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Adaptations of Muscle Tissue of Rats Submitted to Aerobic and Anaerobic Physical Training in Different Ergometer Models

Adaptaciones en Tejido Muscular de Ratas Sometidas a Entrenamiento Físico Aeróbico y Anaeróbico en Diferentes Modelos de Ergómetros

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SUMMARY: The objective of the present study was to analyze the effects of four different training models, two aerobic and two anaerobic models, in relation to muscular hypertrophy, by means of morphometric analysis of the muscle cells of the soleus muscle and the extensor digitorum longus muscle (EDL). The animals were divided into five groups, control (C), aerobic training in swimming (ATS), resistance training in water (RTW), aerobic training on a treadmill (ATT), and resistance training in climbing (RTC). The aerobic training was performed at 70 % of the anaerobic threshold for 30 minutes, while the RTW was composed of 3 series of 10 jumps, and the RTC 4 series of climbs, both at 80 % of the maximum load. All training protocols were performed for a total period of 4 weeks, 3 times per week. The diameters of the muscle cells were measured by means of histological slides of the EDL and soleus muscles. For the EDL muscle, there was no difference between the ATS and ATT aerobic training models ($p = 0.20$). However, the RTW presented greater hypertrophy when compared to the RTC ($p < 0.01$). Regarding the soleus muscle, the ATS was responsible for generating greater hypertrophy than the ATT ($p < 0.01$). In addition, the RTC was more efficient at producing hypertrophy than the RTW ($p < 0.01$). In this way, it was concluded that exercise adaptation was according to exercise type, aerobic or anaerobic, and not to the modality used.

KEY WORDS: Resistance training; Swimming; Hypertrophy; Physical endurance.

INTRODUCTION

Physical training generally induces anabolic alterations in body tissues. This effect results from the stress caused during its execution and shows variations according to the modality used (Manchado *et al*., 2006; Castoldi *et al*., 2013; Ozaki *et al*., 2014). Aerobic training presents a low intensity and high volume of work (exercise time), while anaerobic or resistance training presents a high intensity and low volume of work (Castoldi *et al*., 2013).

With regard to muscle tissue, physical training promotes alterations in the structures of muscle cells (sarcoplasm) and in their cellular metabolism (Hood *et al*., 2011). Due to its plasticity, the skeletal striated muscle may undergo modifications in its microscopical components, with increased cross-section of the muscle fiber, by virtue of the increase in contractile units (actin and myosin filaments) in parallel, resulting in an increase in muscle contraction force; this process is called hypertrophy. In addition, microscopic structures, such as nuclei and mitochondria, may undergo variations in size and number, promoted by increased metabolic demand (Yeo *et al*., 2008; Castoldi *et al*., 2013; Ozaki *et al*., 2016).

It is known that aerobic training increases the ramifications of peripheral vessels, and improves aerobic capacity, promoting greater resistance to metabolic fatigue. In contrast, strength training generates an increase in myofilaments of actin and myosin, which promote muscle contraction force (Jambassi Filho *et al*., 2010; Teixeira *et al*., 2011; Castoldi *et al*., 2013).

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However, there is a scarcity of information regarding the different physical training models and their effects. It is known that muscular adaptation is dependent on the ergometer model used (Manchado *et al*.; Castoldi *et al*., 2017a,b). Thus, better understanding of the effects of training in different models and forms of execution, could contribute with researches related to this theme.

Therefore, the objective of the present study was to analyze the effects of four different training models, two aerobic and two anaerobic models, on muscle hypertrophy, by means of morphometric analysis of the muscle cells of the soleus muscle and the extensor digiti I longus muscle (EDIL).

Considering the above, hypothesis H0 was that there would be no difference between the training models themselves, and hypothesis H1 was that the exercise models would promote different adaptations in muscle tissue.

MATERIAL AND METHOD

Animals. Forty Wistar rats were used, 150 days of age. The animals were housed in collective cages of five animals each, under controlled conditions of temperature (22 ± 2 ºC), humidity (50 ± 10 %), and a light/dark cycle of 12 h (7-19 h), with water and feed provided *ad libitum.*

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

Critical Load Test for determination of Anaerobic Threshold (Lan). The Critical Load (CL) test was used to determine the anaerobic threshold of the animals of the ATS group, and the Critical Speed (CS) test for the animals of the ATT group. For the former, a tank with cylindrical tubes 25 cm in diameter, with a water depth of 70 cm was used. For the CS test, a treadmill with individual bays was used.

