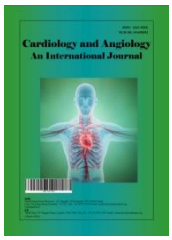

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Physical Training as a Blood Pressure Reducer and a Remodeler of Cardiac Fibers in Spontaneously Hypertensive Rats (SHR)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hypertension is the most prevalent of all cardiovascular diseases, reaching target organs such as the heart. Blood pressure control is critical for preventing organ damage induced by hypertension.
Objective: To analyze blood pressure, heart rate, left ventricular thickness, the percentage of cardiac fibrosis and the percentage of type III collagen in Spontaneously Hypertensive Rats (SHR) submitted to swimming physical training.
Methods: The experimental groups were composed of male Wistar Kyoto (WKY) rats (309-311g),

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which were divided into: 1) Normotensive Sedentary group (SN) (n = 6); 2) Trained Normotensive group (TN) (n = 6); 3) Sedentary Hypertensive group (SH) (n = 6); 4) Trained Hypertensive group (TH) (n = 6). After the end of the protocol, the animals were initially anesthetized to measure blood pressure.
Results: Physical training was responsible for decreasing blood pressure (F = 16,968; p <0.001) and heart rate (F = 10.710; p = 0.004) in the trained groups (normotensive and hypertensive). Moreover, training was responsible for providing an increase in the thickness of the left ventricle (F = 7,254; p = 0.014) and a reduction in the percentage of cardiac fibrosis (F = 16,081; p <0.001). Furthermore, it was observed that the trained group had lower values of type III collagen (F = 13,166; p = 0.002).
Conclusions: Physical swimming training triggered a decrease in blood pressure, heart rate, the percentage of fibrosis and the percentage of type III collagen. In addition, there was also a cardiac remodeling due to the increase in left ventricular hypertrophy.

Keywords: Hypertension; exercise; fibrosis; extracellular matrix; swimming.

1. INTRODUCTION

The practice of physical activity has been shown to be efficient with regard to the treatment and prevention of chronic diseases such as: Cardiovascular Diseases (CVD), diabetes, obesity, among others, which are mainly caused by the sedentary Lifestyle [1]. Cardiovascular Diseases are currently responsible for the highest number of deaths in the world. Among CVDs, Systemic Arterial Hypertension (SAH) is the most important risk factor for the development of cardiac and cerebrovascular complications, being considered an important public health problem worldwide [2].

Systemic Arterial Hypertension is a multifactorial chronic syndrome, caused by both congenital and acquired factors. This syndrome is involved in 50% of deaths from CVD. Blood Pressure (BP) control is necessary to prevent target organ damage induced by SAH [3]. The increase in blood pressure has adverse effects on the heart, with changes in the extracellular matrix, cardiac hypertrophy and cardiac remodeling. Its treatment involves pharmacological and non-pharmacological methods and, among non-pharmacological methods, is physical exercise [4-7].

Sedentary lifestyle is among the risk factors for the development of CVD, thus, sedentary individuals are exposed to an approximately 30% higher risk of developing SAH compared to physically active individuals [8,9]. Swimming is an aerobic exercise that has gained prominence in recent years, given that it has often been prescribed as an adjuvant non-pharmacological measure in cases of SAH. Furthermore, the swimming exercise is more efficient in terms of cardiac hypertrophy induction, due to the increase in volume overload and due to the increase in venous retronum, effects resulting from the aquatic environment. Currently, swimming exercise is prescribed as a physical training protocol involving experimental models [10].

The phase of ventricular hypertrophy is directly related to the accumulation of collagen fibers. Thus, progressive fibrosis is an adaptive response aimed at preserving the contractile capacity of the heart. Activation of fibroblasts appears to be the first step towards culminating in the formation of fibrosis. In the long term, necrosis of myocardiocytes can occur followed by proliferation of connective tissue that will produce matrix componentes extracellular, which is reparative fibrosis. As a result of increased blood pressure, there is a picture of reparative fibrosis in response to cell death. In this way, fibrosis is responsible for tissue hardening, causing a decrease in ventricular compliance. Cardiac fibrosis, which occurs to the detriment of greater fibroblast production, as well as due to ventricular hypertrophy, is directly related to heart dysfunction [4,7]. Fibroblasts are cells responsible mainly for the production and remodeling of the components of the extracellular matrix of the heart [5]. Most of the components of the extracellular matrix are made up of collagen types I and III, which provide structural integrity to the heart muscle and contribute to the proper functioning of the heart [11]. Thereby, the objective of the present study was to to analyze blood pressure, heart rate, left ventricular thickness, cardiac fibrosis percentage and type III collagen percentage in spontaneously hypertensive rats (SHR) submitted to swimming physical training.

