

# Eccentric exercise and stretching for plantar flexor muscles applied during 21 days after immobilization do not modify the non-contractile tissue

*Exercício excêntrico e alongamento para músculos flexores plantares aplicados durante 21 dias após imobilização não modificam o tecido não contrátil*

*Ejercicio excéntrico y estiramiento para músculos flexores plantares aplicados durante 21 días tras inmovilización no cambian el tejido no contráctil*

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**ABSTRACT** | The adaptations of the extracellular matrix, which is closely related to the maintenance of the integrity and performance of the musculoskeletal system, are not widely described in the literature after rehabilitation due to prolonged disuse. The aim of this study was to analyze the perimysial connective tissue area and the cross-sectional area of muscle fibers in soleus and plantaris muscles of immobilized female rats, later rehabilitated due to stretching and eccentric exercise protocols. The expression of the perimysial connective tissue of both studied muscles did not show significant differences after the procedure of immobilization and training. Eccentric training applied for 10 days was enough to recover the area of fibers for the plantaris muscle, while the recovery of the soleus muscle happened only after the 21-days protocol.

**Keywords** | Immobilization; Connective Tissue; Muscle Stretching Exercises; Muscle, Skeletal.

**RESUMO** | As adaptações da matriz extracelular, que está intimamente ligada à manutenção da integridade e do desempenho do sistema musculoesquelético, não estão consensualmente descritas na literatura após recarga por desuso. O objetivo deste estudo foi analisar a área de tecido conjuntivo perimisial e de secção transversa das fibras musculares nos músculos sóleo e plantar de ratas imobilizadas e posteriormente reabilitadas por protocolos de

alongamento e exercício excêntrico. A expressão do tecido conjuntivo perimisial de ambos os músculos estudados não apresentou diferença significativa após o procedimento de imobilização e treinamento. O treino excêntrico aplicado por 10 dias foi suficiente para recuperar a área das fibras para o músculo plantar, enquanto a recuperação do músculo sóleo aconteceu somente após o protocolo de 21 dias.

**Descritores** | Imobilização; Tecido Conjuntivo; Exercício de Alongamento Muscular; Músculo Esquelético.

**RESUMEN** | No figuran descriptas de manera consensual en la literatura las adaptaciones extracelulares, ligadas al mantenimiento de la integridad y del desempeño del sistema musculoesquelético tras inmovilizaciones. En este estudio se evalúa el área del permiso del tejido conjuntivo y de la sección transversal de las fibras musculares del sóleo y del plantar en ratas inmovilizadas y después rehabilitadas siguiendo los protocolos de estiramiento y de ejercicio excéntrico. En el área evaluada de ambos músculos no se observaron significativas diferencias tras el procedimiento de inmovilizarlos y entrenarlos. Aplicados en 10 días, el entrenamiento excéntrico ha sido suficiente para recuperar el área de las fibras para el músculo plantar, mientras que el músculo sóleo ha tardado 21 días en recuperarse.

**Palabras clave** | Inmovilización; Tejido Conjuntivo; Ejercicios de Estiramiento Muscular; Músculo Esquelético.

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## INTRODUCTION

The skeletal muscle represents a complex biological structure composed of contractile and non-contractile elements, which has the ability to adapt to various physiological and pathological stimuli. The immobilization procedure determines unfavorable changes in the muscle, such as muscle atrophy and increase of the intramuscular connective tissue, leading to loss of extensibility and limitation of movements<sup>1-4</sup>.

Facing the deleterious adaptations caused by disuse and responsiveness of the musculoskeletal system, muscle rehabilitation techniques have been developed and improved to alleviate or reverse these adaptations. Responses of the non-contractile tissue regarding immobilization and rehabilitation are little studied in the literature, since the role of these elements became more apparent in the last decades with the improvement of cellular and molecular biology techniques<sup>5</sup>.

