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ORIGINAL PAPER

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Morphology of extensor digitorum longus of Wistar rats after remobilization by vibratory platform

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ABSTRACT

Introduction. In exercise, vibrations are performed in order to produce rapid and short changes in muscle length. These changes are detected by sensory receptors, in response try to dampen the vibratory waves through a modulation of muscle stiffness. However, its effects on the morphology of muscle tissue are still not fully established, especially after long periods of immobilization.

Aim. To compare the effects of the vibratory platform on the remobilization of the extensor digitorum longus (EDL) muscle of Wistar rats with free remobilization.

Material and methods. 20 rats were divided into: CG (Control), IG (immobilized), IFG (immobilization and free remobilization), IPG (immobilization and remobilization with vibratory platform). The immobilization was performed on the pelvic limb for 15 days. The remobilization with vibratory platform was done for 10 minutes daily, for 2 weeks. The EDL was processed for histological analysis of cross-sections.

Results. The area, larger diameter, smaller diameter and fiber density of the EDL muscle of GI presented significant alteration when opposed to CG, IFG and IPG. The density of nuclei of the EDL muscle of IG presented a significant increase when opposed to the others, and IPG also presented a significant increase when compared to CG.

Conclusion. The morphology and morphometry of the EDL muscle tissue were affected, and both free and vibration platform remobilization re-established the morphological aspects of the muscle fiber, without significant differences between the methods.

Keywords. immobilization, skeletal muscle, vibration

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Introduction

The skeletal striated muscle is composed of long, narrow, multinucleated skeletal muscle fibers. Its nuclei are peripherally located in the fiber, below the sarcoplasmic membrane, and have a high capacity of structural and functional adaptation, called neuromuscular plasticity.[1-4] Thus, mass and composition are directly related to function and can be regulated according to workload, activity and pathological conditions.[5-8] In addition, the lack of stimuli can lead to muscle changes, such as immobilization, a therapeutic resource frequently used in the treatment of lesions of the musculoskeletal system.[9]

The greatest effect observed in muscle tissue subjected to long periods of immobilization is muscle atrophy. This process seems to be highly ordered and regulated, characterized by a decrease in the cross-sectional area of the fiber and protein content. These changes result in reduced force production capacity, decreased electrical activation and increased fatigue.[6,10-13] Still, Rocha et al. reported that prolonged immobilization, whether in a shortened or elongated position, seems to reduce the sarcomeres in series number, besides increasing the deposition of intramuscular and perimysium connective tissue, which can reduce the relative strength and muscle dynamics for active movement, leading to functional deficits in the body segment.[14]

In the attempt of a rapid remobilization, several therapeutic meditations have been used, among them physical exercises. However, there are still gaps as to the best type, intensity and period of performance. Thus, it is necessary to search for new modalities and in this sense, exercises that impose hypergravity, due to high acceleration, achieve complex body responses.[15] Whole-body vibration (WBV) has shown good results in the increase of density and acceleration of bone metabolism, besides the increase in strength, balance and muscle power. Still, it can be applied to individuals of various ages and physical conditions, as is the case of those who have limitations to perform exercises that give load.[16-19] In exercise, vibrations are performed in order to produce rapid and short changes in muscle length. These changes are detected by sensory receptors, in response try to dampen the vibratory waves through a modulation of muscle stiffness.[15,20] However, its effects on the morphology of muscle tissue are still not fully established, especially after long periods of immobilization.[20,21]

Aim

Thus, the present study aimed to analyze the morphological effects of the vibratory platform in the remobilization of the extensor digitorum longus (EDL) muscle of Wistar rats compared to free remobilization.

Material and methods

This is an experimental, quantitative study developed at the Laboratory for the Study of Lesions and Physiotherapeutic Resources and at the Laboratory of Structural and Functional Biology of the Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel Campus. The study was previously approved by the Ethics Committee on Research Involving Animals of the Universidade Estadual do Oeste do Paraná.

The experimental model used 20 rats, kept in a 12-hour light-dark photoperiod, temperature between 22-24ºC and, hygiene control, with ad libitum water and feed. After acclimatization of one week, the animals were randomly divided into 4 experimental groups, with 5 rats in each group: CG (Control), IG (immobilized), IFG (immobilization and free remobilization), IPG (immobilization and remobilization with vibratory platform).

