Rev Bras Cineantropom Hum

original article

DOI: http://dx.doi.org/10.5007/1980-0037.2016v18n1p1

Joint immobilization: effects on muscular tissue of obese and malnourished mice

Imobilização articular: efeitos sobre o tecido muscular de camundongos obesos e desnutridos

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Abstract – Although it is a widely used resource for the treatment of musculoskeletal injuries, immobilization causes deleterious effects in muscle tissue after a short period of time. This study aimed to evaluate the gastrocnemius and tibialis anterior muscles of obese and protein malnourished animals under joint immobilization condition. Overall, 28 adult male mice were used (C57 / BL6), being divided into four groups (N = 7): Control Group (CG), Immobilized Control Group (ICG), Immobilized Obese Group (IOG) and Immobilized Malnourished Group (IMG). The immobilization protocol was performed by the use of adhesive tape and plaster. The conditions and obesity and protein malnutrition have been developed through the ingestion of diets specific for each group of animals. The histomorphometric analysis of muscles evaluated area and the diameter of muscle fibers. All immobilized groups showed reduction in the area and diameter of muscle fibers when compared to GC. Comparisons among immobilized groups showed that the area and diameter of muscle fibers of IOG and IMG were lower than ICG. The immobilization protocol caused reduction in muscle trophism in animals, and obese and malnourished animals suffered high losses under condition of muscle atrophy.

Key words: Immobilization; Muscular atrophy; Protein malnutrition; Obesity.

Resumo – *Embora seja um recurso muito utilizado para tratamento de lesões musculoesqueléticas, a imobilização causa efeitos deletérios no tecido muscular após curto período de tempo. O presente estudo teve como objetivo avaliar os músculos gastrocnêmio e tibial anterior de animais obesos e desnutridos proteicamente sob a condição de imobilização articular. Foram utilizados 28 camundongos (C57/BL6) machos adultos, distribuídos em quatro grupos (N=7): Grupo controle (GC), Grupo Controle Imobilizado (GCI), Grupo Obeso imobilizado (GOI) e Grupo Desnutrido Imobilizado (GDI). O protocolo de imobilização foi realizado por meio da utilização de tiras de esparadrapo e faixa gessada. As condições de obesidade e desnutrição proteica foram desenvolvidas por meio da ingestão de dietas específicas para cada grupo de animais. A análise histomorfométrica dos músculos avaliou a área e o diâmetro das fibras musculares. Todos os grupos imobilizados apresentaram redução na área e no diâmetro das fibras musculares quando comparados ao GC. As comparações entre os grupos imobilizados mostraram que os valores do diâmetro e área de fibras dos grupos GOI e GDI foram menores do que o GCI. O protocolo de imobilização provocou redução do trofismo muscular nos animais estudados e os animais obesos e desnutridos sofreram prejuízo elevado na condição de atrofia muscular.*

Palavras-chave: *Atrofia muscular; Desnutrição proteica; Imobilização; Obesidade.*

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Received: 31 July 2015
Accepted: 21 November 2015

INTRODUCTION

About 40% of the human body is composed of skeletal muscle tissue which main function is to regulate the motor responses for the performance of daily physical activities[1,2]. According to Ferreira et al.[3], the structure and function of the muscle tissue are conditioned by factors such as innervation, mechanical load imposed to the tissue, proprioceptive activity and performance of stretching/shortening cycles. The commitment of one of these factors will certainly lead to muscle atrophy.

Although it is a widely used resource for the treatment of musculoskeletal injuries, joint immobilization causes deleterious effects on the affected tissue, such as muscle atrophy, decreased protein content, disturbances in the area / connective tissue of fibers, increased fatigability, loss of strength and contractile activity. However, its physiological, biochemical and muscular understanding still needs to be elucidated[4].

