Exercise Physiology

Effect of high-intensity interval training on the skeletal muscle of spontaneously hypertensive rats

Paulo Henrique Aleixo[1], Robson Chacon Castoldi[1], Francilene Lima Agostinho De Souza[2], Thaoan Bruno Mariano[2], Guilherme Akio Tamura Ozaki[3], Thiago Alves Garcia[3], Lucas Silva Santos[4], Francis Lopes Pacagnelli[2], Regina Celi Trindade Camargo[1], José Carlos Silva Camargo Filho[1]

[1]Universidade Estadual Paulista, "Júlio de Mesquita Filho", Departamento de Fisioterapia, Presidente Prudente, SP, Brasil. [2]Universidade do Oeste Paulista, Departamento de Fisioterapia, Presidente Prudente, SP, Brazil. [3]Universidade Estadual de Campinas, Departamento de Ortopedia e Traumatologia, Campinas, SP, Brasil. [4]Universidade do Oeste Paulista, Departamento de Educação Física, Presidente Prudente, SP, Brasil.

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Abstract - Aim: This study aimed to evaluate the effect of High-Intensity Interval Training (HIIT) on the skeletal muscle of Spontaneously Hypertensive Rats (SHR). **Method:** In total, 20 male rats, SHR, 12 months old, were used, distributed into 2 groups: Control Group (C) and Training Group (HIIT). The training lasted approximately 50 minutes/-day, 5 days/week, for 8 weeks. Systolic blood pressure (BP) was measured at the beginning and end of the study. **Analysis:** The medial gastrocnemius muscle was used to measure the smallest fiber diameter, after which the Shapiro-Wilk normality test was performed, followed by the Mann Whitney test to compare the medians and interquartile intervals (IQI) of the muscle fibers and Student t-test for performance. For analysis of BP, Analysis of Variance - ANOVA was used, followed by Tukey's post-test. All procedures adopted a significance value of 5% ($p < 0.05$). **Results:** The median values for the variable "smallest diameter" of muscle fibers were 29.48 (IQI: 9.96) µm in the C group and 33.45 (IQI: 9.44) µm in the HIIT group ($p < 0.05$). Also, the performance was increased in the trained animal group and blood pressure values decreased significantly at the end of the experiment ($p < 0.05$). **Conclusion:** The HIIT intensity promoted an increase in the median values of the muscle fibers and performance. Finally, a significant decrease was observed in blood pressure variation values.

Keywords: high-intensity interval training, arterial hypertension, spontaneously hypertensive rats, skeletal muscle.

Introduction

Blood pressure (BP) is a worldwide problem and care related to its control and treatment has been discussed by various health agencies. Systolic arterial hypertension (SAH) is among the most relevant diseases in the population and is directly related to a high mortality rate[1,2].

In the period from 1990 - 2013, SAH was responsible for the second-highest number of deaths in the world, totaling 10.4 million cases[3]. Several factors contribute to the development of SAH. Among these, we can mention heredity, sex, ethnicity, age, diet, low physical fitness, and sedentary behavior[4].

This disease can be related to several factors, such as stiffening of the arteries, caused by the accumulation of atheromatous plaque on the walls, left ventricular hypertrophy, and altered heart rate[5,6]. Scientific evidence has consistently shown that physical activity is recommended as a way of preventing, treating, and controlling SAH[7].

Although conventional methods, such as drugs, are used to treat SAH, physical exercise acts as an adjuvant in the control of blood pressure, generating adaptations to the stress caused by the exercises and improving peripheral circulation[8,9].

Physical training (PT) could be a non-pharmacological therapy in the treatment and prevention of SAH since it generates a series of physiological adaptations in the cardiorespiratory system[10]. Among these adaptations, blood vessel dilation increased myocardial contraction force, and, consequently, ejection volume can be highlighted. In addition, in skeletal muscle, PT can result in a higher number of mitochondria, as well as increased contraction strength and protein synthesis[11].

Hypertrophied muscle increases its function with greater efficiency. In this case, the calf musculature has a fundamental role in the regulation of blood pressure, as it acts in the prevention of vascular diseases in the lower limbs (LL)[12,13].

In addition, the conjunction formed by the Gastrocnemius and Soleus muscles forms the Triceps Surae. The contraction of this musculature exerts pressure on the deep vessels of the cardiovascular system, pumping venous blood from the extremities of the lower limbs to the heart[14,15].