Immobilization Protocol

To perform immobilization, the animals were anesthetized (xylazine 15 mg/kg and ketamine 80 mg/kg, intraperitoneally) and immobilized with appropriate material to plaster a body segment, the same: ligature of saturated tissue with dehydrated calcium sulfate, in the form of a white powder, characterizing a plastered bandage. The immobilized experimental groups had the orthosis molded from the abdominal region, just below the last ribs, followed to the right pelvic limb, being placed throughout the extension of the limb so that it remained in extension of the knee joint as well as complete plantar flexion of the ankle, that is, in position of stretching of the extensor long muscle of the fingers. The animals were kept in this position for a period of 15 consecutive days.[1]

Remobilization protocol

Animals of the IFG were freely remobilized in the cage for 15 days receiving water and feed at will. For the IPG, remobilization was carried out on the commercial platform, with a frequency of 60 Hz and vibrations with an amplitude of 2 millimeters. The rats were contained in the platform by an apparatus, built in MDF, in white color, with a total area of 25.4 cm^2 subdivided into eight stalls with an area of 2.4 cm^2 and 26.5 cm in height, where each application was done a caster, so that the same rat did not always remain in the same stall. It was performed for 10 minutes, three days interspersed per week, with rest of two days at the end of the week, for 2 weeks.

Histological processing

As soon as the immobilization was removed, the animals of the IG and the other groups, after two weeks of remobilization, were weighed, anesthetized, euthanized in the guillotine and the EDL were collected for histological analysis.

The muscles were dissected and fixed in 7% formaldehyde, stored in 70% alcohol and followed the routine histological procedure for paraffin emblocation. Cross sections of 7 μm thickness were obtained in microtome and the slides were stained with hematoxylin and eosin for general morphological analysis of muscle tissue, and the results were expressed in morphological plates. The slides were analyzed using a light microscope, in which the morphometric characteristics of the muscle tissue were evaluated (area, largest diameter, smallest diameter (100 fibers analyzed per muscle), number of fibers, nuclei and core ratio per fiber of the EDL muscle (10 observation fields per muscle, each field had an area of 3743 μm^2)).

Statistical analysis

The data analysis was performed with the SPSS 20.0 software, by means of Generalized Linear Model (GLM), and a gamma model was adopted for the variables area, largest and smallest diameters, number of nuclei and fiber/core ratio; for the number of fibers the model was linear. The Sidak post-hoc was used, and in all cases the accepted level of significance was 5%. Cohen's effect size was evaluated, considering variations of <0.2 trivial; 0.2-0.5 small; 0.5-0.8 moderate; > 0.8 large.

Results

Morphological Analysis

CG EDL muscles presented muscle fibers in polygonal format, peripheral nuclei and normal fascicular pattern (Fig. 1A). In the animals submitted to the immobilization period (IG), it was verified a disarray in the tissue organization, with apparent signs of atrophy, some fibers with rounded appearance and increase in fiber density per area analyzed after 15 days of immobilization in elongated muscle position (Fig. 1B).

Free remobilization (IFG) for 15 days restored the morphological characteristics, presenting polygonal fibers and peripheral nuclei (Fig. 1C). In the remobilized group with vibratory platform (IPG), fibers with nuclei were observed in the central region and some fibers with rounded appearance, but with a density closer to CG (Fig. 1D).

Morphometric Analysis

The area, larger diameter, smaller diameter and fiber density of the EDL muscle of IG presented significant difference when opposed to CG, IFG and IPG. The EDL muscle nuclei density of IG presented a significant increase when compared to the others, and IPG also pre-

