

AEROBIC EXERCISE TRAINING CORRECTS CAPILLARY RAREFACTION AND ALTERATIONS IN PROPORTIONS OF THE MUSCLE FIBERS TYPES IN SPONTANEOUSLY HYPERTENSIVE RATS



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ABSTRACT

Aerobic exercise training (ET) has been established as an important non-pharmacological treatment of hypertension, since it decreases blood pressure. Studies show that the skeletal muscle abnormalities in hypertension are directly associated with capillary rarefaction, higher percentage of fast-twitch fibers (type II) with glycolytic metabolism predominance and increased muscular fatigue. However, little is known about these parameters in hypertension induced by ET. We hypothesized that ET corrects capillary rarefaction, potentially contributing to the restoration of the proportion of muscle fiber types and metabolic properties. Twelve-week old Spontaneously Hypertensive Rats (SHR, n=14) and Wistar Kyoto rats (WKY, n=14) were randomly assigned into 4 groups: SHR, trained SHR (SHR-T), WKY and trained WKY (WKY-T). As expected, ten weeks of ET was effective in reducing blood pressure in SHR-T group. In addition, we analyzed the main markers of ET. Resting bradycardia, increase of exercise tolerance, peak oxygen uptake and citrate synthase enzyme activity in trained groups (WKY-T and SHR-T) showed that the aerobic condition was achieved. ET also corrected the skeletal muscle capillary rarefaction in SHR-T. In parallel, we observed reduction in percentage of type IIA and IIX fibers and simultaneous augmented percentage of type I fibers induced by ET in hypertension. These data suggest that ET prevented changes in soleus fiber type composition in SHR, since angiogenesis and oxidative enzyme activity increased are important adaptations of ET, acting in the maintenance of muscle oxidative metabolism and fiber profile.

Keywords: exercise training, hypertension, angiogenesis, muscle fiber type.

INTRODUCTION

Hypertension (HTN) is a multifactorial syndrome characterized by high and sustained levels of blood pressure (BP), considered one of the more relevant risks in the cardiovascular disease (CVD) etiology^{1,2}. Experimental and clinical studies show that dysfunction in the vasomotor tonus and alterations in the microvascular structure are the primary processes in the HTN pathogenesis³⁻⁶. Many studies have shown capillary rarefaction in the skeletal muscle of animals and hypertensive patients³⁻⁶, with increase in the percentage of fast twitch fibers, which present predominance of glycolytic metabolism^{8,12} and classified as type II fibers⁷⁻¹¹. The skeletal muscle presents high plasticity and suffers transition of fiber type due to the alterations in the isoforms of myosin of heavy chain (MHC) in many conditions, such as: disuse, growth, aging, electrical stimulus, exposure to microgravity, physical exercises and CVD^{8,11}.

Considering the alternatives and the higher treatment effectiveness for HTN, aerobic ET has been intensively investigated. Alterations in the life style, such as introduction of regular practice of aerobic physical exercise, have been effective as non-pharmacological measures in the HTN treatment, preventing and reducing high pressoric levels^{13,14}. In the last decades, epidemiological studies have shown the inverse existing relation between the physical fitness level and development of CVD¹⁵. Thus, physical inactivity is associated

with higher risk of development of HTN, where ET is considered a key-component in the prevention and treatment of HTN, contributing to improvement of other factors of cardiovascular risk¹³⁻¹⁵. Studies point effects of the aerobic ET on the microcirculation in spontaneously hypertensive rats (SHR), such as increase in capillary density and capillary: fiber ratio in the skeletal muscle promoting reversion of capillary rarefaction occurred in HTN. Moreover, aerobic exercise normalizes the peripheral vascular resistance to the skeletal musculature and the arteriole wall: lumen ratio¹⁶⁻¹⁸. The restoration of the microvascular network may be a determinant contribution for the effect of BP decrease through reduction of peripheral vascular resistance, which has been shown as responsible for primary HTN in adults^{3,4,16-18}.

Although the therapeutic effect of aerobic ET on BP in HTN has been shown to greatly impact on the recovery of the microvascular network, studies with non-pharmacological approach, such as the ET, concerning regulation of the skeletal muscle fiber types profile, have not been widely explored. Thus, the aim of this study is to verify: 1) the possible alteration in the proportion of skeletal muscle fiber types associated to microvascular damage in spontaneously hypertensive rats (SHR); and 2) the effect of ET on the correction of the capillary rarefaction and on the restoration of the proportion of muscle fiber types in HTN.