2. METHODOLOGY

This work was approved by the Animal Ethics Committee of the Federal University of Triângulo Mineiro (protocol nº166) entitled "Study of cardiovascular autonomic function and renal morphology in spontaneously hypertensive rats submitted to physical training" and complied with the rules of the Brazilian College of Animal Experimentation, which are in line with international animal research procedures. All procedures performed on animals are in accordance with the Guide for the Care and Use of Laboratory Animals standards published by the US National Institutes of Health (1996).

Sampling was done in a simple random way. The samples were selected by lot and thus have the same probability of being chosen. In addition, the sample size was in accordance with the availability of the animals, so that the ethics committee of the proposing institution recommends using the minimum number of animals to obtain statistically valid results.

2.1 Care Protocol for Animals Submitted to Exercise / Training

24 male animals were studied; of these, 12 rats were SHR, matched for age (365.21 days) and average weight (309g), and 12 normotensive rats (Wistar Kyoto - WKY), all matched for age (364.19 days) and the average weight (311g). All animals were supplied by the vivarium of the Physiology Discipline of the Federal University of Triângulo Mineiro (Uberaba, MG, Brazil). They were fed standard laboratory ration and water ad libitum in a temperature-controlled room (22°C). The animals were kept under stable conditions in a vivarium, in 414mm x168mm plastic cages, in a special room, with constant air renewal, with a 12-hour / 12-hour light-dark cycle, lit at 7:00 am, water and standard food at will. During the experiment period, the animals were supervised daily to check for possible deaths. These animals were randomly divided into four groups: Sedentary Normotensive (SN, n = 6); Trained Normotensive (TN, n = 6); Sedentary Hypertensive (SH, n = 6); and Trained Hypertension (TH, n = 6). Physical training was carried out following the swimming protocol, that is, five days / week for nine weeks, with progressive training time each week of 10 minutes in the first week and 120 minutes in the last week of training. The entire procedure was performed in a tank with heated water (30 ± 1◦C) [12]. The exercise protocol was applied in the morning. Sedentary groups were placed in the same tank for a period of one minute, the purpose of which was to observe the effect of physical training and not for a possible change due to aquatic stress (Fig. 1). Before training, the rats were initially weighed on a scale (Filizola). This training was carried out in a tank measuring 100 cm x 50 cm x 60 cm, containing water heated to 30ºC ± 1ºC to a depth of 40 cm, sufficient to prevent the animals from touching the tail on the bottom of the tank.

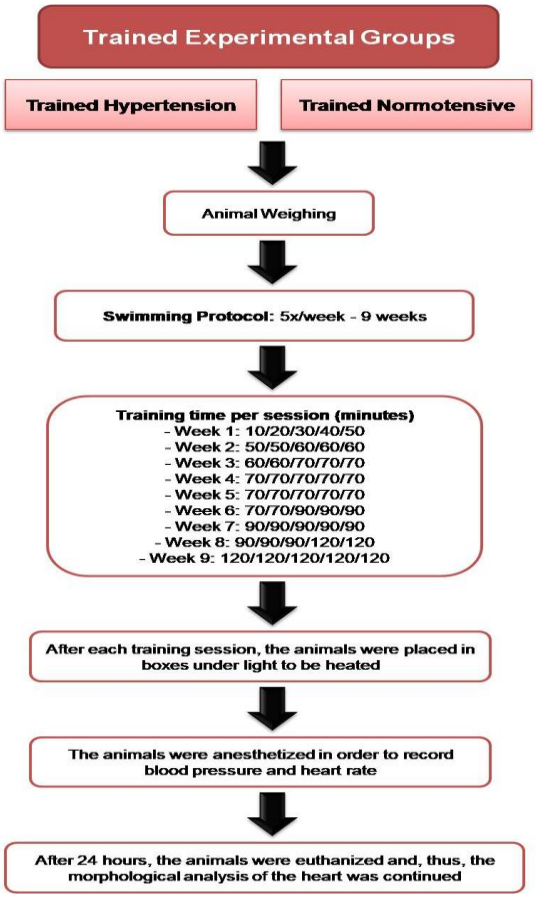

Fig. 1. Flowchart of the physical training protocol for trained hypertensive and trained normotensive groups