The extracellular matrix of the skeletal muscle is composed of collagen, proteoglycans, and metalloproteinases, these structures involve the satellite cells, which are essential in muscle regeneration<sup>5</sup>. In adult Wistar rats, the immobilization procedure significantly reduced the amount of type I collagen and type III collagen did not show quantitative changes, compared with the control group<sup>6</sup>. However, the disuse followed by rehabilitation of passive stretching type applied for 10 days, made up of 10 repetitions of 30 seconds with 30-second intervals, determined a significant increase of type III collagen, compared with the control group<sup>6</sup>.

After four weeks of immobilization and application of stretching in daily sessions or 3 times a week (10 stretches held by 1 minute with 30-second interval), we concluded that the protocols were effective in recovering the cross-sectional fiber area of the soleus muscle, compared with the contralateral muscle and with the control group<sup>7</sup>. Stretching is an important technique capable of reducing the disorganization and aggregation of collagen bundles and preventing the loss of sarcomeres in series of immobilized muscles<sup>1,7</sup>, being recommended to prevent injuries<sup>8</sup>. Stretching programs used by several researchers, after segmental immobilization, are effective in restoring the range of motion to the normality patterns of the joints<sup>6,8,9</sup>.

The development of greater tension in eccentric contractions, compared with the concentric ones, occurs due to the removal of myofilaments and due to the development of passive tension in the elastic

elements, titin and desmin. In addition, it is known that eccentric training determines further development of strength, and this fact is directly associated with the increased myofibrillar protein synthesis and muscle hypertrophy<sup>10-12</sup>. Despite the adjacent anatomical location, on the back of the leg, the muscles elected for this study have different cytoarchitectural and biochemical characteristics. The soleus muscle is composed predominantly of type I fibers, given its constant activation in static posture. The plantaris muscle, however, has low proportion of type I fibers, showing predominantly type IID fibers<sup>13</sup>. The soleus muscle, postural muscle, is still a vigorous decelerator in the support phase, and is potentially enabled during eccentric training, which is the most physiological training after disuse. These results can provide, even if indirectly, knowledge about the election and implementation of rehabilitation techniques adopted in physiotherapy. Therefore, therapeutic protocols, such as stretching and eccentric exercise, promote the morphofunctional reestablishment of the skeletal musculature after situations of disuse.

The aim of this study was to investigate if the performance of 10 and 21 days of eccentric exercises or muscle stretching modify the connective tissue area and the cross-sectional area of muscle fibers, as well as soleus and plantaris muscles of rats submitted to immobilization.

## METHODOLOGY

### Animals and procedures

This study was approved by the Research Ethics Committee of the Ribeirão Preto School of Medicine of the University of São Paulo, under protocol no. 043/2007. Forty-two Wistar female rats with 81 days of age were divided into 8 groups: 1) Immobilized Control (ICG); 2) Control of 10 days (CG<sub>10</sub>); 3) Control of 21 days (CG<sub>21</sub>); 4) Immobilized for 10 days (IG); 5) Immobilized for 10 days and eccentrically trained for 10 days (IETG<sub>10</sub>); 6) Immobilized for 10 days and eccentrically trained for 21 days (IETG<sub>21</sub>); 7) Immobilized for 10 days and stretched for 10 days (ISG<sub>10</sub>); and 8) Immobilized for 10 days and stretched for 21 days (ISG<sub>21</sub>). Control groups of 10 and 21 days were used for a comparison paired by age with the trained groups. For immobilization, the animals

were anesthetized in advance (Ketamine-95 mg/kg and Xylazina-12 mg/kg), the right tibiotarsal joint was placed in maximum plantar flexion and a device composed of a lined structure of steel anatomically accompanied the restriction of the right lower limb<sup>14,15</sup>.

