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ORIGINAL RESEARCH

Aerobic Exercise Practiced Over Time Mitigates the Structural Effects on the Vascular System Caused by the Deleterious Effects of Aging

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Background: Aerobic training has been considered beneficial for determining the detrimental alterations in blood vessels caused by aging.
Objective: Evaluate the relationship between the preventive effects of aerobic exercise and time of practice on cardiovascular health, in aged Wistar rats.
Methods: Wistar rats (16 months) were divided into 3 groups: (1) sedentary (AGED); (2) long-term trained\61 weeks (LTT); and (3) short-term trained\final 8 weeks of life (STT). Body weight, maximum physical capacity, systolic blood pressure (SBP), pulse wave velocity (PWV), plasma nitrite (NO), oxidative stress (TBARS), wall thickness, the wall-to-lumen ratio, and collagen of the thoracic aorta, carotid, and femoral arteries were measured.
Results: Both trained groups showed an increase in physical capacity when compared to the AGED group (p=<0.001 for LTT and p=0.011 for STT), and the LTT group demonstrated higher values when compared to the STT group (p= 0.004). The LTT group presented attenuation of PWV (p= 0.002) and a reduction in the wall thickness and wall-to-lumen ratio of the thoracic aorta (p=0.032 and 0.008, respectively) and carotid arteries (p=0.019 and 0.012, respectively) when compared to the AGED group. The STT group presented a reduction in TBARS compared to the AGED group (p=0.046). Additionally, both trained groups (LTT and STT) presented a reduction in the percentage of arterial collagen compared to the AGED group in the thoracic aorta (p=<0.001 and p=0.001 respectively) and carotid arteries (p= <0.008 and p= 0.041 respectively).
Conclusion: This study demonstrated that long-term training decreased the level of collagen, PWV values, wall thickness, and the wall-to-lumen ratio of the aorta and carotid arteries compared to the AGED group. Moreover, short-term training reduced TBARS and collagen percentage in the aorta and carotid arteries compared to the AGED group.
Keywords: aging, aerobic training, pulse wave velocity, collagen deposition, arteries

Introduction

Aging is considered the main risk factor for cardiovascular disease, and consequently, it is a huge contributory factor in the development of atherosclerosis.[1–5] The effect of aging is very noticeable in the large elastic arteries, observed through an increase in arterial stiffness and the wall thickness of the artery, and a decrease in lumen diameter.[4,6,7]

The middle layer is primarily responsible for the distensibility of the vascular wall and is composed of elastic fibers, smooth muscle cells and collagen fibers.[8] Various structural alterations may be associated with the progressive loss of elastic tissue, and increased collagen levels and calcium deposition, which can compromise arterial stiffening, thicken the arteries, and reduce the vessel lumen.[4,9] Among these changes, we can observe degradation and reorganization of collagen, elastin, and rupture of elastic fibers that contribute to increased arterial stiffening. Factors such as increased TGF-β can stimulate the production of fibronectin and collagen and the decrease in elastin content which is crucial to maintain the elasticity of the arteries.[4,6–9]

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Moreover, proinflammatory cytokines and chemokines accumulate in the arterial wall at the molecular level. At the cellular level, vascular cells change their phenotypes and become senescent, while another subset of cells becomes more rigid. The extracellular matrix demonstrates fibrosis, elastolysis, calcification, amyloidosis, and glycoxidation. Finally, at the tissue level, an increased arterial intima-media thickening (IMT), endothelial dysfunction, arterial stiffening, and elevated blood pressure (BP) can be observed.[4,8,9]

Overall, investigate the changes during the natural aging process of the extracellular matrix is of utmost importance, as studies show that the analysis of arterial stiffness is an excellent predictor of cardiovascular events.[4]

Additionally, many other alterations can compromise arterial function during the aging process. Hemodynamic changes and increased reactive oxygen species (ROS) in the arterial wall promote an imbalance and consequent pro-inflammatory action, endothelial dysfunction, and stiffness. Conversely, nitric oxide (NO) in blood vessels plays an important role in modulating vascular diameter and vascular resistance, acting to relax the vascular smooth muscle, increasing blood flow and regulating blood pressure. With aging, the concentration of NO is reduced due to the low production or the interactions with ROS. Overall, peroxynitrite (ONOO-) is generated, and the reduction in NO bioavailability impairs endothelium relaxation and increases vasoconstriction.[5,10–12]

The regular practice of physical exercise is considered a strategy to counteract the deleterious effects of the natural aging process, acting to reduce many cardiovascular diseases.[4,13] Aerobic training is an important regulator of vascular function, promoting a reduction in heart rate and blood pressure, an increase in maximal oxygen consumption and NO availability, maintenance and/or improvement in endothelial function, and an increase in the antioxidant defense pathways.[14,15] Therefore, the regular practice of physical exercise among middle-aged and older adults is essential to promote successful aging.[16,17]

The above statements lead us to consider that if regular physical exercise is adopted earlier in life, better benefits could be observed throughout the aging process. Studies show that short-term physical training is related to increased endothelial function in the coronary arteries, while long-term interventions are linked to improvement in endothelium-dependent dilation in the aorta and coronary arteries.[18] A study with an animal model using young and aged rats demonstrated that arterial stiffness increases with age, however, aerobic exercise training was able to attenuate the age-related increase in aged rats, with no change in young rats.[19] Furthermore, the authors demonstrated improvement in the endothelial function of coronary arterioles, reversing diastolic dysfunction in the aging heart. In addition, in an animal model using young and aged rats, it was reported that the collagen-elastin ratio of the arterial wall did not change with age and was reduced with physical training.[20] The same authors also showed that the interference of training reduces the wall-to-lumen ratio, decreases wall stiffness, and restores myogenic function in aged coronary arteries.

Although the benefits of physical training for the maintenance and longevity of the aging process are well understood in the literature, there are still some gaps with respect to understanding the relationship between the time of practice and the preventive effects of aerobic training on cardiovascular health. The majority of studies that work with aging instigate a sedentary lifestyle for the animals throughout their lives and only start the physical exercise training around 2 months before euthanasia. Therefore, there is still a lack of studies that evaluate the improvement in arterial stiffening and structural alterations in blood vessels when animals perform exercise throughout their life (from young to adulthood).

Therefore, the purpose of the current study was to evaluate the relationship between the preventive effects of aerobic exercise and time of practice (throughout youth/adulthood until 16 months of age or practiced only from late adulthood until 16 months of age) on the functional, humoral, and structural factors of the arteries of 16-month-old (aged) rats.

