Clinical Biomechanics 29 (2014) 57–62

Contents lists available at ScienceDirect

Clinical Biomechanics

journal homepage: www.elsevier.com/locate/clinbiomech

Structural adaptations of rat lateral gastrocnemius muscle–tendon complex to a chronic stretching program and their quantification based on ultrasound biomicroscopy and optical microscopic images

Carolina Carneiro Peixinho [a,b,*], Natália Santos Fonseca Martins [a,b], Liliam Fernandes de Oliveira [b], João Carlos Machado [a,c]

[a] Biomedical Engineering Program, COPPE, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[b] Laboratory of Biomechanics, Department of Biosciences and Physical Activity, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[c] Post-Graduation Program in Surgical Sciences, Department of Surgery, School of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 17 July 2013
Accepted 6 November 2013

Keywords:
Ultrasound biomicroscopy
Musculoskeletal system
Stretching
Sarcomere

ABSTRACT

Background: A chronic regimen of flexibility training can increase range of motion, with the increase mechanisms believed to be a change in the muscle material properties or in the neural components associated with this type of training.
Methods: This study followed chronic structural adaptations of lateral gastrocnemius muscle of rats submitted to stretching training (3 times a week during 8 weeks), based on muscle architecture measurements including pennation angle, muscle thickness and tendon length obtained from ultrasound biomicroscopic images, in vivo. Fiber length and sarcomere number per 100 μm were determined in 3 fibers of each muscle (ex vivo and in vitro, respectively), using conventional optical microscopy.
Findings: Stretching training resulted in a significant pennation angle reduction of the stretched leg after 12 sessions (25%, $P = 0.002$ to 0.024). Muscle thickness and tendon length presented no significant changes. Fiber length presented a significant increase for the stretched leg (8.5%, $P = 0.00006$), with the simultaneous increase in sarcomere length (5%, $P = 0.041$) since the stretched muscles presented less sarcomeres per 100 μm.
Interpretation: A stretching protocol with characteristics similar to those applied in humans was sufficient to modify muscle architecture of rats with absence of a sarcomerogenesis process. The results indicate that structural adaptations take place in skeletal muscle tissue submitted to moderate-intensity stretching training.

1. Introduction

Muscle stretching is used to enhance range of motion and thus to improve muscular performance by increasing flexibility (Magnusson, 1998; Magnusson et al., 1996) and reducing the risk of injury and pain associated with muscle stiffness, despite inconclusive and controversial scientific evidence.

Several studies with humans monitored acute structural and mechanical adaptations of muscles submitted to stretching protocols. On the other hand, few reports regarding longer periods of flexibility training indicate an increase in range of motion (Magnusson, 1998; Mahieu et al., 2009) and muscle length (Davis et al., 2005; Magnusson et al., 1996), with the increase mechanisms believed to be a change in the muscle material properties or in the neural components. Gajdosik et al. (2007) concluded that the chronic gain in range of motion due to stretching of calf muscle–tendon unit was caused by changes in the viscoelastic properties of tissues involved in a flexibility training lasting 6 weeks, whereas Mahieu et al. (2009) indicated that the gain in range of motion due to proprioceptive neuromuscular facilitation stretching could only be explained by an increase in stretching tolerance. Some authors also suggest a possible increase in the number of sarcomeres in series to explain the right-shift of the length–tension curve peak observed in response to training (Caiozzo et al., 2002; Williams and Goldspink, 1978). However, only one case study in humans confirmed the association of increased number of sarcomeres in series with increased muscle length, after a 4-cm femoral lengthening procedure for leg length discrepancy (Lieber, 2002).

