Brief Communication

Remodeling of elastic layer of aortic artery after training by swimming in spontaneously hypertensive rats

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Abstract

Hypertension is a major risk factor for cardiovascular diseases, in which the elastic properties of arteries are subjected to high pressure levels, and networks of elastic fibers may develop cleft longitudinal, transverse, breaks and fragmentation, and such structural changes (fibrosis and degradation of elastin) may lead to a decrease in the elasticity of the artery. The descending thoracic aortas of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHRs) subjected to physical training through swimming or those of sedentary rats were prepared with hematoxylin–eosin and Verhoff to assess the artery medial. The images were captured with a videocamera coupled to an ordinary light microscope and the images were analyzed with the same program. SHRs showed a larger area of the medial layer of the thoracic aorta ($F = 25{,}764$, $P < 0.001$), and it was observed that rats submitted to physical training through swimming showed a larger area of the thoracic aorta ($t = 3.206$, $P = 0.011$). There was a higher percentage of elastic trained ($F = 6.536$, $P = 0.019$). To conclude, this study aimed to determine the elastic component of the aortic artery in animals that underwent exercise when compared with those that did not perform the activity, and analyze the relationship between the area of the aortic wall in trained and sedentary animals. The principal conclusion is that the rigidity of the aorta is not increased in SHRs subjected to physical training compared with that of trained WKY animals; however, when sedentary SHRs were analyzed there was a decrease in the elasticcomponent, which can characterize the aortic arterial stiffness in SHRs.

Keywords: aortic artery, hypertension, physical training

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Introduction

The mechanical properties of the arteries play an important role in cardiovascular hemodynamic. These large arteries, including thoracic aortas, are made up of three layers: intima, media or middle layer and adventitia, and the middle layer is composed of vascular smooth muscle cells and extracellular matrix such as collagen and elastin. The pathological changes of the arteries have been attributed to adverse changes in endothelial function or the middle layer.[1]

Hypertension is a risk factor for aortic disease and is present in most patients with aortic dissection, abdominal aortic aneurysm and peripheral vascular disease.[2] Hypertension can produce an increase in arterial stiffness,[3] especially when the elastic properties are subjected to high pressure levels.[4,5] The elastic fiber network develops longitudinal cracks, transverse breaks and fragmentation, and such structural changes (fibrosis and degradation of elastin) may lead to a decrease in the elasticity of the artery.[6] There are evidences that exercise training improves conditions of the vessels in spontaneously hypertensive rats (SHRs) subjected to swimming protocol.[7]

The aim of this study was to evaluate the areas of thoracic aortas and the elastic component of normotensive Wistar Kyoto (WKY) and SHRs subjected to physical training through swimming as well as that of the sedentary ones.

Methods

Animal care and exercise-training protocol

Male SHRs and WKY rats that were 245 ± 2 days old were obtained from the breeding facility of the Federal University

of Triangulo Mineiro (Uberaba, MG, Brazil). They were fed the standard laboratory chow and water *ad libitum* while housed (3–5 per cage) in a temperature-controlled room (22°C) and 12-hour dark/light cycles. SHRs are the best models of chronic hypertension and these animals were randomly assigned to four groups: sedentary hypertensive (SH, $n = 06$), exercise-trained hypertensive (TH, $n = 06$), sedentary normotensive (SN, $n = 06$) and exercise-trained normotensive (TN, $n = 06$) rats. Exercise-training was performed by swimming protocol five days/week for nine weeks with a progressive training time every week of 10 min in the first week and 120 min in the last week of the training in a tank with warm water (30 ± 1°C).[7,8] The sedentary animals' groups were put in the same tank for a period of one minute to note the effect of the exercise-training and not for a possible alteration resulting from the aquatic stress. All procedures were followed in accordance with institutional guidelines. This work was approved by the Committee of Ethics in Animals of Federal University of Triangulo Mineiro and the norms of the Brazilian College of Animal Experiments were followed.

