Effects of Aerobic Exercise on the Skeletal Muscle of Rats Exposed to Cigarette Smoke

EXERCISE AND SPORTS SCIENCES

Original Article

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ABSTRACT

Introduction: Smokers have reduction in the muscle mass and in the fatigue resistance and, possibly, physical activity practice contributes positively to this state. Objective: This study had as objective to analyze the adaptations of the skeletal musculature to the interaction between physical activity practice and cigarette smoke exposure. Methods: 32 male rats were divided in groups exposed to the cigarette smoke, exercised (G1) and sedentary (G3), and not exposed to cigarette smoke, exercised (G2) and sedentary (G4). The exposure to the smoke was done by the combustion of 10 cigarettes in an inhalation camera during 30 minutes, twice a day, five days per week, during 15 days; the aerobic exercise consisted of daily walking sessions of 60 minutes on treadmill, once a day, five days per week. After sacrifice, soleus muscle was obtained and its cuts were stained by HE technique and submitted to the hystochemical method of NADH-TR. Results: Group G1 presented more muscle alterations, and also lack of enzymatic activity, the same occurring in G3, but with less intensity; G2 presented normal pattern to exercised fibers, being the fibers of G4 preserved. Regarding the morphometry, there was significant difference for the exercise factor ($p = 0.007$), while no significant differences were observed for the smoke exposure factor ($p = 0.668$) and for the interaction of exposure to smoke and exercise ($p = 0.077$). Conclusion: The interaction between cigarette smoke exposure and physical activity during 15 days in male wistar adult rats, accentuated the histological changes in the soleus muscle, causing an alteration in the enzymatic activity and increase in the fiber diameter.

Keywords: tobacco, exercise, musculoskeletal system, histology.

INTRODUCTION

According to the World Health Organization[1], there are over 100 billion smokers around the globe, and the low cigarette taxes and cost contribute to this addiction continuation. In the 20th century, smoking was responsible for about 100 million deaths; currently, around 5.4 million deaths a year occur due to smoking and it is estimated that in 2030 eight more than million will occur, out of which, over 80% will be in developed countries.

Generally speaking, this high death incidence is a consequence of countless pathologies associated with the deleterious activity of the chemical components inhaled from cigarettes, which are responsible for the development of pharyngitis, bronchitis, pulmonary emphysema, many kinds of cancer, especially lung, and cardiovascular diseases such as coronariopathies and myocardial infarct, among others[1].

Cigarette frequent use is associated with reduction in skeletal muscle mass, especially reducing the transversal section of the oxidative fibers[2] and, consequently, fatigue resistance of the individuals[3,4]. When we consider that preservation of the muscle mass is extremely important to daily life activities performance, such scenario may negatively influence on the quality of life of smokers.

Due to these alterations associated with tobacco, many alternatives are currently proposed with the purpose to interrupt the smoking habit, being physical exercise practice one of them. Studies[5,6] indicate that physical activity practice is associated with reduction of the smoking deprivation symptoms, as well as smoking craving, acting as a distraction from stressful feelings and thoughts reducing anxiety.

On the other hand, physical activity practice is also associated with the onset of skeletal musculature adaptations, which include alterations in their metabolic and structural characteristics, with hypertrophy of the thin connective tissue, alterations in the kind of fibers, broadening of the volume/mitochondrial density ratio, of the oxidative enzymes, of the capillary density, as well as muscle fibers hypertrophy and hyperplasia[7-9].

Therefore, if the adaptation effects of the skeletal musculature exposed to exercise are considered, it is expected that a smoker who practices physical activity benefits from these adaptations, at least preserving his/her muscle mass. However, there is no conclusive evidence on the muscular adaptations during the interaction between physical exercises and chemical components in the cigarette smoke, which makes it a question to the clinical practice.

Thus, the aim of this study was to analyze the histological, histochemical and morphometric alterations in the fibers of the soleus muscle of rats submitted to aerobic physical exercise associated with cigarette smoke exposure.

METHODS

32 Wistar rats (*Rattus novergicus*), aged between 90 and 100 days, placed in collective plastic cages, were used for the experiments. The animals remained in the animal facility under mean temperature of 22 ± 2ºC and 12-hour light/dark cycle, with light cycle starting at 7:00h. The animals were fed with standard food (Supralab®, Supra Alisul Alimentos S/A, Brazil) and water *ad libitum*.

The entire experimental protocol followed the "Ethical Principles in Animal Experimentation", adopted by the Brazilian College of Animal Experimentation (COBEA), and was approved by the Ethics in Research Committee of the Sciences and Technology College of UNESP under the number 121/2006.