The tests were carried out at 4 different intensities: 7, 9, 11, and 13 % of the 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 was performed each day, with a 24-hour interval between sessions, in order to avoid interference from the previous session. In this way, 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. Subsequently, a trend line (linear) was added which defined the anaerobic threshold (Castoldi *et al*., 2013).

Maximum load test. In the RTW and RTC groups, a maximum strength test was performed to define the work intensity of each animal. The animals of the RTC group were submitted to the stress on a stairway (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, with 30 g for each successful climbing attempt. The test was interrupted when the animal failed to climb after three attempts (Leite *et al*., 2013).

In the RTW group, the same test model was performed, and the animals were submitted to aquatic jumps in a PVC tube (30 cm in diameter, 50 cm in height, with a depth of 38 cm), with a load attached to the animal by means of a vest in the region of the torso. This equipment was also used during training (Castoldi *et al*., 2016).

The animals were submitted to a maximum load test, for which an initial load of 80 % of the animals' body weight was used, and 10 % of the body weight added each new series until the animal was unable to perform the test. Failure to perform the test was determined when the animal failed to complete 10 jumps (10 RM). In this way, the previous successfully performed intensity was assumed as the intensity of 10 RM. This test was adapted from the previously proposed climbing model (Leite *et al*.).

The training was started 72 h after the tests, using the same ergometer model. The training sessions were carried out 3 times a week. In the aerobic training, the intensity used was 70 % of the threshold, and anaerobic training was performed at 80 % of maximum intensity.

Sample collection and preparation. Forty-eight hours after the final exercise session, the animals were submitted to euthanasia through anesthetic overdose; ketamine hydrochloride and xylazine hydrochloride intraperitoneally (Garcia *et al*., 2017). The soleus muscles and the extensor digitorum longus muscle (EDL). were collected from the animals.

Histological Processing of Skeletal Striated Muscle. The muscle tissue was immersed in n-hexane solution and cooled in liquid nitrogen (-190 ºC), through the freezing method for unfixed tissues, and later stored in an ultra-low temperature freezer (-75 ºC) (Camargo Filho *et al*., 2011). The 5 mm sections were produced in a cryostat microtome at -20 °C, collected on slides and then stained with hematoxylin-eosin (HE) for immediate analysis of the minimum muscle cell diameter (Castoldi *et al*., 2017b).

Optical Microscopy. The sections submitted to HE staining were observed and photomicrographed under a Nikon® 50i model microscope, coupled to an Infinity 1 camera. Interactive markers for the determination of muscle fiber cross-sections (AST) were carried out using NIS-Elements D3.0 2- SP7 - Nikon® software. In total, 100 muscle fibers were measured on each slide (Castoldi *et al*. 2017b).

Statistical analysis. The results were initially analyzed by the Shapiro-Wilk test to verify the Gaussian distribution of the data. As the body weight variable did not present normal distribution, the Wilcoxon test was applied for intra-group evaluation of the differences between the pre and post training periods.

The values of muscle fiber morphometry (soleus and EDL) also did not present normality, so the Kruskal-Wallis test and Dunn's posttest were applied to evaluate the differences between the groups. The analyses were performed using the IBM-SPSS v.22 software, with a significance level of 5 %.

RESULTS

The analysis of the body weight of the animals (Fig. 1) showed a statistically significant difference between the pre and post periods only in the C (p=0.028) and ATS groups (p=0.028). Thus, the groups that underwent anaerobic training and the group that performed aerobic treadmill training did not present significant changes in weight during the experimental period.

The morphometry of the muscle fibers of the EDL muscle presented a significant increase in the trained groups (ATW, ATT, RTW, and RTC) in relation to group C (p>0.01). The ATW group presented significantly smaller fibers than the RTW and RTC groups (p>0.01). The ATT group also