Surgical procedure and blood pressure and heart rate recording

At the end of the protocol training, two polyethylene catheters (PE-50 welded to PE-10) filled with a saline solution (0.9%) and heparin (500 IU/mL) were implanted into the left femoral arteries of the anesthetized rats (sodium pentobarbital – 40 mg/kg intraperitoneally) for direct recordings of the pulsatile pressure. Thereafter, the polyethylene catheters were exteriorized at the posterior neck region of the animal. The experimental rats received food and water *ad libitum* and were studied one day after the catheter placement. On the day of the experiment, the rats were conscious in their cages and allowed to move freely during the experiments. The arterial catheter was connected to a strain-gauge transducer (P23Db, Gould-Statham), and pulsatile arterial pressure (AP) signals were recorded in baseline conditions over a 30-minute period by a microcomputer equipped with an analog-to-digital converter board (CODAS®, 1000-Hz sampling frequency, Di220 Dataq Instruments, Inc., Akron, OH, USA). During the experimental procedure, the systolic (SAP), diastolic (DAP), mean AP (MAP) and heart rate (HR) were derived from the AP signals. Following that, all animals were euthanized and their thoracic aortas were removed and fixed in formaldehyde 10%. The analysis of blood pressure (BP) was carried out only at the end of the experiments, because the objective was to compare the data of BP and pulse interval of groups subjected to physical training with that of the groups that were not only at the end of the experiments.

Morphological study of the aortic artery

The descending thoracic aortas consisting of the entire segment were harvested between the left subclavian and celiac artery branch points. Tissues were fixed in formaldehyde, embedded in paraffin and transverse sections (5 μm thick) were prepared for Verhoff staining.

To check the elasticity of the middle layers images were captured using a videocamera coupled to an ordinary light microscope with a $\times 40$ objective, which sends images to an analyzer system (KS300, Kontron Zeiss®, Germany). Captured images were viewed on a monitor and recorded for morphometric analyses in the program itself. Verification of the elasticity of the middle-layer area was performed through the demarcation of three measurements of the area per field analyzed in all fields of the court. And the quantification of the elastic middle layer was performed in all fields of the court.

Statistical analysis

Statistic 6.0 (StatSoft, Inc., Scientific Software, Tulsa, OK, USA) was used for statistical analyses. All data are expressed as mean ± SEM. Comparisons of the SHRs and WKY rats, both trained and untrained were analyzed using factorial ANOVA. When significance was found, the Scheffe test comparison was performed. A value of $P < 0.05$ was considered statistically significant.

Results

The SHRs showed a larger area of the medial layer of the thoracic aorta (TH = 127.790 ± 13.999; SH = 134.920 ± 22.653; TN = 96.928 ± 17.269; SN = 95.619 ± 8.313; $F = 25.764$, $P < 0.001$), and it was observed that rats subjected to physical training through swimming showed a larger area of thoracic aorta ($t = 3.206$, $P = 0.011$) (Figure 1).

A higher percentage of elasticity in trained animals ($F = 6.536$, $P = 0.019$) (Figure 2) was observed.

The results obtained for BP and HR after the exercise-training protocol are shown in Figure 3. Note that in Figure 3a, we can see that physical training decreased HR in both groups of the hypertensive and normotensive rats (SH = 418.472 ± 28.756; TH = 335.945 ± 16.844; TN = 322.332 ± 19.625; SN = 341.030 ± 28.046; F = 10.710; $P < 0.05$). In Figure 3b untrained hypertensive rats had

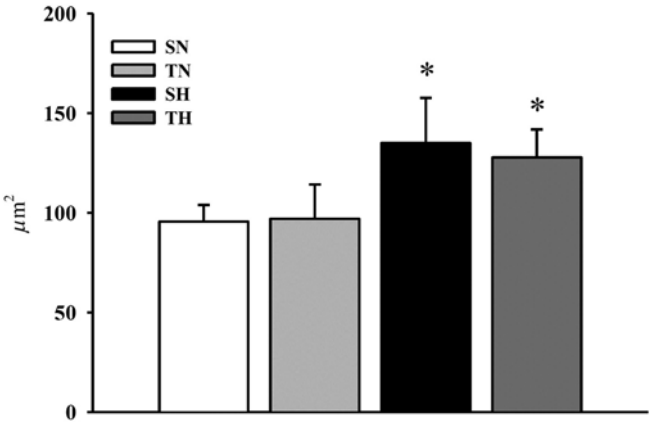

Figure 1 Area of the middle layer of the thoracic aortic artery. $F = 25{,}764$, $P < 0.001$; TH × TN=$t = 3.206$; $P = 0.01$. SN, sedentary normotensive; TN, exercise-trained normotensive; SH, sedentary hypertensive; TH, exercise-trained hypertensive