Methods

This study was performed with 80 male Wistar rats (6 weeks of age / 250–300g), obtained from the Animal Facility of São Paulo State University (UNESP/ Campus of Botucatu, SP, Brazil). The animals were kept in the Animal Facility of the School of Sciences of the São Paulo State University (UNESP/ Campus of Bauru, SP, Brazil), separated into cages of up to 5 animals. All animals had free access to water and food and were kept in a controlled temperature environment (22°C), under a 12h/12h light-dark cycle. All procedures followed the guidelines for the welfare of laboratory animals, according to "The Brazilian College of Animal Experimentation" (COBEA). All procedures were approved by the Ethics

Committee on Animal Use (CEUA) of the São Paulo State University - Bauru, Brazil (protocol # 281/2020) which follows the Brazilian Ethical Principles in Animal Research (CONCEA).

Characterization of the Groups

The animals were divided into four groups (n=20/group), equally matched for body weight and physical capacity. All animals had 60 days of life when the protocol was started. The following groups were formed:

YOUNG: remained sedentary and were euthanized at 4 months of age;
AGED: remained sedentary throughout life and were euthanized at 16 months of age;
LONG-TERM TRAINING (LTT): started aerobic training at the beginning of the protocol (60 days of life) and continued the aerobic training throughout their lifetime until euthanasia at 16 months of age;
SHORT-TERM TRAINING (STT): remained sedentary until 14 months of age and started an aerobic training protocol 8 weeks before euthanasia (16 months of age).

At the end of the procedures, each group was divided into two subgroups for functional and humoral analyses (systolic blood pressure, arterial stiffness, plasma nitrite, and thiobarbituric acid reactive substances) and for histological analyses (wall thickness and wall-to-lumen ratio morphometry and collagen deposition of the thoracic aorta, carotid, and femoral arteries). The number of animals in each group is represented in Figure 1:

Aerobic Physical Capacity Tests

After an adaptation period of 10 days, all animals were subjected to a maximum capacity test (Tmax) to indirectly determine their maximum physical capacity. Briefly, the protocol establishes an increase of 0.3 km/h for every 3 minutes of exercise on a treadmill, with an inclination of 0%, until the animal stops running spontaneously.[21,22] The maximum physical capacity evaluation for the animals in the AGED and LTT groups was carried out at 16 different times (start, 4, 8, 12, 16, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, and 61 weeks), related to the increases in training load of the LTT group, to maintain exercise intensity. The STT group was assessed at three different times (start, 14, 15, and 16 months of age) during the experimental protocol.

Physical Training and Experimental Design

The trained groups underwent aerobic physical training on a treadmill (Inbramed, Millenium, Brazil), at 50–60% of Tmax (moderate intensity) for 1h/day, 5 days a week, for 8 weeks (STT group) or 61 weeks (LTT group), while the non-exercised groups remained sedentary. Figure 2 illustrates the 16-month experimental protocol.

Functional Analysis

Pulse Wave Velocity (PWV)

At the end of the experimental protocol, PWV assessments were carried out as previously described.[23] Briefly, the animals were anesthetized with xylazine hydrochloride (ANASEDAN®, 10mg/Kg), and ketamine hydrochloride, (CEVA and DOPALEN®, 50mg/Kg), at 0.1mg/100g body weight. Two pOpet® probes (Axelife SAS, Saint Nicolas de Redon, France) were positioned, one on a forelimb and one on a hindlimb. The transit time (TT) signal was recorded for 10 seconds using pOpet 1.0 software and the PWV was calculated according to the formula: PWV (m/s) = D (m)/TT (s), where D is the distance between the probes.

Direct Blood Pressure (BP) Measurement

Immediately after the PWV procedure and with the animals still anesthetized, they were positioned in the prone position and an incision and dissection of the left femoral artery were performed. A catheter was introduced for direct BP measurement, as described previously.[24,25] After 24 hours, BP was recorded using LabChart Pro 7 software (ADInstruments, NSW, Australia), in awake rats. Systolic (SBP) and diastolic blood pressure (DBP) were directly measured, and mean BP and heart rate (HR) were calculated from the pulsatile BP measurement.