MATERIAL AND METHODS

Experimental animals

Twenty eight SHR with 12 weeks of age were used for the present study. Twenty eight male Wistar Kyoto (WKY) rats were used as control of the SHR. The animals came from the Central Animal Facility of the Biomedical Sciences Institute of the University of São Paulo (ICB-USP). The rats weighed between 240 and 270g at the beginning of the protocol.

The animals used in this study were kept in plastic cages in groups of three or four animals per cage and separated by experimental group. Room temperature of the animal facility was kept between 22 and 24°C, with controlled light in inverted 12-hour light/dark cycle. Water and food were administered ad libitum.

All procedures were performed according to the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation and this Project was approved by the Ethics in Research Committee of the Physical Education and Sports School of the University of São Paulo (EEFE-USP) (# 2007/35).

Animals identification

The animals were randomly divided in four groups with seven animals in each group, according to the experimental protocol:

- Wistar Kyoto rats (WKY);
- trained Wistar Kyoto rats (WKY-T);
- spontaneously hypertensive rats (SHR);
- trained spontaneously hypertensive rats (SHR-T).

Aerobic exercise training protocol

The swimming ET was performed according to protocol by Fernandes et al.¹⁹. The animals were trained during 10 weeks, 60 min-sessions, once a day, five times a week, with gradual work load increase (weight on the tail in body weight percentage) until reaching 4% of body weight. The protocol used was characterized as low to moderate intensity and long duration training, being effective in promoting cardiovascular adaptations and increasing the muscular oxidative capacity. The rats were identified and weekly weighed for correction of the training overload in relation to body weight increase.

Pre and post the ET period, the animals were submitted to hemodynamic analyses, exercise tolerance test and peak oxygen consumption. After 24 hours from the last training session, the animals were killed by anesthesia with an intraperitoneal injection of sodium pentobarbital (80mg/kg). The necessary samples were collected and stored for histological and biochemical analyses.

Evaluation of the hemodynamic responses

The BP was performed pre and post-ET by tail plethysmography (KENT SCIENTIFIC RTBP1001 system for rats and mice, Litchfield, USA), in the four animal groups. The animals were awake, at rest and were restricted from movement so that the measurements could be taken. In order to avoid measurement and analysis errors, the rats were submitted to a one week-familiarization period with the measurement technique.

The tail BP recording equipment consists of a rubber cuff adapted to the proximal region of the tail, which is connected to the plethysmographer to gradually inflate and deflate the cuff from 1 to 250/300mmHg. In a more distal region of the tail, a pneumatic wrist transducer is attached for detection of the wrist wave passage signals

of BP on the tail artery and recorded in the sign acquisition system. This indirect BP measurement method enables the BP and heart rate (HR) quantification during the entire period of the experimental protocol.

Graded treadmill exercise test

The animals of the four groups were individually placed on the treadmill for the evaluation of the maximal exertion protocol. Immediately after the animal was positioned, the exertion test was initiated. Initial velocity was of 6m/min (with no inclination), followed by velocity increase of 3m/min at every 3min until the maximal velocity sustained by the animals was reached. The criterion for determination of animal exhaustion and test interruption was the moment in which the rat could not run inside the metabolic box with the velocity increase on the treadmill.

This evaluation was pre and post-training period so that the animal's performance response between groups could be compared. Although the treadmill test is not specific to the ET performed in the present study, we used this test to help verify the efficiency of the ET as prediction of better capacity to perform exertion. The time (min), velocity (m/min) and distance run (m) for each rat were compared.

Evaluation of the peak oxygen consumption (VO₂ peak)

After the familiarization week to the metabolic box, the rats were submitted to a progressive test of maximal exertion on treadmill adapted from Brooks and White²⁰, with load increase of 3m/min at every 3min, until exhaustion, for peak VO₂. The peak VO₂ was measured by determination of the oxygen expired fraction (FeO₂) during the progressive exercise test until exhaustion. In this protocol the rats were placed in a metabolic box on the treadmill, which served as mixture chamber of the expired gases. This chamber is connected to a "T" shape tube for acquisition of the air samples (1,000ml/min) to be analyzed at FeO₂ in a gas analyzer. The other way of the "T" shape tube is used for air aspiration in continuous flow (2,500ml/min), regulated by aspiration pump. The front part of the metabolic box has a 2mm opening from the surface, which allows the entrance of the unidirectional room air aspirated by the aspiration pump. The air flow in the metabolic box is of 3,500ml/min.