2.2 Heart Rate and Blood Pressure Records

The SHR start the development of SAH at approximately four weeks of age, presenting tension levels considered hypertensive between the seventh and fifteenth weeks, reaching a plateau between the twenty and the twenty-eighth week. From this age onwards, hypertension stabilizes up to approximately one to 1.5 years of age, when animals begin to

develop congestive heart failure, which is associated with a high mortality rate. Twenty-four hours after the end of the swimming protocol, the animals were initially anesthetized with sodium thiopental (40 mg/kg, i.p.) and then the left femoral artery was cannulated for direct BP recording. Subsequently, the cannulas were externalized to the dorsal region of the rats and they were housed in individual boxes for 24 hours for post-operative recovery to occur. After 24 hours of surgical recovery, all animals were subjected to a baseline BP recording session for 30 minutes. During the experiment, the arterial cannula was connected to a PA transducer, the signal converted by an analogue-digital plate (with sampling frequency - 1000 Hz) and stored in a computer.

2.3 Morphological Study of the Heart

At the end of the registration protocols, all animals were weighed and anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and euthanized with a lethal dose of potassium chloride. Then, the organs were previously placed on aluminum sheets and weighed in a semi-analytical balance (Gehaka). To process the material, fragments from the middle pole of the hearts were used, since the heart was divided into three parts (upper, middle and lower pole). The fragments were included in paraffin wax. The blocks were submitted to a microtomy for the preparation of three slides for each animal with three µm thick incisions and fixed in glass slides (Poli-L Lisina®), one with Picrosirius for quantification of interstitial fibrosis; one with reticulin to quantify type III collagen; and one with hematoxylin eosin for analysis of the left ventricle.

2.4 Left ventricular thickness

The quantification of the ventricle was done by capturing the 24 images of the cardiac incision (myocardium) with the aid of an HP scanjet G4050 scanner, with a resolution of 300dpi and a dual core Pentium computer program. The captured images were visualized and recorded for later use with the image analysis program “Imagej”, these images were calibrated using a ruler graduated in centimeters (cm).

2.5 Quantification of cardiac fibrosis

For the morphometric evaluation of cardiac fibrosis, picrosirius (saturated aqueous solution of picric acid plus 0.1g% sirius red F3B) was used (Direct Red 80, Aldrich®). They were viewed under polarized light, with a 20x objective. The images were captured under a light microscope and analyzed by images from a KS300 analyzer system (KS 300 Carl Zeiss). The entire slide was analyzed. The percentage of fibrosis was recorded in Microsoft Excel and the average of the values was performed to obtain the percentage of existence of fibrosis in the fragment.

2.6 Quantification of type III Collagen

For the morphometric evaluation of type III collagen, reticulin color was used, which was visualized with a 20x objective. The images were captured by light microscopy and analyzed by an Automatic Image Analyzer System KS300 (KONTRON-ZEISS). The entire slide was analyzed. The percentage of type III collagen found in each one was recorded in Microsoft Excel and the values were averaged to obtain the percentage of type III collagen in the fragment.

2.7 Statistical Analysis

Statistics 6.0 (StatSoft, Inc. Scientific Software; Tulsa, OK) was used for statistical analysis. All data are expressed as mean ± SEM. The verification of the normal distribution of quantitative variables was performed using the Kolmogorov-Smirnov test. The effect of physical training (trained or sedentary) and blood pressure (hypertensive or normotensive) on the various parameters measured were assessed using variables that were independent of each other and at the same time, compared between the groups by the “Two Way” ANOVA factorial test, and in case of normal distribution, the Tukey test was used. In this type of distribution, the results were expressed as mean ± standard error (X ± Epm). When the sample distribution was not normal, we used the “Two Way” ANOVA on Ranks test.