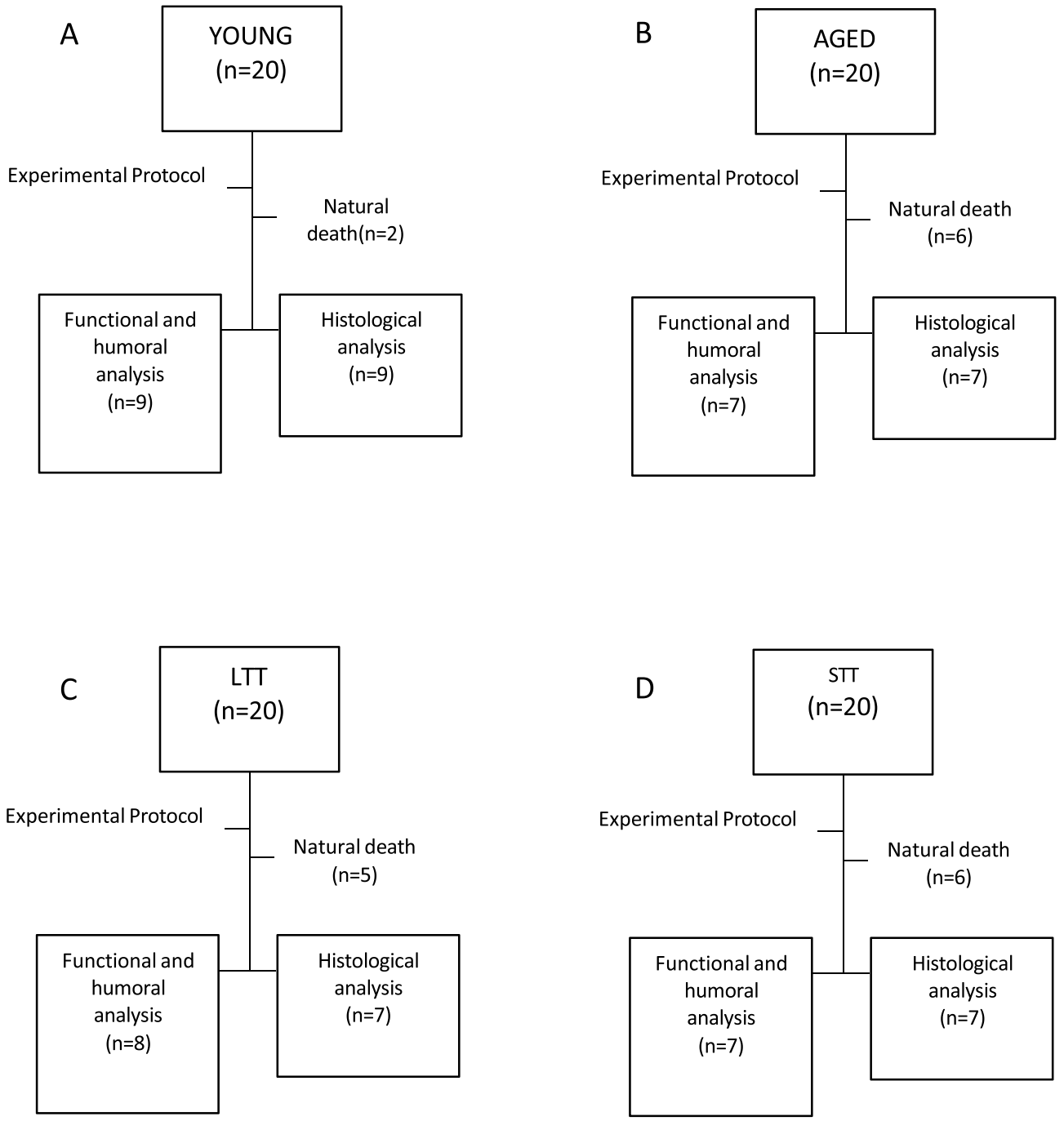

Figure 1 Number of animals in each group before and after the experimental protocol: Panel (**A**) – Young group; Panel (**B**) – Aged group; Panel (**C**) - Long-Term Training group (LTT); and Panel (**D**) - Short-Term Training group (STT).

Euthanasia

The animals were anesthetized with an overdose of xylazine hydrochloride, (ANASEDAN ®, 20mg/kg) and ketamine hydrochloride (DOPALEN®, 160mg/kg), at 0.1mg/100g body weight. After anesthesia, two different methods were used: decapitation for the animals that were used for the humoral analysis, and transcardiac perfusion with 4% paraformaldehyde for the histological technique, which will be described later.

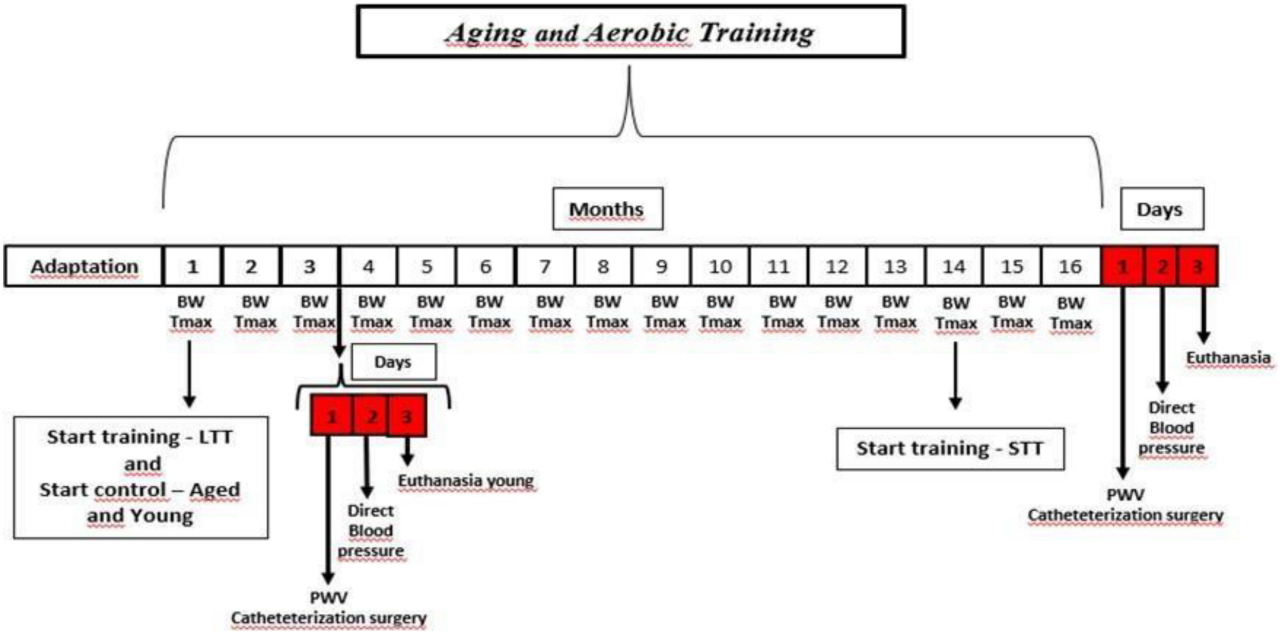

Figure 2 Experimental protocol of 16 months of aging and physical training. Adaptation period (5–10 days), body weight (BW, g), aerobic maximal capacity test (Tmax, s), pulse wave velocity (PWV, m/s), Long-term training group (LTT), and Short-term training group (STT).

Humoral Analysis

Analysis of Plasma Nitrite Concentrations

After euthanasia by decapitation, 5 mL of blood were collected for analysis of plasma nitrite concentrations. The blood was immediately centrifuged at 4,000 rpm for 5 minutes and the plasma was stored in a −80°C freezer. The solution containing the plasma aliquots was analyzed in duplicate for nitrite concentrations, using the ozone-based reductive chemiluminescence method, and was subsequently evaluated by a gas-phase chemiluminescence analyzer (Sievers analyzer Model 280 NO; Boulder, CO, USA), as previously described.[26,27]

Analysis of Thiobarbituric Acid (TBARS)

Analysis was performed to quantify the presence of thiobarbituric acid (TBARS) in the samples. For this, 750 µL of 10% (W/V) trichloroacetic acid (TCA) were added to 250 µL of the plasma sample to trigger the reaction. TCA has the function of denaturing the proteins present and acidifying the reaction medium. This mixture was stirred and centrifuged for 10 minutes at 4,000 rpm. Then, 500 µL of the supernatant were removed and 500 µL of thiobarbituric acid (TBA) 0.67% (W/V) were added, which reacted with the products of lipid peroxidation, forming a pink-colored compound. The mixture was incubated for 18 minutes at 100°C and then cooled on ice, for subsequent absorbance reading at 535 nm in a spectrophotometer (Biospectro).

