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EFFECT OF AEROBIC AND ANAEROBIC TRAINING ON DIFFERENT ERGOMETERS IN RAT MUSCLE AND HEART TISSUES

EFEITO DOS TREINAMENTOS AERÓBIO E ANAERÓBIO EM DIFERENTES ERGÔMETROS NOS TECIDOS MUSCULAR E CARDÍACO DE RATOS

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ABSTRACT

Objective: Analyze the effects of aerobic and anaerobic training on different ergometers on muscle and cardiac hypertrophy in rats. Methods: The animals were separated into the following groups: Control (C), Aerobic Training in Water (ATW), Resistance Training in Water (RTW), Aerobic Training on Treadmill (ATT), and Resistance Training in Climbing (RTC). All training protocols were carried out for 4 weeks, 3 times/week. The cross-sectional area (CSA) of the gastrocnemius muscle cells and the areas of the cardiomyocytes were measured. Results: In the fast-twitch fibers, there was an increase in CSA in the RTW and RTC groups compared to the ATW ($p<0.01$ and $p<0.01$) and ATT groups ($p<0.01$ and $p<0.01$). In the slow-twitch fibers, the ATW and ATT groups demonstrated a lower CSA compared to the RTW ($p=0.03$ and $p<0.00$) and RTC groups ($p<0.01$ and $p<0.01$). In the cardiomyocytes, there was an increase in the area of the RTW and RTC groups compared to groups C ($p<0.01$; $p<0.01$), ATW ($p=0.02$; $p<0.01$), and ATT ($p<0.01$; $p<0.01$). Conclusion: The anaerobic training effectively promotes hypertrophy in the fast-twitch fibers and the cardiomyocytes. ***Level of Evidence V; Animal experimental study.***

Keywords: Resistance training. Myocytes, Cardiac. Muscle Fibers, Skeletal. Physical Endurance.

RESUMO

Objetivo: Analisar os efeitos dos treinamentos aeróbios e anaeróbios em diferentes ergômetros na hipertrofia muscular e cardíaca de ratos. Métodos: Os animais foram separados nos grupos controle (C), treinamento aeróbio em natação (ATW), treinamento resistido em meio aquático (RTW), treinamento aeróbio em esteira rolante (ATT) e treinamento resistido em escalada (RTC). Os protocolos de treinamento foram realizados por 4 semanas, 3 x/semana. Foram mensurados a área de secção transversa (CSA) das células do músculo gastrocnêmio e as áreas dos cardiomiócitos. Resultados: Nas fibras de contração rápida houve aumento da CSA dos grupos RTW e RTC em relação aos grupos ATW (p<0,01 e p<0,01) e ATT (p<0,01 e p<0,01). Nas fibras de contração lenta os grupos ATW e ATT demonstraram menor CSA comparado aos grupos RTW (p=0,03 e p<0,00) e RTC (p<0,01 e p<0,01). Nos cardiomiócitos houve aumento da área dos grupos RTW e RTC em comparação com os grupos C (p<0,01 e p<0,01), ATW (p=0,02 e p<0,01) e ATT (p<0,01 e p<0,01). Conclusão: Os treinamentos anaeróbios promoveram hipertrofia nas fibras de contração rápida e nos cardiomiócitos. ***Nível de Evidência V; Estudo experimental em animais*.**

Descritores: *Treinamento de Resistência. Miócitos Cardíacos. Fibras Musculares Esqueléticas. Resistência Física.*

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INTRODUCTION

Physical training is essential for maintaining quality of life, promoting improvement in the skeletal and cardiac muscular system, which results in better cardiovascular capacity and improvement in the individual's functional capacity, in addition to avoiding the harmful effects of sedentarism.[1–3] However, the adaptations generated are dependent on the training modality used.[3,4]

Training models can be grouped into aerobic and anaerobic training. The first group includes training modalities composed of exercises

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The study was conducted at the Laboratory of Biomaterials in Orthopedics (UNICAMP), in partnership with the Laboratory of Histology and Histochemistry of São Paulo State University "Júlio de Mesquita Filho" (FCT/UNESP), where all experimental procedures were performed.