After immobilization, the animals of IETG<sub>10</sub> and IETG<sub>21</sub> ran downhill on a treadmill for a period of 10 and 21 days, respectively. The treadmill exercise protocol began with 10 minutes a day, with daily increments of 5 minutes, until completing 40 minutes, with an average speed of 17 m/min and downward slope of 16 degrees. The exercise was conducted for three consecutive days, followed by one day of rest<sup>16-18</sup>. The animals of ISG<sub>10</sub> and ISG<sub>21</sub> were subjected to stretching protocols for 10 and 21 days, respectively. The stretching technique was obtained by maximum dorsiflexion, held by duct tape<sup>19</sup>. The stretching protocol followed the same eccentric training intervals, starting with 10 minutes of daily stretching for 3 consecutive days, with 1-day interval. There were daily increments of 5 minutes, until 40 minutes of stretching was completed. The animals were euthanized by overdose of anesthetics, immediately after the last protocol session, and the soleus and plantaris muscles were removed. The fragments were involved in talcum powder, frozen in liquid nitrogen, and stored in a freezer at -80 °C.

### Histological processing and morphometric analysis

Histological sections of 5 µm thick were obtained in cryotome Leica CM 1850 UV (-25 °C) (Leica Instruments GmbH, Heidelberg, Germany) and subsequently stained with modified gomori trichome stain. Morphometric analysis was performed with the aid of QualiView software – Atonus software (Atonus Engenharia de sistemas LTDA, São José dos Campos, São Paulo, Brazil) whose images were captured on digital video camera Leica DFC 300FX, connected to a microcomputer, using the light microscope Leica DM 2500. The connective tissue area was analyzed from three random fields, from the blades of soleus and plantaris muscles at 40× objective. The total area of each field was 91860.51 µm<sup>2</sup>, composed of connective tissue and muscle fibers. To determine the area of the perimysial connective tissue, all the muscle fibers from each field (total area of the fibers in the field) were initially measured.

The measurement of the fibers was manual and they were examined individually by the same evaluator. Subsequently, from the total area (3×91860.51 µm<sup>2</sup>), the total value of the area of fibers obtained in the three fields was subtracted.

### Statistics

The PROC MIXED procedure of the SAS program was used, multiple comparisons of the means of connective tissue areas were made among the different groups using a heteroscedastic linear regression model (which considers groups with unequal variances), considering a Bonferroni correction. The information of repeated measurements of the area for each animal was introduced in the model by random effects. The normality assumption of the model's residuals was verified by graphic methods. For the inferences, we considered a significance level of 5%.

## RESULTS

### Morphological and morphometric analysis

The results indicated that no group showed significant change in the perimysal connective tissue area of soleus and plantaris muscles (Figure 1 and 2). On the other hand, the mean of the cross-sectional area of the soleus muscle from the IG reduced significantly when compared with the ICG (ICG × IG;  $p < 0.0001$ ). Both eccentric training and stretching protocols applied for 10 or 21 days increased the mean of muscle fiber areas (IG × ISG<sub>10</sub>; IG × ISG<sub>21</sub>; IG × IETG<sub>10</sub>; IG × IETG<sub>21</sub>;  $p < 0.0001$ ). However, only the eccentric training held for 21 days was effective in restoring the cross-sectional area of the fibers, compared with the controls (CG<sub>21</sub> × IETG<sub>21</sub>; NS) (Table 1).

In the plantaris muscle, the period of immobilization significantly reduced the cross-sectional area of muscle fibers (ICG × IG;  $p < 0.0001$ ). Both eccentric training and stretching protocols applied for 10 or 21 days increased the mean of muscle fiber areas (IG × ISG<sub>10</sub>; IG × IETG<sub>10</sub>; IG × ISG<sub>21</sub>; IG × IETG<sub>21</sub>;  $p < 0.0001$ ). We also observed that in this muscle, the eccentric training applied for 10 and 21 days was effective in restoring the cross-sectional area of muscle fibers. (CG<sub>10</sub> × IETG<sub>10</sub>; CG<sub>21</sub> × IETG<sub>21</sub>; NS) (Table 2).

