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Effects of Concurrent Training on Muscle Fibers of Wistar Rats Submitted to Standard and Hypercaloric Diets

Efecto del Entrenamiento Concurrente en las Fibras Musculares de Ratas Wistar Sometidas a Dietas Normal e Hipercalórica

Robson Chacon Castoldi[1,4]; Paulo Henrique Aleixo[1]; Alysson Christian Jesus Pereira[1]; Sergio Ruiz Ferreira[1]; Thiago Alves Garcia[2]; Guilherme Akio Tamura Ozaki[2]; Tatiana Emy Koike[1]; Danilo Antônio Corrêa Pinto Júnior[3];Patrícia Monteiro Seraphim[2]; William Dias Belangero[4]; José Carlos Silva Camargo Filho[2]

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SUMMARY: To analyze the effects of concurrent training (CT) on the muscle fibers of Wistar rats submitted to standard and hypercaloric diets. In total, 40 rats were used, divided into 4 groups: Sedentary Group (GS); Exercise Group (GE), Obese Sedentary Group (OS) and Obese Exercise Group (OE). The animals performed a CT protocol consisting of: muscle strength training and aerobic training, carried out 3 times a week for 45 days. The smallest diameter of muscle fibers (MDF) was analyzed to evaluate muscle hypertrophy. It was observed that the OE group presented a significant decrease in MDF, compared to the OS group (OE=77.41 µm vs. OS=98.58 µm). In addition, the animals that performed CT demonstrated muscle hypertrophy (GE=74.39 µm vs. GS=72.13 µm). In conclusion, the CT with a standard diet promoted an increase in MDF while CT with a hypercaloric diet resulted in a decrease.

KEY WORDS: Training; Muscles; Hypertrophy; Rats; Obesity.

INTRODUCTION

After the Industrial Revolution, which took place in England in the nineteenth century, there was a steady increase in the consumption of processed foods and in the following century, the emergence "fast food" networks. In addition, there are reports that foods in preserved and/or processed forms are not healthy, as they contain preservatives and high caloric and sodium values, and may contribute to an increase in overweight and obesity indices (Jarosz & Rychlik, 2008).

According to the World Health Organization (WHO, 2008), obesity affects about 600 million people worldwide and is defined as a disease in which excess accumulated fat may impair health. In the majority of cases, there is an energy imbalance, due to the energy intake being greater than the needs of the organism (Francischi *et al*., 2001).

It can be seen in the literature that obesity reduces life expectancy, as it is related to medical complications such as diabetes mellitus type 2, respiratory complications, and hypertension, among others (Lawrence & Kopelman, 2004). Obesity kills around 300,000 people each year in the USA, and nearly 100,000 in Brazil (WHO).

Although it is very difficult to identify the reasons for the great increase in obesity, the main causes are increased consumption of products with high energy values which are rich in saturated fats and sugars. Moreover, physical inactivity is considered an important cause of debility, decreased quality of life and premature death (Nahas, 2010).

Regular physical exercise is one of the principal ways to prevent obesity. Exercise can cause a negative energy balance due to increased energy expenditure, providing greater control of body mass (Pinto *et al*., 2011).

[1] Department of Physical Education. Universidade do Oeste Paulista – UNOESTE, Brazil.
[2] Department of Physical Therapy. Universidade Estadual Paulista "Júlio de Mesquita Filho" – FCT/UNESP, Brazil.
[3] Universidade de São Paulo. Instituto de Ciências Biomédicas, Departamento de Fisiologia e Biofísica, Brazil.
[4] Faculty of Medical Sciences. Universidade Estadual de Campinas – UNICAMP, Brazil.

According to Fleck & Kraemer (2006), strength exercises can result in changes in motor performance, muscular strength, body composition and, consequently, body aesthetics. Physical exercises are classified as aerobic or anaerobic, depending on their intensity and duration (Castoldi *et al*., 2013).