Poor eating habits are associated with several health hazards, including obesity, whose indexes have grown in recent decades, leading to a serious public health problem in Brazil and worldwide[5]. According to De-Farias[6] and Kimbal et al.[7], obesity often interferes in the relationship of the insulin hormone with the mechanisms of protein synthesis and degradation in the muscles. Considering that insulin plays a fundamental role in the synthesis and inhibition of proteolysis, in obese people, due to disorders in the insulinemic profile, they may show negative balance regarding the formation and degradation of body muscle mass.

Malnutrition is characterized as a disease that results from insufficient food intake and energy and nutrients or even inadequate biological utilization of the ingested food. Whereas the incidence of malnutrition is increasing worldwide, such a condition has been considered a serious social problem and emerges as a worrying disease in the public health context[8].

Muscle tissue is the most important source of protein available in the body; however, in cases of severe malnutrition, this tissue is considerably reduced. Thus, during total or partial fast, there is loss of body protein in order to provide the conversion of amino acids into energy, resulting in total body weight loss[9,10].

Based on the observations above, this study aimed to evaluate the skeletal muscle tissue of obese animals and those submitted to protein malnutrition under joint immobilization condition. It was considered that both morbidities associated with the use of immobilization may show cellular and molecular interactions that could enhance musculoskeletal disorders.

The characterization of muscle tissue through experimental techniques was aimed at assessing the risk/benefit that the joint immobilization protocol provides for obese patients and those under protein malnutrition, as they are routinely at risk of being through procedures of this nature.

This research presented experimental scientific information in order to contribute to the clinical practice in cases in which carriers of the aforementioned morbidities have to be submitted to joint immobilization.

METHODOLOGICAL PROCEDURE

Experimental design

The project was approved by the Ethics Research Committee on Animal Use of the State University of Campinas (CEUA/Unicamp), under No. 2872-1 of 10/04/2012.

Overall, 28 male adult mice were used (C57 / BL6) (105 days) from the animal facilities of CEMIB/UNICAMP, kept in standard plastic cages under temperature of 22°C with light/dark cycle of 12 hours receiving *ad libitum* water and diet specific for each group.

Animals were divided into four groups as follows: Control Group (CG, n = 7), Immobilized Control Group (ICG, n = 7), Immobilized Obese Group (IOG, n = 7) and Immobilized Malnourished Group (IMG, n = 7). Immobilization of the pelvic limb was held on the 91st day of life and maintained until the end of the experiment (105th day).

Obesity and protein malnutrition induction

To induce obesity, soon after weaning, animals received normal protein diet (14% protein) until they reached 45 days of age. Then, up to the end of the experiment (105th day), mice received low-protein and high-fat diet, containing 34% fat. However, to induce protein malnutrition, after weaning, animals received low-protein diet containing 6% protein. The intake of this diet remained until the end of the experimental period (day 105)[11].

Body weight of animals and weight of retroperitoneal and perigonadal adipose tissues were used as variables for evaluation and characterization of obese and malnourished groups.

Immobilization Protocol

To perform the immobilization protocol, the following steps were followed: animals were previously anesthetized with a mixture of Ketamine (50mg/kg body weight) + xylazine (8mg/kg of body weight). Subsequently, the right hind limb was previously trichotomized and immobilized with hypoallergenic micropore (Cremer®). Then, immobilization was carried out with strips of waterproof adhesive tape - Cremer® (3 cm wide), which included pelvis, hip, knee (both in length) and ankle (plantar flexion). Subsequently, a strip of plaster Cremer® (2 cm in width and 10 cm in length) was moistened and applied without excessive torque pressure around the animal's limb in order to prevent it from destroying immobilization performed with adhesive tape. Daily, immobilization was checked and, when damaged, was replaced. Immobilization was maintained for a period of two weeks (14 days).

Euthanasia and material collection

At the end of the experimental treatment (105th day), animals were anesthetized by carbon dioxide inhalation and euthanized by decapitation. Subsequently, dissection and removal of the gastrocnemius and tibial anterior muscles were performed, which were weighed and measured. Then,

retroperitoneale perigonadal adipose tissues were also dissected, isolated and weighed on analytical scale (Shimadzu, Model AUW220D).

