Original Article

Effects of resisted exercise on brown adipous tissue in C57BL/6 mice

Efeitos do exercício resistido sobre o tecido adiposo marrom em camundongos C57BL/6

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ABSTRACT | OBJECTIVE: To verify the effects of stair training and physical activity on brown adipose tissue histomorphometry in C57BL / 6 mice. **MATERIALS AND METHODS:** Sample composed of 16 mice, randomly divided: control (n = 4), stair exercise with electrical stimulus (n = 4), stair exercise (n = 4) and physical activity in an enriched environment (n = 4). A Group of physical activity in an enriched environment was performed in a box with toys. Ladder exercise group and ladder with electrical stimulus were performed with vertical ladder. With the ladder's use, the animals performed six sets, eight repetitions with 90-second intervals between sets, with ten sessions. In the stairway exercise with electrical stimulation, the animal was encouraged to climb using a steel plate at the base of the stairs, with an electric current of 20V intensity and 45Hz frequency. Brown adipose tissue collection was performed in the scapular region and stained with Hematoxylin-Eosin (HE). The level of significance of the analyzes was 95% ($p < 0.05$). **RESULTS:** There was no significant difference when comparing the TAM cell size compared to the tissue collected from the mice in the four groups. **CONCLUSION:** Physical activity and resistance exercise did not promote morphometric differences in the TAM of C57BL/6 mice.

KEYWORDS: Resistance training. Physical activity. Electrical stimulus. Histology. Metabolism.

RESUMO | OBJETIVO: Verificar os efeitos do treinamento de escada e atividade física na histomorfometria do tecido adiposo marrom em camundongos C57BL/6. **MATERIAIS E MÉTODOS:** Amostra composta por 16 camundongos, divididos aleatoriamente: controle (n=4), exercício de escada com estímulo elétrico (n=4), exercício de escada (n=4) e atividade física em ambiente enriquecido (n=4). Grupo de atividade física em ambiente enriquecido foi realizada em caixa com brinquedos. Grupo exercício de escada e escada com estímulo elétrico foram realizadas com escada vertical. Com a utilização da escada os animais realizaram 6 séries, 8 repetições com intervalos de 90 segundos entre séries, sendo 10 sessões. No exercício de escada com estimulo elétrico, o animal foi estimulado a subir usando uma placa de aço na base da escada, com uma corrente elétrica de 20V de intensidade e 45 hz de frequência. A coleta de tecido adiposo marrom foi feita na região escapular e manchado em Hematoxilina-Eosina (HE). O nível de significância das análises era 95% ($p < 0.05$). **RESULTADOS:** Não houve diferença significativa no comparativo do tamanho da célula de TAM em comparação com o tecido recolhido dos camundongos dos quatro grupos. CONCLUSÃO: A atividade física e o exercício resistido não promoveram diferenças morfometricas no TAM dos camundongos C57BL/6.

PALAVRAS-CHAVE: Treinamento resistido. Atividade física. Estímulo elétrico. Histologia. Metabolismo.

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Introduction

Resistance training (RT) has many followers worldwide, for having a low injury rate and an increase in critical physical capacities such as strength, power, and muscular endurance.[1,2] By providing a training method adaptable to the practitioner, TR is a widely used procedure with the most diverse objectives and physical fitness levels. It can have important effects on the maintenance of skeletal muscle, metabolism, and cardiovascular functions, standing out for improving the body composition and increase protein synthesis.[1,2]

Multilocular tissue, also known as brown adipose tissue (TAM), plays a central role in thermogenesis and energy expenditure regulation of energy expenditure[3], having the primary function of oxidizing lipids for the production of heat, production of anti-inflammatory substances, hormones that are related to improving insulin sensitivity and cardiovascular protection.[4] TAM deposits occur in small mammals and newborn humans. The evolutionary reason for the abundance of TAM at birth, both in rodents and in humans, is based on the need to maintain body temperature after an abrupt reduction in room temperature after birth.[5]

The TAM can vary from a light brown and can reach a reddish-brown due to the abundant vascularization and mitochondria. These are rich cytochromes that give them a reddish color.[6] The connective tissue forms septa between the lobes of adipose cells; each cell is supported by reticular fibers and collagens, a rich blood network, the cells being more closely connected, and the capillaries than the white adipose tissue.[7] The main signal for the activation of brown adipocytes is reducing body temperature below the thermoneutral (23ºC). Thermal signals are analyzed by specific central nervous system areas (CNS), which promote stimulation of the sympathetic innervation of TAM.[5]

The animal model is widely used to answer questions and explore hypotheses that are often not feasible in studies with humans, allowing for deepening the knowledge about the neurobiology of diseases.[8]

