.8	*I–IV*	0.67	*III–IV*	0.23

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

Table 2 – ***Comparative analysis of aortic wall thickness values (measured in μm) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	149.6	6.16	150.25	18.49	124.82	189.11	*I–II–III–IV*		0.73	
II	146.14	3.79	145.75	11.39	131.67	162.38	*I–II*	0.64	*II–III*	0.34
III	142.17	4.27	135.64	12.81	128.41	160.12	*I–III*	0.48	*II–IV*	0.5
IV	140.65	7.02	138.24	21.08	117.15	175.06	*I–IV*	0.35	*III–IV*	0.66

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

Table 3 – ***Comparative analysis of serum malondialdehyde values (measured in nmol/mL) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	4.34	0.06	4.39	0.18	4	4.53	*I–II–III–IV*		0.0007	
II	3.87	0.35	3.8	1.06	2.64	5.85	*I–II*	0.22	*II–III*	0.0006
III	5.7	0.24	5.48	0.74	4.91	7.11	*I–III*	0.0005	*II–IV*	0.1
IV	4.77	0.37	4.66	1.12	3.16	5.96	*I–IV*	0.28	*III–IV*	0.05

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

Table 4 – ***Comparative analysis of serum thiol-based groups values (measured in nmol/mL) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	0.1	0.006	0.11	0.01	0.07	0.12	*I–II–III–IV*		0.0003	
II	0.17	0.009	0.16	0.02	0.12	0.21	*I–II*	0.0002	*II–III*	0.28
III	0.18	0.004	0.18	0.01	0.16	0.2	*I–III*	0.0001	*II–IV*	0.6
IV	0.17	0.019	0.17	0.05	0.11	0.3	*I–IV*	0.0008	*III–IV*	0.16

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

Table 5 – ***Comparative analysis of aortic homogenate malondialdehyde levels (measured in nmol/mg protein) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	0.66	0.06	0.69	0.2	0.28	0.9	*I–II–III–IV*		0.002	
II	0.96	0.24	0.65	0.72	0.28	2.34	*I–II*	>0.99	*II–III*	0.38
III	0.56	0.05	0.52	0.15	0.36	0.87	*I–III*	0.25	*II–IV*	0.06
IV	1.64	0.24	1.55	0.73	0.72	2.92	*I–IV*	0.003	*III–IV*	0.002

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

Table 6 – ***Comparative analysis of aortic homogenate thiol-based groups values (measured in nmol/mg protein) in the studied groups***

Group	Mean	SE	Median	SD	Min.	Max.	*p*			
I	0.07	0.01	0.06	0.04	0.02	0.15	*I–II–III–IV*		0.06	
II	0.06	0.01	0.07	0.03	0.02	0.1	*I–II*	0.8	*II–III*	0.05
III	0.03	0.003	0.03	0.01	0.02	0.05	*I–III*	0.05	*II–IV*	0.69
IV	0.06	0.007	0.05	0.02	0.03	0.09	*I–IV*	0.57	*III–IV*	0.01

SE: Standard error; SD: Standard deviation; Min.: Minimum value; Max.: Maximum value.

The analysis of correlation showed that, in animals subjected to exercise, MDAaoh strongly correlated with MDAs and also with arterial wall thickness. In trained and supplemented animals, MDAaoh inversely correlated with arterial wall thickness suggesting a modification induced by the supplement administrated. There was also a strong and direct correlation between MDAaoh and both SHs and SHaoh groups. In sedentary supplemented

rats, SHs correlated with both diameter of SMCs and arterial wall thickness suggesting that the increase of SHs noted in these conditions may be involved in vascular wall structure modifications. Levels of MDAaoh, an indicator of local oxidative stress, strongly and inversely, correlated with SMCs dimensions (Table 7).