The animals were randomly divided in four groups of eight animals, namely: G1, exposed to cigarette smoke and exercised; G2, not exposed to the smoke and exercised; G3, exposed to the smoke and sedentary; and G4, not exposed to the smoke and sedentary.

In order to expose the animals to the cigarette smoke, an inhalation chamber built based on the experiments by Cendon[10] was used. This chamber consisted of an aluminum and glass structure (100 x 44 x 44cm) hermetically closed, divided in two compartments by a dark glass partition with five narrow passages of one inch of diameter. One of the compartments is used for the cigarettes burning, which were displayed on a wooden holder, while the other compartment was used for accommodation of eight animals which were in a cage. A compressed air source with 10l/min flow which enables the cigarettes burning and the smoke conduction to the exposure compartment was connected to the compartment for cigarette burning. This later compartment has a hole for air draining through which exhaustion was performed.

The cigarette smoke exposure protocol was composed of two phases, adaptation and the exposure itself. In the adaptation, the G1 and G3 animals were exposed to the burning of two cigarettes for 30 minutes, twice a day, for five days. On the second phase, the animals were exposed to the smoke of burning of 10 cigarettes, with duration of 30 minutes[10-12], twice a day, with a total of 20 cigarettes per day. Such procedure was performed five days a week, during 15 days. The animals from G2 and G4 groups were exposed to compressed air, following the same protocol described before.

The cigarettes used were commercially bought, producing in each burning (according to the manufacturer): 0.7mg nicotine, 8mg tar and 9mg carbon monoxide. This product was chosen due to its low cost in the market and for being a highly popular brand among smokers.

The training protocol was performed on treadmill for small animals, always in the morning shift and after the cigarette smoke exposure. The animals from G1 and G2 groups were submitted to five days of adaptation, with five, 15, 30, 45 and 60-minute sessions. Exercise sessions on treadmill of 60 daily minutes, five times a week, during 15 days, at 9.75 meters per minute velocity were subsequently performed, totalizing 585 meters at each 60-minute session[13].

24 hours after the last session, the animals were sacrificed by overdose of sodium pentobarbital by intramuscular administration. Subsequently, the soleus muscle was removed from the right pelvic limb and its fragments frozen by the immersion in N-Hexaneat -70ºC system and stored in a Nitrogen container at -182º[13]. Histological sections were obtained through cryostat microtome (HM 505 E Microm, Germany) following suitable techniques and care.

The slides were made with 8µm slices and stained by the hematoxylin-eosin (HE)method[13]and were used for evaluation of the following morphological characteristics: shape, position of the nuclei, inflammatory infiltrate, presence of phagocyte process and presence of necrosis[14]. Additionally, they were used to measure the smallest diameter of 120 fibers per animal, by computer imaging analysis system using the software *Image Pro-Plus* (Media Cybernetics, Silver Spring, MD), respecting the criteria previously established in the literature[13]. The demonstration of the nicotinamide adenine dinucleotide tetrazolium redutase activity (NADH-TR), which indicates the presence of oxidative activity, was used to verify the amount of phormazane in the sarcoplasm of the muscle fibers[15].

The results observed on the mounting were analyzed both qualitatively and quantitatively. Concerning the qualitative analysis, observation and description of the histological and histochemical findings was performed. Quantitative analysis of the morphological characteristics described above was performed from the frequency analysis, in which the presence or absence of these characteristics was verified on the mounting. The values obtained in the morphometry are presented as mean and standard deviation and were compared by the two-way analysis of variance, where the factors set were the exercise and the exposure to the cigarette smoke; the Bonferroni test was used for the multiple comparisons of the means. P values lower than 0.05 were considered significant.

RESULTS

Qualitative analysis of the mounting showed that the G1 animals presented muscle fibers with angular rounded borders, polymorphic and atrophic fibers, sarcolemmaunder degeneration, fibersin phagocytosis process, inflammatory infiltrate and necrosis (figure 1a). In the G2 animals, the majority of the fibers presented alteration in their shape, and there was presence of polygonal, rounded, angular and polymorphic fibers; moreover, atrophic fibers with sarcolemma degeneration and inflammatory infiltrate, but not much remarkable, have been identified (figure 1c).

Conversely, the G3 animals presented regions with normal aspect and other regions with angular, rounded and polymorphic fibers, besides presence ofsarcolemma degeneration and some atrophic fibers and in phagocytosis process (figure 1e). G4 animals presented fibers with alterations only in shape, presenting normality characteristics; it is worth mentioning that, in all studied groups the nuclei were on the periphery of the muscle fiber, and no central nuclei were present.