The rat was placed in the metabolic box for a rest period of 30 minutes for recording of the basal status and subsequently the test was initiated with velocity of 3m/min. During each stage (3 min) of the performed exercise, the FeO₂ of the gas contained in the metabolic box air was analyzed. The expired fractions of the last 30 seconds of each stage were considered for determination of the peak VO₂ of each stage.

When exhaustion was reached, the rat was kept in the metabolic box for approximately 3 min and the expired fractions were recorded to verify the animal's recovery as well as the functioning of the analyzers.

The VO₂ was calculated through the following mathematic formula: $VO_2 = \text{air flow} \times (FiO_2 - FeO_2) / \text{body weight}$.

Where: $VO_2 = \text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, Air flow = 1,000ml/min (analyzer) + 2,500ml/min (aspiration pump) = 3,500ml/min, FiO_2 = inspired oxygen fraction (room air), FeO_2 = expired oxygen fraction (mixture box), body weight = kg.

Histochemical evaluation of the skeletal muscle

The soleus skeletal muscle was dissected and carefully extracted and mounted on mounting dough tissue tek-based (to maintain the tissue in the correct position pre-freezing) by the tendinous region.

Subsequently to the mounting on the tissue tek-based dough, the soleus was immersed in isopentane (crioprotector which avoids artefacts in the samples) and after that in liquid nitrogen for freezing, where they were kept until the cuts were performed. After the 10 μ m cuts performed in Cryostat Microm HM505E (Zeiss, Walldorf, Germany) were obtained, reactions adapted from Brooke and Kaiser²¹, which enabled the evaluation of the Myosin ATPase enzyme activity through solutions with different pHs (4.3 and 10.3) were performed with the goal to perform the fibers typing and capillaries marking.

Determination of the transversal section area and types of muscle fibers

The images were acquired with amplification of 200x in a 20x objective. The images acquisition was processed in a computer, connected to a video system through an image program (Image-Pro Plus; Media Cybernetics, Silver Spring, MD). 10 fields of each histological cut were analyzed in an attempt to evaluate the tissue as a whole. The transversal section area was calculated by each type of muscular fiber in μm^2 .

In order to identify the types of fiber by myosin ATPase in pH 10.3 (alkaline), the dark fibers were characterized as type IIA, the grey ones as type IIX and the white ones as type I. In the pH 4.3 (acid), the marking of the types of fiber is contrary to the alkaline one, being used for analysis confirmation in pH 10.3.

Analysis of the capillary: fiber ratio

The capillary ratio per fiber of the soleus muscle was evaluated through histochemical reaction for myosin ATPase in pH 10.3, as described by Sillau and Banchemo²². Basically, after the histological cuts in cryostat are acquired, the ATP present in the incubation medium of the histochemical reaction is hydrolyzed by the endothelial ATPase of the capillaries which is revealed by the sulfite deposition. Once visualized, the capillaries were quantified by the analysis of 10 random and not overlapped fields, with amplification of 200x, using a morphometric computer system (Leica Quantimet 500, Cambridge, UK). The calculation of the capillary ratio per fiber was performed with the total number of capillaries divided by the total number of fibers counted in the same field. Only vases with diameter smaller than 12 μ m were quantified.

Evaluation of the activity of the citrate synthase enzyme

In order to evaluate the activity of the citrate synthase enzyme, the soleus muscle was homogenized at 4°C in extraction buffer (pH 7.4) containing Tris-base (50mM) and EDTA (1mM). The samples were centrifuged at 3,000g during 15 minutes at 4°C and the supernatant was used for the enzymatic kinetics. The protein quantification in the homogenized was performed according to the Bradford method.

The enzyme activity was determined according to Alp et al.²³, from the quantification of the complex made between the coenzyme A and the 5,5'ditio-bis 2 nitrobenzoic acid (DTNB), added to the medium, forming a yellow complex. The assay buffer consisted of Tris-base (100mM), DTNB (0.4mM), acetyl-CoA (1.24mM) and Triton X-100 1% (v/v) to which the homogenized was added. The reaction was initiated by the addition of oxaloacetate (18.9mM) to the medium and the Reading was performed at 25°C during a 10-minute interval, in 412nm with the use of the Victor (Victor3 1420 Multilabel Counter/ PerkinElmer, MA, USA). The result of the enzymatic activity was expressed in $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$ values.