Analysis of the Morphometry and Collagen Deposition of the Thoracic Artery, Carotid Artery, and Femoral Artery

For the analysis of morphometry and arterial collagen, the animals were euthanized using the anesthetic procedure described above and, immediately after cardiac arrest, a ventral incision was made in the midline of the thoracic region, with an opening through the thorax to expose the heart. The animals were perfused through the left ventricle by inserting a needle into the left ventricle and immediately afterwards the right atrium was opened to drain the blood. Perfusion was carried out using a peristaltic pump, with ~100 mL of sterile saline solution and then ~500 mL of 4% paraformaldehyde solution buffered with PBS (in an exhaust hood). After the perfusion procedure, dissection was carried out to remove the vessels: median thoracic aorta, carotid, and left femoral arteries, and the arterial segments were post-fixed in 4% buffered paraformaldehyde solution for 48 hours. The tissues were then dehydrated in an increasing sequence of ethanol and xylene (70%, 95% (2x), 100% (3x), and xylene (3x)) and embedded in paraffin. Next, cross-sections of 5 µm (morphometry) and 7 µm (collagen) were made and three slides were prepared for each tissue sample, each with nine

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semi-serial sections, totaling twenty-seven sections/tissues. The sections were mounted between a slide and a coverslip and then stained using the Weigert method and hematoxylin-eosin to reveal the cellular components;[22,28] and Picro-Sirius red to differentiate the type I collagen fibers (stained bright red).[28,29] For image analysis and photography, a Leica DM 4 B microscope (Leica Microsystems, GmbH, Wetzlar, Germany) was used at 2.5x magnification for morphometric analysis of the thoracic aorta, carotid, and femoral arteries and 20x and 40x magnification for the collagen deposition area of the thoracic aorta, carotid, and femoral arteries respectively. All offline analyses (ImageJ software) were carried out blindly to avoid any misinterpretation.

Statistical Analysis

Descriptive statistics were reported as mean and ± standard deviation (SD). For the analysis of inductive statistics, the Shapiro–Wilk test was used to assess the normal distribution of the data. The Student's *t*-test and one-way analysis of variance (ANOVA) with Tukey's post-test were used for data with normal distribution ($p < 0.05$), otherwise, Kruskal–Wallis analysis was used to detect these differences, with a significance level of $p < 0.05$. The data were analyzed using the statistical package SigmaPlot 12.0 (Systac Software, Inc., San Jose, CA, USA).

Result

Effects of Aging

Table 1 presents a comparison of body weight, physical capacity, hemodynamics, and vascular function and structure between the Wistar young control and Wistar aged control groups.

Table 1 Comparison of Body Weight, Physical Capacity, Hemodynamics, Vessel Function, and Structure Between the Young Control Wistar and Aged Control Wistar Groups

	YOUNG	AGED	P
Body Weight (g)	420.60±42.16	600.70±72.87	<0.001*
Physical Capacity (s)	632.87±117.39	490.70±84.17	0.009*
SBP (mmHg)	103.76±7.09	100.15±9.65	0.363
PWV (m/s)	4.55±0.37	5.13±0.61	0.065
Plasma Nitrite (µmol/mg)	0.603±0.09	0.449±0.17	0.074
TBARS (µmol/mg)	0.22±0.02	0.25±0.02	0.109
Aorta			
% Collagen	9.03±1.44	12.15±1.55	0.005*
Wall thickness	102.58±3.38	140.89±8.28	< 0.001*
Wall-to-lumen ratio	0.066±0.0042	0.091±0.0078	< 0.001*
Carotid			
% Collagen	10.59±2.99	13.49±2.12	0.116
Wall thickness	47.10±7.51	65.59±8.70	0.007*
Wall-to-lumen ratio	0.074±0.011	0.09±0.017	0.028*
Femoral			
% Collagen	21.85±5.09	20.53±5.67	0.706
Wall thickness	60.46±7.53	71.72±10.77	0.161
Wall-to-lumen ratio	0.171±0.055	0.167±0.061	0.934

Note: *p<0.05.

Abbreviations: Body weight, physical capacity, systolic blood pressure (SBP), pulse wave velocity (PWV), Plasma Nitrite, thiobarbituric acid reactive substances (TBARS), histological analysis of arteries, collagen deposition in both sedentary groups: young (n=5-10) and AGED (n=5-10). 7 µm-thick sections were stained with Picrosirius Red for collagen data and 5 µm-thick arterial sections were stained with hematoxylin and eosin for vessel morphometry (wall thickness and wall-to-lumen ratio) in aorta, carotid, and femoral arteries. Significance.

The AGED group showed an increase in body weight at the end of the experimental protocol compared to the YOUNG group (p=<0.001). The physical capacity of the AGED animals demonstrated a decrease compared to the YOUNG group (p=0.009). The hemodynamic analyses and structural function of the YOUNG animals did not demonstrate statistical differences for the variables of systolic blood pressure (SBP), pulse wave velocity (PWV), plasma nitrite, and thiobarbituric acid reactive substances (TBARS) compared to the AGED group. Conversely, the structural analyses demonstrated an increase in collagen deposition in the arterial aorta (p=0.005), and an increase in the Wall thickness and Wall-to-lumen ratio in the aorta (p=<0.001 and p=0.007, respectively) and carotid arteries (p= <0.001 and p=0.028, respectively).

Body Weight and Physical Capacity

Table 2 presents the body weight values of the animals at 1 month, 14 months, and 16 months of age, during the experimental protocol.

The LTT group had a lower body weight at 14 months compared to the AGED group (p= 0.036). The STT group had a lower body weight compared to the AGED group (p=0.027) at the end of the experimental protocol. There were no statistical differences between the AGED and LTT groups (p=0.338) at the beginning of the protocol. The evaluation of the maximum physical capacity of the animals that underwent the aging process, at 14 months, showed that the LTT group presented increased physical capacity compared to the AGED group (p<0.001) and STT group (p<0.001) when this group started the training protocol. However, at the end of the 16-month experimental protocol, both the LTT and STT groups demonstrated an increase in physical capacity compared to the AGED group (p<0.001 and p=0.011, respectively). Additionally, the LTT group also presented higher physical capacity values compared to the STT group (p=0.004).

Systolic Blood Pressure and Arterial Stiffness Measurement

Figure 3A presents the systolic blood pressure values at the end of the protocol, demonstrating that late or lifelong physical training did not promote benefits in systolic blood pressure when compared to the older group (p=0.227). The pulse wave velocity (PWV) values, performed at the end of the 16-month aging protocol, for the AGED, LTT, and STT groups, are shown in Figure 3B. The LTT group presented attenuated PWV values when compared to the AGED group (p=0.002).