Table 1 shows the result of the quantitative analysis of the histological alterations observed in the muscle fibers of the studied groups. The results state that the G1 animals presented the highest frequency of histological alterations, including concerning the presence of phagocytosis and inflammation to G3. In the G2 animals the alterations found were more related to the fibers shape.

In G1, fibers with weak reactive activity were present in the NADH-TR reaction mounting while absence of reaction was observed in some of them (figure 1b). Regarding G2, normal reaction pattern was verified for the majority of the fibers, and in a few of them increase

Table 1. Frequency analysis of the morphological characteristics onset of the fibers of the soleus muscle in the animals of each studied group.

Characteristics	G1	G2	G3	G4
Roundedfiber	88%	63%	100%	75%
Angular fiber	100%	100%	100%	88%
Polymorphic fiber	100%	100%	100%	38%
Atrophic fiber	88%	50%	88%	–
Inflammatory infiltraes	100%	38%	50%	13%
Fagocyted fiber	100%	13%	88%	–
Necrosis	75%	13%	88%	–
Sarcolemma injury	88%	63%	88%	–

of enzymatic activity, characterized by amorphous phormazane in subsarcolemmal and central position aggregates was observed, which indicates the mitochondria position (figure 1d).

In the G3 animals it was verified that the majority of the fibers presented amorphous phormazane aggregates in subsarcolemmal position; however, fibers with loss of enzymatic activity in some section regions and irregular distribution of the reaction product were present (figure 1f).

According to the results obtained in the morphometry (table 2), it was verified that the animals exercised with (G1) or without (G2) cigarette smoke exposure presented higher transversal section measurements compared with the control group (G4), while the group only exposed to presented the lowest values. Nevertheless, the bifactorial analysis of variance identified significant difference only for the exercise factor ($p = 0.007$), indicating that despite the different values obtained to smoke exposure ($p = 0.668$) and its interaction with exercise ($p = 0.077$) they have not been the determinant factors of these differences.

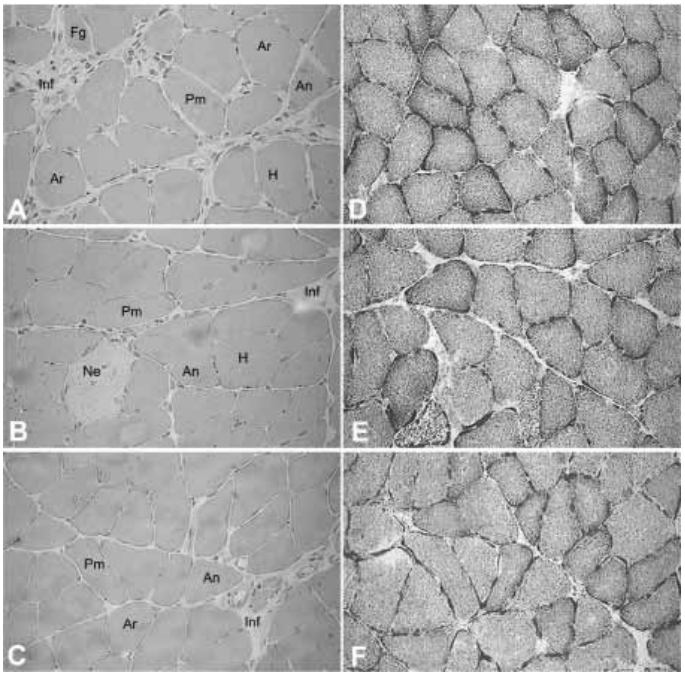

Figure 1. Transversal section of the soleus muscle of rats A) Exposed to cigarette smoke and exercised; B) Exercised; C) Exposed to cigarette smoke. HE, 500x.Rounded fiber (Ro); angular fiber (An); polymorphic fiber (Pm); hypertrophic fiber (H); inflammatory infiltrate(Inf); necrosis (Ne). D) Exposed to cigarette smoke and exercised; E) Exercised; F) Exposed to cigarette smoke. NADH-TR, 500x.

Table 2. Morphometry values (µm).

	Mean ± SD	CI – 95%	
		Minimum	Maximum
G1	41.47 ± 10.06	40.79	42.14
G2	39.54 ± 8.23	38.98	40.09
G3	35.21 ± 7.51	34.73	35.68
G4	38.19 ± 7.06	37.68	38.71

SD = standard deviation; CI = confidence interval.