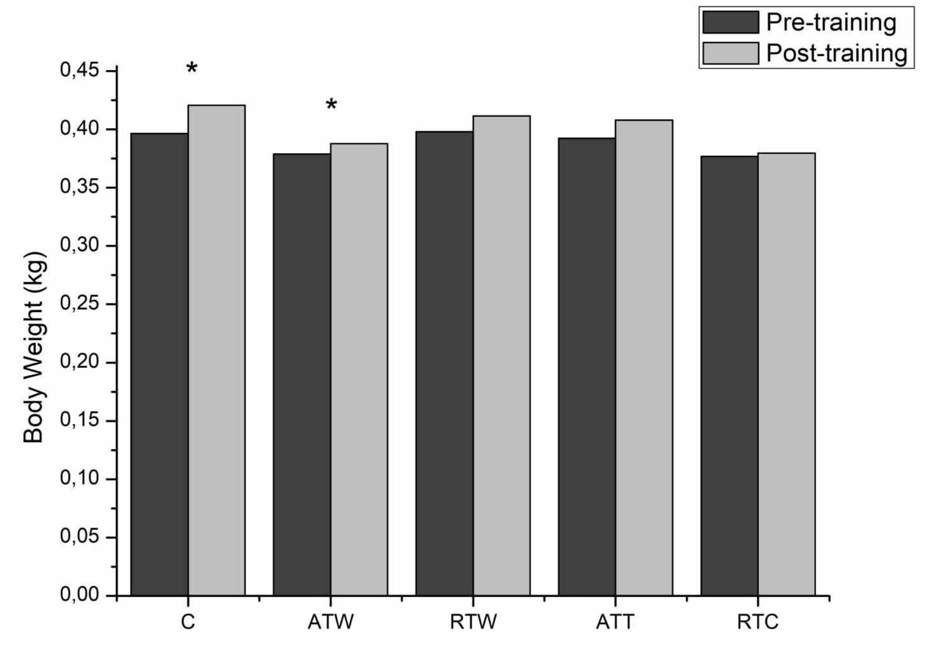

Fig. 1. * Statistically significant difference between the pre- and post-training periods.

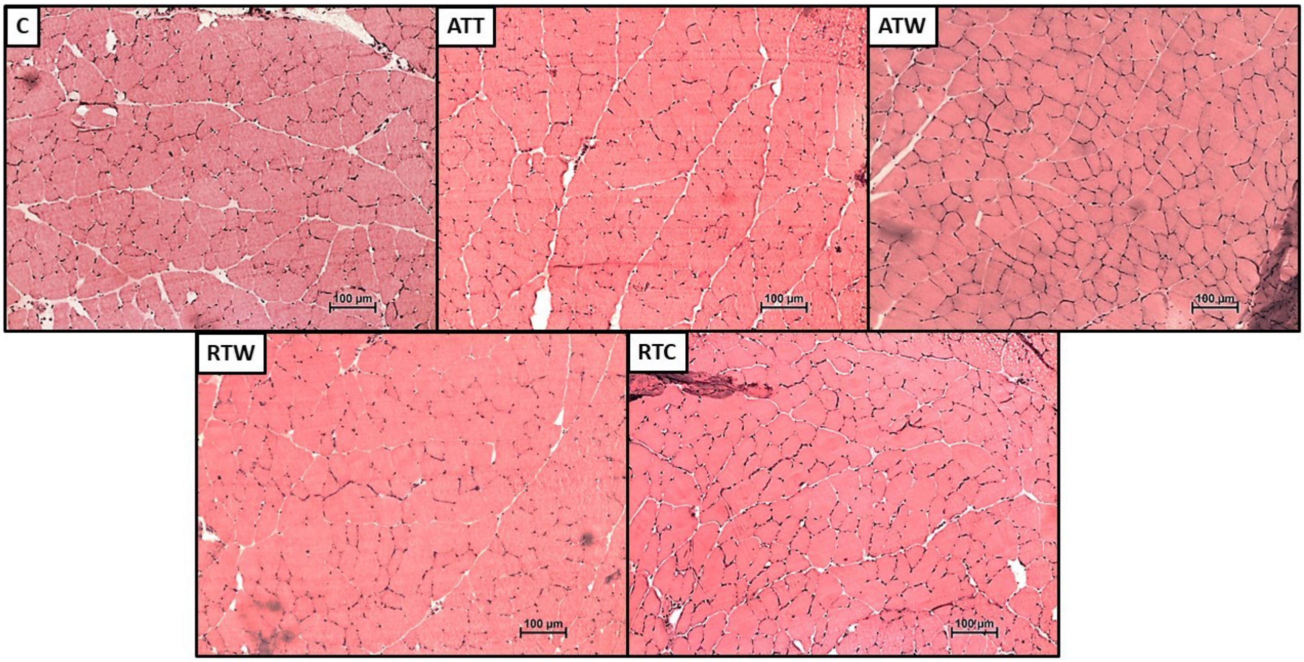

Fig. 2. Cross-sectional image of the EDL muscle, magnification 100x, 100 mm scale bar. C: Control, ATT: Aerobic Training on Treadmill, ATW: Aerobic Training in Swimming, RTW: Resistance Training in Water, RTC: Resistance Training in Climbing.