Table 2 Body Weight and Physical Capacity Parameters During the 16-month Protocol in All Age Groups

	AGED	LTT	STT	p
Body Weight (g)				
Month 1	259.50±18.27	251.70±19.93	–	0.273
Month 14	613.50±70.13	543.00±54.49*	551.30±53.64	0.036
Month 16	600.70±72.87	546.10±53.91	526.90±43.79*	0.028
Physical Capacity (s)				
Month 1	560.70±79.30	616.40±160.59	–	0.338
Month 14	515.80±113.43	920.60±132.56*	536.50±94.16^{+}	<0.001
Month 16	490.70±84.17	882.20±172.53*	674.20±117.24*$^{+}$	<0.001

Notes: Values of body weight and physical capacity; groups: aged (n=10); long-term training (LTT, n=10); short-term training (STT, n=10). Significance: * vs AGED; p<0,05 and $^{+}$ vs STT; p< 0.05.
Abbreviations: DBP, diastolic blood pressure; HE, hematoxylin-eosin; HR, heart rate; LTT, long-term training; NO, nitric oxide; NS, non-statistical; ONOO-, peroxynitrite; PWV, pulse wave velocity; ROS, oxygen species; SBP, systolic blood pressure; STT, short-term training; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TMAX, maximum capacity test.

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Plasma Nitrite Concentrations and Thiobarbituric Acid Reactive Substances (TBARS)

Figure 4 demonstrates the lack of statistically significant differences between the groups for nitrite (NO2-) concentrations (Figure 4A). Figure 4B presents the values of thiobarbituric acid reactive substances (TBARS). At the end of the experimental protocol, the STT group showed lower TBARS values when compared to the AGED group (p= 0.046), which remained sedentary.

Morphometry of the Wall Thickness and Wall-to-Lumen Ratio of the Thoracic Artery, Carotid Artery, and Femoral Artery

Aorta Artery

Figure 5 presents the wall thickness (A), wall-to-lumen ratio (B), and histological sections stained in H.E. (C) of the aortic artery. The LTT group showed lower arterial wall thickness values when compared to the AGED group (p=0.032). The same relationship affected the wall-to-lumen ratio variable (AGED vs LTT, p= 0.008).

Carotid Artery

Figure 6 presents the wall thickness (A), wall-to-lumen ratio (B), and histological sections (C) stained in H.E. of the carotid artery. The LTT group showed a lower arterial wall thickness and arterial wall-to-lumen ratio when compared to the AGED group (p=0.019 and p=0.012, respectively). However, no significant differences were found in the STT group.

Femoral Artery

Figure 7 presents the wall thickness values (A), wall-to-lumen ratio (B), and histological sections stained in H.E (C) of the femoral artery. No significant differences were found between the groups for these variables (A, p=0.418; B, p=0.392).

Collagen Concentrations in the Thoracic Artery, Carotid Artery, and Femoral Artery

Figure 8A presents the % of collagen deposition area in the aortic artery and histological sections stained with picrosirius red. In the aortic artery, both aerobic physical training programs (LTT and STT) showed a decrease in the percentage of collagen compared to the AGED group (p= <0.001 and p= 0.001, respectively). Figure 8B presents the % of collagen deposition area in the carotid artery and the histological sections stained with picrosirius red. In the carotid artery, it can be observed that both aerobic physical training programs (LTT and STT) led to a decrease in the percentage of collagen

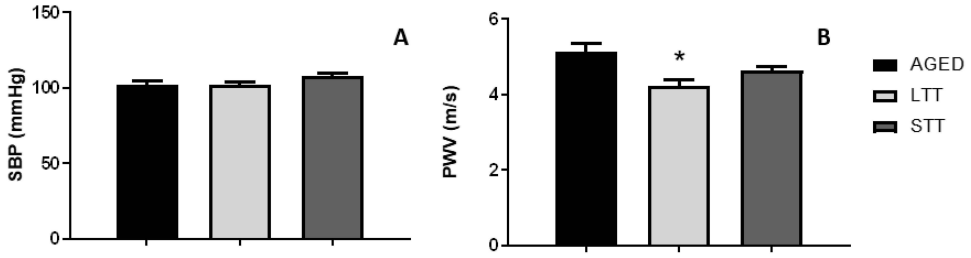

Figure 3 Systolic blood pressure (SBP - figure (**A**); pulse wave velocity (PWV - figure (**B**); Groups: aged (n=8 (**A**) and n=10 (**B**)); Long-term training (LTT, n=6 (**A**) and n=10 (**B**)); Short-term training (STT, n=6 (**A**) and n=10 (**B**)). Significance: * vs AGED; p< 0.05.

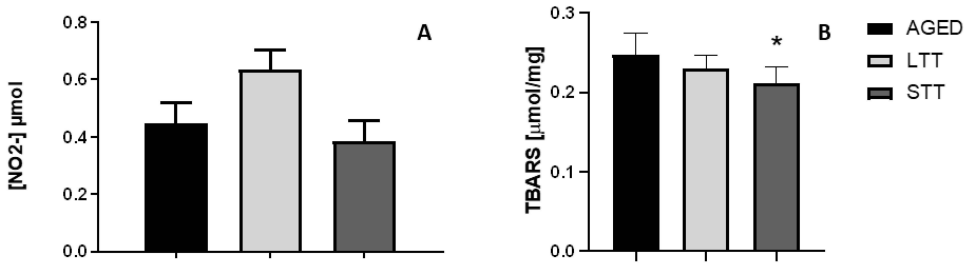

Figure 4 Plasma nitrite (figure (**A**) and thiobarbituric acid reactive substances (TBARS) (figure (**B**); Groups: AGED (n=6 (**A**) and n=7 (**B**)), Long-term training (LTT, n=6 (**A**) and n=7 (**B**)); Short-term training (STT, n=5 (**A**) and n=7 (**B**)). Significance: * vs AGED; p< 0.05.