Statistical analysis

The data were analyzed through two-way ANOVA analysis of variance (ET and HTN as independent factors) to compare the values of the groups and Tukey test as post hoc (Statistica software, StatSoft, Inc., Tulsa, OK, USA). All results were presented in mean \pm standard error of mean (SEM) and a $p < 0.05$ of significance was adopted for all experiments.

RESULTS

Hemodynamic parameters: blood pressure and heart rate

The BP values expressed in millimeters of mercury (mmHg) and the HR expressed in beats per minute (bpm) pre and post-ET are summarized in table 1. In pre-ET, it can be observed that the SHR groups presented higher levels of SBP compared to WKY groups, indicating that the HTN was established. There were no HR alterations between groups.

Post-ET, it is observed that the swimming ET was able to reduce the SBP of the SHR-T group (162 \pm 4.4mmHg) compared with the SHR group (207 \pm 5.5mmHg), with none SBP alteration in the control animal, WKY and WKY-T groups. Moreover, rest bradycardia was observed in the trained animal groups, reducing hence the HR values of these groups when compared with the groups kept sedentary in the same experimental period (post-ET- WKY: 393 \pm 12; WKY-T: 322 \pm 14; SHR: 407 \pm 11; SHR-T: 338 \pm 8bpm).

Table 1. Hemodynamic parameters.

	SBP, mmHg	HR, bpm
Pre ET		
WKY	127 \pm 3.0	390 \pm 12.2
WKY-T	124 \pm 1.6	393 \pm 8.2
SHR	184 \pm 3.9*	409 \pm 7.7
SHR-T	184 \pm 2.9*	415 \pm 6.5
Post ET		
WKY	132 \pm 3.9	393 \pm 11.8
WKY-T	131 \pm 3.7	322 \pm 14.2 ^F
SHR	207 \pm 5.4* [§]	407 \pm 11.2
SHR-T	162 \pm 4.4* ^{†§}	338 \pm 7.8 ^F

Values expressed in mean \pm SEM. Results of systolic blood pressure (SBP) and heart rate (HR) were obtained Pre and Post ET period in Wistar Kyoto rats (WKY), WKY trained rats (WKY-T), spontaneously hypertensive rats (SHR) and trained SHR (SHR-T). * $P < 0.001$ vs WKY and WKY - T; [†] $P < 0.01$ vs SHR Post ET; [‡] $P < 0.01$ vs WKY and SHR Post ET; [§] $P < 0.05$ vs SHR and SHR-T pre ET. ET: exercise training, bpm: beats per minute.

Exercise tolerance test

The exercise tolerance test was one of the parameters evaluated for efficiency of ET. The results of the test performed before and after the 10 weeks of experimental protocol are presented in figure 1. Figures 1A, 1B and 1C evidenced that the velocity values (WKY: 30 \pm 1.5; WKY-T: 30.5 \pm 0.9; SHR: 31.8 \pm 0.7; SHR-T: 31.5 \pm 1 $\text{m}\cdot\text{min}^{-1}$), time (WKY: 27 \pm 0.5; WKY-T: 27 \pm 0.6; SHR: 28 \pm 0.6; SHR-T: 27 \pm 0.3min) and distance run (WKY: 475 \pm 17.5; WKY-T: 467 \pm 22; SHR: 508 \pm 28; SHR-T: 478 \pm 10m), respectively, were similar between groups pre-ET. However, the trained animals significantly increased velocity (WKY: 28.5 \pm 0.7; WKY-T: 37 \pm 1; SHR: 31 \pm 1.2; SHR-T: 39.5 \pm 0.9 $\text{m}\cdot\text{min}^{-1}$), time (WKY: 24 \pm 0.4; WKY-T: 31 \pm 0.9; SHR: 26 \pm 1; SHR-T: 35 \pm 0.6min) and distance run (WKY: 381 \pm 15; WKY-T: 601 \pm 3.5; SHR: 449 \pm 32; SHR-T: 739 \pm 36.8m) in the post-ET test.