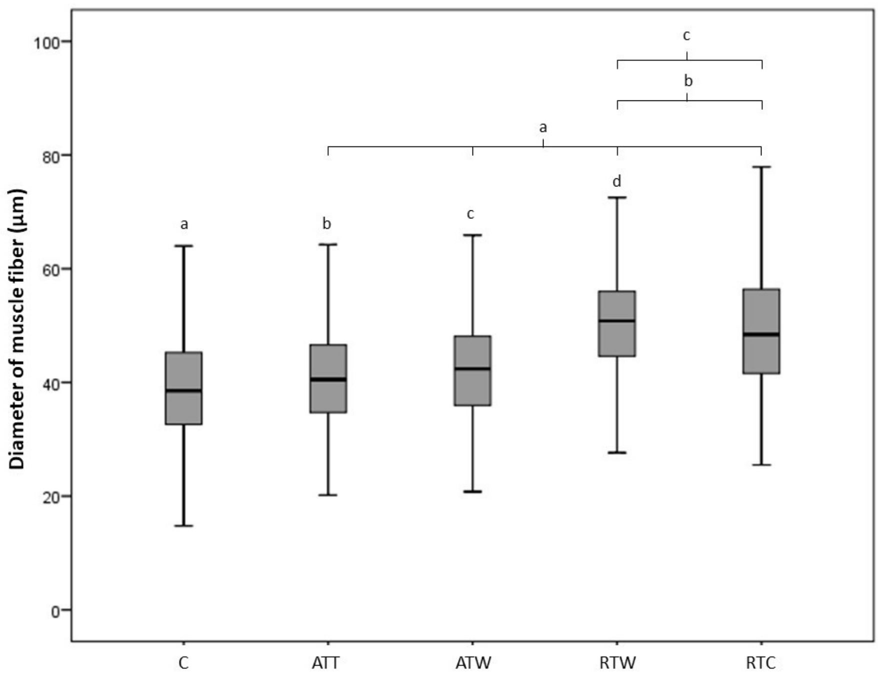

Fig. 3. Box plot with EDL muscle morphometry values. a Significantly different from ATS, ATT, RTW, and RTC. b Significantly different from RTW and RTC. c Significantly different from RTW and RTC. d Significantly different from RTC. C: Control, ATT: Aerobic Training on Treadmill, ATS: Aerobic Training in Swimming, RTW: Resistance Training in Water, RTC: Resistance Training in Climbing.

presented smaller fibers than the RTW and RTC groups (p>0.01). The RTW group demonstrated a significant increase in muscle fibers in relation to the RTC group (Figs. 2 and 3).

In relation to the soleus muscle, there was a significant increase in the fibers of all the trained groups (ATS, ATT, RTW, and RTC) in relation to group C (p>0.01). The ATS group presented a significant increase in relation to the ATT and RTW groups, and a significant reduction in relation to the RTC (p>0.01). The ATT group presented a significant reduction in relation to the ATS, RTW, and RTC groups (p>0.01), and the RTW group presented a significant reduction in relation to the ATS and RTC groups (p>0.01) (Figs. 4 and 5).

