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ULTRASOUND BIOMICROSCOPY AND MAGNETIC RESONANCE IMAGES IN THE CHARACTERIZATION OF RAT MUSCLE SUBMITTED TO STRETCHING TRAINING – A PRELIMINARY STUDY

[1]Natália Santos da Fonseca Martins, [1]Carolina Carneiro Peixinho, [1,2] Liliam Fernandes de Oliveira,[1,3]João Carlos Machado
[1]Biomedical Engineering Program, Rio de Janeiro, Brazil
[2]Department of Biosciences Physical Activity, EEFD/UFRJ, Rio de Janeiro, Brazil
[3]Post-Graduation Program in Surgical Sciences, FM/UFRJ, Rio de Janeiro, Brazil; e-mail: natalia_sfm@hotmail.com

SUMMARY

This pilot study aimed to test the potential use of imaging techniques, ultrasound biomicroscopy (UBM) and magnetic resonance (MRI), to quantify muscle architecture parameters and evaluate, in vivo, structural adaptations of rat lateral gastrocnemius (LG) submitted to chronic stretching protocol with characteristics similar to those used in humans. After 20 daily static stretching sessions, both UBM and MRI images were acquired. Muscle architecture parameters such as muscle thickness (MT), pennation angle (PA), anatomical and physiological cross-sectional area (ACSA and PCSA) were measured in images of the elongated right hindlimb and compared with the contralateral member, used as control. The results show reductions of approximately 15% in MT, 17% in PA, 11% in CSA and 10% in PCSA of the GL muscle of the treated leg. These values indicate that the intensity of the applied protocol was sufficient to cause structural changes in this muscle. This preliminary study indicates the potential of MRI and UBM images in the, in vivo, quantification and tracking in vivo parameters of muscle architecture of rats submitted to chronic stretching training.

INTRODUCTION

Muscle architecture refers to structural characteristics of muscle fascicles, such as the fibers arrangement in relation to the axis of force generation [1]. Furthermore, muscle function can be understood by the characterization of its architecture [2]. Noninvasive image techniques, such as ultrasound and MRI are used in studies that analyze muscle architecture parameters as pennation angle (PA), muscle thickness (MT), anatomical and physiological cross-sectional area (ACSA and PCSA), and the course of adaptations in conditions of increasing use or disuse in health or disease and in response to different rehabilitation programs and training [3].

Ultrasound biomicroscopy (UBM) is a technique to generate high-resolution images that uses frequencies from 40 to 100MHz and shows potential for acquiring images of skeletal muscle tissue of rats, allowing the quantification of muscle architecture parameters [4]. MRI, also a noninvasive method, can be used likewise in the clinical evaluation of skeletal muscle injuries. Changes in proton longitudinal and transverse relaxation (T1 and T2) properties have been used to study muscle activation during exercise and in evaluation of muscle regeneration in rats and humans [5]. Both techniques have shown their applicability and it is suggested their use in longitudinal studies concerning the adaptations of skeletal muscle, such as flexibility training.

Although scientific evidences are inconclusive and controversial, stretching is used as a method to increase the range of motion and the flexibility [6] and to decrease risk of injuries and pain associated with muscle stiffness. Few studies monitored structural adaptations of muscles submitted to a long-term stretching training in humans. But so far fewer studies used a stretching protocol specific to humans in rats [7].

Thus, this pilot study aimed to investigate the potential of imaging techniques (MRI and UBM) to measure and estimate parameters of muscle architecture and evaluate, in vivo, structural adaptations of lateral gastrocnemius muscle of rats submitted to a chronic stretching training similar to what is used in humans.

METHODS

One Wistar male rat (3 months, 260g) was submitted to a static stretching protocol of the right triceps surae whereas the contralateral hindlimb was used as control. The study was approved by the Ethics Committee for Animal Research Institution.

Stretching sessions were performed daily and five times a week, during 30 days. The stretching sessions consisted of 10 repetitions lasting one minute of a static position of 180 degrees of hip flexion, full knee extension and maximum ankle dorsiflexion with rest interval of 30s in a relaxed position.