DISCUSSION

It was observed that the animals submitted to cigarette smoke, exercised or not, presented higher incidence of muscular alterations (table 1) from the experimental model used and according to the histological findings of the different groups. Moreover, it was verified that smoking altered the oxidative pattern, and its interaction with exercise resulted in distinct responses (figure 1).

In fact, previous studies have reported that smoking influences the muscle characteristics and can change the fibers percentage and size, as well as the muscular oxidative activity[2,16,17]. It is believed that there are two mechanisms involved in the injury process caused by smoking, the vasoconstrictor activity promoted by nicotine and the carboxyhemoglobin increase (COHb) in the blood, being both related to low tissue oxygenation[2].

In situations of low oxygen supply to the body, the muscle tissue responds in a trial to adapt and, therefore, suffers some important histological alterations, such as fiber atrophy and fagocytosis[15]. Such aspects were present in the muscle fibers of this study, and their relation with low oxygenation could be verified from the analysis of the NADH-TR mounting.

Concerning the enzymatic reaction pattern, fibers with activity loss and irregular distribution of the reaction product have been observed in the animals submitted to cigarette smoke inhaling. Additionally, it could be observed that the animals only exposed to smoke (G3) presented large quantity of amarphous phormazane aggregates in subsarcolemal position, which indicates mitochondrial dislocation closer to the cellular nucleus.

However, muscular alterations have been also observed in the animals exercised only (G2), despite its occurrence lighter than in the G3 animals. Such alterations, mainly characterized by alterations in the cellular shape and inflammatory infiltrates, may be considered normal alterations to exercise.

Previous research which used histological evaluation of the soleus muscle fibers of animals submitted to stress by stress by swimming exercise[18] and treadmill[13] have also identified the shape alterations in the muscle fibers and presence of muscular inflammatory process, identified as adaptation mechanisms of the muscle tissue to the exercise[18,19].

Concerning the NADH-TR histochemical reaction, it was verified that these animals presented increase of the oxidative activity, presenting enzymatic response pattern similar to the one found in the animals only exposed to the cigarette smoke. Despite this similarity, one can suppose that the adaptation mechanisms are distinct; in the case of the exercised animals, the enzymatic alterations occur due to the increase in the metabolic demand[20], while in the G3 animals the acceleration of the respiratory chain

may be understood as a response to the deficit in energy supply derived from low oxygenation, leading to the need of an optimum level of mitochondrial activation[2,16].

The G1 animals were submitted to both stress situations; therefore, more remarkable adaptations are necessary. Such fact may have led to mitochondrial overload, resulting in weak reactive activity and even oxidative activity loss. Furthermore, such stressing stimuli differences may also justify the distinct histological and morphometric responses found in the different studied groups.

Concerning the morphometric responses, it was observed that the exercised animals presented the highest values compared with the sedentary ones, and exercise only was the factor which influenced the alterations in the fiber size. Thus, it can be stated that the animals from the exercised groups presented muscle fibers hypertrophy, regardless of the exposure to cigarette smoke factor.

Physical exercise practice is associated with alterations in size and quantity of muscle fibers, and its practitioners present higher fiber diameter values[13,21]. Generally speaking, muscular hypertrophy is an adaptation of the muscles to physical overload, which, on its turn[21], results in amplification of the number of contractile elements[s] in parallel and increase of the maximum tension the skeletal muscle may produce.

Speaking of the adaptation responses to smoking, previous studies[2,16,17] verified tendency to muscular atrophy aiming better oxygen contribution by the small capillaries located in the membrane. In spite of the presence of atrophic fibers in this study, the exposure to smoke factor did not significantly influence the size of the muscle fibers.

It is worth mentioning that although the hypertrophic situation is positive to the body, the animals exercised and exposed to the cigarette smoke were submitted to this interaction for just 15 days and, therefore, it is not known if this effect goes on after chronic exposure to this interaction, since it has been more aggressive to the skeletal muscle even in this short exposure period.

Furthermore, studies add that the effects of the cigarette smoke in the muscle fibers of Wistar rats depend on the exposure amount, and higher doses have proved to cause higher adaptation responses [2,16]. Thus, further studies which analyze the effect of the exercise and exposure to cigarette smoke interaction in the musculature of rats for longer periods and under different dosing are suggested. In addition to that, in this study only one muscle of oxidative characteristic has been analyzed, when muscles with other metabolic characteristics may be used in order to verify possible action selectivity of this interaction.

To sum up, in this research it was verified that the interaction between cigarette smoke inhaling and exercise, performed during 15 days in male adult Wistar rats, stressed the histological alterations of the soleus muscle, leading to alteration of the enzymatic activity and increase of the muscle fibers diameter.

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