Original article

Effects of aerobic exercise on soleus muscle of rats with metabolic syndrome

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Abstract

Introduction: Studies have shown a strong relationship between physical activity and the presence of cardiovascular risk factors such as hypertension, insulin resistance, diabetes, dyslipidemia and obesity that characterize the metabolic syndrome. Moreover, the practice of regular physical activity has been recommended for the prevention and treatment of this syndrome. **Objectives**: The aim of this study was to analyze by histomorphometric techniques, the effect of aerobic exercise in the soleus muscle of rats with metabolic syndrome. **Methodology**: A total of 15 male Wistar rats, 150 days old, divided into three groups (n = 5): sedentary, control (C); metabolic syndrome (MS) and trained, metabolic syndrome (TMS). The induction of MS was performed using fructose in the drinking water of animals. From the 9th week of induction, animals in the Training groups underwent exercise treadmill belt (Imbramed TK-01) with moderate intensity (50-70% of maximum speed achieved in the stress test). Physical training was conducted for nine weeks, with a frequency of 5 times per week, for about 60 minutes. The procedures were approved by the Ethics Committee of the São Judas Tadeu University (protocol Nr 060/2007). At the end of the experiment the animals were euthanized by decapitation. The right soleus muscle was sectioned, fixed and treated for conducting conventional histology, and the slides stained by HE and Picrosirius methods. Photomicrographs of 10 fields per animal were captured by light microscope, transferred to the image analysis program (Software Axio Vision, Zeiss). We measured the cross-sectional areas of muscle fibers and to analyze the volume densities of muscle fibers, capillaries, and interstitial collagen fibers, was used stereological method (252 points). The statistical analysis used was ANOVA One Way and Tukey test ($p < 0.05$). **Results**: The MS group showed a significant decrease in the cross section area of the muscle fibers, the volume density of the fibers, and increased capillary and interstitial collagen fibers. With training there was an increase in the volume density of collagen fibers, interstitial and capillary. **Conclusion**: Our data show that the metabolic syndrome causes changes in the soleus muscle in all parameters studied and that aerobic exercise of moderate intensity was not able to minimize major changes caused by metabolic syndrome.

Keywords: metabolic syndrome, skeletal muscle, stereology, aerobic exercise, morphometry.

1 Introduction

Metabolic syndrome (MS), also known as insulin resistance syndrome, is characterized by signs and symptoms caused by the same mechanism but dependent on different causes, where insulin resistance occurs before other manifestations and may be determinant of this syndrome (HAFFNER, VALDEZ, HAZUDA et al., 1992).

The prevalence of this syndrome has increased in recent years together with increasing life expectancy of the population whose lifestyle habits emphasize physical inactivity and inadequate diets.

The metabolic alterations caused by the MS can trigger disorder in glucose transport into cells of mammals. It is known that serum glucose uptake is essential for survival and much of this is captured by glucose-insulin dependent organs, primarily skeletal muscle. However, any imbalance in peripheral glucose uptake may induce glucose intolerance, leading to a negative feedback of muscle and liver glycogen on the activity of glycogen synthase (PAN, LILLIOJA, KRIKETOS et al., 1997; OAKES, COONEY, CAMILLERI et al., 1997; DE FRONZO, 1997).

Skeletal muscle constitutes approximately 45% of body weight, being the largest organ system of humans, and is the main site for processing and storage energy, being the final destination of the primary support systems involved in the exercise, such as cardiovascular and pulmonary (SANTOS, 2001).

Muscle plasticity observed in contractile, metabolic and morphological properties of the fibers in response to a particular stimulus, allows it to adapt to different functional demands and metabolic changes caused by MS and aging.

In general, these changes follows a pronounced decreased in physical activity, which causes changes in fiber type towards fast to slow, reducing contractile activity and also selective loss and remodeling of motor units. Reducing the number of sarcomeres in series and proliferation of connective tissue leads to higher stiffness, which makes it more resistant to the muscle force stretching (MINAMOTO, 2004).