Preparation of the material and histomorphometric analysis

After dissection, the ventral segment of the gastrocnemius and tibialis anterior muscles were fixed in 10% buffered formalin solution for 24 hours at 22°C. Protocol was followed with subsequent washing of samples, subsequent dehydration in alcohol baths, diaphanization with xylene baths and routine process of inclusion in paraffin.

After these steps, cross sections with thickness of 7mm were performed with a microtome (Leica® model RM2125RT). Subsequently, sections were stained with hematoxylin-eosin technique and completion of slides with cover slips in Canada Balm.

For histomorphometric study of images, they were captured using optical microscope (Olympus® model BX53) and a camera (Olympus® model QCOLLOR5) coupled to it. All images were captured at 10X objective.

This particular study on muscles quantified the area and diameter of muscle fibers with the aid of software (Image_J). The areas and diameters of 225 fibers of tibialis anterior and gastrocnemius muscles per animal were thus obtained: 15 fibers per field, three fields per section, a total of five sections per animal. A lattice grid was used to define the field and the choice of 15 fibers was performed randomly. Muscle fibers of seven animals per group were quantified (N = 7), totaling 1575 fibers in each group.

Statistical treatment

Data normality was verified by the Shapiro-Wilk test. In case of normal data, analysis of variance (One-way ANOVA) and the "post hoc" Tukey test for multiple comparisons were used. This form of analysis was applied in the following variables: weight of the gastrocnemius and tibialis anterior muscles (mg), body weight (g), weight of the retroperitoneal and perigonadal adipose tissues (mg).

In case of non-normality of data, the nonparametric Kruskal-Wallis was used, followed by "post hoc" Dunns for multiple comparisons. This form of analysis was applied to the area and diameter of muscle fibers. Statistical significance was set at $p < 0.05$, using as analysis resource the GraphPadPrism version 5.00 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com).

RESULTS

In a study previous to the immobilization procedure, when compared to the 91st days, IOG presented weight (g) 18% higher than CG, while IMG presented weight 7.4% lower than the control group ($p < 0.05$, Table 1). ICG showed no statistical difference when compared to CG ($p < 0.05$, Table 1).

In comparison made on the day of sacrifice (105th day), ICG and IMG showed, respectively, a decrease of 6.7% and 16.5% in body weight (g) when

compared to the control group (p <0.05, Table 1). IOG presented body weight 16.9% higher compared to the control group (p <0.05, Table 1). When comparing immobilized groups, it was observed that IOG showed body weight 25.3% higher compared to ICG. IMG showed reduction of 10.5% in body weight when compared to ICG (p <0.05, Table 1).

Results related to the retroperitoneal adipose tissue revealed an amount 42.4% higher in the retroperitoneal adipose reserve of IOG when compared to GC, and an amount 68.1% higher when compared to ICG (p <0.05, Table 1). IMG showed no significant difference in the retroperitoneal adipose reserves in relation to GC and ICG (p <0.05, Table 1).

The results observed in the perigonadal adipose tissue revealed that IOG had perigonadal fat reserves 23.2% higher than the GC and 27.7% higher than the ICG (p <0.05, Table 1). However, IMG showed rate 32.1% below the amount of perigonadal fat tissue in relation to CG and 29.6% lower in relation to ICG (p <0.05, Table 1).

Table 1. Body weight (g) at the 91st day, Body weight (g) at the 105th day, weight of retroperitoneal and perigonadal adipose tissues (mg) evaluated according to the specific diet ingested by each group.

Groups	Body weight (g) 91st day	Body weight (g) 105th day	Retroperitoneal adipose tissue (mg)	Perigonadal adipose tissue (mg)
CG	25.5±0.4	25.4±0.2	85.56±5.6	254.1±7.9
ICG	25.6±0.6	23.7±0.2*	72.49±1.8	245.1±11.4
IOG	30.1±0.5*†	29.7±0.4*†	121.9±87.9*†	313.1±14.5*†
IMG	23.6±0.6*†	21.2±0.3*†	68.11±4.6	172.5±13.9*†

GC = control group; ICG = Immobilized Control Group; IOG = Immobilized Obese Group; IMG = Immobilized Malnourished Group; * statistically significant values compared to GC; † Statistically significant values in relation to ICG (p <0.05). Values expressed as mean ± standard error.