Thinking about the TR, several methodologies include voluntary climbing exercise, climbing apparatus, climbing, isometric training, excavation, and ladder with electrical stimulation.[9-14] Given the possible impact of TAM on the energy balance, interest in finding ways to increase this tissue's thermogenic activity grows. In this context, physical exercise begins to be tested as a non-pharmacological method of activating TAM.[15]

TAM's presence was observed in adult humans under circumstances of chronic exposure to cold or by adrenergic hyperexcitation in individuals who had pheochromocytoma (tumor of the adrenal gland medulla)[5]. The study by Marken Lichtenbelt16 showed that the relatively high metabolic activity of TAM in young men was found in 96% of individuals exposed to the cold (16°C). From the studies by Virtanen[17], it was suggested that TAM activity in humans could be regulated in the same way as in rodents. The literature shows that endurance physical exercises practiced regularly stimulate TAM in vitro and lean humans and control animals.[15]

Given the recognized influence of exercise and diet on TAM[18,19], we realized the need to verify the hypothesis of the different proposals for activity and physical exercise in TAM morphology. Hence, this study's objective was to verify the effects of training of ladder and physical activity in brown adipose tissue histomorphometry in C57BL / 6 mice.

Materials and methods

Study characterization and ethical care

The present study was experimental, analytical, prospective, and with a quantitative approach. The ethics committee submitted and approved animal experimentation and welfare (CEEBEA / Unimontes, process number 131/2017). Performed with 16 healthy, female animals, aged between 10 and 12 weeks, the average body weight of 20 ± 5g, they were housed respecting the 12h light / dark cycle, the average temperature of 22 ± 2 ºC and with low sound level.

All animals were randomly assigned to a control group (n = 4), stair exercise with electrical stimulation (n = 4), stair exercise (n = 4), physical activity (n = 4). The animals were housed in groups of 4 animals in autoclavable polypropylene boxes with dimensions of 414 x 344 x 168 mm, with a galvanized steel lid and containing stainless steel separators (Zootech, model ZT 375). All the boxes were lined with wood shavings, which were changed three times a week.

Experimental format

There were two moments of familiarization with the proposed activity and physical exercise to avoid compromising the experimental protocols. The control group did not perform any intervention, being in the same housing conditions at the time of interventions in the other groups. The physical activity group's condition was performed in a closed and enriched environment (plastic box) 60 cm long, 30 cm wide, and 45 cm high. This environment was composed of a seesaw, wheel, balls, and tunnels. The ladder exercise group's condition was performed on a vertical ladder 110 cm high, 18 cm wide, 2 cm between the steps, and 80° of inclination. The condition of the stair exercise group with electrical stimulation was performed using the staircase with electrostimulation. In the groups using the ladder, the animals performed six sets of eight repetitions with ninety-second intervals between the sets, and the resistance obtained by the exercise was the animal's own body weight. The proposal was adapted according to the literature. In the ladder exercise with electrical stimulation, the animal was encouraged to climb due to the presence of a steel plate at the base of the ladder, to which an electric current of 20 volts intensity and 45 Hz frequency was applied to the four legs of the animal.[14]

Ten sessions of each experimental intervention were performed, 24 hours after the last session, the animals were sacrificed, first anesthetized with ketamine/xylazine (75 mg/kg and 5 mg/kg of body weight, respectively) and, subsequently, euthanized by cervical dislocation to perform the collection of brown adipose tissue in the scapular region.[14]

Histomorphometry of brown adipose tissue

After collecting brown adipose tissue in the scapular region after euthanasia of the animals, they were then fixed in formalin and included in paraffin. Then they were subjected to seven µm sections stained in Hematoxylin-Eosin (HE). The sections were then examined and photographed using the Olympus BX50 microscope (Olympus Optical). The TAM's transversal areas were determined using the Image J software (Scion, Frederick, MD). The measurements were performed in at least three distinct fields selected and from each cross-section.[20]

Twelve histological slides were analyzed in Hematoxylin-Eosin (HE) staining of each group; 3 images of each slide and one slide of each animal were taken.