Within a training program, aerobic resistance exercise and strength exercise may be associated, denominated concurrent physical training (CT) (Castoldi *et al*., 2013). There have been many questions about the effectiveness with respect to the influence of one on the other (Häkkinen *et al*., 2003; Laursen, 2010).

However, one of the major controversies regarding CT is the gain or decrease in muscular strength. While the creator of CT points to a decrease in physical capacity (Hickson, 1980), current findings show that, depending on the way it is used, it can be an effective way to increase performance (Castoldi *et al*., 2013; Machado *et al*., 2014). Furthermore, the effects of high-calorie diets have not been explored in conjunction with this type of training.

Thus, the aim of this study was to analyze the effects of CT on muscle fibers of Wistar rats fed standard and hypercaloric diets.

MATERAL AND METHOD

In total, 40 male Wistar rats were randomly divided into four groups: Sedentary Group (GS); Exercise Group (GE); Obese Sedentary Group (OS) and Obese Exercise Group (OE), all animals were fed standard food and water ad libitum. In addition, the obese groups received a hypercaloric diet (cafeteria diet) which was composed of ham, bacon, sausage, filled biscuits and soda (Ribot *et al*., 2008), starting after weaning and lasting 60 days. At the end of this period, the animals were submitted to CT for 45 days. This study was approved by the CEUA of the University of Oeste Paulista – UNOESTE (Protocol 2742).

Strength Training. The strength training model proposed by Tamaki *et al*. (1992) was used, in which a vest adapted for this type of exercise was placed on the animal above a metal platform. Next, electrical stimulation was applied to the tail of the animal (10 V, duration 0.3 s, with 2 s interval) via the platform (Fig. 1).

Due to this stimulation, the animal performed a jumping movement, consisting of four sets of 10 repetitions, lifting the load coupled to the device, mimicking the "squat" movement performed by humans in a weight machine (Uchiyama *et al*., 2006).

Body weight was measured for prediction of training intensity, which was initially set at 50 % and gradually increased, for adaptation, ending the training with an average of 70 to 75 % of total body mass (Barauna *et al*., 2005). This procedure was performed three times a week on non-consecutive days, followed by the aerobic training protocol.

Aerobic training. The aerobic training was developed on a treadmill three times a week, performed shortly after the end of the strength training (jumps) described above. The initial time was 5 minutes during the first week, increasing progressively (5 min/session) up to 45 minutes in the final weeks. The training was performed at a speed of 9.75 meters per minute, over a period of 45 days.

Striated Skeletal Muscle. Seventy-two hours after the final training session, the animals were subjected to an overdose of ketamine and xylazine, 40 mg/kg body weight, via intraperitoneal injection, following the method proposed by Seraphim *et al*. (2001). Next, the muscles of the posterior pelvic right limb, with specific functions in the mechanical movement of jumps, were collected, in this case the gastrocnemius muscle in the lateral portion.

Histological processing of the Striated Skeletal Muscle. The muscle tissue was immersed in n-hexane cooled in liquid nitrogen by the not fixed tissue freezing method and then stored in a freezer at an ultra-low temperature (-80 º C) (Castoldi *et al*., 2015). Cuts of 5 µm were produced in a microtome cryostat at -20 °C, collected on slides and then stained with the hematoxylin-eosin (HE) and Toluidine Blue methods (Castoldi *et al*., 2013).

Optical microscopy. The cuts subjected to coloration were photomicrographed using a Nikon® brand microscope, model H550S. For analysis of the images an Infinity 1 camera was used. The markings for determining the length of the smallest diameter of the muscle fibers were performed using the software (Auxio VisionRel 4.8 - Carl Zeiss® and NIS-Elements D3.0 - SP7 - Nikon®); 200 muscle fibers from each animal were observed, following the protocol model used by Castoldi *et al*. (2015).