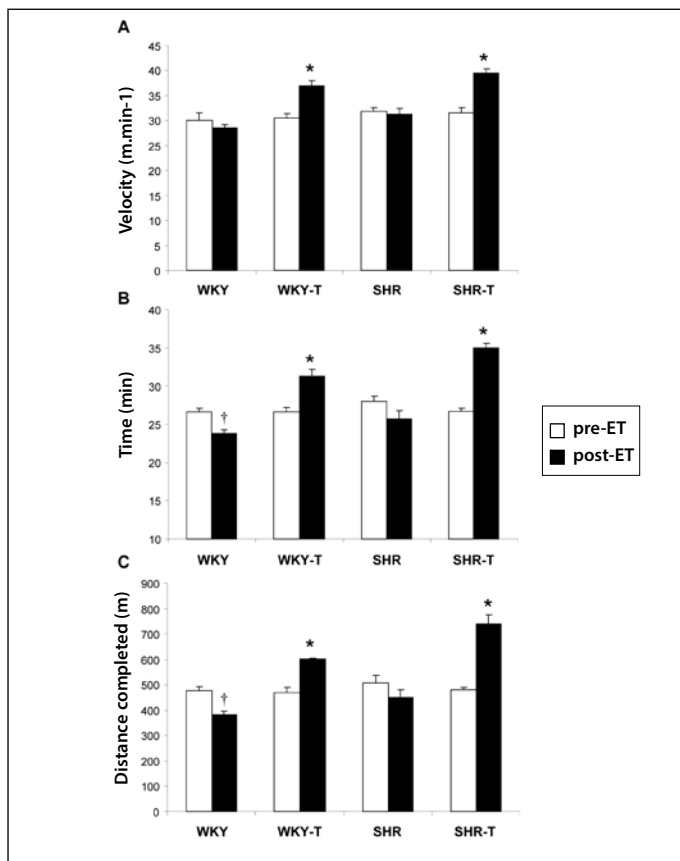


Figure 1. Exercise tolerance test. Velocity (A), time (B) and distance run (C) pre and post ET. The results are expressed as mean \pm SEM. * $P < 0.05$ compared to pre-ET and WKY and SHR post-ET; † $P < 0.05$ compared to pre-ET and WKY-T and SHR-T post-ET.

Measurement of the peak oxygen consumption

Figure 2 shows the VO_2 peak of the animals pre and post the experimental protocol. In the pre-ET period it is observed that all groups presented the same mean level of VO_2 peak (pre-ET- WKY: 69 ± 3.5 ; WKY-T: 69 ± 2.5 ; SHR: 72 ± 2 ; SHR-T: $73 \pm 36 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$); however, post-ET the efficiency of the training can be observed with the increase of VO_2 in the groups which trained (WKY-T and SHR-T) and decrease in the control groups (WKY and SHR) (post-ET- WKY: 58 ± 2.5 ; WKY-T: 78 ± 4 ; SHR: 61 ± 2 ; SHR-T: $84.5 \pm 2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Measurement of the activity of the citrate synthase enzyme

Figure 3 shows that there was an increase in activity of the citrate synthase enzyme in the soleus muscle of rats from the control and trained hypertensive groups compared with the sedentary control groups (WKY: 86 ± 12 , WKY-T: 120 ± 11 , SHR: 76 ± 9 and SHR-T: $144 \pm 15 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$).

Determination of the transversal section area, types of fiber and capillary: fiber ratio in skeletal muscle

Morphological analyses after the histological processing revealed important alterations in the skeletal muscle microcirculation induced by ET in normotensive and hypertensive rats. Figure 4 shows the results obtained through histochemical evaluation of the soleus skeletal muscle through myosin ATPase reaction.

As expected, capillary rarefaction was observed in the SHR group compared with the WKY group. Conversely, the ET was effective in increasing in 47% the number of capillaries through the analysis of the capillary ratio per fiber in the WKY-T group and corrected the

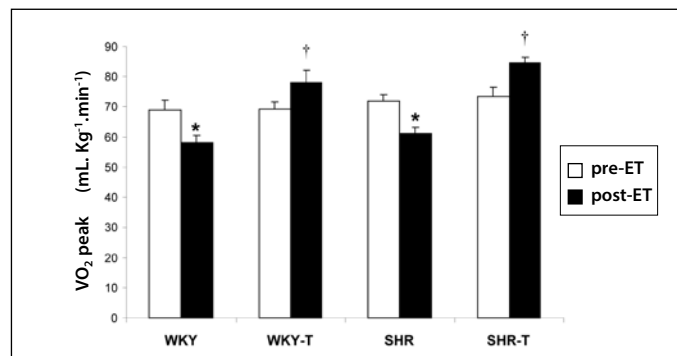


Figure 2. Pre and post-ET peak oxygen consumption (VO_2). The results are expressed as mean \pm SEM. * $P < 0.05$ compared pre-ET and WKY-T and SHR-T post-ET; † $P < 0.05$ compared to pre-ET and WKY and SHR post-ET.