Among the different forms of exercise, the endurance modality is commonly prescribed for the treatment of SAH[1,9]. However, some studies have shown that high-intensity interval training (HIIT) can promote physiological alterations that are similar or better than those caused by low and medium-intensity exercise[16,17].

HIIT is a form of training with high intensity and short duration stimuli. These stimuli are interspersed with pauses that can be active or passive[18]. The concern regarding this method, however, is in relation to which populations can perform this modality since training should be prescribed with greater caution to people with heart disease or in an at-risk group, such as hypertensives and older adults. In this sense, physiological parameters should be established according to the needs of the individual[19].

As HIIT is an intense form of exercise, one option to test its use is in studies with animal models. Spontaneously hypertensive rats (SHR) genetically develop SAH from 4 to 20 weeks of age, due to several physiological factors of the species, such as increased BP and cardiac remodeling, factors that do not occur with Kyoto animals[20,21].

The advantage of studies with the use of animals is that they enable high control of variables and the possibility of using invasive methods as a form of analysis. In addition, the results obtained can be extrapolated to human beings, thus preserving the integrity of the human being[11,22].

When prescribing HIIT, several methods are adopted to establish safe parameters in relation to the control or decrease in BP. This study can corroborate with the literature, relating arterial hypertension with HIIT and its effects on the skeletal muscles of hypertensive patients. Thus, the current study aimed to evaluate the effects of HIIT on the skeletal muscle of spontaneously hypertensive rats (SHR).

Method

Sample

Twenty male rats, SHR, were used, 12 months of age, with a mean weight of 550 grams. The animals were subdivided into two groups: Control Group (C) [n = 10] and Training with HIIT Group (HIIT) [n = 10].

The animals remained in groups of 5 animals per box (polyethylene), with a controlled ambient temperature (22+2 °C) and brightness (12-hour light/dark cycle and inverted cycle), with free access to water and feed (laboratory rat feed). The study was developed according to the ethical standards and principles of animal experimentation after approval by the Ethics Committee on the use of animals (CEUA - 4115).

Stress and performance test

The animals were initially submitted to adaptation to training, consisting of running on a treadmill at a speed of 6 meters per minute (m/min.), lasting 5 minutes, for 5 days. After the adaptation period, the animals were submitted to the maximum speed test to assess the physical capacity of each animal.

The test started at a speed of 6m/min with increments of 3 m/min every 3 minutes until the exhaustion of each animal[23,24]. The animals were considered exhausted when they were unable to run even after stimulation. The speed obtained in the test determined the training prescription. This protocol was performed at the beginning of the study and after 4 weeks of training[24] and the performance was obtained through the distance determination.

HIIT Training

The training protocol was performed on 2 treadmills adapted for rodents (model TK 1, IMBRAMED), for approximately 50 minutes/day (40-60 min), 5 days/week, for 8 weeks. The training was performed in an inverted cycle, adapted from Haram et al.[25]and Moreira et al.[26], and took place from 14h to 14h50min.

Each training session consisted of three phases: warm-up, HIIT, and cool-down. The warm-up included 5 minutes at 60% of the exhaustion speed. The HIIT was started at 95% of the speed reached in the exhaustion test (21 m/min) for 4 minutes, interspersed with 65% of the exhaustion speed for 3 minutes. The training was repeated five times during the first and second weeks.

In the third and fourth weeks, the HIIT was performed at the same speed as the first week (95% of exhaustion speed), however, repeated 6 and 7 times per session, consecutively. After the end of the fourth week of training, a new stress test was performed to reevaluate the exhaustion speed and the training load was adjusted.

In the fifth and sixth weeks, the HIIT was carried out with an adapted protocol and the animals performed training at a speed of 23 m/min for 4 minutes, interspersed by 12 m/min for three minutes, repeated 7 times.

In the seventh week there was an increase in the speed of HIIT corresponding to 15% and in the eighth week to 18% of the maximum speed, performed for 4 minutes, interspersed by 65% of the maximum speed for three minutes, repeated 7 times[25,26]. The details of the HIIT and the experimental design are described below[27] (Figure 1).

Systolic blood pressure

The tail-cuff plethysmography method was used to obtain blood pressure control. The systolic blood pressure was verified using the tail-cuff (Narcobio system®, model709-0610, International Biomedical, Inc, USA), before and after the training period. One week before the beginning of the experiment the animals were adapted to the use of this device. The difference in blood pressure values was obtained through the mean BP and the delta value Δ: (final value−initial value).