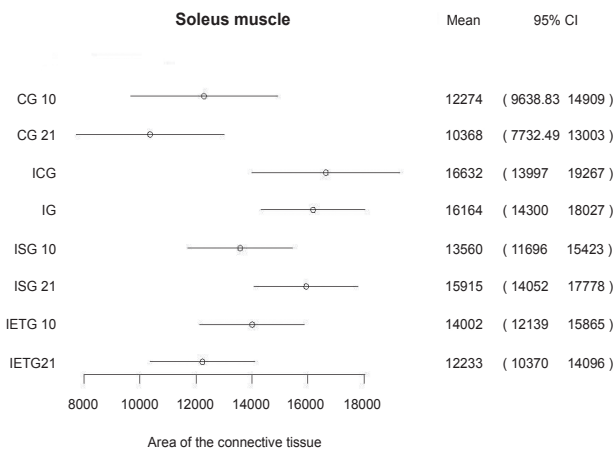


Figure 1. Mean of the connective tissue area with 95% confidence intervals of the soleus muscle from different groups

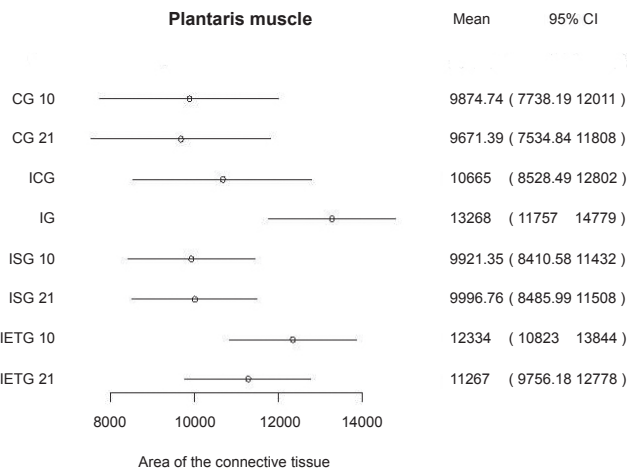


Figure 2. Mean of the connective tissue area with 95% confidence intervals of the plantaris muscle from different groups

Table 1. Mean of the muscle fiber area from experimental groups of the soleus muscle

Groups	Mean of the muscle fiber area
CG <sub>(10)</sub>	1882.93 (1722.42 - 2043.43)
CG <sub>(21)</sub>	2001.59 (1840.77 - 2162.41)
ICG	1793.32 (1633.21 - 1953.44)
IG	1380.84* (1222.55 - 1539.14)
ISG <sub>(10)</sub>	1607.74# (1449.43 - 1766.05)
ISG <sub>(21)</sub>	1819.63# (1661.29 - 1977.98)
IETG <sub>(10)</sub>	1815.31#□ (1656.94 - 1973.68)
IETG <sub>(21)</sub>	1922.38#○ (1763.99 - 2080.77)

\*p <0.001 when compared with the ICG  
 #p <0.001 when compared with the IG  
 □p <0.001 when compared with the ISG<sub>(10)</sub>  
 ○p <0.001 when compared with the ISG<sub>(21)</sub>

Table 2. Mean of the muscle fiber area from experimental groups of the plantaris muscle

Groups	Mean of the muscle fiber area
CG <sub>(10)</sub>	2895.59 (2705.8 - 3085.38)
CG <sub>(21)</sub>	2718.56 (2529.11 - 2908.01)
ICG	2824.9 (263.51 - 3015.29)
IG	1470.59* (1293.81 - 1647.37)
ISG <sub>(10)</sub>	2038.06# (1860.73 - 2215.4)
ISG <sub>(21)</sub>	2356.79# (2178.97 - 2534.61)
IETG <sub>(10)</sub>	2361.4#□ (2182.81 - 2539.99)
IETG <sub>(21)</sub>	2562.94#○ (2384.2 - 2741.68)