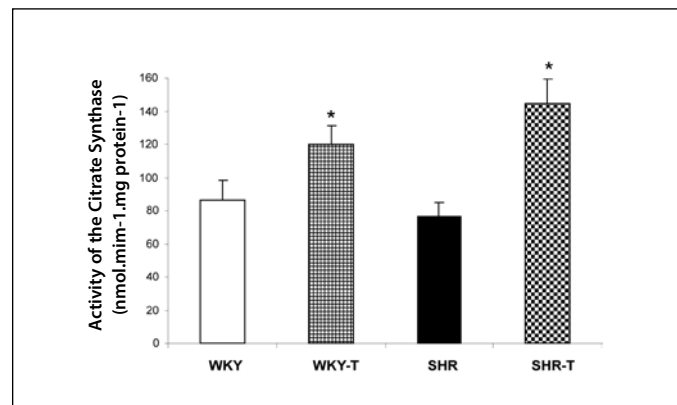


Figure 3. Activity of the citrate synthase enzyme in the soleus muscle represented in $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1}$ values. Results expressed as mean \pm SEM. * $P < 0.05$ compared with WKY and SHR.

capillary rarefaction in the SHR-T group when compared with WKY group (WKY: 1.2 ± 0.06 ; WKY-T: 1.8 ± 0.04 ; SHR: 0.7 ± 0.02 and SHR-T: 1.1 ± 0.04 # of capillaries/muscle fiber) (figure 4A).

Alteration in the transversal section area in the different types of fiber, such as type I (WKY: 2.987 ± 52 , WKY-T: 3.053 ± 152 , SHR: 2.884 ± 145 and SHR-T: $2.939 \pm 109 \mu\text{m}^2$), type IIA (WKY: 2171 ± 44 , WKY-T: 2.167 ± 20 , SHR: 1.982 ± 107 and SHR-T: $2.149 \pm 47 \mu\text{m}^2$) and type IIX (WKY: 1.846 ± 169 , WKY-T: 1.851 ± 65 , SHR: 1.770 ± 160 and SHR-T: $1.731 \pm 144 \mu\text{m}^2$) has not been observed in the soleus muscle of the four studied groups (figure 4B). However, the ET was effective in recovering the proportion in distribution of the types of fibers in the SHR-T group, reducing the percentage of type IIA fibers (type IIA – WKY: 4.8 ± 1.5 ; WKY-T: 2.7 ± 1 ; SHR: 18.5 ± 1.4 and SHR-T: $11 \pm 0.9\%$) and type IIX (type IIX – WKY: 1.1 ± 0.2 ; WKY-T: 0.88 ± 0.1 ; SHR: 3.9 ± 0.4 and SHR-T: $1.9 \pm 0.5\%$) over the increase in percentage of type I fiber (type I – WKY: 92.7 ± 1.5 ; WKY-T: 96.5 ± 1.1 ; SHR: 77.5 ± 1.8 and SHR-T: $87.2 \pm 1.3\%$), leveling with the control animal (figure 4C). These alterations can be observed in figure 4D by the images representing the histological cuts of the soleus muscle for each studied group, by the histochemical characterization of the myosin ATPase activity.

DISCUSSION

In the present study, the effect of the aerobic ET on the structural and metabolic alterations of the skeletal musculature associated to primary HTN was evaluated. The main results of the study show that aerobic ET on the HTN: 1) reduces SBP and induces rest bradycardia; 2) increase exercise tolerance; 3) increased VO_2 peak; 4) increased the citrate synthase enzyme activity; and 5) corrected the