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of low and moderate intensity and higher volume. The second group includes exercises with high intensity and lower volume.[3,5,6] Anaerobic training presents greater stimulus for muscle hypertrophy, since it can promote synthesis of contractile elements, which consequently increases the contraction force.[3,5,6] Aerobic training presents greater stimulus for resistance to fatigue, with greater oxidative capacity, an increased number of mitochondria, and improved blood perfusion.[6,7]

In cardiac muscle tissue, aerobic training is known to promote physiological hypertrophy and improve parameters such as ejection volume and reduced peripheral resistance. Anaerobic training is seen as a valuable tool to promote adaptations in the cardiovascular system, such as hypertrophy of cardiomyocytes and increased thickness of the left ventricle.[8,9]

Muscle hypertrophy occurs in both skeletal muscle and cardiac muscle, and is an important factor for improving contraction strength and improving functional capacity in both tissues.[1,2] Thus, evaluating the effects of different models of aerobic and anaerobic exercises is relevant so that health professionals can develop specific treatment programs to meet the therapeutic objective and improve physical capacities.

Therefore, the current research aimed to analyze the effects of aerobic and anaerobic training using different ergometers on skeletal and cardiac muscle hypertrophy.

MATERIAL AND METHODS

Animals

In total, 40 male Wistar rats, 150 days old, were used. The animals were kept in collective cages with five animals, under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 10%), and a 12-hour light/dark cycle (7-19h), with water and feed provided ad libitum.

The study was previously approved by the Ethics Committee for the use of animals– CEUA of the São Paulo State University (FCT/UNESP), São Paulo, Brazil (protocol number 03/2014).

Experimental Groups

The animals were divided into five groups according to the independent variables:

- Control (C, n=12): The animals remained in the cages and were euthanized in a paired way with the other experimental groups.
- Aerobic Training in Water (ATW, n=7): The animals were subjected to the critical load test to determine the training load, then underwent aerobic training in water for 30 minutes, three times a week, with an intensity corresponding to 70% of the anaerobic threshold and water temperature of 30° C (±1).
- Resistance Training in Water (RTW, n=7): The animals were submitted to the test of 10 Maximum Repetitions (10RM) to determine the training intensity, then performed jump training in water, three times a week, composed of 4 series of 10 jumps with overload corresponding to the intensity of 80% of 10 RM test and water temperature of 30° C (±1).
- Aerobic Training on Treadmill (ATT, n=7): The animals were submitted to the critical speed test on a treadmill, and then underwent training for 30 minutes, three times a week, with an intensity of 70% of the anaerobic threshold.
- Resistance Training in Climbing (RTC, n=7): The animals were subjected to a maximum workload test to determine the training intensity. Subsequently, the animals performed training consisting of 4 series of climbing, three times a week, with an intensity corresponding to 80% of 1RM.

One week before starting the tests, to determine the training load, the animals, except group C, performed adaptation to the ergometers. In the ATW group the animals performed 10-20 minute efforts, without load; in the ATT group the animals performed 10-20 minute efforts with a speed of 10-15 m/min; in the RTW group the animals performed 1x of 10 jumps with 50 % of body weight; and the RTC group performed 3 climbs with a load of 50% of the body weight. Adaptation is important for animals to become familiar with the exercise, without physiological adaptations to training.[4,10]

Critical Load Test to determine the Anaerobic Threshold (Lan)

The Critical Load (CL) test was performed to determine the anaerobic threshold of animals in the ATW group, and the Critical Speed (CS) test for animals in the ATT group. For the first, a tank with cylindrical tubes 25 cm in diameter was used, with water at a depth of 70 cm. For the CS test, a treadmill with individual lanes was used.