\*p <0.001 when compared with the ICG  
 #p <0.001 when compared with the IG  
 □p <0.001 when compared with the ISG<sub>(10)</sub>  
 ○p <0.001 when compared with the ISG<sub>(21)</sub>

## DISCUSSION

After periods of immobilization in shortened position, important adaptations occur, such as muscle atrophy and changes in the intramuscular connective tissue<sup>1,3</sup>. Only few studies investigated the response of the intramuscular connective tissue regarding the interventions of disuse and rehabilitation<sup>1-4</sup>. Coutinho et al.<sup>14</sup> noted that after four weeks of immobilization, there was disorganization and reduction of the aggregation state of collagen bundles for the soleus muscle.

On the other hand, Järvinen et al.<sup>1</sup> and Józsa et al.<sup>20</sup> observed a sharp increase of the connective tissue in both peri-endomysial regions, in the soleus muscle, of female rats after three to four weeks of cast immobilization<sup>1,20</sup>. However, Mattiello-Sverzut et al.<sup>21</sup>, also in the soleus muscle, did not observe changes in the perimysial connective tissue area in adult animals after 14 days of immobilization. Similarly, Benedini-Elias et al.<sup>15</sup> also did not find significant differences in the perimysial connective tissue after examining the soleus muscle of rat pups subjected to disuse during 10 days.

The differences in the results on the expression of the perimysial connective tissue triggered by immobilization appear to be related to time and to the type of functional constraint. Also, the perimysial connective tissue is mostly composed of type III collagen and, as demonstrated by Cação-Benedini

et al.<sup>6</sup>, the immobilization procedure applied for 10 days does not cause quantitative changes in type III collagen compared with the control group. It is well known that the skeletal muscle can manifest different patterns of adaptations in different stages of life<sup>22</sup>. Scientific studies debate about the collagen synthesis in different physiological and experimental conditions. Karpakka et al.<sup>23</sup> observed that the markers of collagen synthesis decreased during the reduction of muscular activity.

As for the mean of the muscle fiber area, the immobilization determined, in the muscles, a significant reduction in the area of fibers compared with the controls. Cação-Benedini et al.<sup>6</sup> and Cornachione et al.<sup>12</sup> also observed a significant reduction of the diameter of soleus muscle fibers, after 10 days of immobilization using identical device with different analysis technique. The physical training adopted after situations of disuse determines muscle regeneration by a highly structured process, which depends on the interaction of factors related to tissue degeneration and regeneration<sup>5</sup>.

Extracellular matrix elements, previously considered structural and passive, are essential in muscle regeneration post-injury, since the extracellular matrix releases growth factors for cell signaling required in this process<sup>5</sup>. The regulation of collagen synthesis, a major component of the extracellular matrix, is responsible for healing the muscles, avoiding its excess<sup>5</sup>. For that reason, studies that analyze the behavior of contractile and non-contractile elements of the skeletal muscle, confronting different therapeutic procedures post-immobilization, are relevant.

Stretching and eccentric exercise promote muscle adaptations; Cornachione et al.<sup>24</sup> observed an improvement in the trophism of soleus and tibialis anterior muscles, previously subjected to disuse after 21 days of running downhill on a treadmill. Regarding the stretching exercise, we did not observe satisfactory results for the area of fibers in the muscles studied. Mattiello-Sverzut et al.<sup>21</sup> demonstrated that manual stretching, for 10 consecutive days, being 10 sets of 15 seconds each, was not able to increase the diameter of the fibers of the soleus muscle, compared with the immobilized group. The eccentric exercise program held downhill for 21 days recovered the muscle fiber area of the muscles studied adopting similar characteristics to those observed in control conditions.

Associated with the application of rehabilitation protocols, the animals were kept in cage, being able to walk freely using all the limbs. The weight discharge

in the limbs gives the muscle a mechanical tensile load, which determines trophic tissue adaptations that reflect, under the morphological optics, as nuclear centralization, fiber splitting and lobulation, and moth-eaten fibers<sup>6</sup>. However, some studies show the inability of the stretching exercise in increasing the cross-sectional area of the muscle fibers<sup>18</sup>. Thus, in this study, the free training may have represented an additional stimulus that favored the changes observed in the cross-sectional area of muscle fibers in the group stretched, in particular.

