Original article

Evaluation of rats' soleus muscle submitted to remobilization protocol with therapeutic ultrasound associated with static stretching

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Abstract

Despite the deleterious effects, immobilization, is still often used in the treatment of musculoskeletal disorders, even with the occurrence of muscular atrophy, its reversal is a major challenge to rehabilitation. The aim of this study was evaluate the tropism of rats soleus muscle submitted to remobilization with static stretching, preceded by ultrasound, thermal and non thermal. We used 28 rats divided into four groups: G1-immobilized and remobilized with static stretching, G2-remobilized with stretching preceded by ultrasound 1.0 $W.cm^{-2}$; G3 – ultrasound at 0.5 $W.cm^{-2}$, G4 – ultrasound with 0.2 $W.cm^{-2}$. All animals were immobilized in plantarflexion, producing shortening of the right soleus muscle for 15 days. For the groups subjected to remobilization with ultrasound doses were used according to the group, for 3 minutes, for 10 days with an interval of 2 days after the 5th treatment. After treatment with ultrasound (or not for the G1), the animals were subjected to 3 sets of 30 seconds, with 30 seconds interval between them, of static stretching of the soleus. The soleus were dissected, weighed and processed for preparation of histological slides in cross section, and evaluated the smallest diameter of 100 fibers per muscle. There was significant reduction in weight between left and right muscles in all groups, for diameters G3 showed no difference. Conclusion: stretching with or without ultrasound, was unable to reverse the deleterious effects of immobilization on muscle weight, but in the mean dose there was protective effect on the diameter of the fibers.

Keywords: skeletal muscle, muscular atrophy, muscle stretching exercises, ultrasonic therapy, physical therapy modalities.

1 Introduction

Skeletal muscles respond to different stimuli, including immobilization (BURKHOLDER and LIEBER, 1998; DECOSTER, SCANLON, HORN et al., 2004; IKEDA, YOSHIDA, MATAYOSHI et al., 2004; AOKI, MIYABARA, SOARES et al., 2006). The inactivity causes significant muscle remodeling, including loss of myofibrillar proteins and changes in metabolic activity (STEVENS, WALTER, OKEREKE et al., 2004). Despite the deleterious effects, immobilization is still often used in the treatment of musculoskeletal disorders, even with the occurrence of muscle cells atrophy and loss of extensibility (SAKAKIMA, 2004). The occurrence of muscular atrophy during immobilization and it recovery, presenting a major challenge to rehabilitation (STEVENS, WALTER, OKEREKE et al., 2004, DIAS, CANCELLIERO, DURIGAN et al., 2006).

The immobilization, especially when the muscle is maintained in shortened position, resulting in reduced protein synthesis and significant reduction in cross section and mass, which can be correlated with a loss of myofibrillar proteins (GOLDSPINK, 1977; THOMASON, BIGGS and BOOTH, 1989; THOMASON and BOOTH, 1990). This loss of weight, according Mozdiak, Pulvermacher and Schultz (2001), occurs because immobilization and disuse induce apoptosis causing a reduction in the number of myonuclei and DNA content, leading to muscle atrophy and loss of weight.

Goldspink (1999) and Shah, Peters, Jordan et al. (2001), argue that stretching is a powerful stimulant for protein synthesis and muscle hypertrophy. The intracellular pathways, which are supposed to be activated by stretching, reach the core of the muscle fiber, where gene activation occurs. According to Ikeda, Yoshida, Matayoshi et al. (2004), repetitive static muscle stretching increases the amount of myogenin mRNA in skeletal muscle, which is among the myogenic transcription factors, and plays an important role in the formation of muscle tissue.

The therapeutic ultrasound possesses physical, biophysical and therapeutic actions, being target of investigations because of its important roles in metabolic processes and also to repair cell damage. The main effect involved in ultrasound therapy is the acoustic stream, which produces unidirectional movement of fluids along and around cell membranes. It can act in modifying the local environment of the cell, altering concentration gradients in the vicinity of extracellular membrane, affecting the diffusion of ions and molecules through the membrane, it could be responsible for changes in potassium and calcium cells content, thereby producing anabolic effects, and also by possible thermal effects. However, some effects are still controversial in the literature regarding the therapeutic use of the modality (TER HAAR, 1999; JOHNS, 2002). Its action on muscle tissue, has proved useful, mainly by thermal effects in order to gain of extensibility (WESSLING, DEVANE and HYLTON, 1987; KNIGHT, RUTLEDGE, COX et al., 2001; COSTA, COSTA, MENDES et al., 2006; USUBA, MIYANAGA, MIYAKAWA et al., 2006; BERTOLINI, BARBIERI, MAZZER, 2009). However, on muscle mass, Lopes, Bertolini, Martins et al. (2005) and Nussbaum and Locke (2007), observed no changes in muscle fiber cross section and mass, respectively, yet the latter authors report that there was an increase in heat shock proteins, with the use of ultrasound thermal.