Over a period of aerobic training, changes occur according with the type and characteristic of the muscle fibers, evoking additional gain in strength (BUCCIO, VINAGRE, CAMPOS et al., 2005). In the absence of physical activity, there is a decrease in the diameter of type I muscle fibers of the soleus muscle (THOMPSON, JOHNSON and SHOEMAN, 1998).

Numerous studies have shown that in both humans and animals, aerobic or resistance physical exercises, cause beneficial effects on all tissues, preventing chronic and degenerative diseases improving body composition (KAKIYAMA, SUGAWARA, MURAKAMI et al., 2005; GOLDSTEIN, LOCAIS and TOTH, 2004; SUVORAVA, LAUER and KOJDA, 2004; LEITÃO, LAZZOLI, OLIVEIRA et al., 2000)

Thus, fitness and diverse exercises practicing have been therapeutic used to maintain organ function, resulting in better quality of life and lower rates of death from factors associated with Metabolic Syndrome.

The aim of the present study was to analyze by histomorphometric techniques, the effect of aerobic exercise in the soleus muscle of rats with metabolic syndrome.

2 Animals and procedures

A total of 15 male Wistar rats, 150 days old, weighing approximately 400 g were studied. The animals were housed in individual cages in a temperature-controlled room (22-24 °C) with 12-hours dark-light cycle. The procedures used in this research were approved by São Judas Tadeu University Ethical Committee (protocol Nr 60/2007), according to the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, DC, 1996). The rats were divided into 3 groups (n = 5): Control, sedentary (C); sedentary, Metabolic Syndrome (MS) and trained, Metabolic Syndrome (TMS). For all groups it was given commercial feed reference to rats (Nuvital®) and water ad libitum. Metabolic syndrome was induced by means of overloading of fructose in the drinking water (D - fructose, 100 g/l) for 9 weeks.

Animals in the TMS group underwent running exercise on a treadmill (Imbramed TK-01), with moderate intensity (50-70% of maximum running speed achieved in testing effort - with speed and load variation from 0.3 to 1.0 km/h, with 1 km/h corresponds to approximately 60% of maximum effort achieved in maximal exercise test performed in the 4 weeks of training). Physical training was conducted for 9 weeks, 5 times per week, for about 60 minutes each session.

2.1 Preparation of tissue samples

At the end of the experiment the animals were euthanized by decapitation. An incision was made in the posterior region of the right leg. The right soleus muscle was removed and sectioned at mid-point through the cross section major axis of the fibers. Muscle fragments obtained by cutting isotropic and uniform randomly remained in solution of 10% formaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 hours at 4 °C. After washing, the segments were dehydrated in 70% ethanol, 95% and 100%, cleared in xylene and embedded in paraffin blocks forming. Through a microtome, ten, 6-µm thick, nonconsecutive conventional histological sections were cut perpendicularly to the longitudinal direction of the muscle fibers, and stained, respectively, by Hematoxylin-Eosin and Picrossirius before they were examined under light microscope and polarized light.

2.2 Stereology

Photomicrographs were captured by digital processing and analysis of images were performed in a computer in the Morphometric and Immunohistochemical Laboratory of the São Judas Tadeu University. The system consists of a Zeiss microscope, which is coupled to a Sony video that captures the images of histological slides, and transfers to a computer equipped with specific software for quantitative analysis (Axio Vision, Zeiss). Using the system test lines and points (252 points) with Image J software, it was obtained a volume density of the components of muscle (muscle fibers, connective tissue and capillary) and through the program Axio Vision it was measured, in 10 fields per animal, the muscle fibers transverse section area.

2.3 Statistical analysis

Results are shown as mean and standard deviation. One-way Analysis of variance (ANOVA), and post-hoc Tukey tests were duly applied in data analysis. The level of significance for all tests was set at $p < 0.05$.