The tests were performed using 4 different intensities: 7, 9, 11, and 13% of body weight for the CL and 0.9, 1.2, 1.5, and 1.8 km/h for the CS. The intensities were randomized and one performed each day, with an interval of 24 hours between each session, in order to avoid interference from the previous session. Thus, the exercise time of each animal until fatigue was obtained for each intensity. Subsequently, the data were multiplied by the inverse of the time limit and plotted on a scatter plot. Finally, a trend line (linear) was added, from which the anaerobic threshold was defined.[7,10]

Maximum load test

Maximum strength tests were performed in the RTW and RTC groups, in order to define the work intensity of each animal. The animals in the RTC group were submitted to efforts on the stairs (1.1 x 0.18 m, 2 cm space between the steps, 80° inclination), with a load corresponding to 75% of the body weight of each animal, adding 30g to each successful climbing attempt. The test was stopped when the animal failed to climb after three attempts.[11]

In the RTW group, the same test model was performed, with the animals submitted to aquatic jumps in PVC tubes (30 cm in diameter and 50 cm in height, and 38 cm deep). The load was tied to the animal by means of a vest in the region of the torso. The same equipment was also used during training.[12]

The animals in the RTW group were subjected to the maximum load test, for which an initial load of 80% of the animals' body weight was used, with 10% of the body weight being added in each new series, until the animal was unable to perform the test. Failure to perform the test was determined when the animal could not complete 10 jumps (10 RM). The final completed intensity was assumed as an intensity of 10 RM. This test was adapted from the previously proposed climbing model.[11]

The training started 72 hours after the tests, using the same ergometer. The training was performed 3 times a week; in aerobic training the intensity used was 70% of the threshold, and the anaerobic training was performed at 80% of the maximum intensity.

Sample collection and preparation

Forty-eight hours after the final exercise session, the animals were euthanized by anesthetic overdose of ketamine hydrochloride and xylazine hydrochloride via the intraperitoneal route.[13] The right gastrocnemius muscles of the animals were collected.

Histological Processing of the Gastrocnemius Muscle

The muscle tissue was immersed in n-hexane solution and cooled in liquid nitrogen (-190) by the method of freezing unfixed tissues, and later stored in an ultra-low temperature freezer (-75° C). The 5 μm sections were produced in a cryostat microtome at -20° C, collected on slides, and then histochemical staining of nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) was used, which indicates the presence of oxidative activity, for analysis of type I and type II muscle fibers.[14,15]

Histological Processing of the Cardiac Muscle

Samples of cardiac muscle tissue were fixed in a 10% buffered formaldehyde solution for a period of 48 hours. After fixation, the tissue was embedded in paraffin blocks, before obtention of histological sections of 4 micrometers. These histological sections were stained on a slide with Hematoxylin-Eosin (HE) solution to measure sectional areas of cardiomyocytes.

Microscopic Analysis

The slides of skeletal and cardiac muscle tissue were photographed using a Nikon® microscope, model 50i, attached to an Infinity 1 camera. Analyses were performed from the images: in the skeletal muscle, the cross-sectional areas of 100 muscle fibers of each type (type I and type II) were analyzed; and in the heart, areas of 50 cardiomyocytes of the left ventricles were analyzed. All analyses were performed using NIS-Elements D3.0 2 software- SP7 - Nikon®.

Statistical analysis

The results were initially analyzed by the Shapiro-Wilk test to verify the Gaussian distribution of the data. The values of the cross-sectional morphometry of the muscle fibers and the area of the cardiomyocytes did not demonstrate normality, so we proceeded with the Kruskal-Wallis test and Dunn's post hoc, to assess the differences between the groups. The analyses were performed using IBM-SPSS v.22 software, with a significance level of 5%.

RESULTS

In the gastrocnemius muscle, statistical analysis showed an increase in the diameter of type II muscle fibers in the RTW group (117.59 μm $\pm$ 0.70) compared to groups C (109.85 μm $\pm$ 0.03; $p<0.01$), ATW (107.96 μm $\pm$ 0.79; $p<0.01$), and ATT (110.68 μm $\pm$ 0.64; $p<0.01$); the RTC group (119.75 μm $\pm$ 0.70) also presented greater muscle fibers in relation to the ATW ($p<0.01$) and ATT groups ($p<0.01$). In addition, the ATW group presented a significant decrease with group C (109.85 μm $\pm$ 0.77; $p = 0.03$). (Figure 1)