To check the components separately (training or pressure), and to compare the results two by two, in cases of normal distribution and similar variances, the Student's t test (t) was used for comparison between two groups. In this type of distribution, the results were expressed as mean ± standard error (X ± Epm). When the distribution was not normal, or when it was normal, but with non-similar variances, the Mann-Whitney test (T) was used for comparison between two groups. In this type of distribution, the results were expressed in median and minimum and maximum values (Med - Min - Max). Differences

in which the probability ("p") is less than 0.05 (5%) were considered statistically significant.

3. RESULTS

In the TH group, there was a decrease in Mean Arterial Pressure (MAP) when compared to the SH group (F = 16,968; p < 0.001) (Table 1) (Fig. 2A). Regarding heart rate, it was lower in trained groups than in sedentary groups (F = 10,710; p = 0.004) (Table 2) (Fig. 2B). Furthermore, in the trained groups, a lower percentage of cardiac fibrosis was observed in relation to the other groups (F = 16,081; p < 0.001) (Table 3) (Fig. 2C).

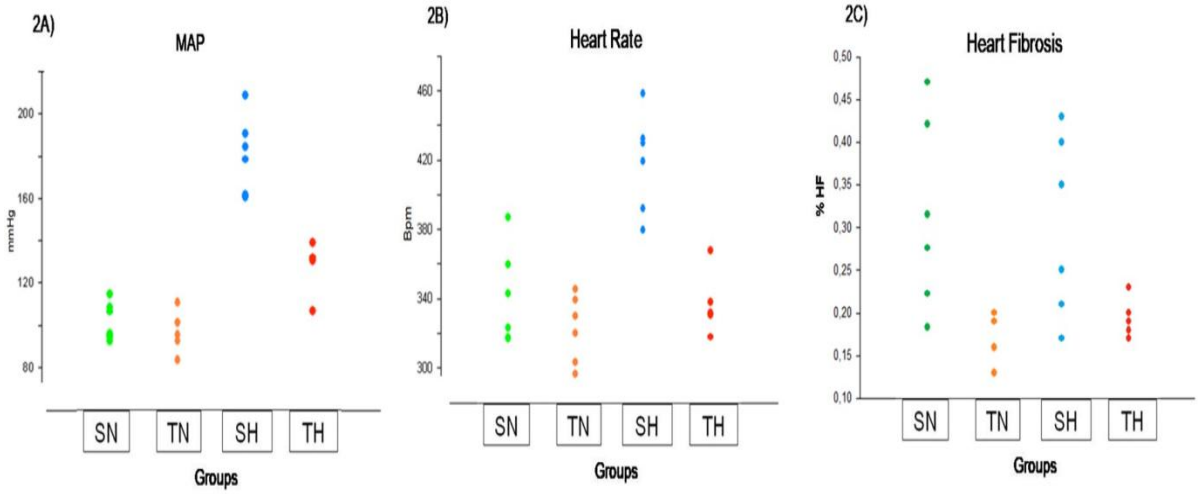

Fig. 2. Relationship of mean arterial pressure (2A), heart rate (2B) and percentage of cardiac fibrosis (2C) inrelation to the groups (SN: sedentary normotensive; TN:trained normotensive; SH: sedentary hypertensive; TH: hypertensive trained)

Table 1. Comparison of Mean Arterial Pressure (MAP) in relation to the association of analyzed components (Two Way Analysis of Variance)

Components	Mean Arterial Pressure (mmHg)	
	F	P
Pressure	109,648	0.001
Training	24.734	0.001
Pressure x Training	16.968	0.001

Table 2. Comparison of Heart Rate (HR) in relation to the association of analyzed components (Two Way Analysis of Variance)

Components	Heart Rate (bpm)	
	F	P
Pressure	21,796	< 0.001
Training	26,937	< 0.001
Pressure x Training	10,710	0.004

Table 3. Comparison of Cardiac Fibrosis (CF) in relation to the association of the analyzed components (Two Way Analysis of Variance)

Components	Heart Rate (bpm)	
	F	P
Pressure	0,114	0,739
Training	16,081	< 0.001
Pressure x Training	0,0555	0.816