Table 7 – ***Statistical correlation analysis between morphological and biochemical parameters in the studied groups***

Parameters		Group I		Group II		Group III		Group IV	
φ SMCs	AWT	0.54	***	0.14	*	0.2	*	0.48	**
	MDAs	0.04	*	0.28	**	-0.1	*	0.21	*
	SHs	0.5	**	0.49	**	-0.15	*	0.61	***
	MDAaoh	0.25	**	-0.08	*	0.03	*	-0.71	***
	SHaoh	-0.53	***	-0.39	**	0.49	**	-0.32	**
AWT	MDAs	0.42	**	0.45	**	-0.25	*	0.49	**
	SHs	0.13	*	0.02	*	-0.4	**	0.65	***
	MDAaoh	-0.28	**	0.78	***	-0.55	***	-0.19	*
	SHaoh	-0.23	*	-0.48	**	0.03	*	0.06	*
MDAs	SHs	0.41	**	0.39	**	0.2	*	0.13	*
	MDAaoh	0.19	*	0.61	***	0.2	*	-0.01	*
	SHaoh	0.35	**	0.06	*	0.2	*	0.05	*
SHs	MDAaoh	0.65	***	0.16	*	0.56	***	-0.33	**
	SHaoh	-0.05	*	0.08	*	-0.21	*	-0.08	*
MDAaoh	SHaoh	0.02	*	-0.06	*	0.6	***	0.2	*

φ SMCs: Smooth muscle cells diameter [μm]; AWT: Aortic wall thickness [μm]; MDAs: Serum malondialdehyde [nmol/mL]; SHs: Serum thiol-based groups [nmol/mL]; MDAaoh: Aortic homogenate malondialdehyde [nmol/mg protein]; SHaoh: Aortic homogenate thiol-based groups [nmol/mg protein]; *: Slight correlation; **: Moderate correlation; ***: Strong correlation.

Discussions

In our study, moderate physical exercise determined aortic wall SMCs hypertrophy and transformation in rhomboid cells, with an enhanced accumulation of polysaccharides in superficial layers and a thickening of elastic fibers. In aortic tissue homogenates, insignificant decrease in MDA levels and significant decrease in SH groups were found in the presence of physical exercise, while in the serum, MDA values diminished in parallel to an increase in SH group levels. Significant correlations were registered between MDAs and MDAaoh levels, as well as between aortic wall thickness and MDAaoh values, which suggest the implication of oxidative stress in vascular wall cells hypertrophy.

In sedentary animals supplemented with Daily Formula, there were no significant morphologic changes compared to non-supplemented sedentary animals, SMCs and elastic fibers had normal aspects. The polysaccharide content decreased in smooth muscle fibers, in parallel to an increase in MDA and SH groups.

In animals subjected to moderate physical exercise and supplemented with Daily Formula, muscle fiber shape, polysaccharide accumulation and elastic fiber thickness were similar to those observed in non-supplemented trained animals. The diameter of smooth muscle fibers was significantly increased and, in aortic tissue homogenates, MDA and SH groups decreased, while in the serum, the levels of MDA and SH groups were significantly increased. Thus, moderate physical exercise combined with Daily Formula supplementation induced a systemic pro-oxidant effect, with the concomitant mobilization of endogenous antioxidants, while in aortic tissue homogenates, changes were favorable, but without statistical significance.

Lipid peroxidation increased significant in sedentary animals treated with vitamins compared to animals subjected to physical exercise, which received vitamins suggesting a pro-oxidant effect of sedentariness.

It has been suggested that cardiovascular risk reduction caused by exercise may be explained by its favorable effect on cardiovascular risk factors and also by a direct, complex, vascular action [32].

Shear stress induced by exercise acts *via* endothelial cells by nitric oxide release and also by cell to cell interaction, transforming vascular SMCs from contractile to synthetic cells characterized by a rhomboid shape. Mechanical forces leading to the stretch of vascular SMCs enhance the production of extracellular matrix and contractile proteins [33, 34]. The activation of the sympathoadrenal system with the increase in circulating catecholamines and subsequent vascular SMCs hypertrophy has also been correlated with exercise [35].