Weight and histomorphometric data of gastrocnemius muscle are reported in absolute values and in percentage in Table 2 for a better understanding of the variations in the course of the text.

In the observation of the gastrocnemius muscle mass, CG presented muscle weight 10.1% higher when compared to ICG. However, IOG and IMG showed values 5.9% and 23.1% lower than the CG (p <0.05, Table 2). In the observation of immobilized groups, IMG showed reduction of 14.5% in muscle mass compared to ICG (p <0.05, Table 2).

The fiber area of ICG was 43.7% lower than CG, while IOG and IMG presented, respectively, fiber area 49.6% and 59.7% lower than CG (p <0.05, Table 2). When compared immobilized groups only, statistical differences were observed in muscle fiber area of IOG and IMG compared to ICG. The fiber area of IOG was10.5% lower compared to ICG, similarly, the fiber area of IMG was 28.4% lower when compared to ICG (p <0.05, Table 2).

Studying the diameter of the gastrocnemius muscle fibers, immobilized ICG IOG and IMG groups showed, respectively, a decrease of 21.1%, 29.7% and 36.7% in the diameter of their fibers when compared to GC (p <0.05, Table 2). When analyzing immobilized groups only, there was a decrease of 10.8% in IOG and 19.7% in IMG compared to ICG (p <0.05, Table 2).

Table 2. Muscle mass (mg), area (μ^2) and fiber diameter (μ) of the gastrocnemius muscle

Groups	Weight (mg) Gastrocnemius muscle	Fiber area μ^2 Gastrocnemius muscle	Fiber diameter μ Gastrocnemius muscle
CG	131.9±1.3	1722 (1558 - 1977)	62.9 (56.1 – 69.3)
ICG	118.6±1.5*	970 (866 – 1125)*	49.6 (44.6 – 55.1)*
IOG	124.1±1.6*	868 (745 – 998)*†	44.2 (39.6 – 49.2)*†
IMG	101.4±1.3*†	694 (618 – 800)*†	39.8 (36 – 44)*†

CG = control group; ICG = Immobilized Control Group; IOG = Immobilized Obese Group; IMG = Immobilized Malnourished Group; * statistically significant values compared to GC; † Statistically significant values in relation to ICG ($p < 0.05$). Weight of gastrocnemius muscle (g) expressed as mean ± standard error. Fiber area and diameter of gastrocnemius muscle expressed as median with 1st and 3rd quartiles.

The tibialis anterior muscle followed the same variation of the gastrocnemius muscle in the analysis of the area and diameter of muscle fibers, demonstrating significant differences in the comparisons among groups ($p < 0.05$, Table 3).

In the observation of the anterior tibial muscle mass, only IMG decreased significantly by 10.9% compared to the control group and compared to ICG ($p < 0.05$, Table 3). The other groups showed no statistically significant differences when compared to each other.

The values found showed a decrease of 43.8%, 52.1% and 47% in the area of muscle fibers of ICG IOG and IMG, respectively, when compared to the control group ($p < 0.05$, Table 3). Analyzing immobilized groups only, IOG had a decrease of 5.6% and IMG of 14.6% when compared to ICG ($p < 0.05$, Table 3).

In the study of the diameter of fibers of anterior tibial muscles, it was observed that ICG, IOG and IMG showed a decrease of 24.1%, 26.2% and 31.8%, respectively, compared to the control group ($p < 0, 05$, Table 3). The analysis of immobilized groups showed a reduction of 2.8% and 10.1% of IOG and IMG, both in relation to ICG ($p < 0.05$, Table 3).