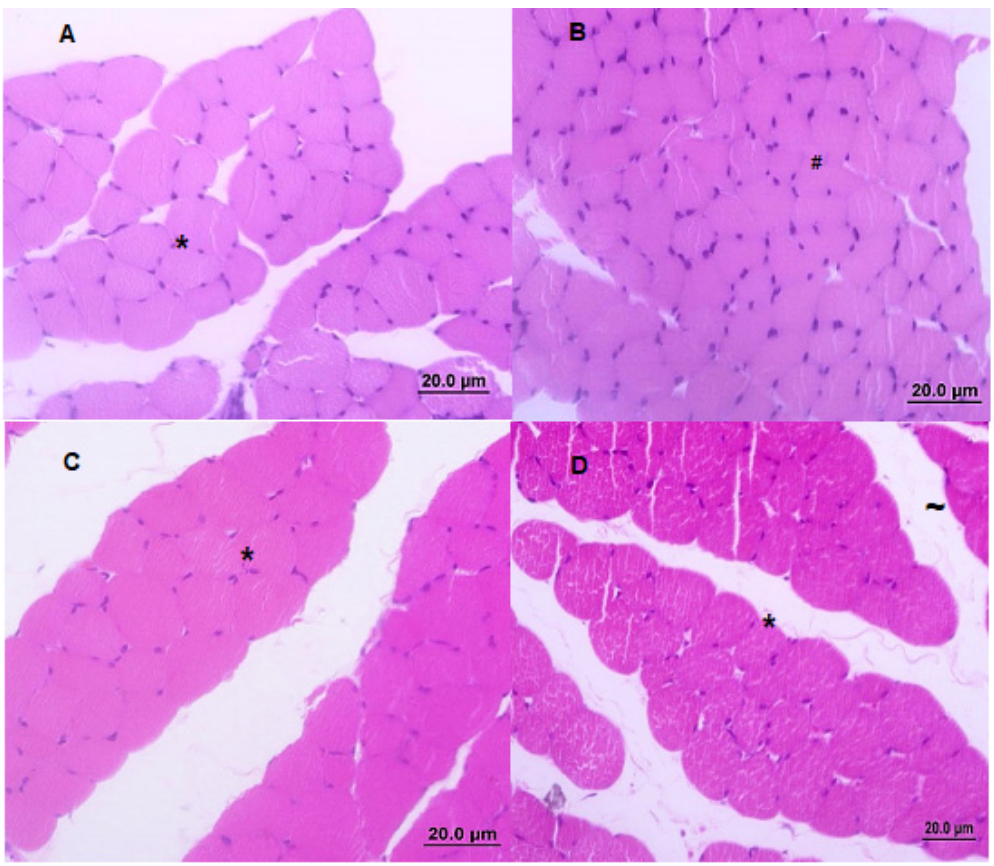

Fig. 1. Photomicrographs of the extensor long muscle of the fingers of Wistar rats, cross-section; hematoxylin and eosin staining. (A), control group (CG), presenting muscle fibers with polygonal shape, nuclei in peripheral position (*). (B), immobilized group (GI), presenting atrophic fibers (#). (C), free remobilization group (GIL) and (D), vibratory platform remobilization group (GIP), fibers with size similar to the control group (*), and central nucleus (~) in GIP (D).

Table 1. Analysis of Morphometric Characteristics. The data are expressed as mean ± standard deviation, below are indicated the Effect Sizes when comparing with the CG. Similar letters indicate no significant differences (p<0.05). CG (control), IG (immobilization), IFG (free remobilization), IPG (remobilization by vibratory platform)

	CG	IG	IFG	IPG
Fiber area	58.23 ± 5.71[a]	41.55 ± 2.28[b] 3.84	54.47 ± 12.58[a] 0.38	57.18 ± 5.64[a] -0.19
Larger diameter	20.54 ± 2.36[a]	14.67 ± 0.78[b] 3.34	20.87 ± 4.72[a] 0.09	20.07 ± 1.96[a] -0.22
Smaller diameter	13.43 ± 1.28[a]	9.45 ± 0.68[b] -3.88	14.11 ± 2.86[a] 0.31	16.33 ± 3.64[a] 1.06
Fiber number	49.13 ± 11.43[a]	101.52 ± 16.72[b] 3.66	53.18 ± 16.22[a] 0.29	59.70 ± 10.65[a] 0.96
Nuclei number	89.37 ± 15.19[a]	175.3 ± 30.77[bc] 3.54	107.76 ± 24.13[ad] 0.91	128.44 ± 32.29[cd] 1.55
Nuclei/fiber ratio	1.75 ± 0.09[a]	1.72 ± 0.11[a] -0.30	2.19 ± 0.20[b] 2.84	2.1 ± 0.20[b] 2.26

sented a significant increase when compared to CG. The fiber nucleus ratio showed a significant increase when comparing IFG and IPG with CG and IG (Table 1).

Discussion

The area, diameter, and diameter of the EDL muscle of IG showed a decrease and fiber number showed a significant increase when compared to CG, IFG and IPG, and the largest effect sizes were found in the comparisons between CG and IG. Such characteristics pointed to a picture of muscular atrophy with immobilization for two weeks. Muscles with a predominantly extensor function present a high degree of adaptation to atrophic stimuli, which was evidenced in the present study.[22]

The nuclei number in IG showed a significant increase when compared to the other groups, however, it was consistent with the increase in the amount of fibers observed per area analyzed, since the ratio of nuclei for fibers had no statistical difference when compared to the control group, again the effect size was greater between the control and immobilization groups. Ferreira et al. stated that muscle atrophy is accompanied by a reduction in the average number of myonuclei per fiber and that apoptosis seems to be underlying this regulated elimination of myonuclei.[23] In the present study, there was no decrease in the nuclei number compared to CG, but it was lower when compared to IFG and IPG.