There is still a gap regarding the effects of therapeutic ultrasound on muscle mass in the muscles after a period of immobilization. Therefore, the purpose of this study was to evaluate the soleus muscle mass of rats subjected to remobilization protocol with static stretching, preceded by therapeutic ultrasound, thermal and non thermal.

2 Materials and methods

2.1 Experimental groups

We used 28 male albino Wistar rats, with 10 ± 2 weeks old, obtained from the Central Animal Vivarium at the State University of Paraná (UNIOESTE). The study was conducted according to international standards of ethics in animal experiments, approved by the UNIOESTE Animal Ethics Committee and Practical Classes under report numbers 0309. The animals were grouped and kept in plastic cages in controlled conditions, with light/dark cycle of 12 hours, temperature 23 ± 2 °C, with access to water and food ad libitum.

The animals were randomly divided into four groups:

- G1 (n = 7): animals in this group had immobilized his right ankle in maximum plantar flexion for 15 consecutive days, in order to maintain the soleus muscle in shortened position. After the immobilization period, the animals were subjected to passive stretch of soleus muscle daily for 10 days, with two rest days between the 5th and 6th therapies;
- G2 (n = 7): this group was also subjected to immobilization. After this period, the animals were subjected for more two weeks of treatment with therapeutic ultrasound 1 MHz, 1.0 W.cm^{-2}. Then we performed the static stretching protocol. The procedures were performed similarly to G1;
- G3 (n = 7): this group was subjected to immobilization and remobilization, similar to previous groups. The intervention was similar to G2, but with a dose of 0.5 W.cm^{-2};
- G4 (n = 7): this group was subjected to immobilization and remobilization, similar to previous groups. The intervention was similar to G2 and G3, but with a therapeutic dose of 0.2 W.cm^{-2}.

2.2 Immobilization protocol

To conduct the study we used as immobilization apparatus, the model described by Coutinho, Gomes, França et al. (2002), which aims to shorten the soleus muscle, for this, the tibio-tarsal joint was immobilized in maximal plantar flexion. The animals were observed daily during 15 days of detention in order to repair possible damage to the apparatus. After removal of immobilization, rats were weighed and submitted to the trichotomy of the right posterior region of the triceps surae (soleus).

2.3 Ultrasound therapeutic protocol

To perform the therapy with ultrasound device was used Sonopuls Ibramed®, which had valid calibration certificate during the research period. The frequency used was 1.0 MHz, ERA, 1 cm^2, and a dose of 1.0, 0.5 and 0.2 W. cm^{-2}, respectively, at G2, G3 and G4, for 3 minutes on the region of the right hind limb soleus for 10 therapies, with an interval of two after the 5th treatment. To implement it we used a PVC retainer to immobilize the animal.

2.4 Stretching protocol

Subsequent to treatment with ultrasound, static stretch was initiated. To perform the technique of stretching in soleus muscle, the tibio-tarsal joint was maintained manually in maximum dorsiflexion during the entire period of stretching, which consisted of three sets of 30 seconds with a rest interval of 30 seconds between sets (KONNO, ALVES, BERTOLINI et al., 2007) During the stretch animals were kept on PVC retainer.

2.5 Histological analysis

At the end of the period of remobilization, all animals were euthanized by guillotine decapitation. Soon after, the right and left soleus muscles were isolated, cleaned and weighed on an analytical balance (Shimadzu®). Were positioned on a Styrofoam mold, fixed with needles on their edges, and remained in formalin 10% until preparation of histological sections, in cross-section of 10 µm, after embedding in paraffin. Then were mounted on the histological sections and subsequently stained with hematoxylin and eosin (HE).

The slides were observed in common light optical microscope (Olympus®), digital camera (EDC-s) attached and a 10x objective lens to perform the digitization of images of muscle fibers transverse sections. Then the images were analyzed using Image-Pro Plus® 3.0, as the smaller diameter of 100 fibers per muscle (BRITO, CAMARGO FILHO, VANDERLEI et al., 2006).

2.6 Data analysis

Data were evaluated by comparing the results obtained in the left soleus (intact) and right (subject to immobilization), among the animals of the same experimental group using the Student *t*-test, and comparison between groups was performed using one-way ANOVA, with pos hoc of Tukey, and considered significant $p < 0.05$.

3 Results

In comparing the left soleus muscles weight with the rights, it was possible to observe differences in all groups; when comparing between the groups there was significant difference of the G2 right with the G4 ritht (Figure 1).

Comparing the diameters, it was observed that only G3, no significant difference when comparing with the right side with the left. When comparing between groups, to the right side there was no significant difference in any time, but the left side there was a significant difference between G1 and G2 (Figure 2).