On the other hand, several animal studies investigated long-term stretching interventions based on methodological approaches that are unacceptable for human application. In this sense, animal models using muscle immobilization at a lengthened position for days or weeks or also employing limb lengthening techniques demonstrated an increase in the number of sarcomeres in series and in the total fiber length (FL) (Caiozzo et al., 2002; Elsalanty et al., 2007; Kurihashi et al., 2006; Williams and Goldspink, 1978). Additionally, moderate elongation sustained for 30 min/day was sufficient to reduce or reverse the fiber shortening that accompanied the immobilization in a shortened position in rat muscles (Williams, 1990). However, contrasting results

* Corresponding author at: 2030, Horácio Macedo Ave, Technology Center, COPPE/UFRJ, Block H, room 327, Rio de Janeiro, RJ 21941-914, Brazil.
E-mail addresses: carolina@peb.ufrj.br, peixinho.carol@gmail.com (C.C. Peixinho).

0268-0033/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.clinbiomech.2013.11.002

including significant increase in fiber and sarcomere length, with no increase in the number of sarcomeres in series, have been reported in a study of tibial lengthening in goats (Elsalanty et al., 2007).

Although there is great effort to understand the adaptation response of skeletal muscle to stretching, there are still unanswered questions regarding this type of training, such as the role of neurophysiological and biomechanical components in stretching skeletal muscle as well as the differentiation between adaptations of contractile and non-contractile components in the muscle–tendon unit.

Functional characteristics of the skeletal muscle are highly influenced by muscle architecture: a terminology used to define certain structural features such as muscle fiber insertion angle (pennation angle — PA), the FL and muscle thickness (MT) (Koryak, 2008; Lieber, 2002). Functional demands, such as stretching, imposed on skeletal muscle lead to muscle architecture rearrangement, revealing the force correspondence with muscle length and contraction speed. Therefore, muscle architecture quantification becomes one way to analyze muscle function, as already explored in experimental models of increased use and disuse of human muscles that demonstrated the high degree of plasticity for the muscle architecture parameters, initially measured in cadavers and thereafter by non-invasive image techniques such as ultrasound and magnetic resonance imaging (Maganaris et al., 1998; Martin et al., 2001; Narici, 1999).

Ultrasound biomicroscopy (UBM), a technique employing ultrasonic frequencies from 40 to 100 MHz to generate high-resolution images, has been used in the imaging of very superficial layers of human tissue (e.g. skin and eyes) because of the limited wave penetration depth caused by higher ultrasound frequencies. It has also been used to quantify changes in rat skeletal muscle architecture during a regeneration process (Peixinho et al., 2011), indicating its applicability in the tracking of longitudinal adaptations of rat skeletal muscle tissue, as in the case of flexibility training.

The present study was planned to quantify structural characteristics during the training of rat muscle subjected to chronic stretching, as an attempt to clarify the mechanisms of adaptive processes of skeletal muscle tissue due to elongation. PA, MT and tendon length (TL) were quantified, in vivo, through UBM images acquired during different intervals of training. In addition, FL and the number of sarcomeres in series per 100 μm of fiber were determined, ex vivo and in vitro respectively, based on optical microscopic images.

2. Methods

2.1. Animals

Eleven *Wistar* male rats (age: 3–4 months, mass: mean = 280.91, SD = 18.90 g) were randomly distributed into two groups: a stretching group (SG, n = 6) submitted to a static stretching of the triceps surae muscles and a control group (CG, n = 5). Architectural parameter measurements were performed in UBM images of both legs acquired before and after 6, 12, 18 and 24 stretching sessions for SG and four times with a two-week interval for the CG. The animals were euthanized after 8 weeks and the lateral gastrocnemius (LG) muscles from both legs were extracted for ex vivo and in vitro analysis. All protocols were in compliance and approved by the Institutional Care and Animal Use Committee of Federal University of Rio de Janeiro. The animals were maintained with appropriate circadian cycle and diet considering the Guide for Care and Use of Laboratory Animals (National Institutes of Health).

