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ADAPTATIONS OF RAT MUSCLES SUBMITTED TO A CHRONIC STRETCHING PROGRAM

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SUMMARY
This study aimed to characterize the chronic adaptations of the lateral gastrocnemius (LG) muscle of rats induced by a stretching training by means of in vitro quantification of muscle fiber length (FL) and number of sarcomeres/100µm (NS). The rats underwent static stretching of the triceps surae muscles 3 times a week during 8 weeks. FL presented a significant increase for the stretched leg, with the simultaneous increase in sarcomere length deduced from a diminished NS. A stretching protocol with characteristics similar to those applied in humans to increase the range of motion was sufficient to increase the fiber length of rat muscle with the absence of a sarcomerogenesis process.

INTRODUCTION
Muscle stretching is usually applied aiming the enhancement or recovery of articular range of motion, improving muscular performance by the increase of flexibility [1,2,3] and the reduction of the risk of injury and pain associated with muscle stiffness. Although its extensive application in rehabilitation and athletic fields, flexibility training presents inconclusive and controversial data in the scientific literature.

There are several investigations regarding the acute effects of stretching stimulus to the muscle-tendon unit. However, reports related to longer periods of training in humans, although less common presented, have demonstrated increased range of motion [1,2,4,5,6] and muscle length [3,7] as long-term results for a chronic regimen of flexibility training. Common explanations for the gains in range of motion associated with stretching are changes in the muscle material properties and structure or in the response of neural components, as increased stretch tolerance. It is reported a right-shift of the length-tension curve peak as a result of this type of training, indicating a possible increase in the number of sarcomeres in series [8,9]. Animal studies investigated the effects on muscle caused by long-term stretching interventions, but with methodological approaches of high intensity stretching such as immobilization and osteogenesis distraction techniques, that cannot be applied to humans. Most of these studies show a fiber length increase associated with a higher number of sarcomeres in series [11,12,13,14,15].
This study aim to assess fiber length and sarcomeres adaptation to a long-term stretching protocol, similar to those applied with humans, to elucidate possible mechanisms that justifies the consequences of flexibility trainings applied in patients and athletes.

METHODS
Eleven Wistar male rats (3-4 months, 280,91± 18,90 g) were randomly distributed in one of two groups: one submitted to a protocol of static stretching of triceps surae muscles three times a week during 8 weeks (SG, n=6) and a control group (CG, n=5). All protocols were in compliance and were approved by the Institutional Care and Animal Use Committee of Federal University of Rio de Janeiro. The stretching protocol, applied 3 times a week for 8 weeks, was designed to resemble flexibility training prescribed for humans[1,2,3,9,13]. The protocol consisted of 10 sequences of 60 seconds stretching and rest interval of 30 seconds, with the stretching caused by a static position of hip flexion (180°), full knee extension and maximum dorsiflexion of the right member (Figure 1).

Figure 1: Static stretching protocol of triceps surae muscles consisted of maintaining the position of hip flexion, knee extension and maximum dorsiflexion during 60 seconds.

After 24 stretching sessions, the animals were euthanized by anesthetic overdose and the lateral gastrocnemius muscles of both legs were dissected out and subjected to a protocol for separation of individual fibers and quantification of the length and number of sarcomeres [15]. After fixation, the distance between the muscle-tendon junctions was measured for each muscle using micrometer calipers. Photomicrographs were made of 3 fibres from each muscle and the number of sarcomeres along 100µm of the fibres determined counting each sarcomere using image processing software (ImageJ; National Institute of Health, Maryland, USA) (Figure 2). Sarcomere length was assumed homogeneous throughout the whole fiber length.

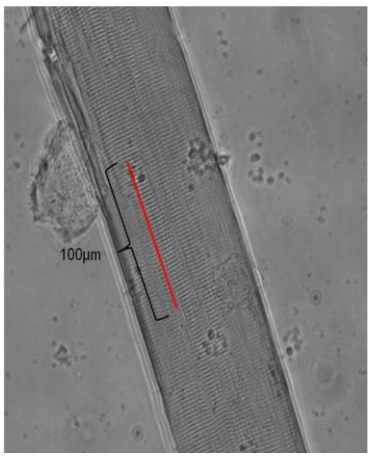

Figure 2: Microscopic image of a single fiber processed to count the number of sarcomeres in a length of 100µm (red line).

RESULTS AND DISCUSSION

Comparisons of the parameters between left and right legs presented significantly higher fiber length for the stretched leg (11.30 ± 0.03 mm) in comparison to the non-stretched one (10.50 ± 0.06 mm) for SG ($p = 0.000006$), as well as a significantly lower sarcomere linear density (#sarcomeres/100µm) of 0.53 versus 0.56 ($p = 0.041$), respectively. No significant difference was found for any of the parameters in the CG.

The increase of 8.5% of FL, as well as the increase in sarcomere length, evidenced by a smaller sarcomere density in the LG muscle fibers of the SG stretched leg, demonstrated that the stretching protocol applied was sufficient to alter muscle structure.

Previous animal studies employing prolonged muscle immobilization at a lengthened position or limb lengthening techniques such as distraction osteogenesis demonstrated an increase in the number of sarcomeres in series and in the total fiber length [8,9,11,12,13,14,15]. Only one case study in human [16] reported longer fiber lengths due to the increase in the number of sarcomeres in series after high intensity stretching interventions. However, other animal studies report sarcomeres lengthening but no sarcomerogenesis [11,12]. An example is the work of Elsalanty et al [11], which showed that an increase of FL (36.7%) resulted from lengthened sarcomeres with no increase in the number of sarcomeres in series.

In a review of such studies, Caiozzo et al. [8] propose 3 mechanisms of muscle fiber adaptation to stretch: increased sarcomere length, increased number of sarcomeres in series, or both. According to the authors, there is the possibility that sarcomere neogenesis would only occur when sarcomere length exceeds a set point during distraction or stretching. After this set point, cellular or molecular events would take place to add sarcomeres in series in muscle fibers, returning sarcomere to its optimum length. The temporal sequence of such events, the limit of sarcomere length after which sarcomerogenesis would initiate and the magnitude of stretch stimulus are still questions to be answered.

CONCLUSIONS

Our results show an increase in sarcomere length, coherent with the stretching protocol employed, much less intense than distraction or immobilization interventions, but very close to those applied to humans. If it is a final adaptation or yet an intermediate phase for future sarcomere neogenesis is still to be investigated.

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