EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

EVALUATION OF HISTOMORPHOMETRIC PARAMETERS OF RAT'S SOLEUS, SUBMITTED TO JUMP REMOBILIZATION IN THE AQUATIC ENVIRONMENT

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ABSTRACT

Introduction: The muscular tissue is able to respond to stimuli such as immobilization that induces hypotrophy, altering muscle performance and it is important to find methods that aim to reverse these deleterious effects in the post-immobilization period. Objective: The aim of this study was to evaluate transverse and longitudinal histomorphometric parameters of the soleus muscle fibers of rats immobilized in shortened position and submitted to remobilization by jumping in water. Methods: 24 rats divided into 3 groups were used: G1 – remobilized freely, G2 – remobilized with jumps daily, and G3 – jumps on alternate days. Immobilization and remobilization occurred in 2 weeks for the right limb. The variables analyzed were: muscle mass, muscle fiber diameter, length and sarcomeres in series estimate along the muscle. Results: There was reduction in muscle mass for both groups. Concerning diameter, there was difference in G1 and G3. No significant differences were observed for muscle length; however, for the sarcomeres in series estimate significant changes were found in all groups. Conclusion: The protocol used presents partial activity against the deleterious effects of immobilization.

Keywords: muscles, immobilization, exercise.

INTRODUCTION

Muscle tissue is able to respond to different internal or external stimuli such as hormonal changes, exercises, nutrition, denervation, electrostimulation, immobilization, among others[1, 2]. Because of this characteristic, muscle tissue is considered the most mutant among biological tissues in a way that it is able to respond to either normal or altered conditions by adapting itself morphologically and functionally[3].

Focusing on immobilization effects on muscle tissue, we may observe that it results in reduction of sarcomeres in series number as well as lesser muscle strength, complacency and loss of movement amplitude[5]. Short periods of time, from seven to ten days, are enough to cause important changes in the morphometry of soleus and gastrocnemius muscles of rats and mice[4,5]. According to Kasper *et al.*[6], fibers type I have lower adaptation comparing to type II and that is the reason they are affected more. Then, we may say that soleus muscle for being predominantly constituted of tonic fibers, suffers more in terms of mobility restrictions[7].

According to Koh and Tidball[8], keeping the number of sarcomeres in series in skeletal muscle is important to developing and muscle functioning. They state that the mechanical stimulus is responsible for such regulation and that it would be involved in releasing growth factors and enhancing the increasing of the number of sarcomeres in the muscle tissue. Besides that, immobilizing in stretched or shortened muscular position alters the number of sarcomeres in a way that changes the normal length-tension relation producing maximum strength in an abnormal spot in the amplitude of the physiological movement which impairs the muscle performance[9]. Thus, the objective of the present study is to evaluate transverse histomorphometric parameters as well as longitudinal aspects of soleus fibers in rats immobilized in stretched position and later remobilized submitted to jump remobilization in water medium.

MATERIALS AND METHODS

Characterization of samples and groups

In this study, 24 male Wistar rats aging 10 ± 2 weeks taken from the vivarium of State University of Western Paraná. This study followed international animal testing regulations and ethics and was approved by the Committee on Ethics and Animal Testing of State University of Western Paraná under no. 1.110. The 24 rats were grouped and kept in polypropylene cages placed in controlled environment with 12 hour cycle of darkness and light at 25 C degrees and provided with water and food *ad libitum*. The rats had their right hind leg immobilized in maximum plantar flexion for 15 consecutive days in order to keep the soleus muscle in maximum shortened position. Later, they were divided in three groups of 7 rats each to undergo therapeutic intervention:

• Group 1 (G1; n = 10): after the immobilization, rats were remobilized without any intervention.
• Group 2 (G2; n = 10): after the immobilization, rats were submitted daily to jump remobilization for two weeks according to remobilization protocol.

• Group 3 (G3; n = 10): remobilization similar to group 2, but the jumps were done every other day for two weeks.

Immobilization Protocol

In this study, immobilization apparatus designed by Coutinho *et al*.[10] was used to shorten the soleus muscle. The joint ankle joint was immobilized in maximum plantar flexion. Animals were observed on a daily basis during 15 days of immobilization to avoid any damage to the apparatus.

