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Acute effects of stretching exercise on the soleus muscle of female aged rats

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ABSTRACT

It has been shown that stretching exercises can improve the flexibility and independence of the elderly. However, although these exercises commonly constitute training programs, the morphological adaptations induced by stretching exercises in aged skeletal muscle are still unclear.

Objective: To assess the acute effects of passive mechanical static stretching on the morphology, sarcomerogenesis and modulation of important components of the extracellular matrix of the soleus muscle of aged female rats.

Methods: Fifteen old female rats with 26 months were divided into two groups: stretching ($n = 8$, SG) and control ($n = 7$, CG): The stretching protocol consisted of 4 repetitions each of 1 min with 30 s interval between sets. Stretching was performed on the left soleus muscle, 3 times a week for 1 week. After three sessions, the rats were anesthetized to remove the left soleus muscle, and then euthanized. The following analyses were carried out: muscle fiber cross-sectional area and serial sarcomere number; immunohistochemistry for the quantification of collagen I, III and TGFβ-1.

Results: a decrease in muscle fiber cross-sectional area of the SG was observed when compared to the CG ($p = 0.0001$, Kruskal–Wallis); the percentage of type I collagen was significantly lower in the SG when compared to the CG ($p = 0.01$, Kruskal–Wallis), as well as the percentage of TGFβ-1 ($p = 0.04$, Kruskal–Wallis); collagen III was significantly higher in the SG than in the CG ($7.06 \pm 6.88\%$ *vs* $4.92 \pm 5.30\%$, $p = 0.01$, Kruskal–Wallis).

Conclusion: Although the acute stretching induced muscle hypotrophy, an antifibrotic action was detected.

1. Introduction

According to the World Health Organization (WHO), the 65 or over age group is growing faster than any other segment of the population (WHO, 2011). Changes of the organic physiological systems in the human body related to aging are important and rapidly expanding public health issues (Peixoto et al., 2004).

In older adults, changes in skeletal muscle composition are associated with an increase in fibrosis, a loss of muscle mass denominated as sarcopenia, and a decrease in force and flexibility which can lead to dependency, morbidity and mortality (Visser and Schaap, 2011; Birbrair et al., 2013). Thus an understanding of the biological mechanisms involved in skeletal muscle adaptation and its response to exercise is essential to sustain and improve the quality of life in older individuals.

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Sarcopenia is a useful feature of muscle degradation, and has been defined as a reduction in muscle fiber diameter, number of fibers, quantity and quality of muscle proteins (actin and myosin) and increase muscle fibrosis (Birbrair et al., 2013; Zhong et al., 2007). Sarcopenia can be accelerated by a variety of factors including inactivity, poor nutrition and chronic diseases (Thomas, 2007; Meng and Yu, 2010).

The aging process includes structural changes such as increases in collagen levels, changes in the elastic fiber system, increases in fat infiltration into the skeletal muscle, and decreases in collagen turnover with an accumulation of collagen cross-links (Kragstrup et al., 2011). These modifications can lead to increases in endomysium and perimysium, and slow muscles seem to be more affected, changing the mechanical properties of the skeletal muscle (Gao et al., 2008; Seene et al., 2012).

Changes in extracellular matrix (ECM) with age are associated with an increase in cross-links, advanced age-glycation of the proteins and changes in the expression of the matrix metalloproteinases (MMPs) (Wu et al., 2011). Such modifications can contribute to impaired musculoskeletal function since ECM controls the passive elastic response and force transmission (Kragstrup et al., 2011).

The ECM of skeletal muscle is composed primarily of type I and III fibrillar collagens, located especially in the epimysium and endomysium, respectively (Kragstrup et al., 2011; Koskinen et al., 2002; Calvi et al., 2012). However, changes in collagen synthesis occur with age, in which there is a considerable increase in the deposition of type I collagen in the muscle tissue in relation to type III collagen, leading to the muscle stiffness characteristic of the elderly (Kragstrup et al., 2011; Calvi et al., 2012). This change could be due to a reduced mechanical efficiency of muscle growth and increased TGFβ (tissue growth factor), which enhances the deposition of collagen and fibrotic tissue in the muscle (Li et al., 2013).

However, it has been shown that stretching exercises can improve the range of motion, flexibility, torque, balance, well-being and independence in the elderly (Feland et al., 2001; Gajdosik et al., 2005; Cristopoliski et al., 2008; Bird et al., 2009; Batista et al., 2009; Gallon et al., 2011). In young rats, stretching exercises can prevent the proliferation of connective tissue and increase the serial sarcomere number and muscle fiber cross-sectional area (Coutinho et al., 2004, 2006; Cação-Benedini et al., 2013).