4 Discussion

The immobilization is a procedure commonly used as treatment in musculoskeletal injuries (LIMA, CAIERÃO, DURIGAN et al., 2007) and/or connective tissue disorders (GOMES, CORNACHIONE, SALVINI et al., 2007), but there may be several deleterious effects due to immobilization in the soleus shortening, such as muscle atrophy, fiber area and number of sarcomeres in series decreased, and increase of connective tissue, resulting in rapid muscle stiffness (CAIERÃO, TEODORI and MINAMOTO, 2007). Therefore, in this study sought to assess a form of rat soleus muscle remobilization, associating with therapeutic ultrasound in non-thermal and thermal doses to static stretching.

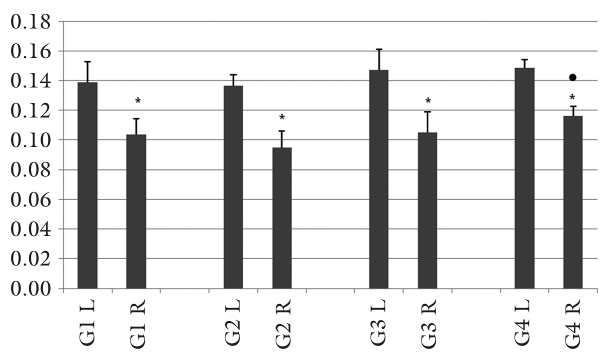

Figure 1. Graphic representation of means and standard deviations of different groups with respect to the left soleus muscles weight (L) and right (R), according to the different groups. *Statistically significant difference when compared with the contra-lateral. ●Significant difference when comparing with G2R.

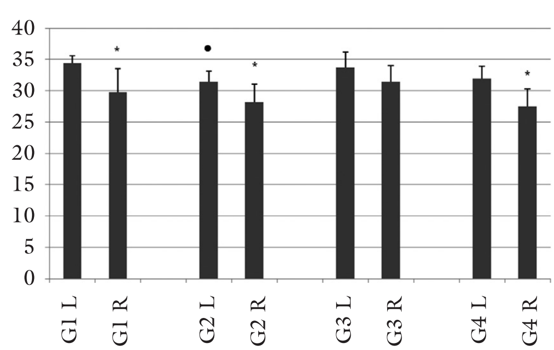

Figure 2. Graphic representation of means and standard deviations of different groups with respect to the muscle fibers diameters of left soleus muscles (L) and right (R), according to the different groups. *Statistically significant difference when compared with the contra-lateral. ●significant difference when comparing with G1L.

According to Goldspink (1977), the muscle disuse leads to atrophy, because the immobilization of a muscle in shortened position leads to a smaller muscle, since it is metabolically expensive for the organism to maintain a larger muscle than is physiologically necessary. To minimize these effects, uses up resources such as stretch and contractile activity, either by active contraction or electrical stimulation. However, the present study, we could see signs of muscle atrophy, decreased muscle mass, for the four study groups, indicating that the static stretching alone was not effective in restoring muscle mass, similar to the contralateral side.

Short periods of immobilization, such as a week is enough to produce significant changes in muscle (LIMA, CAIERÃO, DURIGAN et al., 2007), producing significant reduction in muscle cross-sectional area (SHAH, PETERS, JORDAN et al., 2001). In this study, were evaluated by muscle 100 fibers in the smaller diameter, because according to Brito, Camargo Filho, Vanderlei et al. (2006), this number of fibers is an adequate and effective methodology for this type of analysis. As observed, except for G3, all other groups have succeeded in reducing the fiber diameter.

It is known that stretching of skeletal muscle is very important not only to increase range of motion, but also when it is kept for long periods, has anabolic effects, stimulating the synthesis of proteins (GOLDSPINK, 1977; GOMES, COUTINHO, FRANÇA et al., 2004) inducing muscle hypertrophy and hyperplasia (CAIERÃO, TEODORI and MINAMOTO, 2007). Furthermore, the therapeutic ultrasound is commonly used in rehabilitation because it's thermal and nonthermal effects. It is likely that the mechanical (not thermal) provide benefits in the repair of skeletal muscle (MARKERT, MERRICK, KIRBY et al., 2005), as producing changes in membrane permeability and stimulation of transport by stimulating the proliferation of satellite cells, with formation of new fibers or can assist in the repair of a focal lesion in the early stages of regeneration (KARNES and BURTON, 2002). Thus, it is believed that in our results, the ultrasound therapeutic in threshold dose for thermal effects (JOHNS, 2002), may have produced catalytic effects on muscle recovery, when combined with stretching. However, please note the limitation of the study only evaluated the diameter of muscle fibers, not identifying whether or not recruitment of satellite cells or other mechanisms, which are suggestions for future studies.

5 Conclusion

In this study, just the stretching was not enough to reverse the deleterious effects of immobilization, as well as its association with ultrasound in high or low doses, however, using a medium dose, we observed a small protective effect on the diameter soleus muscle fibers.

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