Table 3. Muscle mass (mg), area (μ^2) and fiber diameter (μ) of the tibialis anterior muscle

Groups	Weight (mg) Tibialis anterior muscle	Fiber area μ^2 Tibialis anterior muscle	Fiber diameter μ Tibialis anterior muscle
CG	40.5±1.1	1202 (1101 – 1340)	51.9 (48 – 56.4)
ICG	40.5±0.9	675 (622 – 742)*	39.4 (35.7 – 42.5)*
IOG	41.5±0.7	637 (593 – 710)*†	38.3 (35.2 – 41.8)*†
IMG	36.1±1.1*†	576 (498 – 648)*†	35.4 (33 – 38.7)*†

CG = control group; ICG = Immobilized Control Group; IOG = Immobilized Obese Group; IMG = Immobilized Malnourished Group; * statistically significant values compared to GC; † Statistically significant values in relation to ICG ($p < 0.05$). Weight of tibialis anterior muscle (g) expressed as mean ± standard error. Fiber area and diameter of tibialis anterior muscle expressed as median with 1st and 3rd quartiles.

DISCUSSION

About 40% of the mammalian body is composed of skeletal muscle tissue and its remodeling process, mass and composition is an ongoing process for adapting the functions imposed to the tissue in daily tasks. Therefore,

in case metabolic and structural changes occur in muscle tissue, there will be, as a result, imbalance between the conditions imposed to the muscle in its daily tasks. In this context, the use of joint immobilization ultimately generates a commitment in the homeostasis of muscle fibers[12].

In this study, the gastrocnemius and tibialis anterior muscles were prioritized because they have different characteristics and anatomical proximity. The immobilization protocol used led to loss of gastrocnemius muscle mass in all immobilized groups compared to the control group. However, the weight of the tibialis anterior muscle was significantly reduced only in IMG when compared to GC.

Based on the results obtained, it was observed that the tibialis anterior muscle suffered less damage compared to the gastrocnemius muscle. This can be explained by the fact that the tibialis anterior muscle has remained in the elongated position during the immobilization period and, according to Williams and Goldspink[13], this position favors a gradual atrophy of muscle tissue. In agreement with our results, Durigan et al.[14] observed that immobilization for different periods and methods results in muscle atrophy, ranging from 15% to 70%, depending on the animal model used and muscle fiber evaluated.

The results expressed in Tables 2 and 3 showed reduced cross-sectional area and diameter of the muscle fibers of skeletal muscles in immobilized groups compared to the control group. This indicated that there was loss and, consequently, muscle tissue atrophy, which is consistent with findings of different researchers in relation to skeletal muscle submitted to immobilization[15].

Reinforcing the results of this research, Slimani et al.[16] demonstrated that gastrocnemius and tibialis anterior muscles, when immobilized, had higher percentage of muscle tropism compared to their controls and greater commitment of the gastrocnemius muscle. This episode can be explained by at least two hypotheses: 1) difference of anatomical position between muscles and 2) difference of fiber types.

Regarding the anatomical position, muscles whose actions are antigravity, as in the case of gastrocnemius muscle, located in a posterior position in relation to the anterior tibial muscle, showed higher degree of atrophy in disuse situations as in joint immobilization[17]. In relation to difference in fiber types, Talmadge[18] found that type I fibers have less ability to adapt when compared to type II fibers and are thus more intensely affected by disuse situations.

Although the present study shows limitations for not individually assessing types I and II muscle fiber, it was verified that the greatest damage occurred to the gastrocnemius muscle, as it is a mixed muscle and with predominance of type I fibers, while the tibialis anterior muscle presents predominance of type II fibers. However, detailed studies on this issue should be conducted in order to provide greater scientific information on the atrophy of type I and II muscle fibers in periods of joint immobilization.

Comparing immobilized groups with each other, it was observed that

the weight of the gastrocnemius and tibialis anterior muscles decreased significantly in IMG when compared to ICG, while the weight of these muscles in IOG did not show statistically significant differences compared to ICG. However, when the cross-sectional area and diameter of fibers were analyzed, both IOG and IMG showed significant reduction of these variables compared to ICG.