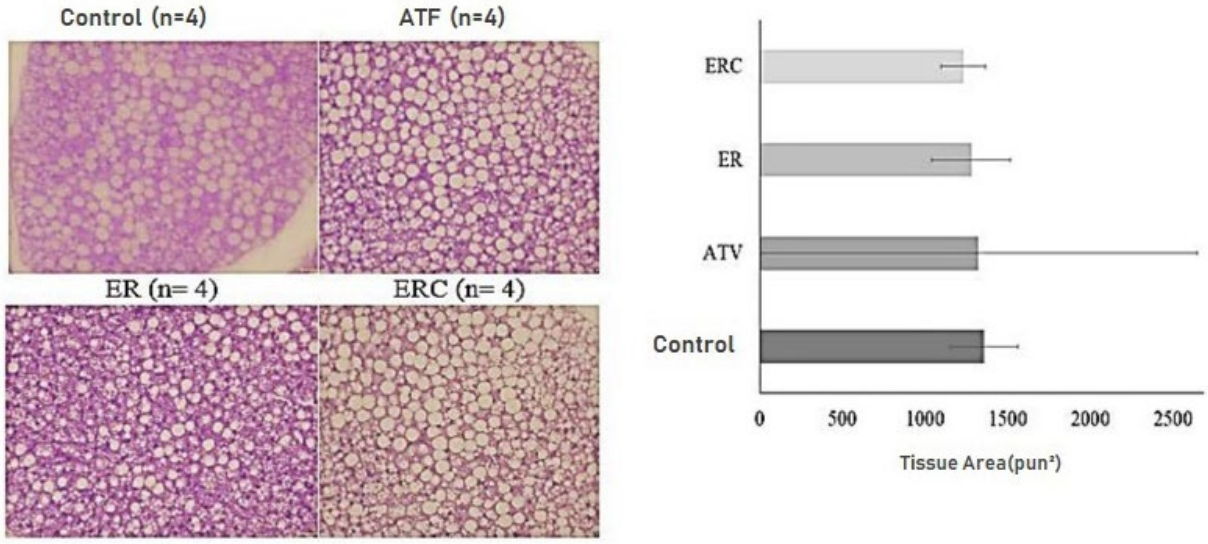
Data analysis

All data are expressed with the mean and standard deviation. The Shapiro-Wilk test was performed to verify normality, then the analysis of variance (ANOVA) with Bonferroni adjustment was selected for inferential verification of the dependent variables to analyze the dependent variables. All statistical procedures were performed using the Statistic Package for the Social Sciences (SPSS) 20.0 for Windows program. The level of significance adopted in all analyzes was set at 95% ($p < 0.05$).

Results

In figure 1, we have the comparison between the samples of brown adipose tissue in mice of the control group, ATV (physical activity in an enriched environment), ER (resistance exercise), and ERC (resistance exercise with shock). There was no significant difference ($p < 0.05$) between groups in the histomorphometric analysis of brown adipose tissue.

Figure 1. Histomorphometric analysis of brown adipose tissue from the scapular region of C57BL / 6 mice. No significant difference was found (p <0.05) between groups



Discussion

Current evidence points to TAM's importance in the control of metabolism in humans and its similarity with the TAM of rodents and studies in animal models of obesity that show a defective activity of this tissue and the consequent deficit in energy expenditure.[21]

A study carried out by Slocum[22] with C57 mice, submitted to a high-fat diet and running on a treadmill for seven days, found that exercise was able to promote weight loss, reduction of body fat, and the histological analysis of TAM showed that the brown adipocytes showed a more color stronger and greater number of mitochondria when compared to sedentary controls with a high-fat diet.

In a study with mice undergoing TAM transplantation, he found that the increase in the mass of this tissue improved glucose homeostasis and insulin sensitivity, accompanied by a decrease in fat mass. The transplant also attenuated the deleterious effects of the high-fat diet, and it was observed that these improvements were volume-dependent. That is, the greater the mass of transplanted tissue, the better the responses analyzed.[4]

In Leite[21] study, the eight-week physical exercise attenuated the development of fat mass and the expression of white adipose tissue formation (TAB) proteins; showed a protective effect against glucose intolerance and insulin resistance. It was also observed to reduce circulating LPS (Lipopolysaccharide), TNF-α (Tumor necrosis factor α), and free fatty acids. In addition, the data also demonstrate the positive effect of exercise on the insulin signaling pathway, increase in the mass of brown adipose tissue (TAM), and the expression of proteins involved in the thermogenesis process. Finally, it was found that the exercise was able to attenuate macrophage infiltration in TAM and promote greater polarization of type M2 macrophages (Alternative macrophage activation in TAM).[21]

Because of the evidence related to the theme, we realized that it has not yet been possible to show the real impact of the different formats of activity and physical exercise on the morphometric changes in brown adipose tissue. Other proposals with different experimental designs and better evaluation formats may clarify the doubts left here.

Conclusion

In this study, we concluded that physical activity and resistance exercise did not promote morphometric differences in the brown adipose tissue of C57BL / 6 mice when verified in stair training with electrical stimulus.

Authors 'contributions

Lopes JVN participated in data collection and animal management. Andrade GS participated in the statistical design and writing of the article. Machado FSM participated in animal management. Sousa BVO participated in the statistical design and laboratory analysis. Rodrigues VD guided and coordinated the research.

Competing interests

No financial, legal or political conflicts involving third parties (government, companies and private foundations, etc.) have been declared for any aspect of the submitted work (including, but not limited to, grants and funding, participation in advisory council, study design, preparation manuscript, statistical analysis, etc.).

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