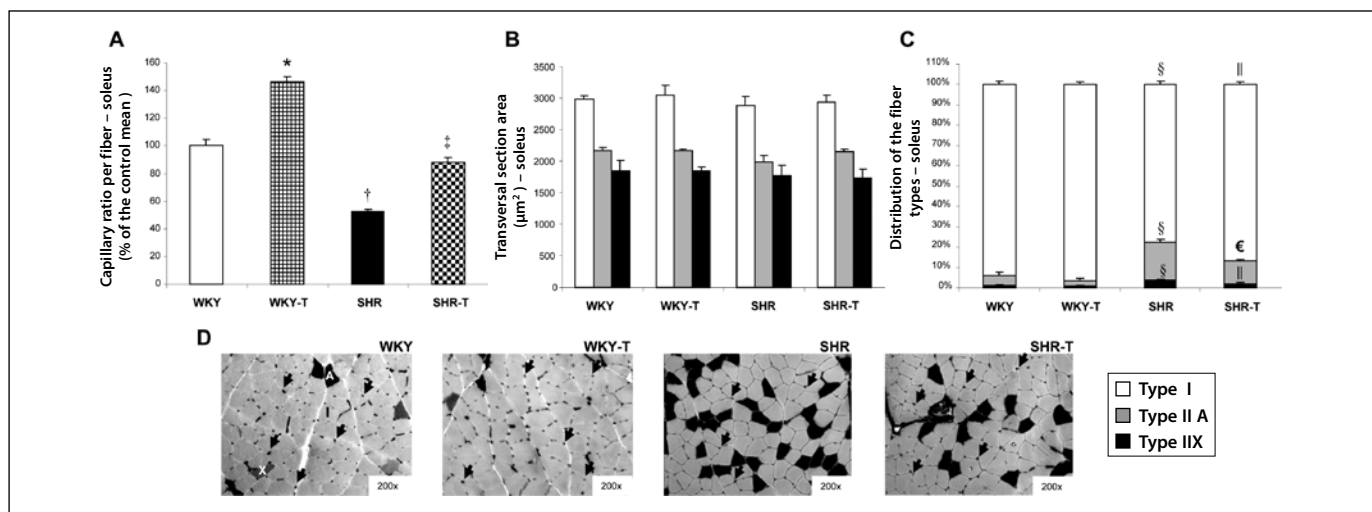


Figure 4. Histochemical characterization of the soleus by the myosin ATPase reaction. Capillary: fiber ratio (A), transversal section area of the type I, IIA and IIX muscular fibers (B), fiber types distribution (D) and images representative of the histological cuts of transversal sections of the muscle in the pH 10.3. The arrows in the images point to the capillaries and symbologies I, A and X represent the type I, IIA and IIX fibers, respectively. Data are presented in mean \pm SEM. * $P < 0.05$ vs. WKY, SHR and SHR-T; † $P < 0.05$ vs. WKY, WKY-T and SHR-T; ‡ $P < 0.05$ vs. WKY-T and SHR; § $P < 0.001$ vs. WKY, WKY-T and SHR-T; ¶ $P < 0.01$ vs. WKY, WKY-T, SHR.

capillary rarefaction recovering the proportion in the distribution of skeletal muscle fiber types.

In order to determine whether the used ET protocol was effective in producing aerobic adaptations, the main training physiological markers were measured. Improvement in aerobic work capacity represented by higher exercise tolerance and VO_2 peak, concomitant with increase of skeletal oxidative muscular activity and presence of rest bradycardia are the most legitimate skeletal and cardiac muscle adaptations of aerobic conditioning^{19,24}.

The first studies which suggested the preventive effect of aerobic ET in the high BP controle and treatment along with the first evidence of BP reduction in hypertensive individuals who regularly practiced physical exercise date from the 60's²⁵.

As expected, we observe that hypertensive groups presented high BP levels compared with the normotensive groups in the beginning of the experimental protocol. Nevertheless, at the end of 10 weeks of ET, the efficiency of low-intensity and long duration training in reducing the SBP of the SHR-T group compared with the SHR group was observed. These results agree with the ones found in the literature, confirming the efficiency of the aerobic ET in reducing BP both in genetically hypertensive animals and hypertensive humans^{16-18,26}.

Increase of peripheral vascular resistance, the one responsible for maintenance of the high pressoric levels in primary HTN, is a consequence of structural and functional alterations in the microcirculation, which regulate the blood flow and pressure³⁻⁶. Studies show that BP reduction induced by ET in SHR was correlated with normalization of both vascular wall: lumen ratio and more remarkable increase of the capillary: fiber ratio. in the skeletal muscle¹⁶⁻¹⁸. According to previous studies¹⁶⁻¹⁸, the results of the present study confirm that swimming ET normalizes capillary rarefaction in the skeletal muscle of SHR, which contributes to the reduction of the high total peripheral resistance promoting increase of parallel conductivity of the microcirculation, thus facilitating the passage of the blood flow due to the increase of the number of vessels of the skeletal musculature. Furthermore, the ET increases the capillary: fiber ratio in the skeletal muscle of trained normotensive rats as demonstrated in many studies¹⁶⁻¹⁸.