Statistical Analysis. After obtaining the data, the Shapiro-Wilk normality test was performed. As the data did not present normal distribution, the Kruskal-Wallis test was used to detect differences between the groups. All procedures adopted a significance of less than 5 % ($p < 0.05$) and were performed using SPSS 22.0 software.

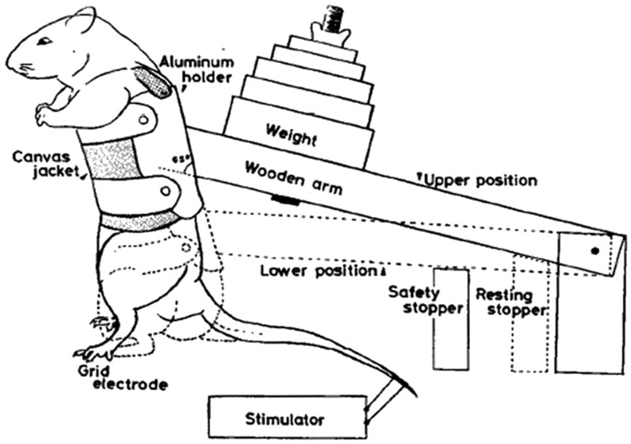

Fig. 1. Physical strength training model proposed by Tamaki *et al*., (1992).

RESULTS

After analyzing the data, it was found that the CT trained animals presented a reduction in body weight compared to the sedentary groups. Furthermore, it was noted that the diet significantly increased the body weight of the animals (Figs. 2 and 3).

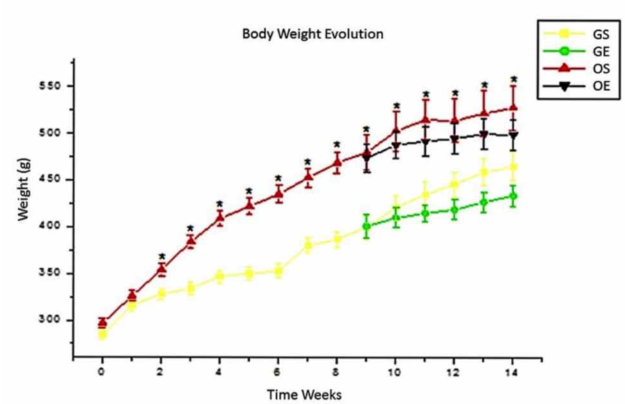

Fig. 2. Evolution of body weight in the different groups of animals. (*): Statistical significance of 5% ($p<0.05$).

