



Histomorphometric alterations of muscle soleus provoked by drawn out immobilization: experimental study with wistar lineage rats

Alterações histomormofétricas do músculo sóleo provocadas pela imobilização prolongada: estudo experimental com ratos da linhagem wistar

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Abstract

Objective: This study has as objective to analyze the effect of joint immobilization of the soleus muscle of posterior members of rats on morphometric profile view, at periods of 21 and 45 days. **Materials and methods:** Ten male *Rattus novergicus albinus*, Wistar variety, were used, separated into two groups (group

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I and II), of 5 animals each, with the first group submitted to immobilization for 21 days and the second for 45 days. The experiment control was acquired from the right contralateral member of each animal. The immobilization of the left posterior member was done by adapted orthosis. The morphometric analysis of soleus was by non-serial cross sections, 8µm thick. **Results:** From the obtained images, the muscle fibers areas were analyzed, including the form of the fibers, comparing with the control group. In both the periods of immobilization cross sections of staple fibres had reduction of the section area, front to the comparison to its respective member has controlled, with significant important ($p < 0.0001$), especially it enters the experimental groups of 21 and 45 days. **Conclusion:** It can be concluded that immobilization to articulate of rats per 21 and 45 days modifies the section area more transversa of staple fibres of the sóleo muscle and deleterious effect of more significant the cellular components for drawn out periods.

Keywords: Immobilization. Rats. Skeletal muscle.

Resumo

Objetivo: Este estudo teve como objetivo verificar os efeitos da imobilização articular no músculo sóleo de ratos, por meio de uma análise histomorfométrica, em períodos de 21 e 45 dias. **Materiais e métodos:** Foram utilizados 10 *Rattus norvegicus albinus machos*, variedade Wistar (3 a 4 meses, massa corpórea entre 250–300 g). A amostra foi dividida em dois grupos (G1 e G2), com 5 animais em cada, sendo o primeiro grupo submetido à imobilização por 21 dias e o segundo por 45 dias. O controle do experimento foi obtido a partir do membro contralateral direito do respectivo animal. A imobilização do membro posterior esquerdo foi feita por meio de uma órtese adaptada. A análise histomorfométrica do sóleo foi realizada por meio de cortes transversais não seriados de 8 µm de espessura. **Resultados:** Foram analisadas, por meio das imagens obtidas, a forma e a área das fibras musculares comparando-as com o grupo-controle. Foi observada a presença de fibras musculares com morfologias distintas, evidenciadas pelos diferentes contornos celulares, predominando fibras com formato poliédrico. Em ambos os períodos de imobilização houve redução da área de secção transversa das fibras, frente à comparação ao seu respectivo membro contralateral, com diferenças significativas ($p < 0,0001$), especialmente entre os grupos experimentais. **Conclusão:** Pode-se concluir que a imobilização articular de ratos por 21 e 45 dias altera a área de secção transversa das fibras do músculo sóleo, sendo as alterações mais significativas evidenciadas em períodos mais prolongados de restrição ao movimento.

Palavras-chave: Imobilização. Ratos. Músculo esquelético.

Introduction

Generally, in the clinical practice it is observed a tendency to precocious immobilization of a body segment (1, 2) due to orthopedic or neurological affections, such as ligament ruptures, fractures and muscle lesions (3, 4).

The immobilization of a body segment comprehends local rest, continuous and rigid (5) with morphological, physiological and biochemical manifestations in the skeletal musculature (6, 7, 8, 9, 10) observed in animals (11) as well in human beings (12).

The restriction to movement by immobilization, because of its non-contribution to homeostatic maintenance in anabolic and catabolic reactions,

converges to alterations of trophic character (4), and, yet can be distinguished by the strength decreasing, by the muscle size, the fiber area (13), the extensibility (14), the resistance of ligaments and tendons, contracture formation, electrical activity alteration (15) and reduction of the number of sarcomeres in series resulting in muscle rigidity during the first week of immobilization (16).

In this sense, Gomes et al. (17) add that the muscle disuse also promotes the decrease in the density of capillaries and the spreading of the intramuscular connective tissue both in the perimysium and in the endomysium, besides promoting an increasing of collagen fibers of the tissue (18), reduction of glycogen reserves, increase in fatigability (19), decrease in

size and number of mitochondria, increase in lactate concentration with the work (20) and lastly, skeletal muscle hypertrophy (19, 21).

According to Appel (22) and Glass (23), hypertrophy may be defined as a consequence of the subtraction in the muscle protein synthesis and/or development of it, and the decrease of availability of energetic substrates. Taking into account that the causes are distinguished among age, gender, muscle group (extensor/flexor), fiber type, length, immobilization time and position in which the muscle is immobilized (22).