Skeletal muscle

Forty-eight hours after the end of the experiment, the animals were euthanized with an overdose of anesthetics (ketamine hydrochloride and xylazine hydrochloride), administered intraperitoneally[28]. Next, the Medial Gastrocnemius muscle was extracted and immersed in 10% formaldehyde solution[18,28].

After this procedure, the skeletal muscle was processed, embedded in paraffin, and histological sections of 5 μm thickness were made. The slices were produced transversely, in the ventral portion of the muscle, in a rotating microtome, and stained with hematoxylin-eosin (HE)[22,28,29].

Optical microscopy

The slices were observed and photomicrographed using a Nikon® microscope, model 50i. An Infinity1 camera was used to analyze the images. The markings for determining the smallest diameter of the muscle fiber passing through the center were made using the software (AuxioVisionRel 4.8 - Carl Zeiss® and NIS-Elements D3.0 - SP7 - Nikon®)[30].

In total, 10 slices of 5 μm were produced and 120 muscle fibers were measured per animal, according to the protocol established by Dubowitz[30,31]. After obtention of the images of the muscle fibers, the smallest diameter was measured, as shown in Figure 2[30,31].

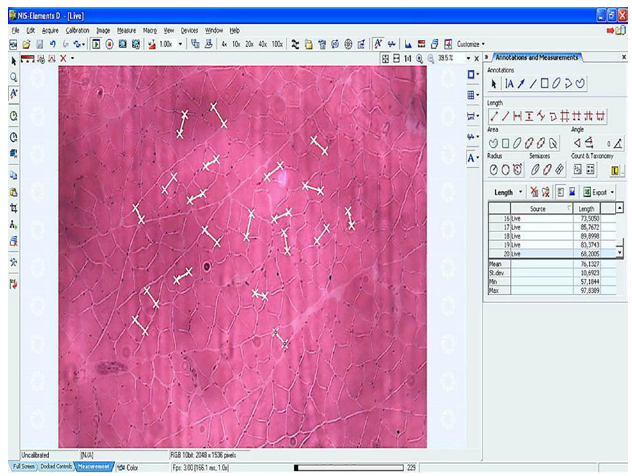

Figure 2 - NIS-Elements D3.0 - SP7 - Nikon® Software and measurement of the smallest fiber diameter.

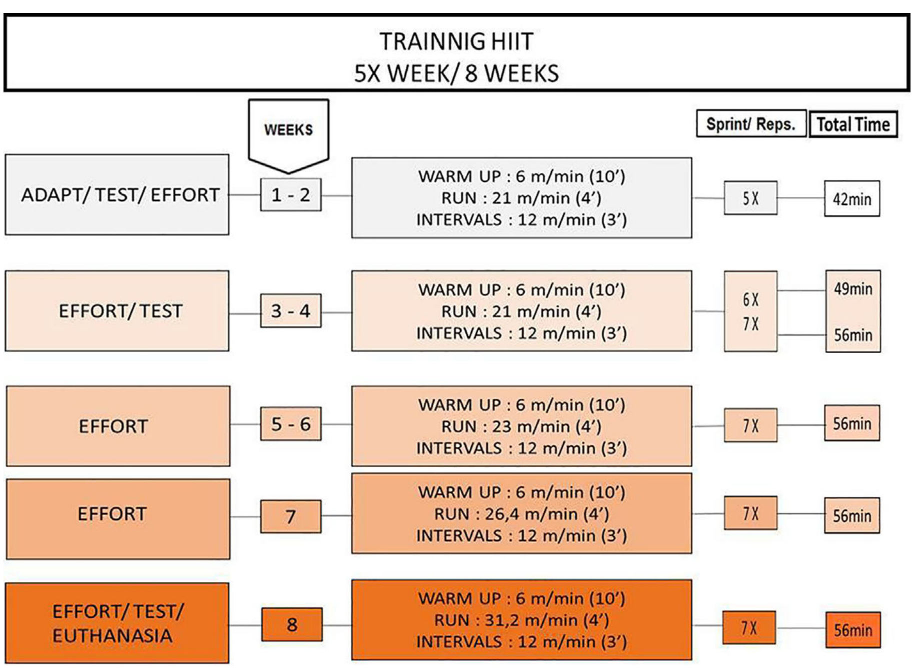

Figure 1 - Training Protocol used in the present study.