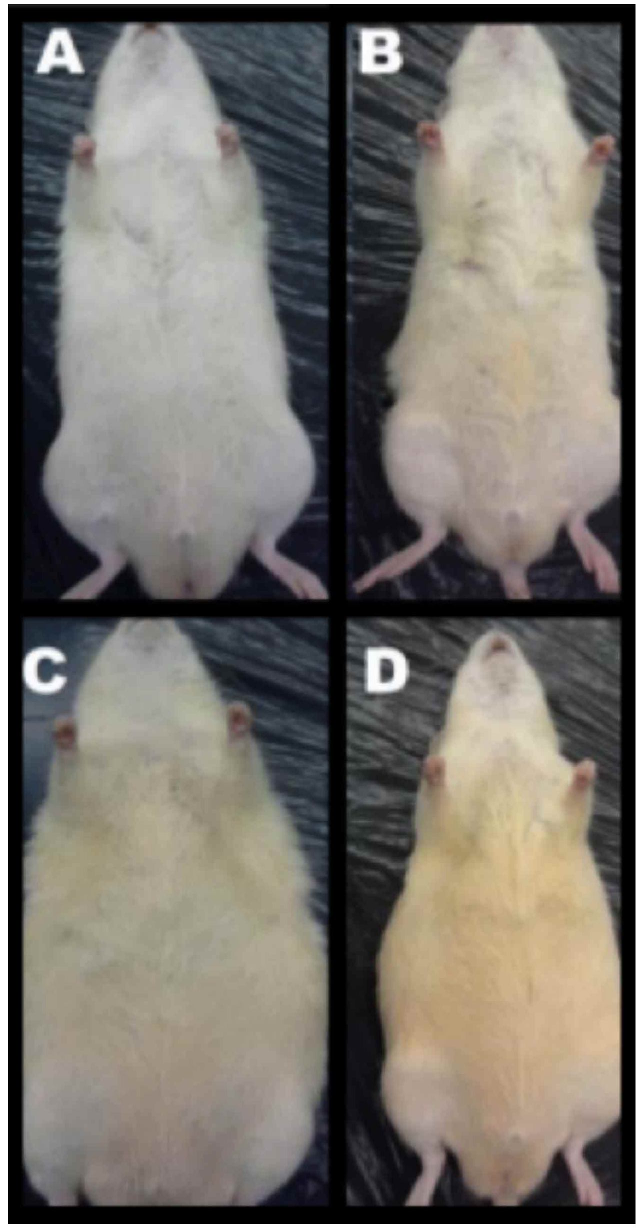

Fig. 3. Representation of body mass in different groups of animals. (A): Sedentary Group; (B): Exercise Group; (C): Obese Sedentary Group; (D): Obese Exercise Group.

When analyzing the adipose tissue separately, there was a significant increase in the OS and OE groups ($p <0.05$). Although the CT influenced the decrease in the value of the OE group, this reduction was not statistically significant ($p> 0.05$). Furthermore, it was observed that the CT significantly influenced only the reduction in the GE group for this variable (GS vs. GE) (Fig. 4).

It was found that the animals subjected to both the normal and CT diet protocols presented similar median values for the variable when comparing between them and with the GS group (mean). It was also noted that the OS group demonstrated a significant increase in median compared to the OE group (OS=98.58 µm vs. OE=77.41 µm) (Figs. 5 and 6).

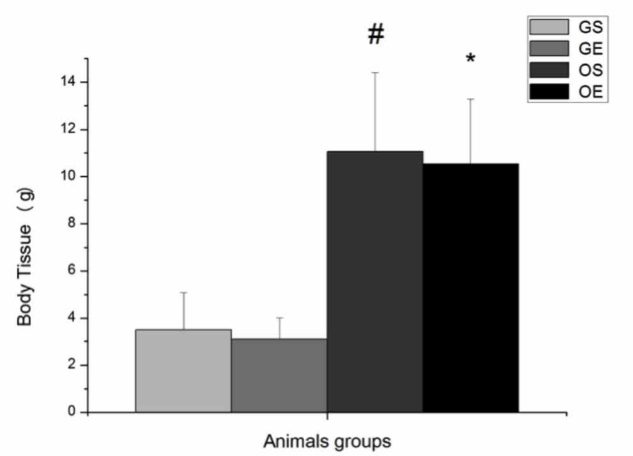

Fig. 4. Comparison of diameter between the groups of animals. (*) Statistical difference between OE, GS and GE; (#) Statistical difference between OS and GE. Kruskal-Wallis test with Dunn's post test (p <0.05). Legend: GS: Sedentary Group. OS: Obese Sedentary Group. GE: Exercise Group. OE: Obese Exercise Group.

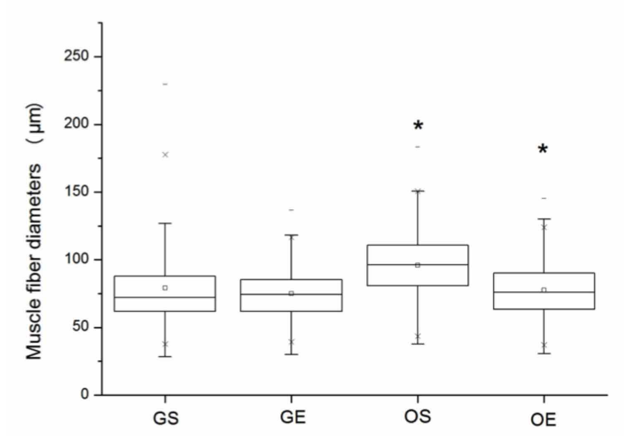

Fig. 6. Comparison of the muscle fiber diameters between the groups of animals. (*). Difference based on comparisons with the GS group. Kruskal-Wallis test with Dunn's post test (p <0.05). Legend: GS: Sedentary Group. OS: Obese Sedentary Group. GE: Exercise Group. OE: Obese Exercise Group.