Studies in humans have indicated that collagen synthesis in the muscle can increase in response to acute exercise (Langberg et al., 1999; Heinemeier et al., 2003, 2007; Moore et al., 2005; Miller et al., 2005). Wu et al. (2011) reported that the ability to feel and respond to mechanical stimulus is decremented in the elderly. Studies with animal models have shown that exercises do not prevent the increase in collagen production due to aging (Gosselin et al., 1998). Kragstrup et al. (2011) found that aging increases type I collagen and decreases type III collagen levels in skeletal muscle. In addition, in young male rats it was observed that daily short bouts of stretching after immobilization induced molecular reorganization of the collagen bundles and muscle fiber hypertrophy (Coutinho et al., 2006). Nevertheless, studies on the effects of stretching exercises on the skeletal muscle of aged rats are scarce.

Despite the fact that stretching exercises are commonly included in training programs for the elderly, the exercise training-induced histomorphological adaptations still remain unclear, especially with respect to skeletal muscle (Birbrair et al., 2013; Bird et al., 2009). Thus, the objective of this study was to investigate the acute effects of stretching on the morphology and on the collagen and TGFβ concentrations in the soleus muscle of aged rats.

2. Material and methods

This study was carried out with female Wistar rats according to the international ethics standard for animal experiments, and approved by the Ethics Committee on animal use of PUCPR (protocol no. 732/2012).

Female rats (26 weeks old) were kept in the bioterium in standard plastic cages under controlled environmental conditions (luminosity: bright/dark 12/hours cycle) with free access to food pellets and water. The rats were divided into 2 groups: Control (CG, $n = 7$); and Stretching (SG, $n = 8$), subjected to the stretching protocol. All the rats were subjected to euthanasia after the one-week period of experimentation.

To determine the sample number of this experiment, a sample number of at least 6 individuals were considered per experimental group, since a homogeneous population of laboratory animals was studied (Zar, 1998). In this arrangement each organism would have a16% probability of presenting a distinct event. In addition, the number of animals was small because it concerns aged animals which are fragile to manipulation, specifically to anesthesia, and thus the authors proposed this number of animals as the minimum necessary to achieve the scientific goal.

2.1. Stretching protocol

For passive stretching of the left soleus muscle, the animal was previously weighed (using *Mettler/Toledo* scales, Curitiba, Paraná), and anaesthetized by inhalation (isoflurane 5% for 2.5 l O_2 min^{-1}). The animal was then positioned on the stretching apparatus (Fig. 1) with the tibio-tarsal joint in maximum dorsal flexion to stretch the soleus muscle (Peviani et al., 2007), using a force of 0.48 N ± 0.04 applied by the apparatus to promote muscle stretching, monitored by a load cell.

The stretching protocol consisted of a set of 4 repetitions (Gallon et al., 2011; Kerrigan et al., 2003; Cristopoliski et al., 2009; Taylor et al., 1990) of 1 min each (Feland et al., 2001; Gallon et al., 2011; Cristopoliski et al., 2009; Watt et al., 2011a,b) with a 30 s interval between each repetition (Kerrigan et al., 2003; Stauber and Willems, 2002), controlled *via* a chronometer (*Technos*) (Peviani et al., 2007; Stauber and Willems, 2002). The stretching protocol was carried out once a day in the morning, three times a week (Monday, Wednesday and Friday) that is with at least one day of interval between each stretching protocol, for a one week period (Gallon et al., 2011; Peviani et al., 2007; Garber et al., 2011; Kamikawa et al., 2013).

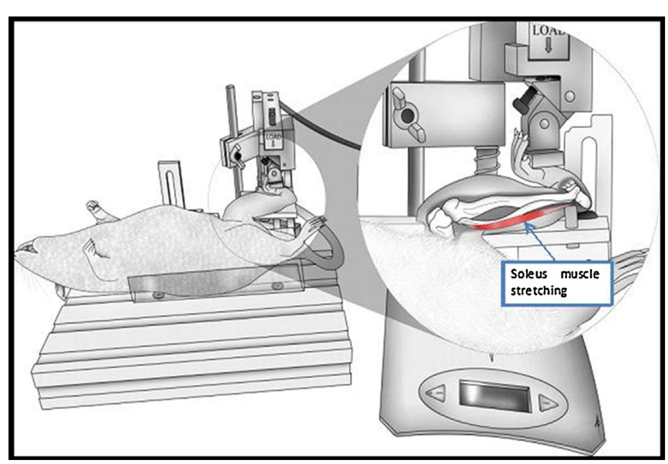

Fig. 1. Stretching position of rat on the apparatus.