Statistical analysis

The Shapiro-Wilk test was performed to verify the normality of the data, followed by the Mann-Whitney test for the comparison between medians of muscle fibers and the Student t-test for performance. For analysis of BP, Analysis of Variance - ANOVA was used, followed by Tukey's post-test. All procedures adopted a significance value of 5% ($p < 0.05$).

Results

With hematoxylin-eosin (HE) staining, it was possible to analyze different morphologies, such as the muscle cells (dark arrows) and cell nuclei (light arrows) between the different groups of animals (Figure 3).

A significant increase was observed in the median values for the variable "smallest diameter" in the HIIT group, 33.45 (IQI: 9.44) µm, compared with the C group, 29.48 (IQI: 9.96) µm (Figure 4). Also, it was observed an increase in performance in the HIIT group ($p < 0.05$) (Figure 5).

Finally, blood pressure values presented a significant decrease between groups, when comparing the beginning and end of the experiment ($p < 0.05$) (Table 1).

Discussion

The findings of the current study showed that high-intensity interval training (HIIT) promoted a significant increase in the median values of the smallest diameter variable in the muscle fibers of the Medial Gastrocnemius of spontaneously hypertensive rats (SHR) ($p < 0.05$). In addition, a significant decrease in systolic blood pressure was observed ($p < 0.05$).

Adaptations in the calf muscles have important effects on venous return and, consequently, on blood pres-

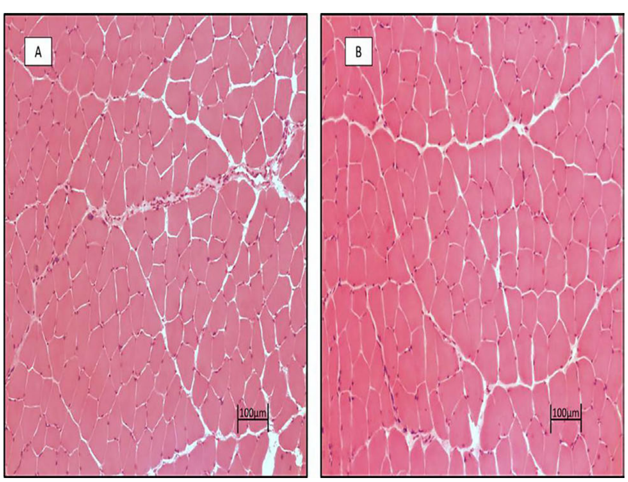

Figure 3 - Muscle fibers from bcontrol and HIIT training after eight weeks in rats. (A): Control Group; (B): HIIT training Group. Hematoxylin-eosin (HE) staining.

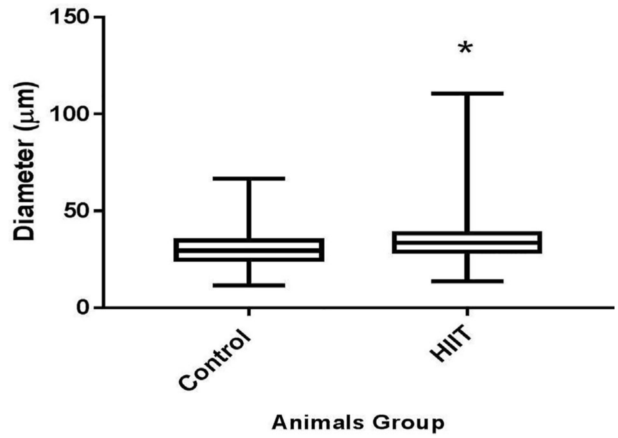

Figure 4 - Comparison between medians between control and HIIT training rats after eight weeks. (*) Statistical difference presented by the Mann Whitney test with a significance value of 5% ($p < 0.05$). Control Group (SHR rats, n = 10). HIIT training Group (SHR rats, n = 10). Values exposed in medians and interquartile intervals (IQI).