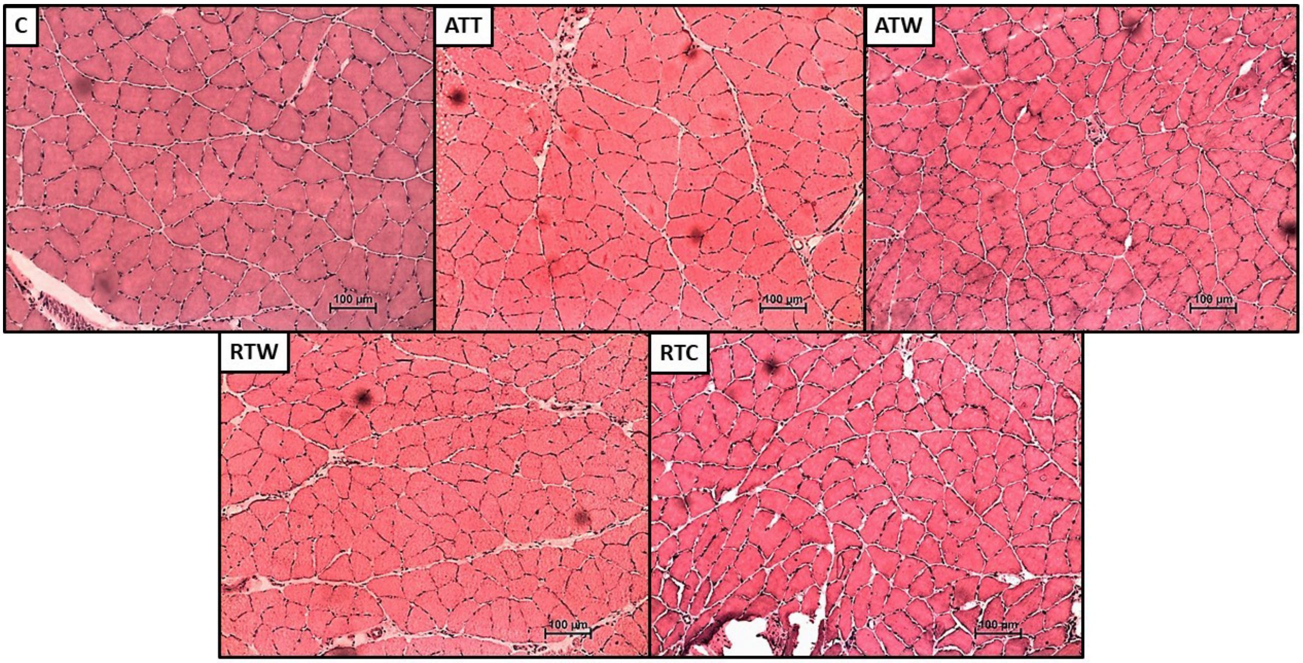

Fig. 4. Cross-sectional image of soleus muscle, 100x magnification, 100 mm scale bar. C: Control, ATT: Aerobic Training on Treadmill, ATS: Aerobic Training in Swimming, RTW: Resistive Training in Water, RTC: Resistance Training in Climbing.

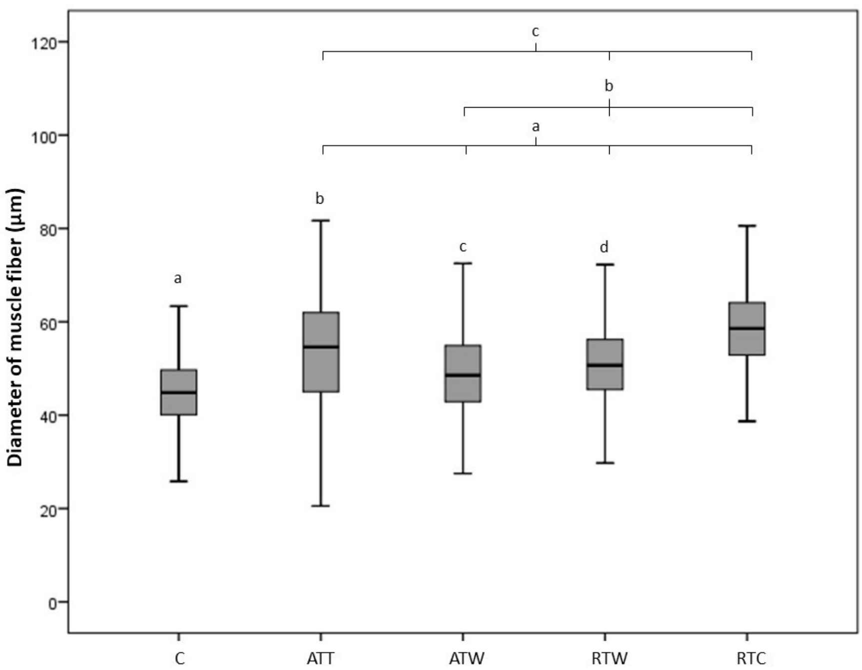

Fig. 5. Box plot with soleus muscle morphometry values. a Significantly different from ATS, ATT, RTW, and RTC. b Significantly different from ATT, RTW, and RTC. c Significantly different from ATS, RTW, and RTC. d Significantly different from ATS and RTC.

DISCUSSION

The results showed that physical training was effective in promoting muscle hypertrophy in all trained groups in relation to group C. In the EDL muscle there was no difference between the ATS and ATT aerobic training models. However, resistance training with water jumps demonstrated greater hypertrophy when compared to climbing training (RTC). Regarding the soleus muscle, aerobic training in the aquatic environment (ATS) was responsible for generating greater hypertrophy than treadmill training (ATT). In addition, climbing training (RTC) was more efficient for producing hypertrophy than water jumping (RTW).