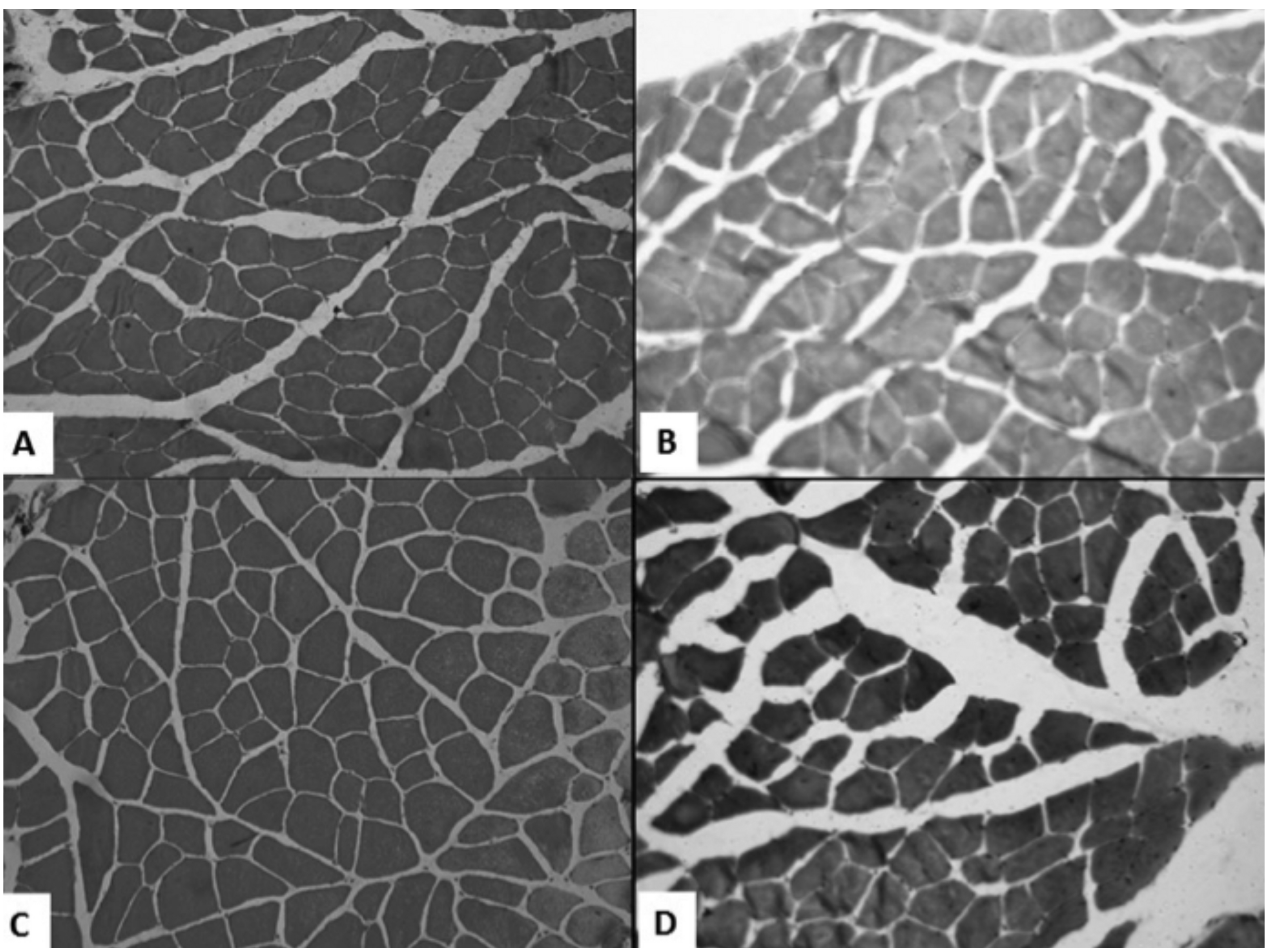

Fig. 5. Histological cuts stained with hematoxylin and eosin and toluidine blue in the different animal groups. 10X magnification. (A): Sedentary Group. (B): Obese Sedentary Group. (C): Exercise Group. (D): Obese Exercise Group.

DISCUSSION

The present study analyzed 40 rats submitted to concurrent training (CT) for 45 days with three training sessions per week, which aimed to analyze the effects of CT on muscle fibers of Wistar rats which were fed standard and hypercaloric diets. The results demonstrated no significant differences between the muscle fibers of the exercise and standard diet group (GE) and the sedentary and standard diet group (GS). Among the groups submitted to the hypercaloric diet, it was verified that the trained animals presented a decrease in the diameter of muscle fibers ($p < 0.05$).

An increase was observed in visceral adipose tissue in both groups treated with the hypercaloric diet. However, the animals that underwent CT presented lower values for this variable, although not statistically significant ($p > 0.05$).

The gastrocnemius muscle was chosen for analysis since it is located in the lower region of the body and its principal function is support and locomotion. Furthermore, studies related to muscle hypertrophy suggest that concurrent activities, whether in water or on land, promote hypertrophy of muscle fibers (Castoldi *et al*., 2013; Manchado *et al*.).

According to Nader (2006), the first author to describe the CT methodology was Hickson, in 1980, and at that time, CT was applied in humans and it was found that physical strength capacity decreased, while VO_2 max remained unchanged. However, in that study, high-intensity training was performed; aerobic near the VO_2 max value performed 6x/week and strength training at 80 % of 1RM applied 5x/week, both with a 10-week duration.