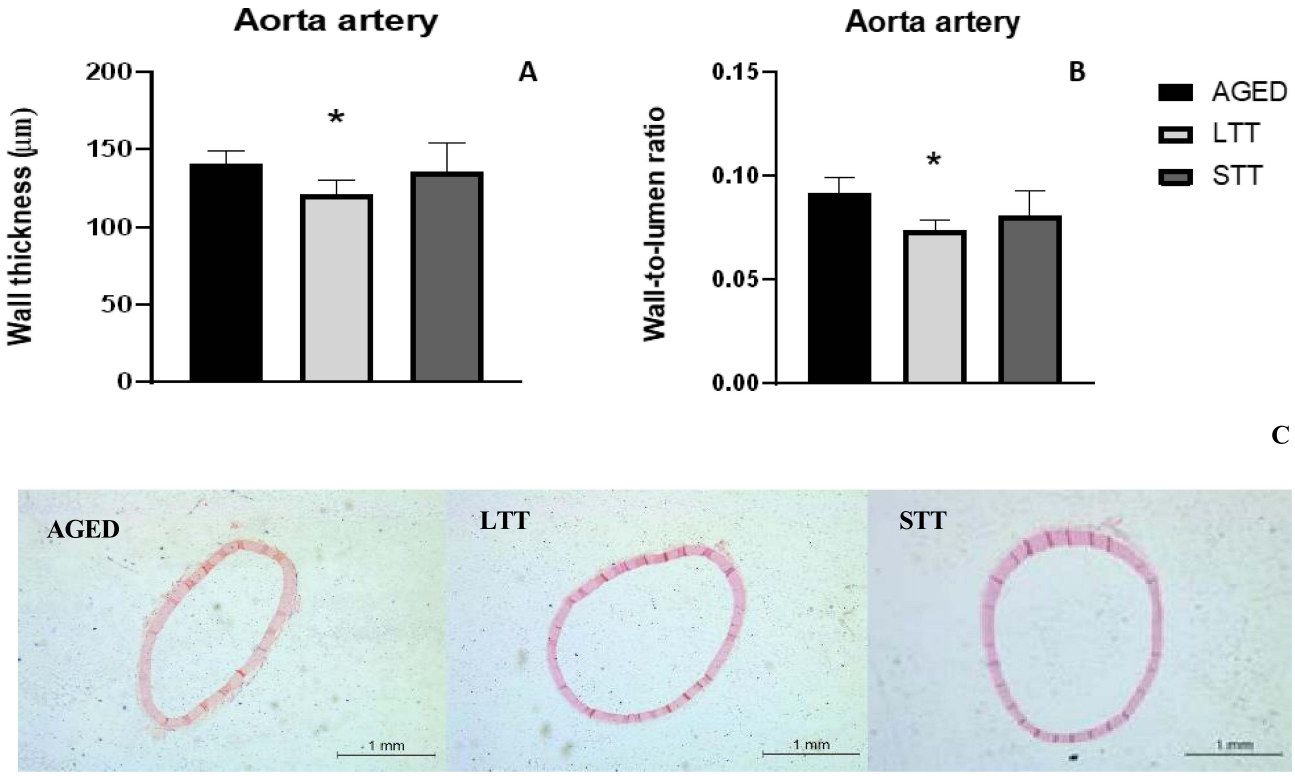

Figure 5 Wall thickness of the thoracic aorta (**A**), wall-to-lumen ratio (**B**), and representative photos of histological sections (5µm) stained with Hematoxylin and eosin (HE), at the end of the protocol (**C**); Groups: AGED (n=6); Long-term training (LTT, n=5); Short-term training (STT, n=5). Significance: * vs AGED; p< 0.05.

compared to the AGED group (p= <0.008 and p= 0.041, respectively). Figure 8C presents the % of collagen deposition area in the femoral artery and the histological sections stained with picrosirius red. No significant differences were found between the groups for this variable (p=0.899).

Discussion

The main results found in the current study were that vascular remodeling was observed in aged (16 months of age) Wistar rats when compared with young rats even though PWV was still preserved. However, training throughout life was able to prevent vessel remodeling and collagen deposition, which probably helped to improve arterial stiffness. This result was not found in the short-term training group.

In the first part of the current study, we sought to evaluate the possible functional, structural, and humoral alterations induced by aging. It was observed that BP and PWV were similar between young and aged groups, however, some vascular changes were present in the aorta and carotid of the aged rats. Although PWV presented values 13% higher in the aged rats compared to young rats (NS), it has been shown that arterial stiffness is a common consequence of the aging process.[30] In agreement, it was possible to observe in the current study that the AGED group presented some structural alterations, such as higher aortic collagen deposition, higher aortic and carotid wall thickness, and wall-to-lumen ratio. Compared with the young group, aged rats presented an increase of 35% in aortic collagen deposition, which is in accordance with the results shown by Fabrício et al and Gioscia-Ryan et al.[23,31] In addition, the aged group demonstrated changes in the morphometry of the vessels. An increase of 37% was found in the aortic wall thickness and 39% in the carotid wall thickness. In addition, the wall-to-lumen ratio was higher in the aorta (+38%) and carotid artery (+22%), compared with young rats. However, despite the presence of these structural alterations in the aorta and carotid arteries, the PWV was still preserved (p=0.06), suggesting that these aged rats developed some vascular remodeling in order to maintain an adequate perfusion, as shown by Jensen et al.[32]