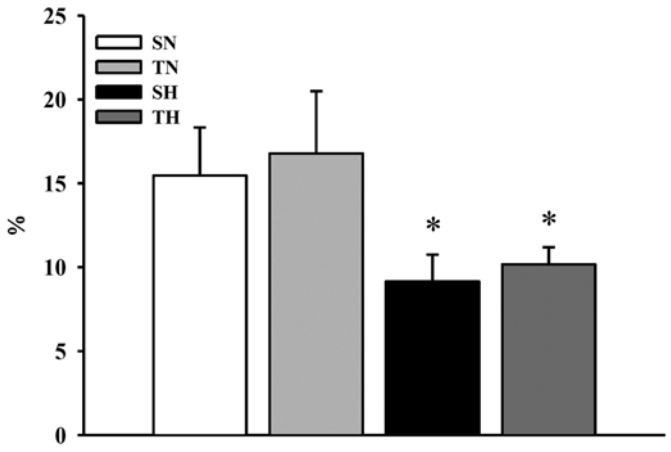

Figure 2 Percentage of elasticity of the middle layer of the thoracic aortic artery. $F = 6{,}536$; $P = 0.019$. SN, sedentary normotensive; TN, exercise-trained normotensive; SH, sedentary hypertensive; TH, exercise-trained hypertensive

a higher average BP pressure compared with those of both sedentary and trained normotensive groups (SH = 181.005 ± 18.115; TH = 132.017 ± 14.311; TN = 97.793 ± 9.194; SN = 102.395 ± 8.898; $F = 16.968$; $P < 0.05$).

Discussion

Aerobic training is recognized as an effective non-drug intervention to reduce BP in humans and SHRs.[9] This study examined the impact of physical training on the vascular injury caused by hypertension in the middle layers of the arteries of the thoracic aortas of SHRs and WKY, which demonstrated that exercise-training reduced the degradation of the elasticity and improved the condition of the vessels in both the SHRs and WKY. In this study, the means for obtaining arteries of the thoracic aortas were maintained as shown by morphological investigations.

The expression of hypertension in humans is similar to the genetically determined hypertension found in SHRs. The SHRs are the best experimental models of arterial hypertension. Both have a polygenic origin and are influenced by various environmental factors, the cardio-circulatory control is multifactorial, and some pressure adjustment mechanisms are not present in both situations.[10] Changes are observed in both experimental animals[11] and in hypertensive patients,[12] especially when physical training is conducted at low to moderate intensity contributing to lower rates of cardiovascular mortality[13,14] and attenuation in hypertension.[15]

Physical training can reduce high BP, the attenuation of the total peripheral resistance and/or decreased cardiac output, which would result from inhibition of sympathetic nerves and decreased vascular response after exercise.[16] Furthermore, the increased release of nitric oxide, prostaglandins and adenosine are also factors related to changes in vascular response after exercise-training and plausible explanations for the reduction in BP after this training.[17]

The hemodynamic marker of primary hypertension is the persistent increase in peripheral vascular resistance, which can be determined by means of different combinations of these factors. Thus, the mechanisms that promote balance between the pressure and non-pressure factors induce changes in the caliber of arteries and arterioles and deserve special attention. They participate primarily in muscle contraction regulating lumen or muscle thickness, covering a greater or lesser part of the lumen, or both.[18] In our study we showed that the SHRs had a larger area of the middle layer and exhibited an increase in the volume, featuring vessels of patients with hypertension. However, trained SHRs and WKY showed more elasticity in their vessels when compared with those of sedentary animals, possibly showing a lower degradation of this component, and hypertensive patients showed morphological degradation of this component and often also an increase in the extracellular matrix.[1,3]

Factors determining the properties of the aortic wall elasticity (e.g. relative proportion and/or interaction between smooth muscle cells and the extracellular matrix) may change the internal diameter and wall stress. Another possibility is that a structural disruption of the media leads to mechanical changes of the aortic wall in older SHRs. It has been demonstrated recently that the network of elastin plays an important role in maintaining the elastic properties of the aorta in the SHRs, not through variations of their total amount, but by increasing the amplitude of its anchorage on