An UBM system (Vevo 770; Visual Sonics, Toronto Canada) with center frequency of 40 MHz, was used for acquiring the longitudinal images of the lateral gastrocnemius (LG). The animal was anesthetized with xylazin (10-15 mg/Kg) and ketamine (50-75 mg/Kg) and positioned in ventral decubitus at the equipment platform, with the ankle immobilized in neutral position. Measurements of MT and PA were obtained from the UBM images. A 7 Tesla magnetic resonance machine, with 210 bore actively screened refrigerated magnet system (Varian, Inc. NMR Systems, Palo Alto, California) was employed to acquire images of the hindlimb. The animals were placed in the prone position and fixed in a appropriate structure for the exam. Contrast injection (gadoteric acid 0.5 mmol / mL) was used to improve the quality of images. A sequence spin-echo axial T1-weighted was used. Data acquired with a matrix encoding 192 x 192 pixels, field of view of 50x 80 mm, in 30 slices with 1mm in thickness, pulse repetition time of 800ms and echo time of 15.5 ms. Measurements of ACSA for LG were obtained from the MRI images and used subsequently in the estimation of PCSA, according to [8]:

$$PCSA = CSA \cdot \cos(PA). \qquad (1)$$

The area quantified corresponds to 1/4 distal of the hindlimb. A free software (*ImageJ*; National Institute of Health, Maryland, USA) was used for measurements and quantification of the muscle architecture parameters, as depicted in Figure 1. MT corresponds to the length of the vertical line and PA to the angle formed by the one line over the deep aponeurose and the other line over the muscle fascicle. CSA was determined over the muscle cross-section delineated by the yellow contour. Measurements were performed three times in each image and the average of them was used for analysis.

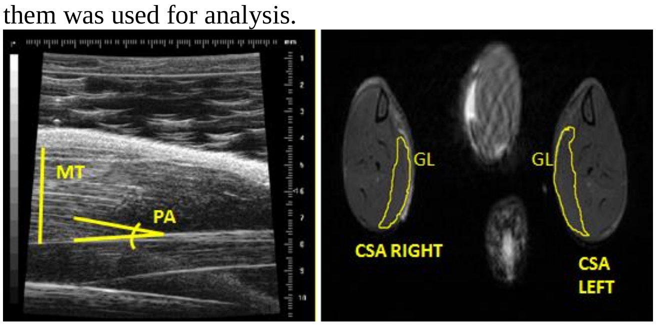

Figure 1: Measurements made by the software in images of UBM (left) and MRI (right), respectively.

RESULTS AND DISCUSSION

UBM provided images with high resolution and sufficient good quality to measure MT and PA, as well as MRI images allowed quantification of CSA and estimation of PCSA, using equation (1), of both hindlimbs (elongated and control). The absolute results comprise a reduction of the architecture parameters from the elongated hindlimb in relation to corresponding parameters of the control member of approximately 15% in MT, 17% in PA, 11% in CSA and 10% in PCSA. The decrease of values of MT after the stretching sessions suggests that the intensity of the applied protocol was sufficient to promote structural changes in this muscle. In a previous study, Peixinho et al. [7] applied a stretching protocol with characteristics similar to those used in humans to increase the range of motion and reported an adaptation of the muscle architecture of rats, reducing approximately 25% of PA values after 12 stretching sessions. It seems to be, so far, the only report found in the literature accompanying the parameters of MT and PA during a chronic stretching training, showing that when a muscle is stretched and subjected to a load of deformation (tension), the pennation angle is decreased. The PA is directly related to the amount of contractile tissue per muscle cross-sectional area (ACSA) and therefore it influences the amount of force that the muscle can produce. The ACSA is proportional to PCSA, which is also a very important parameter in estimating muscle strength. Initially, the lower the ACSA and, the lower the PCSA, with a PA reduction could suggest a decrease of maximum muscle force. However, this cannot be conclusive without the FL measure [8]. Previous studies employing animal stimuli elongation of high intensity, such as immobilization in the extended position for prolonged periods showed structural changes, such as a decreased CSA, with a concomitant increasing of the fiber length (FL) and the number of sarcomeres in series [9]. In another study with goats, FL and the number of sarcomeres in series also increased after limb lengthening, however, mass and PCSA remaining unchanged [10].

Our results are consistent with a possible FL increasing, with the addition of sarcomeres in series, reported by in vitro studies with animals and a case study in human. However, in our study, the curvature of fascicles and the insufficient field of view provided by the UBM machine prevented FL measurements. Such limitation can be solved by an imaging processing specific to this purpose.

CONCLUSIONS

This preliminary study indicates the potential of imaging techniques (UBM and MRI) for measuring and estimating muscle architecture parameters and its structural adaptations when muscles are submitted to a long-term stretching training with characteristics similar to those used in humans. The combination of these two images techniques allows a broader and more complete conclusion regarding muscle function, requiring further investigation about the subject.

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