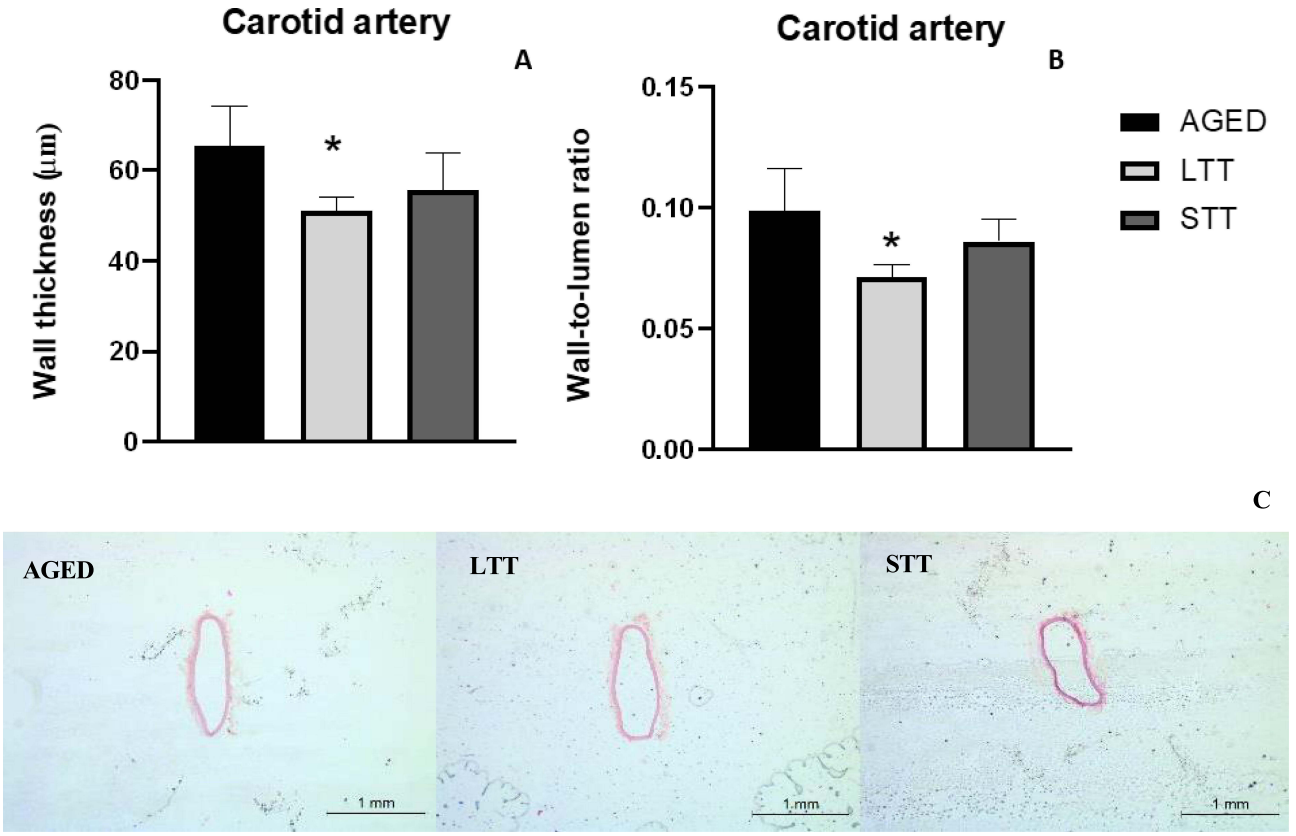

Figure 6 Carotid aorta wall thickness (**A**), wall-to-lumen ratio (**B**), and representative photos of histological sections (5μm) stained with Hematoxylin and eosin (HE), at the end of the protocol (**C**); Groups: AGED (CI, n=5); Long-term training (LTT, n=5); Short-term training (STT, n=4). Significance: * vs AGED; p< 0.05.

In the present study, values of blood pressure were similar between groups, as also demonstrated by HOTTA et al.[19] In agreement, values of plasma TBARS and nitrite plasma content were not significantly altered by aging.

Although PWV was not significantly higher (+13% vs young), aged rats already presented some features of vascular remodeling, suggesting that, if no changes were made, the perfusion could be compromised earlier. Therefore, in the second part of this study, we sought to understand the relationship between the time of the practice of aerobic training and its preventive effects on cardiovascular health. We then compared the effects of physical training practiced continuously throughout young/adulthood until older age (training for 14 weeks) or practiced only from adulthood to older age (short-term 8 weeks). The current study took into consideration the functional, humoral, and structural factors in different arteries of Wistar rats after 16 months of life. It is important to report that both training times increased the physical capacity of the animals.

In the current study, 2 months of training (STT group) in aged rats was not enough to reduce PWV. Conversely, the LTT group presented lower values of PWV compared with sedentary AGED rats and STT, suggesting that training throughout life attenuated the increase in arterial stiffness induced by the aging process. Reduction of PWV by exercise can be associated with lower oxidative stress, as shown by Seals et al,[33] who demonstrated that regular training interventions in middle-aged men caused an increase in carotid artery compliance associated with lower systemic oxidative stress. In agreement, the study by FLEENOR et al, [34] using a voluntary wheel running training protocol in older male mice aged 29 to 32 months, showed attenuation in the levels of carotid stiffness associated with a reduction in oxidative stress. However, the results of TBARS, which represents lipoperoxidation (oxidative damage), in the present study, were lower only in the STT group.

A similar result was observed in the study of NI et al, [35] who showed that a combined training program, lasting more than 4 weeks, induced a slowdown in cellular lipid peroxidation in the bodies of older adult individuals. Unexpectedly, this result was not observed in the group that trained throughout life, which may have been affected by a decrease in antioxidant

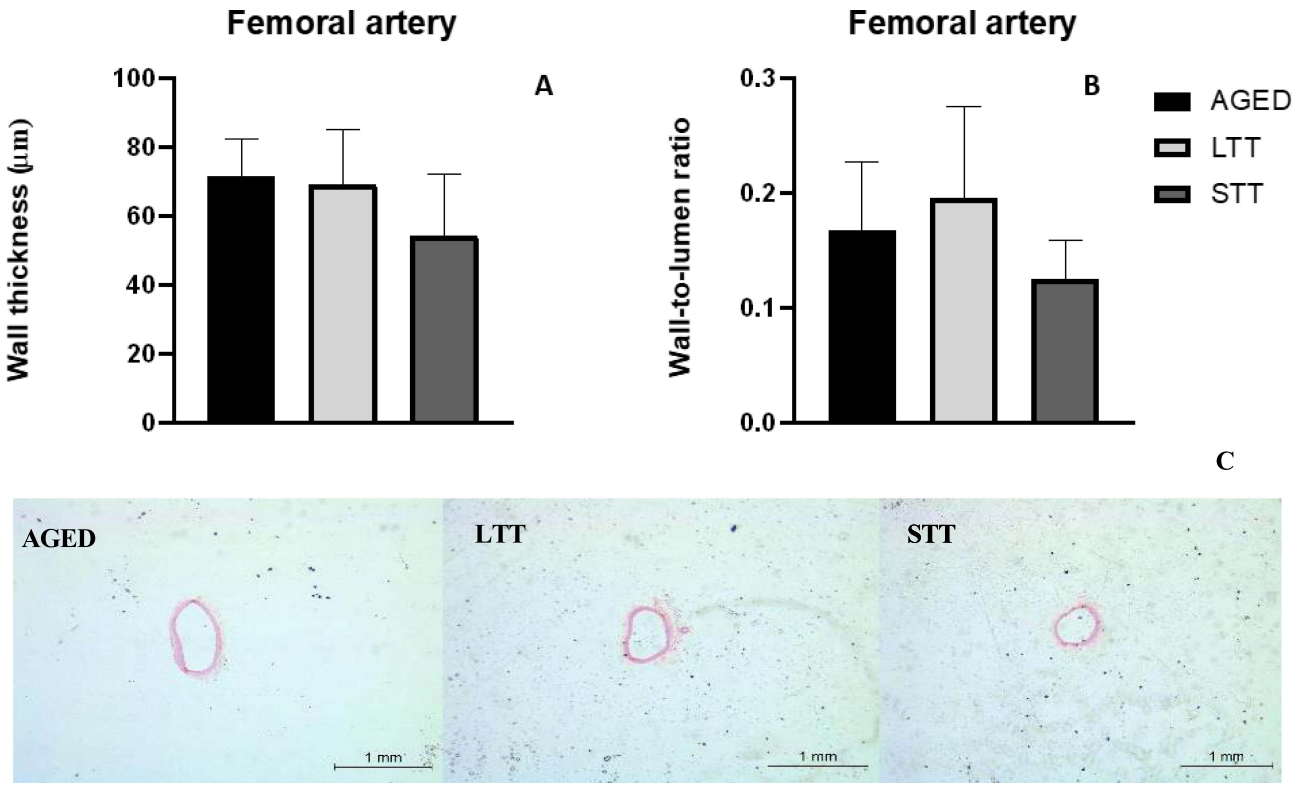

Figure 7 Wall thickness of the femoral aorta (**A**), wall-to-lumen ratio (**B**), and representative photos of histological sections (5µm) stained with Hematoxylin and eosin (HE), at the end of the protocol (**C**); Groups: AGED (CI, n=4); Long-term training (LTT, n=5); Short-term training (STT, n=3). Significance: * vs AGED; p< 0.05.