## CONCLUSION

The perimysial connective tissue does not suffer quantitative changes after 10 days of immobilization associated or not with rehabilitation. The eccentric training program of 10 days only restores the area of muscle fibers for the plantaris muscle. The soleus muscle requires 21 days of rehabilitation to achieve its basic characteristics.

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## REFERENCES

1. Järvinen TH, Józsa L, Kannus P, Järvinen TL, Järvinen M. Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles: an immunohistochemical, polarization and scanning electron microscopic study. *J Muscle Res Cell Motil.* [Internet]. 2002 [acesso em 10 out. 2013];23(3):245-54. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/12500904>
2. Williams PE, Catanese T, Lucey EG, Goldspink G. The importance of stretch and contractile activity in the prevention of connective tissue accumulation in muscle. *J Anat.* [Internet]. 1988 [acesso em 10 out. 2013];158:109-14. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1261981&tool=pmcentrez&rendertype=abstract>
3. Kannus P, Jozsa L, Järvinen TL, Kvist M, Vieno T, Järvinen TA, et al. Free mobilization and low- to high-intensity exercise in immobilization-induced muscle atrophy. *J Appl*



- Physiol. [Internet]. 1998 [acesso em 10 out. 2013];84(4):1418-24. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/9516212>
4. Kannus P, Jozsa L, Kvist M, Järvinen T, Järvinen M. Effects of immobilization and subsequent low and high-intensity exercise on morphology of rat calf muscles. *Scand J Med Sci Sports*. [Internet]. 1998 [acesso 10 out 2013];8(3):160-71. Disponível em: <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0838.1998.tb00187.x/abstract>
  5. Karalaki M, Fili S, Philippou A, Koutsilieris M. Muscle regeneration: cellular and molecular events. *In Vivo*. [Internet]. 2009 [acesso 10 out. 2013];23(5):779-96. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/19779115>
  6. Cação-Benedini LO, Ribeiro PG, Gomes AR, Ywazaki JL, Monte-Raso VV, Prado CM, et al. Remobilization through stretching improves gait recovery in the rat. *Acta Histochem*. [Internet]. 2013 jun [acesso 4 jul 2013];115(5):460-9. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/23265777>
  7. Coutinho EL, Gomes AR, França CN, Oishi J, Salvini TF. Effect of passive stretching on the immobilized soleus muscle fiber morphology. *Braz J Med Biol Res*. [Internet]. 2004 dez [acesso 10 out. 2013];37(12):1853-61. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15558192>
  8. Weldon SM, Hill RH. The efficacy of stretching for prevention of exercise-related injury: a systematic review of the literature. *Man Ther*. [Internet]. 2003 ago [acesso 26 set 2013];8(3):141-50. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S1356689X03000109>
  9. Kisner C, Colby LA. Exercícios terapêuticos: fundamentos e técnicas. 3ª ed. São Paulo: Manole; 1998.
  10. Krentz JR, Farthing JP. Neural and morphological changes in response to a 20-day intense eccentric training protocol. *Eur J Appl Physiol*. [Internet]. 2010 set [acesso 18 set 2013];110(2):333-40. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20495928>
  11. Barroso R, Tricoli V, Ugrinowitsch C. Adaptações neurais e morfológicas ao treinamento de força com ações excêntricas. *R Bras Ci e Mov*. [Internet]. 2005 [acesso 10 out 2013];13(2):111-22. Disponível em: <http://portalrevistas.ucb.br/index.php/RBCM/article/viewFile/632/643..>
  12. Cornachione AS, Cação-Benedini LO, Chesca DL, Martinez EZ, Mattiello-Sverzut AC. Effects of eccentric exercise in rehabilitation of phasis and tonic muscles after leg immobilization in rats. *Acta Histochem*. 2014;116(8):1216-24.