Considering these results, it was observed that muscle tissue of animals from IOG and IMG groups suffered severe damage when submitted to the immobilization protocol. Based on these results, it was suggested that animals that fed with high-fat (IOG) and low-protein diets (IMG) have developed mechanisms that have adversely affected muscle trophism, which can cause more damage to the homeostasis of the muscles studied when compared to ICG.

Kimball et al.[7] reported that the loss of muscle mass is a common feature in the diabetic / obese state, food restriction or even in prolonged physical inactivity, as in cases of joint immobilization.

Based on the above, it was observed that both conditions imposed and the resulting interaction between immobilization/obesity and immobilization/malnutrition proved to be decisive factors for the worsening of muscle atrophy in IOG and IMG groups.

For the development of skeletal muscle and maintenance of muscle mass in individuals, there needs to be insulin supply and adequate amount of contractile activity[19]. The effects of insulin on protein metabolism are especially important in muscle tissue. Thus, the anabolic effects of this hormone are reinforced by its anti-catabolic actions. However, insulin inhibits proteolysis, suppresses the release and inhibits oxidation of essential amino acids[20].

Given these characteristics, it is believed that animals from IOG and IMG groups suffered more severe damage to the muscle tissue because in addition to being affected by immobilization condition, usually when in obesity and protein malnutrition conditions, animals present disturbances in the mechanisms of supply and use of the insulin hormone, which enhances the muscle catabolic state.

Particularly in skeletal muscle, insulin resistance condition creates a compromised gluconeogenesis mechanism, with consequent lack of energy substrate for the muscle to make its shortening/stretching cycle. Thus, muscle affected by these conditions may trigger an insulin resistance atrophy process[20].

Corroborating the findings of this research on protein malnutrition, experimental studies have shown that the offspring of rats exposed to this procedure showed a decrease in the area and diameter of the gastrocnemius and tibialis anterior muscle fibers[21]. Similarly, other studies have shown that the offspring of rats exposed to protein malnutrition during pregnancy and lactation showed alterations in the number and proportions of muscle fibers, as well as insulin resistance in adulthood[22].

In the case of association between obesity, protein malnutrition and joint immobilization, the muscles analyzed showed increase in atrophy

in relation to each isolated condition. Therefore, the joint immobilization procedure of an obese or malnourished patient must be careful, especially in relation to the consequences that immobilization causes to metabolism which, by themselves, in conditions of obesity and malnutrition, generates a significant muscle involvement.

CONCLUSION

Based on the histomorphometric results, it was concluded that the immobilization protocol led to reduced muscle trophism in the animals studied. In the case of associations between obesity, protein malnutrition and immobilization, the muscles analyzed showed reduced muscle trophism, indicating that animals under these conditions showed high muscle damage.