3 Results

3.1 Muscle fibers cross section area (A [mf])

The cross section area of the muscle fibers of the MS group showed a significant decrease (13%) compared to the group C. In the trained animals (TMS) it was found that exercise attenuate the decrease in cross section area of the muscle fibers (Figure 1).

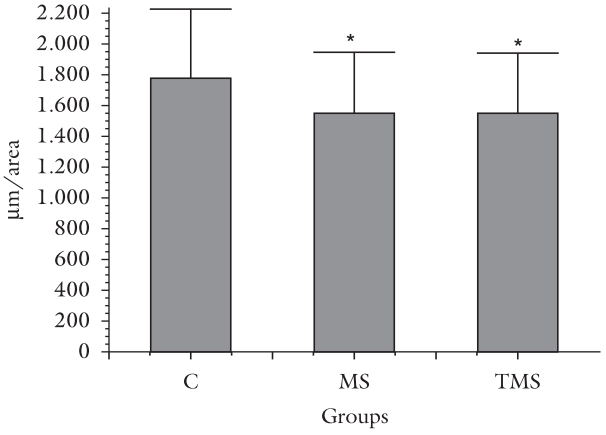

Figure 1. The cross section area of the muscle fibers (A [mf]) of the soleus in the sedentary control (C), sedentary metabolic syndrome (MS) and trained, metabolic syndrome (TMS). Values represent Mean ± SD. *p < 0.001 *vs* C.

3.2 Muscle fibers volume density (Vv [mf])

As for the volume density of muscle fibers, our results show a significant decrease (24%) in the MS group when compared with control group. No difference was observed between groups control and trained metabolic syndrome (Figure 2).

3.3 Volume density of collagen fibers (Vv [cf])

As for the volume density of collagen fibers, our data show that MS group showed significant increase (47%) when compared to C group. When we analyze the TMS group we found that training increased the density volume of collagen fibers by 78% compared with the C group and 21% in relation to MS group (Figure 3).

3.4 Volume density of the interstitium (Vv [int])

The density of the interstitial volume was significantly higher in groups MS (53%) and TMS (88%) compared with group C. We found that the training increased the interstitium to the MS group (Figure 4).

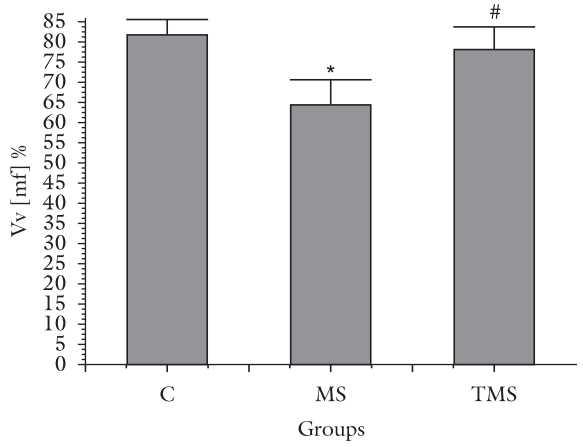

Figure 2. Volume density of muscle fibers (Vv [mf]) of the soleus in the sedentary control (C), sedentary metabolic syndrome (MS) and trained, metabolic syndrome (TMS). Values represent Mean ± SD. *p < 0.001 *vs* C; #p < 0.001 *vs* MS.