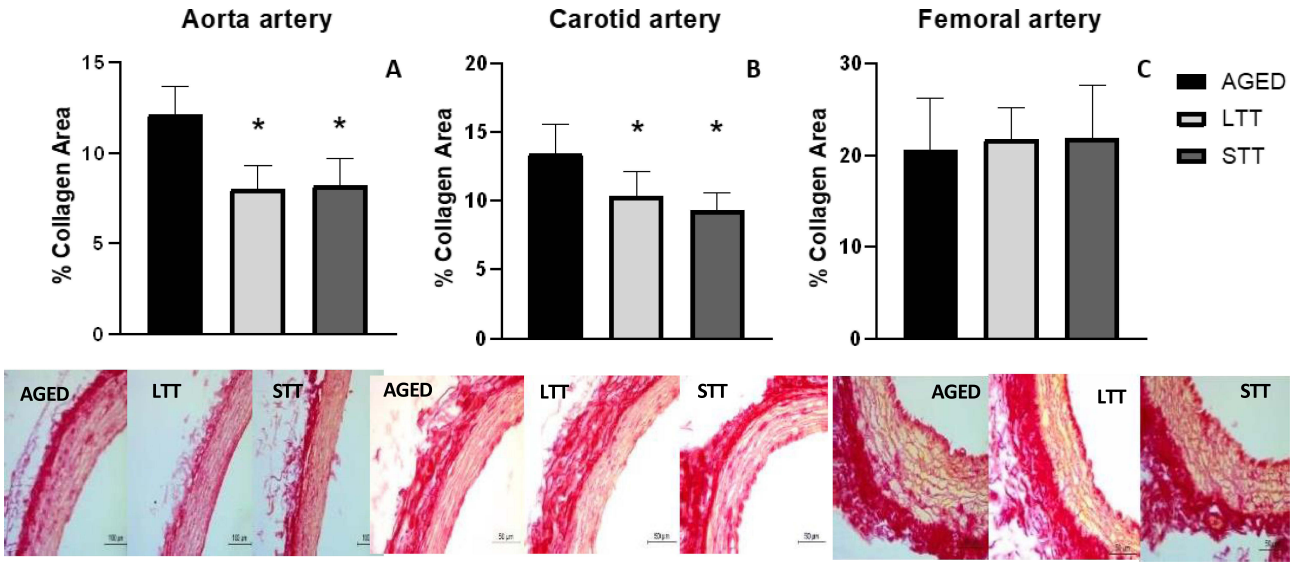

Figure 8 Values of collagen deposition area of histological sections (7µm) stained with picrosirius-red: aorta artery (%, figure (**A**), carotid artery (%, figure (**B**), and femoral artery (%, figure (**C**) in all aged groups at the end of the protocol. Groups: AGED (n=7); Long-term physical training (LTT, n=5); Short-term physical training (STT, n=5). Significance: * vs AGED; p< 0.05. Red staining represents the collagen fibers.

enzymes, as pointed out by BOVERIS and NAVARRO. [36] These authors showed a biphasic effect of exercise, since it decreases the mitochondrial content of oxidation, TBARS, and carbonyls, and increases antioxidant enzyme activities by 15–20% after 24 weeks of training, however, this effect almost disappeared in older rats after 50 weeks of training. We emphasize that the LTT group ended the training protocol at 61 weeks. It is also important to note that the animals used in

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this study were considered normotensive and it is suggested that humoral factors did not have a major influence on the results. The lack of reduction in TBARS in the LTT group was followed by no significant alteration in nitrite concentration, even though there was a trend to an increase. Therefore, further studies are necessary to understand the effects of training on TBARS and nitrite concentration and its association with improvements in arterial stiffness.

Blood pressure was not affected either by age (as shown in the first part of this manuscript) or by the two exercise protocols with different times. Although this response was expected, since the rats were normotensive, it is in accordance to the study by HOTTA et al[19] who also showed no change between their experimental groups of young and older normotensive rats.

Since our rats did not present higher oxidative stress and the nitric oxide bioavailability was similar between groups, we analyzed the morphometry of the vessels and one component of the extracellular matrix to understand the different effects observed by the different timing protocols of exercise.

Our findings showed that aged rats had a higher percentage of aortic collagen deposition, however, both exercise protocols, long-term and short-term, were able to attenuate this increase in aorta and carotid arteries. This lower collagen deposition, induced by exercise can be explained by lower SNA [37] or a lower TGF-beta fibrotic pathway. [38–40] Similarly, the study by Gu et al, [30] which assessed the effects of chronic aerobic exercise training in aortic stiffening and endothelial dysfunction in young (03 months) and aged (23 months) rats, showed that exercise training promotes a reduction in aortic stiffening due to the reduction in collagen concentration and an increase in elastin concentration.

Nevertheless, when the morphometric analysis of the arteries was investigated, we found that only the long-term aerobic exercise training protocol attenuated the increase in aortic and carotid vessel thickness and the wall-to-lumen ratio. These results agree with the studies of HANNA et al[20] who showed that increases in the wall thickness and stiffness of the coronary resistance arteries, and the increased wall-to-lumen ratio induced by age, were reversed by physical training, restoring the contractile function of vascular smooth muscle in aged coronary resistance arteries. We may speculate that the short-term exercise protocol was not long enough to change the vessel structure.

In summary, the results of the current study may suggest that the practice of physical training throughout life proved to be more effective in mitigating the harmful effects of aging on vessels. We demonstrated that only lifelong training attenuated the arterial stiffness and this response was associated with improvement in the vessel structure since collagen deposition was attenuated in both training protocols. It is important to emphasize that even though training later in life did not attenuate PWV, it should be encouraged, mainly for individuals who have never previously practiced any exercise, since it increased physical capacity, decreased aortic and carotid collagen deposition, and attenuated the levels of plasmatic TBARS.

Conclusion

The results of the current study suggest that 16-month-old Wistar rats already presented vascular remodeling when compared with young rats, but PWV was still preserved. However, training throughout life seems to be better than short-term training later in life, mainly because it was able to prevent vessel remodeling and collagen deposition, which probably helped to improve arterial stiffness.

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Disclosure

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