REFERENCES

1. Zhang P, Chen X, Fan M. Signaling mechanisms involved in disuse muscle atrophy. Med Hypotheses 2006;69(2):310-21.
2. Pelizzari C, Mazzanti A, Raiser AG, Lopes STA, Graça DL, Salbego FZ, et al. Estimulação elétrica neuromuscular de média frequência (russa) em cães com atrofia muscular induzida. Ciênc Rural 2008;38(3):736-42.
3. Ferreira R, Neuparth MJ, Ascensao A, Magalhaes J, Duarte J, Amado F. Atrofia muscular esquelética. Modelos experimentais, manifestações teciduais e fisiopatologia. Rev Port Ciên Desp 2004;4(3):94-111.
4. Haddad F, Roy RR, Zhong H, Edgerton VR, Baldwin KM. Atrophy responses to muscle inactivity. II. Molecular markers of protein deficits. J Appl Physiol 2003;95(2):791-802.
5. Rocha SV, Cardoso JP, Dos-Santos CA, Munaro HLR, Vasconcelos LRC, Petroski EL. Sobrepeso/obesidade em professores: prevalência e fatores associados. Rev Bras Cineantropom Desempenho Hum 2015;17(4):450-9.
6. Farias JM. Effects of physical exercise in molecular parameters of the route of obesity and insulin signaling. Rev Bras Cineantropom Desempenho Hum 2014;16(5):588-95.
7. Kimball SR, Vary TC, Jefferson LS. Regulation of protein synthesis by insulin. Annu Ver Physiol 1994;56:321-348.
8. Monteiro CA. A dimensão da pobreza, da fome e da desnutrição no Brasil. Estud Av 2003;17(48):7-20.
9. Alves AP, Dâmaso AR, Dal-Pai V. Efeito da desnutrição protéicapré e pós-natal sobre a morfologia, a diferenciação e o metabolismo do tecido muscular estriado esquelético em ratos. J Pediat 2008;84(3):264-271.
10. Amaral RB, Martins CEC, Junior AHL, Painelli VS. Can leucine supplementation attenuate muscle atrophy? A literature review. Rev Bras Cineantropom Desempenho Hum 2015;17(4):496-506.
11. Batista TM, Ribeiro RA, Amaral AG, De-Oliveira CA, Boschero AC, Carneiro EM. Taurine supplementation restores glucose and carbachol-induced insulin secretion in islets from low protein diet rats: involvement of Ach-M3R, Synt 1 and SNAP- 25 proteins. J Nutr Biochem 2012;23(3):306-12.
12. Paixão APS, Alves EGL, Bitencourt ACV, Junior JDC, Vasconcelos AS. Padronização da técnica de imobilização do membro pélvico para estudo da atrofia muscular esquelética em ratos. J Bras Ciên Ani 2011;4(7):274-91.
13. Williams P, Goldspink G. Connective tissue changes in immobilized muscle. J Anat 1984;138(Pt 2):343-50.

14. Durigan JLQ, Cancelliero KM, Dias CNK, Silva CA, Guirro RRJ, Polacow, MLO. Efeitos da imobilização articular aguda nos músculos do membro posterior de ratos: análise metabólica e morfométrica. Fisioter Pesqui 2006;13(2):38-45.
15. Santos-Junior FFU, Alves JSM, Machado AAN, Carlos PS, Ferraz ASM, Barbosa R, et al. Alterações morfométricas em músculo respiratório de ratos submetidos à imobilização de pata. Rev Bras Med Esporte 2010;16(3):215-8.
16. Slimani L, Micol D, Amat J, Delcros G, Meunier B, Taillandier D, et al. The worsening of tibialis anterior muscle atrophy during recovery post-immobilization correlates with enhanced connective tissue area, proteolysis, and apoptosis. Am J Physiol Endocrinol Metab 2012;303(11):1135-7.
17. Caiozzo VJ, Haddad F, Baker MJ, Henrrick RE, Pritto N, Baldwin KM. Microgravity induced transformations of myosin isoforms and contractile properties of skeletal muscle. J Appl Physiol 1996;81(1):123-32.
18. Talmadge RJ. Mechanical properties of rat soleus after long-term spinal cord transection. J Appl Physiol 2002;93(4):1487-97.
19. Luciano E, Mello MAR. Atividade física e metabolismo de proteínas em musculo de ratos diabéticos experimentais. Rev Paul Educ Fis1998;12(2):202-9.
20. Chingui LJ, Braquinho RP, Severi MTM, Da-silva, CA. Comportamento quimiometabólico do músculo sóleo na fase aguda da imobilização articular. Fisioter Pesq 2008;15(2):194-9.
21. Alves AP, Damaso AR, Dal-pai V. Efeito da desnutrição protéica pré e pós-natal sobre a morfologia, a diferenciação e o metabolismo do tecido muscular estriado esquelético em ratos. J Pediat 2008;84(3):264-71.
22. Toscano AE, Manhães-de-Castro R, Canon F. Effect of a low-protein diet during pregnancy on skeletal muscle mechanical properties of offspring rats. Nutrition 2008;24(3):270-8.

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