Remobilization Protocol

After taking the immobilization apparatus out, the G1 animals only touched water to guarantee the same treatment among the experimental groups and minimize stress caused by the contact with the water. G2 animals were submitted to jump remobilization in water medium with overload of 50% of their weight as presented in studies by Rogatto et al[11]. To make the overload, two lead weighs were joined by a Velcro® band which was positioned on the anima thorax because this device would not impair any movements. The jumps were performed in 4 series of 5 jumps each, with an interval of 3 minutes among each series daily during 15 days in the afternoon. Concerning G3 animals, interventions were similar to G2 but jumps were performed every other day during 15 days. The jumps were performed in a 200-liter round water tank, 50 cm depth and water temperature kept between 31°C and 33°C and checked by Incoterm® thermometer. Before the training, all animals were weighd to verify how much weigh would be used as overload.

Preparation of microscope slides and histological analysis

At the end of the experimentation, animals were weighed and euthanized by decapitation in guillotine. Then, right and left soleus muscles were dissected, cleaned and weighed on an analytical scale Shimadzu®. After that, the soleus muscles were fixed on a flat board on which the length of the relaxed muscles was evaluated through a vernier caliper (Mitutoyo®).

Later, muscles were submerged to 10% formol and cut longitudinally which medial part was analyzed transversally and the lateral part was analyzed longitudinally.

Before preparing the microscope slides to analyze the transversal variables, soleus muscles were dehydrated and the samples were paraffin-embedded. Then, transversal cuts were made using rotary microtome at 7μm thickness and were stained with hematoxylin and eosin (HE stain). After that, the samples were viewed through an optical microscope Opton®, 10x magnification, and images were captured and digitalized to have the fibers diameters measured by program Image-Pro-Plus 3.0®.

Lateral part of soleus muscle was taken out from the 10% formol solution after 3 hours to perform the longitudinal analysis. They were submerged in 30% nitric acid for 72 hours and after that they were stored in 50% glycerol solution. The histological slides consisted of five isolated fibers from tendon to tendon e mounted on slides containing varnish. These slides were viewed through an optical microscope Opton®, 10x magnification, and images were captured and digitalized to check an estimated number of sarcomeres in series along the muscle. So, the counting was done along 50μm in 6 distinct grids reaching up to 300μ. The calculus to reach the total estimate of sarcomeres in series along the muscle was made through cross-multiplication.

Analytical Statistics

The variables in the study were evaluated by comparing results from left and right soleus muscles among animals belonging to the same experimental group and among other groups using ANOVA testing with repeated and unidirectional measures respectively. $p < 0.05$ was considered significant.

RESULTS

Based on the analysis of transversal parameters of immobilized soleus muscles and their muscle mass, there was a significant difference for both groups when comparing left muscle (control) to right muscle (experimental) ($p< 0.05$). Comparing the diameter of muscle fiber, G1 and G3 presented the right muscles of soleus smaller than the left ones ($p< 0.05$).That was not found in G2 animals ($p>0.05$) (table 1).

Table 1. Results of muscular mass and muscle fiber diameter relating to the group studied and comparing values from right soleus muscle (RSM) and left soleus muscle (LSM).

		RSM	LSM
Muscular Mass	G1	0.1249 ± 0.0118 g	0.1561 ± 0.0235 g*
	G2	0.1340 ± 0.0224 g	0.1678 ± 0.0224 g*
	G3	0.1217 ± 0.0241 g	0.1525 ± 0.0283 g*
Muscle Fiber Diameter	G1	14.05 ± 1.98 μm	23.01 ± 3.94 μm*
	G2	18.53 ± 2.34 μm	18.56 ± 4.87 μm
	G3	13.37 ± 3.06 μm	19.49 ± 4.82 μm*

* Significant statistical difference.

Comparing the longitudinal parameters found relating to muscle length, there was no significant difference ($p> 0.05$). However, the total estimate of sarcomeres along the muscle has shown significant difference in all groups when comparing to right and left muscles ($p< 0.05$) (table 2).

DISCUSSION

Skeletal muscle atrophy has been a great problem for patients that undergo movement restrictions caused by surgeries, joint diseases or cast immobilization[12] resulting muscle hypotrophy, reduction of muscle cross-section area and muscle strength even during short periods of time like fifteen days[13-15]. Tonic muscles like soleus present a greater hypotrophy. That is the reason why devising resources to help people have their optimal movements back is extremely important besides the existing electrical stimulation and physical exercises[17-22]. Physical exercise is beneficial to muscle recovery as stated by Malysz *et al.*[23] in his study on muscle recovery of

Table 2. Results on muscle length and total number of sarcomeres in soleus muscle relating to the group studied and comparing values from right soleus muscle (RSM) and left soleus muscle (LSM).

