MR CLARISSA MÜLLER BRUSCO (Orcid ID : 0000-0002-2013-1652)

MR RÉGIS RADAELLI (Orcid ID : 0000-0002-1521-2124)

Article type : Original Article

Title

The effects of flexibility training on exercise-induced muscle damage in young men with limited hamstrings flexibility

Authors

Clarissa M. Brusco¹, Anthony J. Blazevich², Regis Radaelli¹, Cintia E. Botton¹, Eduardo L. Cadore¹, Bruno M. Baroni³, Gabriel S. Trajano⁴, Ronei S. Pinto^{1, 2}

Affiliations

¹ Exercise Research Laboratory, School of Physical Education, Physioteraphy and Dance, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

² Centre for Exercise and Sports Science Research, School of Exercise and Health Sciences, Edith Cowan University, Joondalup, Australia

³ Graduate Program in Rehabilitation Sciences, Federal University of Health Sciences of Porto Alegre, Brazil

⁴School of Exercise & Nutrition Sciences, Queensland University of Technology, Brisbane, Australia

Running head

Flexibility training effects on muscle damage

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/sms.13068

Corresponding author contact details

Clarissa Müller Brusco Exercise Research Laboratory (LAPEX) Federal University of Rio Grande do Sul (UFRGS) Rua Felizardo, 750 – Bairro Jardim Botânico CEP: 90690-200 Porto Alegre – RS, Brazil Phone: +55 51 33085845 E-mail: clarissa.brusco@gmail.com

Abstract

Adaptations to 6 weeks of supervised hamstring stretching training and its potential impact on symptoms of eccentric exercise-induced muscle damage (EIMD) were studied in 10 young, untrained men with limited hamstrings flexibility. Participants performed unilateral flexibility training (experimental leg; EL) on an isokinetic dynamometer while the contralateral limb acted as control (CL). Hip range of motion (ROM), passive, isometric and concentric torques, active optimum angle, and biceps femoris and semitendinosus muscle thickness and ultrasound echo intensity were assessed both before and after the training. Additionally, muscle soreness was assessed before and after an acute eccentric exercise bout in both legs (EL and CL) at post-training only. Hip ROM increased (p<0.001) only in EL after the training (EL= 10.6° vs. CL= 1.6°), but no changes (p>0.05) in other criterion measurements were observed. After a bout of eccentric exercise at the end of the program, isometric and dynamic peak torques and muscle soreness ratings were significantly altered at all time points equally in EL and CL. Also, active optimum angle was reduced immediately, 48 h and 72 h post-exercise, and hip ROM was reduced at 48 and 72 h

equally in EL and CL. Finally, biceps femoris muscle thickness was significantly increased at all time points, and semitendinosus thickness and echo intensity significantly increased at 72 h, with no significant differences between legs. The stretching training protocol significantly increased hip ROM, however it did not induce a protective effect on EIMD in men with tight hamstrings.

Keywords: forces decrease, stretching exercises, constant-angle stretching, passive range of motion.

Introduction

Exercise-induced muscle damage (EIMD) commonly occurs subsequent to the execution of unaccustomed physical activity. It may be influenced by the type, volume and intensity of the exercise, and is particularly prominent when it involves eccentric muscle contractions [1]. Several physiological markers are commonly used to indicate EIMD, including a prolonged reduction in muscle force, delayed onset muscle soreness (DOMS), decreased joint range of motion (ROM), and muscular swelling [2]. These symptoms may remain for up to approximately one week, impairing physical performance and increasing injury risk during this period [3]. Therefore, different strategies have been investigated in order to attenuate the symptoms of EIMD, including (among others) acute and chronic pre-exercise muscle stretching.

Flexibility (i.e. muscle stretching) training has been shown to be successful for ROM maintenance and improvement in different populations [4,5]. The mechanisms underpinning the increase in ROM are still unclear; however an increase in "stretch tolerance" and an increase in muscle-tendon unit compliance have been suggested as possible mechanisms [6-8]. Interestingly, participants with more compliant hamstring muscle-tendon units have been shown to exhibit an attenuated response of some muscle damage markers after eccentric exercise (i.e. strength loss, pain, muscle tenderness and an increased creatine kinase concentration in the blood) when compared to participants with stiffer muscle-tendon units [9]. Such findings indicate that individuals with greater flexibility may be less susceptible to EIMD. Thus, it is reasonable to speculate that a flexibility training program might confer a protective effect on the muscle.

Nonetheless, few studies have explored the effect of chronic flexibility training on markers of EIMD [10-12]. LaRoche and Connolly [10] reported that 4 weeks of static or ballistic hamstring flexibility training were associated with a retention of (rather than the typical decline in) ROM and stretch tolerance after submaximal eccentric exercise, indicating some protective effect on EIMD. In addition, Eston et al. [11] reported a trend towards a faster recovery of muscle isometric strength measured at longer hamstring muscle length, but no additional benefits to isometric strength, DOMS or flexibility after 5 weeks of proprioceptive neuromuscular facilitation (PNF) stretching. Nonetheless, Chen et al. [12] found that longer-duration (8 weeks) static and PNF flexibility training programs resulted in decreases in the magnitude, and faster recovery, of knee flexor muscle strength, ROM and DOMS when compared to a non-stretching control group. Taken together, the aforementioned findings are equivocal and do not completely support an association between adaptations to flexibility training and EIMD attenuation.