Caiozzo et al. (24) and Lieber (25) reinforce that uniaxial muscles, whose action is anti-gravity, have a higher degree of hypertrophy in situations of disuse. Simultaneously, Kasper et al. (26) and Talmadge (27) observe that fibers of type I have a better adaptability in relation to fibers of type II, being, therefore, more affected, which makes it possible to confirm that the soleus muscles that are mainly constituted of tonic fibers suffer considerable compromising regards mobility restriction (28).

In experimental range, some studies were directed to the different periods of muscle inactivity, showing that, only one week is enough to promote important sarcomeres adaptations and morphometric alterations in the mechanism of soleus muscles and gastrocnemius of rats (2, 6, 18, 29, 30, 31). In this sense, there are researches that show a reduction of almost 23% of reduction of the gastrocnemius muscle diameter in immobilizations for 14 days (21, 32) or 21 (33).

The literature points out atrophy by immobility varying from 15% to 70% (34) and along two months, a normal muscle may lose up to half of its volume (35).

According to these reports and considering that immobilization is still an option of treatment frequently used, although it brings undesirable deleterious effects to the muscle fibers (36) directly influencing musculoskeletal lesions rehabilitation, this study had as an objective verifying the effects of articular immobilization in soleus muscles from rats, through histomorphometric analysis in periods of 21 and 45 days.

Materials and methods

The present study received approval from the Committee for Ethical Conduct in Animal

Experimentation of the Universidade Estadual de Maringá (CEAE), report 011/2009. A total of 10 male *Rattus navegicus albinus*, Wistar variety (three to four months, body mass between 250–300 g). They were divided into two groups (groups I and II), five animals each, the first group underwent immobilization for 21 days and the second one for 45 days. The control of the experiment was made through the right contralateral limb of the animal.

The animals stayed at Centro Universitário de Maringá (Cesumar) animal house being treated according to the recommendations from *Guide for the care and use of laboratory animals* (37), where they stayed in polypropylene cages (39 cm length x 32 cm width x 16 cm height), covered with wood shaves. The environment was acclimatized and controlled to periodic photo cycles 12 hours light/dark, with water and rat food (Purina™ for rodents) *ad libitum*. The general monitoring of the experimented member happened daily.

The animals were anaesthetized with an anesthetic association of Ketamine at 50 mg/kg⁻¹ combined with 10 mg/kg⁻¹ Xylazine, with the dose of 0.1 mL for each 100 g of weight, so that the animals were deeply sedated for the realization of the left posterior member immobilization. This, through a molded aluminum orthosis to the segment to be immobilized in 90° ankle dorsal extension and flexion (38) which permitted the ambulation with weight unloading. This way, when necessary, it was replaced, respecting our procedure. The immobilization didn't stop the load locomotion with weight unload and the animals feeding inside the cage.

After the experimental period, the animals were sacrificed, according to the immobilization phases, with a lethal dose of thiopental sodium 45 mg/kg (1g) diluted in water (22 mL), applied via intraperitoneal. After that, the dissection of soleus muscle occurred for histological verification.

For histomorphometric analysis, the soleus muscle was withdrawn, being careful so it was possible to maintain the muscle fibers longitudinally disposed in relation to the biggest axis of the fragment, and frozen in liquid nitrogen, following the frozen method of non-fixed tissues (39), for posterior freezer store (-80 °C). Each muscle fragment was withdrawn and subjected to microtomy at -20 °C (Leica CM 1985 cryostat™), μm of thickness, they were flushed with hematoxylin/eosin (HE) and fixed with *Permount* resin.

After the blade preparation, the observation was auxiliated by Olympus BX40 microscope and obtained in photomicroscope BX50, with photographic equipment PM10-AK. Areas of transverse section of 120 soleus muscle fibers by animal were analyzed, thus determined: 10 fibers by field, being 3 fields by cut, and 4 by animal, in a way which were evaluated the shape and the muscle fiber area (40, 41, 42).

Both groups were analyzed through the method of average measurement of larger and smaller diameters by Image Pro-Plus 4.5 with objective of 10X (43). To statistical analysis the t test of *Student*, with significance level of 5%.

Results

It was observed in the controlled group the presence of muscle fibers with polygon outlines and regular fascicular pattern (Figures 1A and 1C). It was observed in the experimental group the presence of distinct outlines (polymorphism) of muscle fibers from soleus muscle. It was verified in this group the rounding and diameter decrease of muscle fibers, as well as the increase of the space occupied by endomysium and perymysium demonstrating a clear increase in the occupied area by the connective tissue (Figures 1B and 1D).