In type I fibers, the ATW group (69.99 μm $\pm$ 0.48) presented a smaller fiber diameter compared to the RTW (72.09 μm $\pm$ 0.49; $p = 0.03$) and RTC groups (72.71 μm $\pm$ 0.48; $p <0.01$); the ATT group (69.31 μm $\pm$ 0.40) presented a smaller fiber diameter compared to the RTW ($p <0.01$) and RTC groups ($p <0.01$). In addition, group C (78.39 μm $\pm$ 0.44) presented a larger diameter compared to the ATW ($p = 0.03$), ATT groups ($p <0.01$), RTW ($p <0.01$) and RTC ($p <0.01$). (Figure 1)

In the morphometry of the cardiomyocyte area, group C (486.68μm2 $\pm$ 8.54) presented a lower value compared to the RTW (550.95 $\pm$ 11.07; $p <0.01$) and RTC groups (591.97 $\pm$ 9.48; $p <0.01$). The RTW group showed a greater area of cardiomyocytes in relation to the ATW (498.12 $\pm$ 8.03; $p = 0.02$) and ATT groups (489.54 $\pm$ 10.30; $p <0.01$). In addition, the RTC group also presented greater area of cardiomyocytes compared to the ATW ($p <0.01$) and ATT groups ($p <0.01$). (Figure 2)

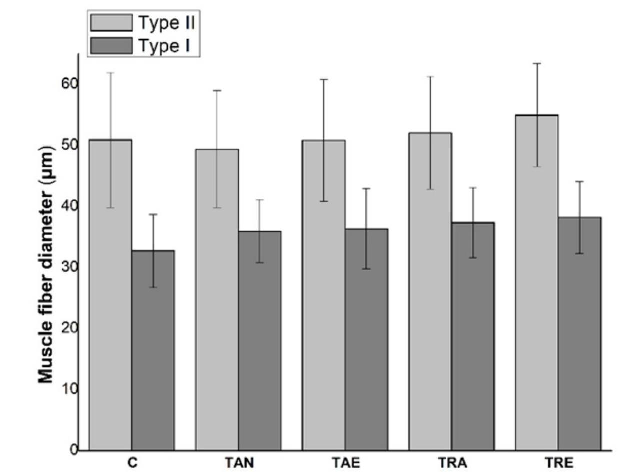
DISCUSSION

The results demonstrated that resistance training in both water jumping (RTW) and climbing (RTC), were more effective for gaining muscle volume in skeletal muscle in type II fibers. However, the cross section of type I muscle fibers was greater in group C. Regarding cardiomyocytes, only the RTW and RTC groups demonstrated hypertrophy.

During the anaerobic exercise, the animals performed vigorous muscle contraction. This type of exercise mainly uses fast-twitch muscle fibers (type II), and in the aerobic exercise they performed less vigorous contractions for a longer period, which preferentially uses slow-twitch muscle fibers (type I). As type I fibers preferentially use the oxidative pathway, and type II fibers preferentially use the glycolytic pathway, the adaptations resulting from exercise may be different in the fast-twitch and slow-twitch fibers.[5,16,17]

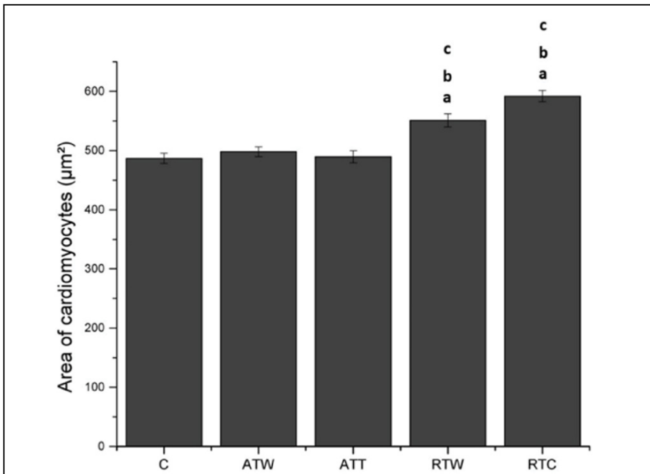
Several authors have demonstrated an increase in the cross-sectional area of muscle fibers from high-intensity, low-volume training.[5,7,18,19] The greater the intensity of the exercise, the greater the stimulus for hypertrophy, promoting greater stimulus to the protein synthesis of the contractile elements responsible for muscle contraction, generating an increase in the volume of muscle fiber.[5,6,17]