In the present study, there were differences in hypertrophy between the aerobic and anaerobic training groups, with the groups submitted to resistance training presenting greater muscular hypertrophy. Both training modalities promote activation of the anabolic pathway in muscle tissue, however of different magnitudes (Ozaki *et al*., 2016), a fact that may explain the lower muscle hypertrophy in the aerobic training group.

Resistance training promotes micro-injuries in skeletal muscle, with alterations in the cytoskeleton, loss of sarcomeres and structural proteins, and necrosis of muscle fibers, among others (Damas *et al*., 2018). These changes may be caused by the mechanical stress induced by the training or activation of the calcium degradation pathway and the inflammatory response (Brook *et al*., 2016; Luciano *et al*., 2017).

Aerobic training presents the ability to increase cellular organelles, such as mitochondria, improving the energy supply to the muscle (Castoldi *et al*., 2017a). In addition, other studies (Klemp *et al*., 2016; Ozaki *et al*., 2016; Schoenfeld *et al*., 2016) have demonstrated an anabolic effect through metabolic fatigue, in this way, the hypertrophy pathway would be triggered by this fatigue, not being dependent on the intensity/load of the exercise (Fink *et al*., 2016).

Analyzing the differences between the resistance training groups, the RTW group presented greater hypertrophy than the RTC in the soleus muscle. However, in the EDL muscle the training model of the RTC group presented greater muscular hypertrophy. In addition, the animals of the RTW group demonstrated lower values when compared to the animals of the ATS group that performed aerobic training.

Regarding the aerobic training, differences between the ATS and ATT groups were not observed in the soleus muscle. However, in the EDL muscle, the ATS group demonstrated a higher cross-sectional value than the ATT, being even larger than the RTW.

This difference in results can be explained by the difference in the predominance of muscle fiber types: the soleus muscle has a predominance of oxidative fibers, whereas the EDL has a predominance of glycolytic fibers; moreover, there is the interference of the biomechanics of movement, for while the soleus muscle is an ankle extensor, the EDL is a toe extender. In this way, the muscles adapted differently to the training, although both demonstrated greater hypertrophy than the animals that performed the aerobic training (Castoldi *et al*. 2017b).

As limitations, analysis of protein-specific quantifications of the hypertrophy pathway, such as Insulin Like Growth Factor (IGF-1) and Mammalian Target of Rapamycin (mTOR), could elucidate the pathway of hypertrophy unleashed by the training.

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CONCLUSION

It was concluded that all the training models promoted muscle hypertrophy. In addition, in the soleus muscle, resistance training promoted greater hypertrophy than aerobic exercises, however, in the EDL muscle, the RTC and ATS presented greater muscular hypertrophy, in this way the adaptation to the exercise was according to the type of exercise, aerobic or anaerobic, and not the modality used.

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RESUMEN: El objetivo del estudio fue analizar los efectos de cuatro diferentes modelos de entrenamiento, dos aeróbicos y dos anaeróbicos en la hipertrofia muscular en ratas, a través del análisis morfométrico de las células musculares de los músculos sóleo y extensor largo de los dedos (ELD). Los animales fueran divididos en cinco grupos: control (C), entrenamiento aeróbico en natación (TAN), entrenamiento resistido en medio acuático (TRA), entrenamiento resistido en escalada (TRE) y entrenamiento aeróbico en estera rodante (TRE). Los entrenamientos aeróbicos fueron realizados a 70 % del umbral anaeróbico, durante 30 minutos, en cuanto los TRA fueron realizados por 3 series de 10 saltos y el TRE, 4 series de escaladas, ambos a la intensidad de 80 % de la carga máxima. Todos los protocolos de entrenamientos fueron realizados tres veces a la semana por un período de 4 semanas. Fueron demarcados los diámetros de las células musculares de los músculos ELD y sóleo por medio de láminas histológicas. En el músculo ELD no se pudo observar diferencia entre los modelos de entrenamiento aeróbico TAN y TAE (p=0,20). Además, el TRA demostró mayor hipertrofia comparado al TRE (p<0,01). Con relación al músculo sóleo, el TAN fue responsable de generar mayor hipertrofia respecto al TRA (p<0,01). De este modo es posible concluir que la adaptación depende del tipo de ejercicio, aeróbico o anaeróbico, y no en función de la modalidad utilizada.

PALABRAS CLAVE: Entrenamiento resistido; Natación; Hipertrofia; Resistencia física.

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