It is known that the angiogenesis represents a primary adaptive response of the skeletal muscle to aerobic ET, contributing hence to the improvement of muscular aerobic capacity (oxygen transportation, provision and extraction)²⁷. On the other hand, many conditions, such as CVD risk factors, lead to alteration in skeletal muscles capillary support and may, consequently, impair the offer of oxygen and nutrients, which is related to alteration in the distribution of the skeletal muscle fiber types towards the increase of type II fibers. The origin of the transition from type I fibers to type II in soleus muscle of SHR still remains little known; however, studies show it is related to capillary rarefaction followed by alterations in the metabolic properties^{11,28}.

Studies show that, when there is a transition between the types of fibers of the skeletal muscle, the different morphological properties of the muscular fiber are changed in the following manner: the capillary density and the activities of the enzymes of the energetic metabolism are early altered during the transition and precede the change in the activity of the myofibrillar ATPase and the contractile characteristics of the muscle^{8,29}.

In mammals, the fibers of the skeletal muscle are usually classified in type I and type II fiber according to the different activities of the myosin ATPase after the pre-incubation in different pHs, and the type II fibers can be subclassified in IIA, IIX/D and IIB. The type II fibers are characterized as being fast twitch with predominance of glycolytic metabolism, while the type I fibers are slow twitch ones with predominance of oxidative metabolism^{11,30}.

There is a lot of evidence in the literature shows that the skeletal muscle of hypertensive individuals, as well as of SHR, contains higher percentage of type II fast twitch, glycolytic fibers compared with their normotensive control⁷⁻¹¹. Interestingly, the results obtained in the composition of the fiber types of the soleus skeletal muscle, the muscle investigates in this study, which presents an average of 90% of type I fibers and 10% of type II fibers, performed both by histochemical myosin ATPase reaction and SDS-PAGE gel electrophoresis for detection of myosins of heavy chain (MHC) for each type of fiber, were positively correlated regardless of the technique applied¹⁰.

According to Bortolotto et al.¹⁰, the main result obtained in their study is that in all stages of hypertension (four, 16 and 24 weeks),

the soleus muscle of SHR presents higher proportion of type II fibers than the soleus muscle of WKY rats, as well as hybrid fibers, the ones which contain two types of MHC in the same isolate muscle fiber, in the case of SHR, greater proportion of IIA+IIX hybrid fibers. The presence of higher proportion of hybrid fibers is an indication of the transition of muscle fiber type in the muscle under consideration.

Similarly to the results exposed above, in the present study, significant alteration in the distribution of the fiber types was observed in the soleus muscle of SHR compared with its control WKY; that is, decrease of slow twitch and oxidative fibers, type I fibers, and simultaneous increase in the percentage of type IIA and IIX fibers parallel to reduction of capillary ratio per fiber of this musculature, as well as slight decrease (12%) of citrate synthase activity.

Recent studies have associated the ET effects with pharmacological treatment. Minami et al.³¹ showed the effects of ET associated or not to treatment with perindopril (angiotensin-converting enzyme inhibitor), on the capillarity and fiber types in the soleus muscle of SHR. The authors observed that chronic treatment with perindopril increases the exercise capacity in untrained animals; however, this effect was not synergic to the exercise capacity acquired as a result of ET alone. On the other hand, treatment with perindopril associated to ET promotes adaptive alterations in the soleus muscle, such as increase of capillary density and percentage of type I fibers³¹. Although no alteration in the composition of types of fiber was observed in the trained SHR and SHR treated with perindopril groups when compared with the sedentary SHR group, the authors observed higher capillariza-

tion in these groups, which may be attributed to improvement in the exercise capacity. A more recently study from the same group showed that pharmacological treatment with a calcium channel blocker (azelnidipine), or an antagonist of type I angiotensin receptor (olmesartan) or even the ET significantly increased capillary density and percentage of type I fibers in the soleus muscle of SHR³². Although the results in the literature are still controversial concerning the alterations in proportion of the types of fiber in response to ET, it was not possible either to observe the comparison between the profile of the types of fiber in the trained SHR group compared with its normotensive control WKY, with the aim to check normalization with the fiber type composition.

Notably, we presented for the first time evidence that aerobic ET corrected the alteration in the composition of fiber types in the soleus muscle of SHR when compared with WKY. This result is probably linked to the increased capillarization and citrate synthase activity observed with ET, since these adaptations are related to changes in fiber type in the skeletal muscle. Altogether, these ET-induced adaptations contribute to the increase of the oxygen consumption and exercise tolerance and decrease of the BP levels observed in the trained hypertensive group.

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