		RSM	LSM
Muscle length	G1	1.99 ± 0.18 cm	2.21 ± 0.18 cm
	G2	1.99 ± 0.20 cm	2.12 ± 0.31 cm
	G3	1.88 ± 0,20 cm	1.99 ± 0.20 cm
Total number of sarcomeres along the muscle	G1	9.560 ± 592.3	10.993 ± 544.4*
	G2	9.456 ± 723.5	10.108 ± 1.188*
	G3	8.609 ± 1.163	9.488 ± 909.6*

* Significant statistical difference.

diabetic Wistar rats that present lesion on the sciatic nerve and were submitted to exercises on a treadmill. However, there is not enough data on scientific literature about using resistant anaerobic exercises for recovery after immobilization. Ju *et al.*[24] analyzed the jump effects in sandbox on Wistar rats remobilization after two-week hindlimb suspended plus intermittent weight support. They observed that five weeks of exercises were enough for the total recovery of the trabecular microarchitecture of the femoral metaphysis and that did not happen with free remobilization.

Results in this study show that functional overload induced by physical exercise in a daily basis may reverse partially the deleterious effects caused by immobilization of medium transverse diameter of muscle fibers but the exercises performed every other day did not have that same effect. So, we may state that this study reinforces research findings on muscular tissue recovery after immobilization as Kannus *et al.*[25] who observed that high intensity exercises as walking on treadmill caused beneficial effects on recovery of fiber cross-sectional area of soleus muscle in rats. Sakakima *et al.*[26] also observed that three-week immobilization period and a six-week remobilization period on a treadmill the effects on recovery of fiber cross-sectional area of soleus muscle were enhanced compared to free remobilization. According to Brito *et al.*[27] 100 samples of muscle fibers are enough to evaluate properly the medium trophism. However, another variable related to muscular trophism, that is, muscle mass has no change in values similar to the non-immobilized sides.

According to Butterfield *et al.*[28] the addition of sarcomeres in series have a great impact on muscular performance because they enhance the contracting speed and potency. As immobilization promotes reduction of sarcomeres[29], in this study we tested the hypothesis of jump as a way to recover longitudinal morphological characteristics of soleus muscle in rats. Even though there are no differences either on immobilized or non-immobilized sides relating to muscle length, and that result found for the croup control, and that finding may suggest that the remobilization period was effective to reverse some deleterious effects of immobilization. Koh and Tidbal[9] reported positive effects of three-week remobilization after four-week immobilization period. Estimated analysis of sarcomeres in series along the muscle has not found advantages on the protocol of physical exercises by jump comparing to free remobilization, though. Losses of sarcomeres in series along the muscular microarchitecture are caused by adjustments in fiber extremities for ideal actin and myosin overposition in myofibrils to perform maximum tension[29], but the optimal length was not reached based on the differences found. Thus, all the groups presented reduction on estimated sarcomeres along the soleus muscle of rats.

Those findings comply with previous studies that used the same model and presented reduction of sarcomeres along the muscle when animals were submitted to remobilization by swimming, low intensity walk on treadmill or series of static stretch[19-21]. Butterfield and Herzog[30] highlighted that tension in the muscle-tendon unit is not the primary source of sarcomerogenesis but it may occur in some cases of eccentric exercise[28] like contractions and disregarded in the present study.

The absence of one group immobilized and having no period of remobilization may be seen as one of the limitations of this study. However, that group was disregarded due to the reduction of studies using animals because the designers of the immobilization method have pointed it as a producer of mass reduction and muscular length[10] and a short period of immobilization around 7 days is enough to decreasing muscular mass, fiber area and number of sarcomeres in series.

CONCLUSION

Physical exercise performed daily acts against deleterious effects caused by immobilization and that was not observed in exercises made every other day relating to muscular mass and muscular fiber diameter. Such effect was also observed in the muscular length of soleus muscles of both groups. However, findings in this study show that the protocol used was not effective on muscle recovery of longitudinal parameters relating to estimated quantity of sarcomeres along the soleus muscle.

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