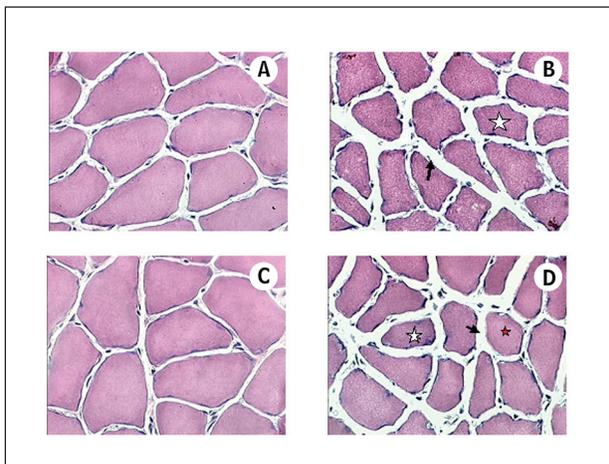


Figure 1 - Photomicroscopes of transverse sections of the middle third from the soleus muscle

Note: A: control group; B: experimental group 21 days; C: control group; D: experimental 45 days. Observe reduction of the muscle fibers area (star), increase of connective tissue (arrow) and rounding (red arrow) of fibers in relation to the control group (HE – magnitude of 320x).

Concerning the histomorphometric analysis, in both periods of immobilization there was a reduction of the transverse section of the fibers under the average calculation of larger and smaller diameters in relation to the comparison to its respective contralateral member (control). This decrease was of 63.8% (38.82 ± 3.86) and of 66.8% (41.65 ± 3.95) in the animals of 21 and 45 days of immobilization, respectively. The values were verified by statistic tests with significant importance ($p < 0.0001$). It is important to emphasize the observation ($p < 0.0001$) of significant importance among the experimental groups of 21 and 45 days, suggesting the progressive exacerbation of hypertrophic degree (muscle volume) when related to the immobilization time (Table 1).

Discussion

The findings of experimental models done so far do not converge to a general agreement referring to the physiological-histochemical alterations provoked by the muscle disuse, having contradictory conclusions. These denote differential percentage of susceptibility to hypertrophy antagonistic functional equivalence in relation to each type of muscle fiber and its specific location (1, 4, 8, 16, 21).

The soleus muscle composed of 84% of fibers of type I was used for being a biarticular muscle and for acting in static posture as dynamic creating tensile and compressive strength in knees and ankle articulations.

The results of the present study point out the existent association between the transverse area of the muscle fiber and the articular immobilization, during 21 and 45 days. Järvinen et al. (44) report that the longer the exposure period, the bigger the number of collagen deposit between the muscle fibers and the reduction of myofibril volume, interfering in the muscle regeneration process and consequently in the rehabilitation. At articular level, this tissue increase conjunct reduces the articular amplitude of movement, which is harmful to synovial fluid production, therefore the articulation lubrication and the nutrition of articular cartilage. The results reached with the histomorphometric analysis show and increase in the occupied space by the endomysium and perymysium from the animals from the experimental group from 21 and 45 days.

Table 1 - Average and standard deviation of the transverse section area (in micrometers) of the analyzed fibers from the soleus muscle from the analyzed animals (groups: control and experimental) and value of p

Experimental period	Control group (n = 5)	Experimental group (n = 5)	p*
21 days	60.83 ± 1.79	38.82 ± 3.86	< 0.001
45 days	62.31 ± 2.87	41.65 ± 3.95	< 0.001

Note: *t test significant to comparison of the average.

As well as Chakravarthy et al. (45) that demonstrated a significant reduction of soleus muscle mass after three weeks of immobilization and Mercier et al. (3) emphasized that through the suspension of weight unloading, there is a reduction of 40% in the soleus muscle weight in the same period. In a study from 2004 (46) the authors point out a muscle weight reduction of 33.87 % in the soleus and of 15.08% in the long finger extensors during the period of 45 days of articular restriction.

Chingui et al. (47) in their studies conclude that the biggest homeostatic compromising occurs in the initial phase of disuse. In this context, it is important to enhance that the determination of the results of studies on immobilization present a multifactorial factor differing regarding the model and used material to promote disuse, the period of immobilization, the articular position and the type of muscle fibers analyzed (27, 28, 30, 36).

Similar to the observation of other authors (48) in the studied samples, we found morphological alterations (presence of diverse outlines of the muscle fibers) and the sharp decline of the muscle fibers diameters after 21 days of immobilization, which corroborates with the findings, Okita et al. (19), Järvinen et al. (49) and Itai et al. (50) report about hypertrophy in the period of three, five and eight weeks, respectively.

As a limitation of the study, we point out the control of the experiment made through right contralateral member from the respective animal, which may have interfered in the movement of the control group initial phase after immobilization.

Conclusion

The articular immobilization of rats for 21 and 45 days alters the transverse section area of the soleus muscle fibers, in other words, it provokes cellular

hypertrophy with significant deleterious effects of cellular components. It is also concluded that the increase of the area of the intermuscular septum indicate a proliferation of the connective tissue of muscle covering and that the biggest alterations are found in the muscle fibers of rats' immobilization for a longer period.

It is suggest experiment studies with group control composed of samples regardless the experimental group.

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