In another instance, Mendes Junior *et al*. (2012), observed the effects of CT in untrained middle aged individuals and found that when performed 3x/week, this approach did not lead to decreased strength or VO2 max compared with the groups which underwent training of each capacity in isolation.

In a way, the findings of Hickson and Mendes Junior *et al*., performed in humans resemble the results of the present study, since the trained animals in the obese group demonstrated lower values for the smallest diameter variable. Moreover, in contrast, the GE animals presented an increase for this variable.

It is worth emphasizing that although an increase in the diameter of muscle fibers was observed, in both cases, there was no statistical difference ($p > 0.05$). It is probable that the experimental diet interfered more in muscle adaptation than the CT protocol, since animals fed the hypercaloric diet (OS and OE) demonstrated a significant increase ($p < 0.05$) compared to the other groups of animals (GS and GE).

Fazelifar *et al*. (2013), observed the effects of CT in obese children. It was found that the protocol used was sufficient to develop increased physiological variables of VO2 max, flexibility and strength. In addition, a reduction in body weight, waist circumference and body fat percentage was found (%BF).

This finding emphasizes the use of physical training as a way to improve physical capacity and, in this case, CT could be one way to aid in the fight against and prevention of obesity. In the present study, a reduction in body mass and visceral fat (epididymal adipose tissue) variables was observed in animals that underwent CT, although not significant from a statistical point of view ($p > 0.05$).

Animal studies have demonstrated increased physical capacity, when performed 3x/week. In the studies of Moret *et al*. (2013), and Spagnol *et al*. (2012), muscle hypertrophy was observed after eight weeks of CT. However, in these studies, swimming exercise at 100 % of the anaerobic threshold was used and water jumps with a load of 50 % of body weight.