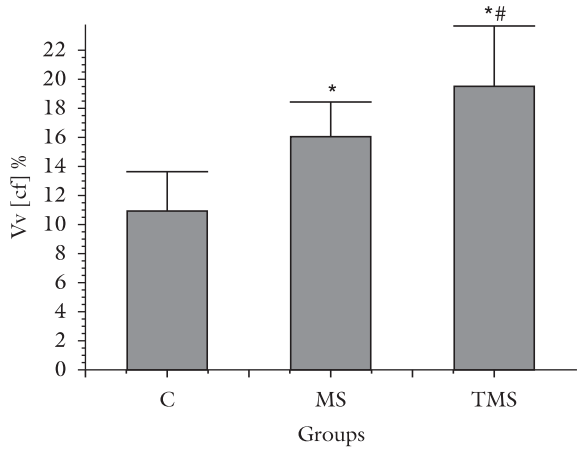

Figure 3. Volume density of interstitium Vv [int] of the soleus in the sedentary Control (C), sedentary Metabolic Syndrome (MS) and trained, Metabolic Syndrome. Values represent Mean ± SD. *p < 0.001 *vs* MS and C; #p < 0.01 *vs* MS.

3.5 Volume density of capillaries (Vv [cap])

The volume density of capillaries was significantly lower in the MS group (61%) compared with the group C. The TMS group managed to reverse the process of capillary loss, which occurred in the MS group (Figure 5).

4 Discussion

Exercise has been claimed for several authors as a promoter of health and wellness, improving functional fitness and contributing favorably with the circulatory, respiratory, immune, and musculoskeletal system among others, reducing the deleterious factors related to physical inactivity. The aim of the present study was to analyze by histomorphometric techniques, the effect of moderate aerobic exercise in the soleus muscle of rats with metabolic syndrome. Our results show that the MS group underwent significant changes in the morphology of the muscle fibers, showing a reduction in cross section area, volume densities of capillaries and muscle fibers in addition to an increase in volume density of collagen fibers compared with the Control group. When comparing the TMS group to the MS group, our results show that training minimized the loss of muscle fibers and capillaries and did not promote change in cross-sectional area of muscle fibers. As for the volume density of collagen fibers, the training caused a significant increase of collagen fibers, leading consequently to an increase in the interstitial space.

Few studies were found linking morphometric aspects of muscle fibers in skeletal muscles of animals with metabolic syndrome, so we will confront our results with studies related to risk factors associated to it like diabetes, aging, hypertension that characterize the metabolic syndrome.

We know that the morphological alterations in skeletal muscle due to aging, includes reducing cross-sectional area, loss in the number of muscle fibers, increase in the interstitial space and connective tissue, leading to a reduction in mechanical efficiency, promoting muscle weakness and fragility (CACCIA, HARRIS, JOHNSON et al., 1979; BUA, McKIERNAN, WANAGAT et al., 2002). In situations of low oxygen supply, muscle tissue responds in an attempt to adapt and therefore suffers some major histological changes,

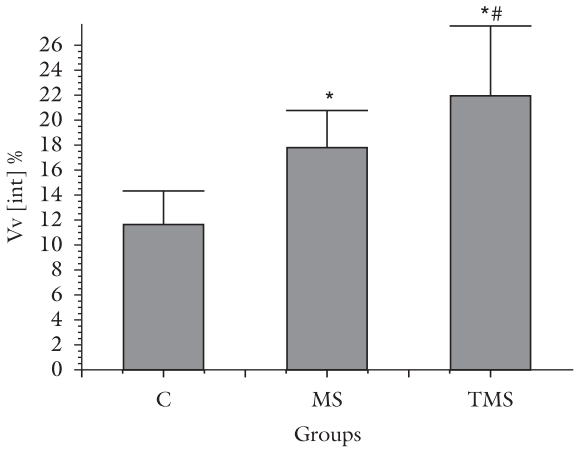

Figure 4. Volume density of interstitium (Vv [int]) of the soleus in the sedentary Control (C), sedentary Metabolic Syndrome (MS) and trained, Metabolic Syndrome. Values represent Mean ± SD. *p < 0.001 *vs* MS and C; #p < 0.001 *vs* TMS.