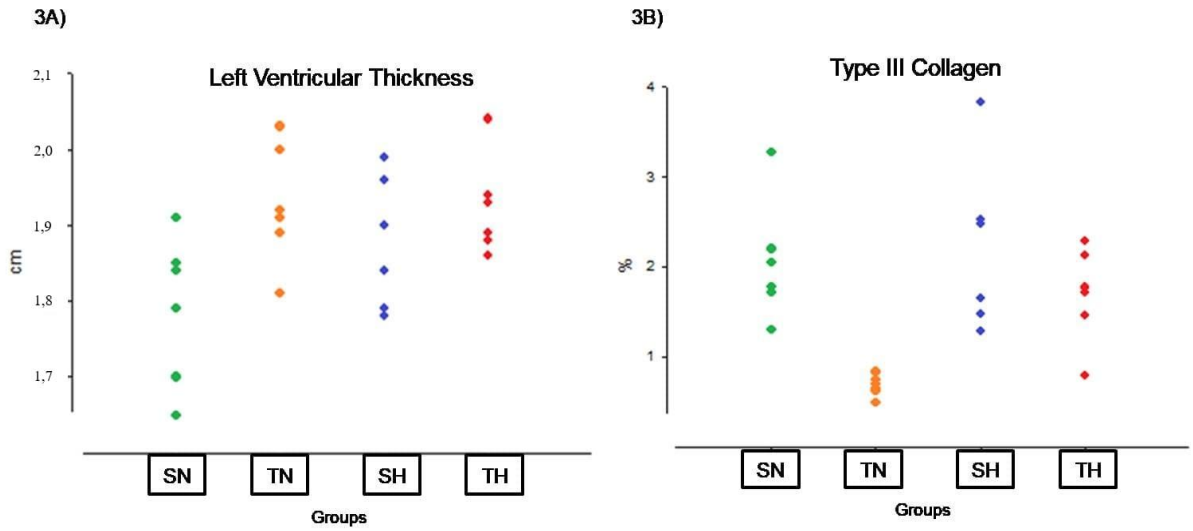

Fig. 3. Comparison of left ventricular thickness (3A) and percentage of type III collagen (3B) between groups (SN:sedentary normotensive; TN: trained normotensive; SH: sedentary hypertensive; TH: trained hypertension)

In addition, the trained groups showed greater thickness of the left ventricle compared to sedentary groups (F = 7,254; p = 0.014) (Table 4) (Fig. 3A). Furthermore, in the trained groups, a lower percentage of type III collagen was observed in relation to the other groups (F = 13.166; p = 0.002) (Table 5) (Fig. 3B).

Table 4. Comparison of the thickness of the left ventricle in relation to the association of the analyzed components (Two Way Analysis of Variance)

Components	Left ventricular thickness	
	F	P
Pressure	1,499	0,235
Training	7,254	0,014
Pressure x Training	1,748	0,201

Table 5. Comparison of type III collagen in relation to the association of analyzed components (Two Way Analysis of Variance)

Components	Cardiac Fibrosis (%)	
	F	P
Pressure	4,992	0,037
Training	13,166	0,002
Pressure x Training	2,693	0,116

Correlations of the relative weight of the heart with the development of cardiac fibrosis were not statistically significant in SN (rS = -0.613; p = 0.272), TN (rS = 0.340; p = 0.576), SH (rS = 0.560; p = 0.247), TH (rS = 0.611; p = 0.197). In addition, the correlations between the relative weight of the heart and the percentage of type III collagen also did not show any significant difference in SN (rS = -0.0908; p = 0.864), TN (rS = 0.162; p = 0.795), SH (rS = 0.206; p = 0.695), TH (rS = - 0.207; p = 0.694).

4. DISCUSSION

The most commonly used model of cardiovascular disease is the SHR, and the WKY is used as the normotensive control. SHRs are descendants of a Wistar male outbred with spontaneous hypertension from a colony in Kyoto, Japan, mating with a female with high blood pressure and then brother mating with sister, continued with the selection for spontaneous hypertension, defined as a

pressure systolic upper 150 mmHg that persists for more than a month. As of 1968, this pure strain of SHRs was developed in the United States. The various colonies of SHR are prehypertensive during the first 6-8 weeks of their lives with systolic blood pressure around 100-120 mmHg. And then, hypertension develops over the next 12-14 weeks [13].