2.2. Procedures

The stretching protocol was designed to resemble flexibility training prescribed for humans (Magnusson, 1998; Magnusson et al., 1996; Morse et al., 2008). Throughout the procedure, the rats were anesthetized with isoflurane (Isofrine; Cristália, São Paulo, SP, Brazil) at 1.5% in 1.5 l/min of O_2 employing an animal anesthesia apparatus (EZ-7000; Euthanex, Palmer, PA, USA) and immobilized on a 37 °C heated bed (T/Pump System; Gaymar, Orchard Park, NY, USA) in a supine position with their arms and left leg fixed by paper tape. The streching protocol session consisted of 60 s, with a rest interval of 30 s, of a static position of hip flexion (90°) with full knee extension and maximum dorsiflexion of the right member (Fig. 1), repeated 10 times. This protocol was performed 3 times weekly during 8 weeks. The animals of both groups were allowed unrestricted ambulation during the 8 weeks.

An UBM system (Vevo 770; VisualSonics, Toronto, ON, Canada) operating at a center frequency of 40 MHz was used (immediately before and after 6, 12, 18 and 24 sessions of stretching) to generate sector images with a 10 × 10 mm field of view and at a frame rate of 34 Hz. Lateral and axial resolutions were 80 and 40 μm, respectively. Prior to image acquisition, the animals were anesthetized with an intraperitoneal injection of ketamine (10–15 $mg \cdot kg^{-1}$) and xylazine (50–75 $mg \cdot kg^{-1}$) and had their leg shaved. They were placed in the ventral decubitus position with the ankle immobilized in an angle of 90°, aided by an external fixation device, keeping the posterior leg free to be assessed with the UBM probe. The UBM machine was always operated by the same individual to acquire longitudinal image planes from both legs of each rat, generating images of LG and calcanear tendon. The images with good fiber and tendon visualization were saved for further analysis.

Ultrasound gel was used to couple the probe to animal skin and minimal pressure was applied to the muscle, minimizing image distortion and errors in measurements. Image processing software (ImageJ; National Institutes of Health, Bethesda, MD, USA) was used for the measurements of PA, MT and TL. The parameters were quantified at anatomical points previously identified (for MT and TL) and at the best visually distinguishable fibers (for PA) by an evaluator with experience in ultrasonographic measurements. MT was calculated as the distance between superficial and deep aponeuroses, considering the site on left side of the ultrasound image, which corresponds to the largest thickness (Fig. 2a). PA was calculated as the angle between the deep aponeurosis and the fascicle (Fig. 2b). TL was measured as the distance between distal and proximal tendon insertions (Fig. 2c).

Three good quality picture frames were selected from the videos recorded by the UBM equipment during each animal examination. The criterion for frame selection considered the image with the fascicles and the aponeuroses well distinguished and suited for parameter quantifications. In each frame, one measurement of the parameter related to the image was performed and the mean of the three measurements was used for further analysis. The reliability of this procedure was evaluated

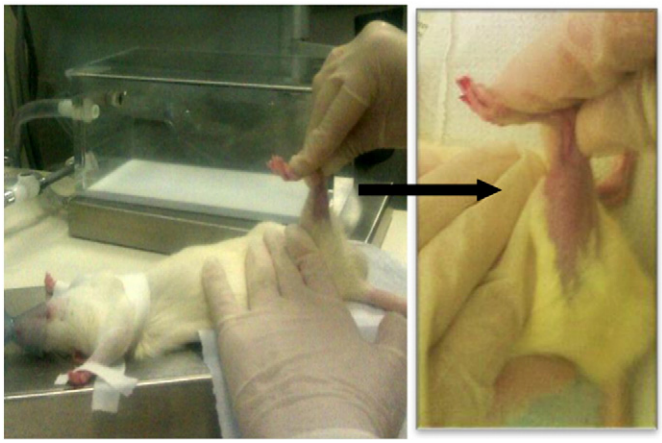

Fig. 1. Static stretching protocol of the triceps surae muscles consisted of maintaining the position of hip flexion, knee extension and maximum dorsiflexion during 60 s for 10 times.