The muscle hypertrophy process is modulated by the mechanical stimulus to which the muscle tissue is submitted, so an exercise with greater overload will promote greater stimulus for muscle hypertrophy.[20] In addition, a study carried out with exercise on a stationary exercise bike[21] that the stimulus for the hypertrophy pathway, and increase in the glucose transporter (GLUT4), occurred with greater magnitude in type II muscle fibers, than in type I muscle



C= Control; ATW= Aerobic Training in Water; ATT= Aerobic Training on Treadmill; RTW= Resistance Training in Water; RTC= Resistance Training in Climbing. a Significant difference in relation to the C group; b Significant difference in relation to the ATW group; c Significant difference in relation to the ATT group.

Figure 1. Values of mean and standard error of the cross section of the fast-twitch fibers (type II) and slow-twitch fibers (type I) of the gastrocnemius muscle.



C= Control Group; ATW= Aerobic Training in Water; ATT= Aerobic Training on Treadmill; RTW= Resistance Training in Water; RTC= Resistance Training in Climbing. a Significant difference in relation to the C group; b Significant difference in relation to the ATW group; c Significant difference in relation to the ATT group.

Figure 2. Values of mean and standard error of the cardiomyocyte area.

fibers, a fact that could explain the hypertrophy observed only in type II fibers in the current study.

In the slow-twitch fibers there was no increase in the cross section of muscle fibers, so the effects of training seem to have occurred only in the fast-twitch fibers. This finding corroborates a previous study that also did not observe hypertrophy in slow-twitch muscle fibers after 4 weeks of training.[18] Aerobic exercise is best known for promoting improvement in oxidative capacity, increased blood perfusion, and the number of mitochondria, generating greater resistance to fatigue.[3,6]

A review study looked at the effectiveness of aerobic training for gaining muscle hypertrophy, and found that there is hypertrophy when the exercise is performed over the long term (9 to 12 weeks).[22] In the current study, only 4 weeks of training were performed, so the training time may have been decisive for the non-adaptation of the slow-twitch muscles to exercise. In addition, the strength gain also depends on neuromuscular adaptation, so the non-increase in the cross section does not mean that there were no adaptations in this tissue.[3,23]

Regarding the cardiac muscle, an increase in cardiomyocytes was observed in the RTW and RTC groups compared to the control group. Although aerobic exercise has the ability to promote cardiomyocyte hypertrophy,[24–26] some authors did not find hypertrophy of cardiac cells after training.[27,28] In addition, strength exercise also demonstrates effectiveness in increasing the ventricle wall and the contraction force of the heart.[8,29]

In the current study, only anaerobic training showed higher values of cardiomyocyte area when compared to aerobic training and group C. This type of training promotes an overload to the cardiovascular system, which through this stimulus remodels itself with alterations such as cellular hypertrophy and an increase in the left ventricular wall, promoting greater ventricular ejection capacity, less peripheral resistance to blood flow through the vessels, and greater efficiency in contraction.[30,31] Thus, anaerobic training may have induced a greater adaptation to exercise through the larger effort required during the execution of this type of exercise.

It should be noted that the current research was carried out with healthy animals; therefore, caution should be exercised when extrapolating these results to humans and to cardiac patients. Research with humans is necessary to verify the effectiveness of this training model and the safe parameters of exercise in these conditions.

The current study collaborates with the literature by investigating the effects of different forms of training on the striated cardiac and striated skeletal muscles of Wistar rats. However, as limitations of the present research we can mention the lack of a performance test at the end of the experimental period, which could demonstrate whether there were adaptations beyond hypertrophy, and the lack of measurement of the left ventricular wall.

CONCLUSION

It was concluded that anaerobic training was effective in promoting hypertrophy in the fast-twitch fibers and in the cardiomyocytes. Furthermore, there was no hypertrophy in the slow-twitch fibers in any of the training protocols. Thus, the adaptation proved to be sensitive to the exercise model, aerobic or anaerobic, and not to the ergometer model used.

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