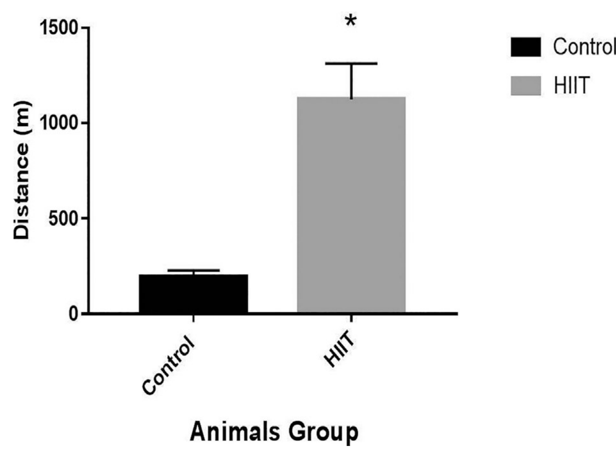

Figure 5 - Comparison of performance between control and HIIT training rats after eight weeks. (*) Statistical difference presented by the Student t-test with an error of 5% (p-value $p < 0.05$). Control Group (SHR rats, n = 10). HIIT training Group (SHR rats, n = 10).

Table 1 - Systolic blood pressure results.

Groups	Blood pressure	Before (mmHg)	After (mmHg)	*p*	*Δ*	*p*
Control	Mean	202.12	223.85		21.73	
	Standard Deviation	25.43	33.29	0.187		0.012
HIIT	Mean	210.80	200.35		−10.45	
	Standard Deviation	18.95	21.27			

Comparison between means in different groups of animals after eight weeks. Analysis of Variance test - ANOVA with Tukey post-test. HIIT: High-Intensity Interval Training. Variation in systolic blood pressure Δ: final value - initial value. Control Group (SHR rats, n = 10). HIIT training Group (SHR rats, n = 10).

sure[32,33]. These muscle gains are important concerning arterial hypertension, as muscle hypertrophy promotes greater vascularization in the tissues, due to the increase in the number of blood vessels (angiogenesis), a fact that allows the better exchange of gas and energy substrates such as fat[34]. This muscle is directly connected to the calf muscle pump which, when contracted, compresses the deep tibial veins and ejects blood back to the heart. This mechanism aids in venous return and, thus, in BP control[35].

This mechanism was observed by Lima et al.[36] in a case study that evaluated the hemodynamic pump musculature (Triceps Sural) of a patient diagnosed 21 years previously with chronic venous insufficiency and concluded that strengthening this region can improve the quality of life and autonomy of individuals with this disease.

It is worth mentioning that in the present study, muscle hypertrophy, an increase in performance, and a significant decrease in blood pressure were found among the groups of trained animals. This finding corroborates the study by Borges[37] who analyzed the systolic and diastolic blood pressure and heart rate of rats.

However, it should be noted that the Borges protocol[37] consisted of a single training session, and the findings demonstrated that the animals which performed an HIIT protocol presented reduced BP values when compared to the continuous training group. In this sense, as it takes less time to perform and results in similar benefits, the use of HIIT could be advantageous.

Ferreira[38] used an HIIT protocol in hypertensive animals and found that this training model demonstrated efficiency in reducing blood pressure. However, although the same training protocol was used, pressure values were compared with the addition of Wistar Kyoto animals, which differs from the present study[20,39].

In humans, several studies suggest that HIIT can improve blood pressure. In a review performed by Kessler[40] positive results were observed with regard to a reduction in blood pressure after 12 weeks of training.

Nemoto[41] analyzed interval training in walking and noted an increase of 17% in isometric knee extension and flexion. In addition, the peak aerobic capacity for cycling increased by 8% and aerobic capacity for walking by 9%. Furthermore, a decrease in blood pressure at rest was observed.

These findings direct attention to the fact that HIIT includes a high intensity of exercise, and is applied individually, which did not occur in the current study. Our findings may support the literature when investigating the effects of an HIIT protocol on skeletal muscle and blood pressure in SHR. However, some limitations should be highlighted, such as the ergometer (treadmill) used, and that the animals performed the training at the mean level of the stress test and, thus, some animals performed different percentages of effort. Future studies that consider other training methods, as well as different forms of ergometers, may collaborate with the present study.

Conclusion

It was observed that HIIT promoted an increase in median values for the variable of the smallest diameter in the muscle fibers of SHR animals. Also, the performance was increased in the trained animal group.

In addition, a significant reduction was observed in blood pressure between the initial and final moments in the HIIT group. These findings suggest that it is possible to use this form of training in the clinical environment for patients with SAH, both for increasing muscle mass and reducing BP.

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Corresponding author

Paulo Henrique Aleixo, Rua Roberto Simonsen 305,
Presidente Prudente, SP, Brasil.
E-mail: Aleixo99@hotmail.com.

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