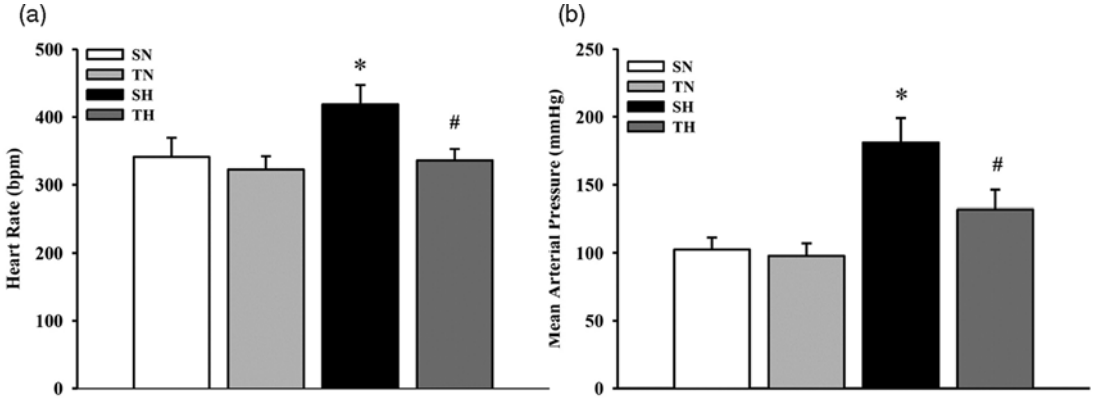

Figure 3 Results obtained for blood pressure and heart rate after exercise training protocol. HR = $F = 10{,}710$; $P < 0.05$. MAP = $F = 16{,}968$; $P < 0.05$. SN, sedentary normotensive; TN, exercise-trained normotensive; SH, sedentary hypertensive; TH, exercise-trained hypertensive

smooth muscle cells.[3,4] The rigidity of the aorta in SHRs is associated with the hardening of the aortic wall, which may not only be explained by an additional increase in BP or its connection to the aortic wall thickening or fibrosis[19], but also by a reduction in the elasticity. This suggests that some other kind of structural disruption of the media (such as changes in the number of muscle elastic lamellae connections or an increase in the proportion of collagen type I–III) leads to the aortic wall mechanical changes in SHR.[19]

It is, however, that hypertension is accompanied by functional changes in the autonomic nervous system, renal renin–angiotensin system, and other humoral mechanisms and endothelial dysfunction. Thus, hypertension results from several structural alterations of the cardiovascular system to stimulation amplify both hypertension and cardiovascular damage as caused.[20]

Essential hypertension is associated with several alterations in the arterial function. Endothelial dysfunction in hypertension leads to an imbalance in the production/release of contractile factors and relaxation and causes a decrease in the generation of nitric oxide/increase reactive oxygen species, thereby increasing the vascular tone, also contributes to the increased permeability leading to vascular subendothelial edema , and increase the expression of adhesion molecules on a consequent increase in leukocyte adhesion to the vascular wall intravascular coagulation accelerating and increasing proliferation of smooth muscle cells, leading to hypertrophy/hyperplasia of the vascular wall. It is thus evident that the endothelium plays a major role in hypertension, controlling vascular permeability, leukocyte adhesion, proliferation of smooth muscle cells, clotting and the balance between endothelial factors.[21]

The objectives of this study were to determine the elastic component of the aortic arteries in animals that underwent exercise when compared with that of those animals that did not perform the activity, and to analyze the relationship between the area of the aortic wall in trained and sedentary animals. The principal conclusion is that the rigidity of the aorta does not increase in SHRs subjected to physical training compared with trained WKY; however, when sedentary SHRs were analyzed there was found a decrease in the elastic component, which can characterize the arterial aortic stiffness in SHRs.

Authors contribution: GPA was involved in quantifying the elastic holding area and the middle layer of the arteries; MMMC in selection and training of animals; PMA in realization of staining and histological procedures necessary; OBN in surgical and animal training; RCRS in assistance in writing the project and reviewing the final article; VJDdS in assistance and supervision of physiological analyses; MAR in assistance and supervision of pathological analyses; DTRSA is the creator of the project, and helped in guiding the students and supervision at all stages.