The aorta is, predominantly, an elastic vascular structure. Aging and various pathological conditions may induce stiffness of the great arteries [6], while regular physical exercise has beneficial effects on vascular distensibility [36]. Vascular elastic properties are dependent mainly on elastin content of the vascular wall extracellular matrix. Elastin is an inert protein, which allows blood vessels to take their initial form after contraction or stretch, with a half-life of decades [37]. Our study showed a slight increase in the thickness of elastic fibers in animals subjected to physical exercise; this morphological change can lead to an increase in vascular distensibility and adaptation capacity to mechanical stress. A recent experimental study, which evaluated the effects of moderate aerobic exercise on arterial wall structure in low-density lipoprotein (LDL) knockout mice with estrogen deprivation found an increase in the volume density of elastic fibers stimulated by moderate exercise [38], suggesting a favorable effect of exercise on arterial wall aging induced by estrogen deprivation. An increase in elastin after exercise has also been reported in male rats compared to sedentary rats [39].

In our study, arterial wall modifications induced by physical training were not accompanied by a significant

change in vascular wall thickness. Previous experimental [38] and clinical [32] studies have reported a favorable role of physical exercise on the vascular wall, with a reduction of intima–media thickness. However, these beneficial effects seem to be more pronounced in peripheral arteries compared to central arteries. Changes in the carotid wall have been found particularly after intense and prolonged exercise [32]. In our study, rats exercised during a short period of time, which might not be sufficient to positively influence arterial wall thickness.

Animals subjected to moderate exercise and supplemented with Daily Formula showed structural changes similar to those observed in non-supplemented, trained animals regarding SMCs morphology as well as polysaccharide accumulation and elastic fiber thickening. These changes are more probably related to physical exercise than to nutritional supplements.

Previous studies regarding the administration of various supplements, including vitamins A, B12, D, K, C, and E, indicate a favorable effect on endothelial function and arterial stiffness, particularly due to the antioxidant effects of these vitamins [40]. Copper, zinc, iron, selenium and manganese contribute to antioxidant defense because they act as co-factors for antioxidant enzymes. Several studies have shown that these minerals may have favorable effects on oxidative stress balance, exercise performance or cardio-respiratory function in trained subjects [41].

It has been reported that, at low intracellular concentrations, reactive oxygen species (ROS) play an essential physiological role in the regulation of vascular tone, as well as cell growth, adhesion and differentiation [42, 43]. However, excessive ROS production has been linked to adverse vascular effects [43]. The association of physical exercise with antioxidant supplements produced contradictory results [44, 45]. Exercise induces oxidative stress, which may stimulate adaptive physiological reactions [22]. Antioxidants may be useful only in excessive oxidative reactions, which may have harmful effects [45]. However, these excessive oxidative responses to exercise are difficult to quantify. Moreover, it has been suggested that antioxidant supplements may have paradoxical effects, attenuating the favorable antioxidant effect of exercise [45, 46].

In our study, the administration, during a short time period, of a combination of vitamins and minerals did not influence vascular structure, either at rest, or after exercise. This may be due to the short period of administration or to a possible interaction between the components, with a decrease in their action. The results can also suggest that vascular remodeling induced by moderate exercise may not be influenced in the presence of vitamin supplements. Moreover, the oxidative stress parameters in trained animals were not significantly modified by the vitamin and mineral supplementation.

Study limitations

One important limitation of our study is the small number of animals included, which reduced the statistical power of the results. The short period of exercise training and vitamin supplementation, which may have been insufficient for the development of significant vascular and oxidative stress parameters modifications, may represent another limitation.

Conclusions

Moderate physical exercise induced morphological changes in the arterial wall, characterized by the transformation of SMCs from fusiform to rhomboid cells, and by a thickening of elastic fibers, accompanied by a trend to reduction of systemic oxidative stress and intensification of antioxidant defense. Two weeks of moderate physical exercise seemed to have no influence on aortic wall thickness. However, the association between oxidative stress markers and aortic thickness suggests the implication of oxidative stress in aortic wall hypertrophy. Supplementation with a vitamin and mineral complex did not significantly change the morphology of the arterial wall, either at rest or after exercise but the modifications of aortic oxidative stress parameters suggest an increase in local antioxidant defense. Further studies are required to assess the usefulness of vitamin and mineral supplementation combined with exercise training for vascular protection.

Conflict of interests

The authors declare no conflict of interests.

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