It is known that hypertrophy is due to the increase in the individual size of muscle fibers, due to increased protein production and the addition of myofilaments, myofibrils and sarcomeres. This process may result from the activation and fusion of satellite cells, resulting in the addition of new myonuclei.[24] In view of the above, it is observed that the findings of the present study are consistent with the literature, because in IFG and IPG an increase in the ratio of nuclei per fiber was observed when compared to IG, which explains the return to baseline values in relation to the cross-sectional area and fiber diameters in IFG and IPG, characterizing muscle hypertrophy and indicating greater muscle metabolism, also pointed by the larger size of effect of these compared to the control.

The present study showed that the fibers of the EDL of the CG presented as polygonal with peripheral nuclei. In IG, degenerative results of immobilization were observed, such as apparent hypotrophy of the muscle fibers. These findings can be explained by Taillandier et al. who state that muscle immobilization can compromise the metabolic homeostasis of muscle fibers, besides causing muscle hypotrophy and changes in the connective tissue of the soleus muscle of rats.[25]

It has been observed that slow muscle fibers (type I), predominantly oxidative, seem to be more vulnerable to disuse atrophy when compared to rapid muscle fibers (type II), due to differences in their metabolismo.[1,23,26] The EDL is predominantly composed of fast fibers type II, however, just as the slow fibers are more vulnerable to immobilization, the muscles with a predominantly extensor function present a greater degree of adaptation to atrophic stimuli than the flexors, which was evidenced in the present study by the apparent atrophy and increase in fiber density per region analyzed in IG.[22]

Similar findings were observed by Polizello et al., who after 14 days of immobilization in position of elongation of the EDL muscle observed atrophy of different types of muscle fibers, in which they justified such findings due to the fact that immobilization in elongation is considered a stimulus to longitudinal tension that does not determine overload in the skeletal muscle, but can induce changes in factors related to myogenic regulation.[27]

In IFG a reestablishment of the morphological pattern found in the animals of the control group (CG) was observed after 14 days of re-mobilization of the animal freely in the cage. Corroborating this study, Polizello et al. showed that the free movement of the animals after

10 days was able to restore the values of diameter and proportion to the values observed in the CG for almost all types of fibers of the EDL muscle of rats immobilized for 14 days.[27] This may have occurred because the discharge of weight in the limbs, exerted by the animal in the cage, provides the muscle with a mechanical tensile load, which, in turn, determines trophic tissue adaptations.[28]

The EDL muscle of the animals treated with vibratory platform presented an apparent return of fiber density to the CG pattern, and also presented some nuclei in the central region, which may be indicative of muscle adaptations. Nuclear centralization suggests that there was sarcolemmal damage and subsequent architectural modification of the costamer proteins, which are of fundamental importance to stabilize the sarcolemma and position the nucleus especially for the EDL muscle.[29,30] These deleterious effects may have occurred owing to the frequency of vibration used in this study. Studies that demonstrate increases in muscle strength using vibration training have employed frequencies between 25 Hz and 45 Hz, requiring studies with different frequencies.[31] In the present study, the frequency of 60 Hz vibrations were used, in which it approaches the limit of the values proven harmful to the musculoskeletal system.

On the other hand, according to Wiggs, these cells with central nucleus can be translators of muscle regeneration, since physical exercise causes a transient inflammatory response, which will have a cytoprotective influence, essential to cell regeneration, translated by the increase in satellite cells.[32,33] Adaptation in the context of skeletal muscle reflects a change in its structure and function in response to stimuli such as physical exercise, immobilization and trauma. The ability of the muscle to respond to these stimuli is based on its regenerative capacity, due to the presence of undifferentiated myogenic cells, known as satellite cells, which are activated and proliferate and/or differentiate themselves through stimulation.[34]

Exercises on vibration platform have the characteristic of increasing the action of gravitational force on skeletal tissues, inducing neuromuscular and neuroendocrine adaptations, showing the possible activation of satellite cells, since the tonic vibration reflex is referred to as the neuromuscular mechanism activated in response to the effect of vibration, resulting in a significant increase in recruitment of motor units.[16,18,21,31,35]

Since the immobilization and consequent muscular atrophy cannot be avoided many times, it is essential for the repair of health and quality of life the recovery of muscle function. In the present study, both free remobilization and vibratory platform were able to restore the normal trophic characteristics of the EDL muscle. However, treatment with the vibratory platform did not promote improvement in relation to free remobilization, but also did not cause deleterious effects, so it could be used in physical rehabilitation programs, for gains in other structures without muscle damage.

Conclusion

It is concluded that immobilization in an elongated position for 15 days affects the morphology and morphometry of the muscle tissue of the EDL muscle, and both free remobilization and vibration platform reestablished the morphological aspects of the muscle fiber, without significant differences between the methods.

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