In the present study this fact was not verified, however, despite the intensity of the jumps being similar (50 % body weight), the modes of exercises were different, in the present study, the exercises were performed in a dry environment. It is known that in a liquid medium, the animal may experience the action of thrust, caused by the counterforce of the water, which may minimize the effects of impact and physical wear (Castoldi *et al*., 2016).

In the present study the animals performed the jumping movement in a dry environment; this fact may have increased the dose of effort, and thus minimized or prevented muscle adaptation (hypertrophy) of the animals. The increase in cross-sectional area of striated skeletal muscle is directly related to the increase in contractile force and neural adaptation, generated by the innervation of motor neurons (Fleck & Kraemer). In this sense, although there was an increase in sarcoplasmic content, such as the number of mitochondria and energy reserves, there was an increase in innervation and muscle fiber recruitment.

It is possible that the adaptation of muscle tissue is more related to the balance between volume and workout intensity than the specific type of training. In other words, a recovery period is necessary for adaptation and performance gain. This fact can be justified by the results presented in studies using frequencies 3x/week.

The use of forms of exercise to control or combat obesity has become important due to the increased prevalence of the disease in the world population in recent years. According to WHO, there are many reasons for this incidence, among them, especially, inappropriate eating habits associated with sedentary lifestyles resulting from modern life, and also genetic, psychological, physiological factors.

It is known that regular physical exercise helps to decrease or maintain body weight, improve glycemia, reduce cardiovascular disease factors, and minimize the need for oral anti-diabetic agents and insulin resistance (Wajcberg *et al*., 1999). When we analyzed the body mass, it was found that the physical training model used was sufficient to stop weight gain in both the obese and the sedentary groups.

Thus, this study contributes to the literature by evaluating the effects of concurrent training on the muscle fibers of Wistar rats submitted to standard and hypercaloric diets. One limitation of the present study was the fact that the strength training load was not adjusted so that there was an overload every week. Furthermore, although the intensity of the aerobic training had a constant increase, it was not adjusted for the different weights of the animals. Future studies analyzing fast and slow twitch fibers may show which training method has a greater influence on muscles.

CONCLUSION

In conclusion, CT caused a decrease in the diameter of muscle fibers in animals fed a hypercaloric diet and an increase in the animals fed a standard diet. Moreover, it was able to stop the weight gain and visceral adipose tissue in the OE group.

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RESUMEN: El objetivo de este trabajo fue analizar el efecto del entrenamiento concurrente (EC) en las fibras musculares de ratas Wistar sometidas a dietas normal e hipercalórica. Fueron utilizadas 40 ratas Wistar, distribuidas en cuatro grupos de animales: grupo sedentario (GS); grupo ejercicio (GE); grupo obeso sedentario (OS) y grupo obeso ejercicio (OE). Los animales realizaron el protocolo del EC compuesto por entrenamiento de fuerza y aeróbico, tres veces en la semana y por 45 días. La media del menor diámetro (MMD) de las fibras musculares fue medida para verificar la hipertrofia muscular. Fue observado que el grupo OE presentó una significante diminución del MMD comparado al grupo OS (OE=77.41 μm vs. OS=98.58 μm). Además, los animales que fueron sometidos al protocolo del EC demostraron hipertrofia muscular (GE=74.39 μm vs. GS=72.13 μm). Se puede concluir que el protocolo del EC con dieta normal tiene como resultado un aumento del MMD, mientras que el EC con dieta hipercalórica tiene como resultado la diminución del MMD.

PALABRAS CLAVE: Entrenamiento; Músculo; Hipertrofia; Ratas; Obesidad.

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Correspondence author:
Robson Chacon Castoldi
Universidade do Oeste Paulista - UNOESTE
Rodovia Raposo Tavares, KM 572, Limoeiro
Presidente Prudente – SP, 19067-175
BRAZIL

E-mail: castoldi_rc@yahoo.com.br

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