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REFERENCES

1 Han WQ, Chen J, Wu LY, Zhu DL, Gao PJ. Different biomechanical properties of medial and adventitial layers of thoracic aorta in Wistar-Kyoto and spontaneously hypertensive rats. *Acta Pharmacol Sin* 2010;**31**:1319–23. Epub 2010 Aug 30
2 Hanson B, Levesley M, Watterson K, Walker P. Simulation of the human cardiovascular system for real-time physical interaction with an assist device. *Conf Proc IEEE Engl Med Biol Soc* 2005; **1**:409–12
3 Laurent S. Arterial wall hypertrophy and stiffness in essential hypertensive patients. *Hypertension* 1995;**26**:355–62
4 Bezie Y, Lacolley P, Laurent S, Gabella G. Connection of smooth muscle cells to elastic lamellae in aorta of spontaneously hypertensive rats. *Hypertension* 1998;**32**:166–9
5 Berry CL, Greenwald SE. Effects of hypertension on the static mechanical properties and chemical composition of the rat aorta. *Circ Res* 1976;**10**:437–51
6 Hayoz D, Rutschmann B, Perret F, Niederberger M, Tardy Y, Mooser V, Nussberger J, Waeber B, Brunner H. Conduit artery compliance and distensibility are not necessarily reduced in hypertension. *Hypertension* 1992;**20**:1–6
7 Abate DTRS, Barbosa Neto O, Rossi e Silva RC, Faleiros ACG, Correa RRC, Dias da Silva VJ, Castro ECC, Reis MA. Exercise-training reduced blood pressure and improve placental vascularization in pregnant spontaneously hypertensive rats – pilot study. *Fetal Pediatr Pathol* 2012;**31**:423–31
8 Tanno AP, Bianchi FJ, Costa Sampaio Moura MJ, Marcondes FK. Atrial supersensitivity to noradrenaline in stressed female rats. *Life Sci* 2002;**71**:2973–81
9 Bozi LHM, Melo SFS, Fontes EPB, Silva KA, Castro CA, Quintão JF Jr, Natali AJ. Stress protein expression in the myocardium of spontaneously hypertensive rats in response to physical training. *Rev Bras Hipertens* 2010;**17**:72–7
10 Trippodo NC, Frohlich ED. Similarities of genetic (spontaneous) hypertension.Man and rat. *Circulation Research* 1981; **48**:309–19
11 Negrão CE, Moreira ED, Santos MC, Farah VM, Krieger EM. Vagal function impairment after exercise training. *J Appl Physiol* 1992;**72**:1749–53
12 Katona PG, Mclean M, Dighton DH, Guz A. Sympathetic and parasympathetic cardiac control in athletes and nonathletes at rest. *J Appl Physiol* 1982;**52**:1652–7
13 Paffenbarger RS Jr, Hyde RT, Wing AL, Jung DL, Kampert JB. The association of changes in physicalactivity level and other lifestyle characteristics withmortality amongmen. *N Engl J Med* 1993; **328**:538–45
14 Muntner P, Roccella HEJ, Whelton PK. The impact of JNC-VI guidelines on treatments recommendations in the US population. *Hypertension* 2002;**39**:897–902
15 Gava NS, V'eras-Silva AS, Negrão CE, Krieger EM. Low-intensity exercise training attenuates cardiac beta-adrenergic tone during exercise in spontaneously hypertensive rats. *Hypertension* 1995;**26**:1129–33
16 American college of sports medicine. Exercise and hypertension. *Med Sci Sports Exerc* 2004;**36**:533–53
17 Rao SP, Collins HL, Dicarlo SE. Post exercise alpha-adrenergic receptor hyporesponsiveness in hypertensive rats is due to nitric oxide. *Am J Physiol Regul Integr Comp Physiol* 2002;**282**:R960–8
18 Michelini LC, Morris M. Endogenous vasopressin modulates the cardiovascular responses to exercise. *Ann N Y Acad Sci* 1999; **897**:198–211

19 Marque V, Kieffer P, Atkinson J, Lartaud-Idjouadiene I. Elastic properties and composition of the aortic wall in old spontaneously hypertensive rats. *Hypertension* 1999;**34**:415–22

20 DBHA. VI Brazilian Guidelines on Hypertension (DBHA). Chapter 1.1: Conceptualization, epidemiology and primary prevention. *Rev Bras Hipertens* 2010;**17**:7–10

21 Carvalho MHC, Nigro D, Lemos VS, Tostes RCA, Fortes ZB. High blood pressure: the endothelium and its multiple functions. *Rev Bras Hipertens* 2001;**8**:76–88

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