  13. Cornachione AS, Benedini-Elias PCO, Polizello JC, Carvalho LC, Mattiello-Sverzut AC. Characterization of fiber types in different muscles of the hindlimb in female weanling and adult Wistar rats. *Acta Histochem Cytochem*. [Internet]. 2011 abr 28 [acesso 8 abr 2014];44(2):43-50. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3096081&tool=pmcentrez&rendertype=abstract>
  14. Coutinho EL, Gomes AR, Franca CN, Salvini TF. A new model for the immobilization of the rat hind limb. *Braz J Med Biol Res*. [Internet]. 2002 nov [acesso 10 out. 2013];35(11):1329-32. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/12426632>
  15. Benedini-Elias PC, Morgan MC, Gomes ARS, Mattiello-Sverzut AC. Changes in postnatal skeletal muscle development induced by alternative immobilization model in female rat. *Anat Sci Int*. [Internet]. 2009 [acesso 12 out 2013];84:218-25. Disponível em: <http://link.springer.com/article/10.1007/s12565-009-0016-3>
  16. Takekura H, Fujinami N, Nishizawa T, Ogasawara H, Kasuga N. Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. *J Physiol*. [Internet]. 2001 [acesso 10 out. 2013];533(Pt 2):571-83. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2278631&tool=pmcentrez&rendertype=abstract>
  17. Hayward R, Ferrington DA, Kochanowski LA, Miller LM, Jaworsky GM, Schneider CM. Effects of dietary protein on enzyme activity following exercise-induced muscle injury. *Med Sci Sport Exerc*. [Internet]. 1999 [acesso 10 out. 2013];31(3):414-20. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/10188746>
  18. Lynn R, Morgan DL. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol*. [Internet]. 1994 [acesso 10 out. 2013];77(3):1439-44. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/7836150>
  19. Gomes ARS, Cornachione A, Salvini TF, Mattiello-Sverzut AC. Morphological effects of two protocols of passive stretch over the immobilized rat soleus muscle. *J Anat*. [Internet]. 2007 mar [acesso 10 out 2013];210(3):328-35. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2100284&tool=pmcentrez&rendertype=abstract>
  20. Józsa L, Kannus P, Thöring J, Reffy A, Järvinen M, Kvist M. The effect of tenotomy and immobilisation on intramuscular connective tissue: a morphometric and microscopic study in rat calf muscles. *J Bone Joint Surg Br*. [Internet]. 1990 [acesso 10 out. 2013];72(2):293-7. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/2312572>
  21. Mattiello-Sverzut AC, Carvalho LC, Cornachione A, Nagashima M, Neder L, Shimano AC. Morphological effects of electrical stimulation and intermittent muscle stretch after immobilization in soleus muscle. *Histol Histopathol*. [Internet]. 2006 set [acesso 10 out. 2013];21(9):957-64. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/16763945>
  22. Alnaqeeb MA, Al Zaid NS, Goldspink G. Connective tissue changes and physical properties of developing and ageing skeletal muscle. *J Anat*. [Internet]. 1984 dez [acesso 10 out. 2013];139(Pt 4):677-89. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1164979&tool=pmcentrez&rendertype=abstract>
  23. Karpakka J, Virtanen P, Väänänen K, Orava S, Takala TE. Collagen synthesis in rat skeletal muscle during immobilization and remobilization. *J Appl Physiol*. 1991;70(4):1775-80.
  24. Cornachione A, Cação-Benedini LO, Shimano MM, Volpon JB, Martinez EZ, Mattiello-Sverzut AC. Morphological comparison of different protocols of skeletal muscle remobilization in rats after hindlimb suspension. *Scand J Med Sci Sports*. [Internet]. 2008 [acesso 12 out 2013];18(4):453-61. Disponível em: <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0838.2007.00720.x/full>