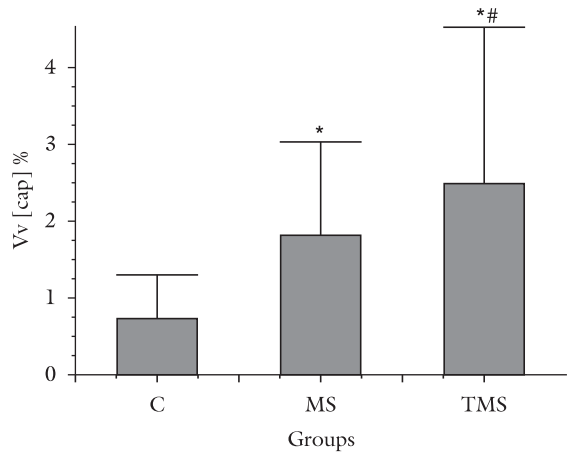

Figure 5. Volume density of cappilaries (Vv [cap]) of the soleus in the sedentary Control (C), sedentary Metabolic Syndrome (MS) and trained, Metabolic Syndrome. Values represent Mean ± SD. *p < 0.01 *vs* C; #p < 0.001 *vs* C.

such as atrophy and fiber phagocytosis (DUBOWITZ and SEWRY, 2007). Similar data were found in the present study in the SM group.

In this context, several authors (ROSA, SILVA, IHARA et al., 2005; KIM, KWAK, LEEUWENBURG et al., 2008; AHMED, MATSUMURA and CHRISTIAN, 2005; NARICI, REEVES, MORSE et al., 2004) studying elderly sedentary animals found decreased area of muscles fibers and increased collagen fibers. However, with the introduction of an aerobic exercise protocol for elderly animals, the deleterious effects were minimized.

Muscular atrophy and necrosis are known complications in diabetes and commonly affect the skeletal muscles. Several authors confirm that analyzes in skeletal muscle of diabetic animals results indicate reduction of fiber diameter with a corresponding increase in the density per unit area and a slight decrease in the length and diameter of the myonuclei (KLUEBER and FECZKO, 1994; AUGHSTEEN, KHAIR and SULEIMAN, 2006; RAN, WANG, ZHAO et al., 2005; TRUJILLO SANTOS, 2003). Furthermore, in animals that perform exercises, these changes are minimized, being observed increase in the volume density of muscle fiber hypertrophy process by which contractile proteins adds to the muscle fibers, contributing to better blood glucose control in diabetics and facilitates weight loss by increasing basal metabolic rate (SANTARÉM, 2005).

In our study we found that the metabolic syndrome promotes significant reduction in the volume density of capillaries, indicating focal degeneration and damage of the structure with increased collagen fibers in response to muscle tissue repair. Similar data were found in spontaneously hypertensive animals (HERNÁNDEZ, TORRES, FINOL et al., 1999; HERNÁNDEZ, TORRES, LOSADA et al., 2008; PREWITT, 2002) and in other pathological conditions such as inflammatory myopathies (FINOL, MULLER, TORRES et al., 1986; VLODAVSKY, LUDATSCHER, SABO et al., 1999), arteriosclerosis obliterans (MAKITIE, 1977), and diabetes mellitus (COHEN and YU-WU, 1983; DOSSO, LEUENBERGER and RUNGGER-BRÄNDLE, 1999). When we analyzed the animals TMS group verified that the exercise was highly effective in the preservation of the capillaries, which can be explained by the fact that the muscle fibers in the soleus muscle predominantes are type I, characteristic of aerobic, ie has a higher degree of capillarization, corroborate our results, Gonçalves, 2011, studying animals who underwent exercise in aging.

5 Conclusion

Our data show that the metabolic syndrome causes changes in the soleus muscle in all parameters studied and that aerobic exercise minimized the morphological changes in the cross section area and volume densities of capillaries and muscle fibers. However, training caused a pronounced increase in the collagen fibers and interstitium. Thus, we consider important to emphasize that exercise, performed so strenuous and inappropriate, can induce deleterious effects on the musculoskeletal system.

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