SHR is the genetic model of experimental hypertension that presents pressure overload and gradually develops hypertension. Blood pressure inheritance is located on chromosomes 1, 10 and 18. When assessing the time course of hypertension in SHR animals, they found that BP increases progressively from 7 weeks of life to 16 weeks, when the plateau is reached, characterizing the sustained phase of hypertension [14].

According to the data obtained, mean arterial pressure was significantly lower in hypertensive rats that underwent the physical training protocol than in hypertensive rats that did not. This finding can be explained by the fact that physical training provides hemodynamic and autonomic adaptations that influence the cardiovascular system, such as the redistribution of blood flow, which becomes elevated in the circulatory perfusion to the muscles in activity [15]. Thus, physical exercise leads to significant reductions in blood pressure [16]. The decrease in blood pressure occurs as a result of the reduction in cardiac output, which occurs as a result of the decrease in heart rate and the sympathetic tone in the heart, due to lesser sympathetic intensification and greater vacancy withdrawal. Furthermore, there is a reduction in plasma noradrenaline, which triggers a reduction in sympathetic nervous activity, inhibiting the release of noradrenaline in sympathetic nerve endings. Another factor that explains the decrease in blood pressure values is the mitigation of total and systemic peripheral vascular resistance as a result of increased vascular compliance [17].

In addition, it was possible to verify that the heart rate values decreased by performing physical training. The group that performed the physical activity protocol, compared to the other groups that did not, had decreasing heart rate values. These data confirm scientific reports that physical training, through swimming intervention for nine weeks, is responsible for triggering the decrease in heart rate values [10,18].

Moreover, our data demonstrated that physical training significantly influenced the increase in left ventricular thickness. Regarding cardiac hypertrophy, data from the literature demonstrate a left ventricle / body weight ratio of around 7% of left ventricular hypertrophy in adult SHR trained with swimming. Cardiac hypertrophy is triggered as a way of responding to aerobic physical training in swimming, which course with increased preload [17]. The groups that did the physical exercise protocol obtained an increase in the thickness of the left ventricle in relation to the other groups. Thus, it can be inferred that physical activity induces cardiac hypertrophy due to the increased preload imposed by the heart [19].

For more, the groups that performed the physical training protocol achieved lower values in terms of the percentage of cardiac fibrosis when compared to the groups that did not perform the protocol. Thus, it is noted the importance of aerobic training in the treatment of arterial hypertension. In addition, physical activity provides numerous benefits for cardiac tissue, among them, the reduction of fibrosis and the number of inflammatory cells, thus promoting cardiac remodeling. In arterial hypertension, there is an increase in the level of angiotensin II, this increase in angiotensin II leads to the formation of cardiac fibrosis due to the stimulation of collagen production. Thus, physical exercise provides: decrease in BP levels, by improving the baroreflex; decreased heart rate; and decreased cardiac output and BP [16,20]. The reduction in BP for physical training leads to a drop in levels of angiotensin II and, consequently, to a lesser stimulation of collagen synthesis [21]. In addition, it was possible to observe the presence of cardiac fibrosis in sedentary normotensive animals. The animals in the present study are around 365 days old, in order to be considered elderly, which justifies the presence of cardiac fibrosis in this group [22].

When the tissue structure, which is damaged by some lesions, collagen is necessary for the defect to repair, thus restoring the organ's structure [23]. There was a decrease in the percentage of type III collagen in trained rats compared to sedentary rats. Furthermore, there was no correlation between the relative weight of the heart with the percentage of cardiac fibrosis and the percentage of type III Collagen [24].

5. CONCLUSIONS

Thus, the regular practice of physical training improves the cardiovascular system, acting directly in the prevention and treatment of several diseases, among them, arterial hypertension, since physical training was responsible for attenuating the pressure levels of hypertensive animals trained in relation to sedentary hypertensive animals. In addition, it was possible to observe that the exercises contributed to the reduction of heart rate and the percentage of cardiac fibrosis, both in the group of normotensive animals and in the hypertensive group. Moreover, there was also a cardiac remodeling due to increased left ventricular hypertrophy in both trill groups, normotensive and hypertensive.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study protocol was reviewed and approved by the Institutional Ethics Review Board.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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