The reasons for these discrepancies may include differences between the stretching protocols used (specially related to type and hold duration of the stretches) and the length of the training periods. Nonetheless, another factor that may influence the consistency of the findings is the control of stretching intensity (joint torque or joint angle) during training sessions. Most studies have used the pain perception to modulate the ROM during training sessions whilst others have allowed the participants to perform the stretches at their own volition. Moreover, it is not clear whether the monitoring of the stretch amplitude (i.e. joint ROM) throughout the training program would result in differences in the gains in flexibility training parameters (i.e. ROM, peak passive torque, stiffness). Use of an apparatus (i.e. isokinetic dynamometer) to perform the stretch would allow absolute control over the ROM throughout the course of the study and evaluate the importance of the control of this variable to training programs.

Another important factor affecting the adaptive response is the current flexibility levels of the participant, where already-flexible individuals may show increased muscle-tendon unit compliance [13] and thus already exhibit some protection prior to the stretching training being performed [9]. Therefore, it may be that an effect of static stretching on EIMD is more evident in individuals who currently exhibit poor flexibility. Therefore, the aims of the present study were to: a) evaluate the chronic adaptations induced by 6 weeks of hamstrings flexibility training; and b) assess whether the flexibility training program confers protection against the symptoms of EIMD in untrained, inflexible young men.

Materials and Methods

Participants

Twenty healthy men volunteered to participate in the present study. As the symptoms of EIMD may be influenced by muscle compliance, and thus levels of flexibility [9], each participant was expected to have a limited hamstring flexibility,

defined as maximum of 80° hip flexion in passive straight-leg raise test (PSLR) [5]. Based on this criterion, six participants were excluded from participation, three further participants were excluded because they were engaged in sports programs, and one participant dropped out prior to the first evaluation. Additionally, after the allocation process three participants who started the study were omitted because they performed exercise involving strong eccentric contractions during the study period. Therefore, ten healthy men (24.4 ± 4.1 years; 78.7 ± 13.4 kg; 176.6 ± 6.6 cm; $17.6 \pm 8.7\%$ body fat) who were not engaged in strength or flexibility training activities for at least three months prior to the study were accepted into the study. All participants were free from functional limitations that could impair their performance, and were instructed to avoid any changes in their diet and physical activity levels (e.g., walking, jogging, and biking) during the course of the study.

The participants were carefully informed of the purpose, procedures and risks of study participation, and written informed consent was obtained from all participants. All procedures were approved by the University Institutional Review Board, and conducted in accordance with the Declaration of Helsinki.

Experimental Design

This study was designed to examine the effect of hamstring flexibility training on exercise-induced muscle damage (EIMD) after maximal eccentric exercise. There is considerable heterogeneity in participants' responsiveness to EIMD [14], however since both limbs of a participant respond similarly to a muscle damaging stimulus [15] and to flexibility training [16,6] the right and left legs of the participants were allocated according to their leg dominance as the experimental (EL) or control (CL) leg; i.e. participants served as their own controls, with some individuals having their dominant leg in the experimental group and others in the control group. EL was exposed to constant-angle muscle stretching training for six weeks, as described below, and CL remained untrained. The adherence to the training program was 99.2%, with only one subject missing one training session. Six participants were assigned their dominant limb as the experimental limb (EL) and four were assigned their non-dominant limb.

This study had a total duration of 8 weeks. In the first week, participants attended to two testing sessions separated by at least 48 h for familiarization and determination of test-retest reliability. When the variation in hip ROM was ≤5% over the test-retest sessions, the participant was considerate familiarized with the test procedure. No participant needed more than two familiarization sessions. The participants were tested for: (a) maximum hip flexion range of motion (ROM), (b) passive knee joint torque during extension, (c) isometric and dynamic knee flexion peak torque, (d) active optimum knee flexion angle, and (e) biceps femoris and semitendinosus muscle thickness and ultrasound echo intensity. From the second to the seventh week of the study, participants performed the flexibility training twice a week (i.e. 12 training sessions). In the last week participants performed a maximal eccentric exercise session designed to induce muscle damage in both legs. All flexibility training measurements completed before the training program were repeated in the last week. In addition, EIMD and DOMS assessments were also completed before and at five time points after the eccentric exercise-induced muscle damage protocol (i.e. pre, 0 h, 24 h, 48 h and 72 h). EIMD was evaluated only after the training program, and not at pre-training, in order to avoid the repeated bout effect, since the symptoms may last several weeks and could influence the results

[17]. The same investigator conducted all the evaluations and the same specific instructions were repeated at all testing time points (Figure 1).

Flexibility training

The participants performed two training sessions per week on nonconsecutive days for six weeks. The knee flexor muscles were stretched using a passive, constant-angle stretch by rotating the knee from flexion to extension on an isokinetic dynamometer (Cybex Norm; Ronkonkoma, NY, USA). The participants were seated with the trunk perpendicular to the seat and with the trunk and the stretching limb stabilized with straps. The thigh rested on a specially constructed device, which elevated it to 30° from horizontal, similar to that adopted in previous studies [18]. The dynamometer's axis of rotation was aligned with the lateral femoral condyle of the stretched limb.

Before each training session, participants warmed up on a cycle ergometer (Movement Technology; SP, Brazil) for 5 min with minimum load at a self-selected cadence. Prior to administration of the stretching procedure, knee ROM was determined by the examiner with the participant seated and stabilized in the dynamometer's chair. With the participant set on the dynamometer chair, the examiner passively and slowly moved the lower leg toward knee extension at a slow, constant speed (~5°/s; the Cybex system is unable to do this automatically), and the movement was terminated when the participant informed the examiner that they reached their maximum tolerable stretch amplitude irrespective of the timing or magnitude of pain onset. The leg was immediately returned to the starting position and the angle achieved that was displayed in the dynamometer' monitor was used as target angle in the training session. The participants were carefully instructed to

remain relaxed and to not offer any resistance to the movement, and they were encouraged to always reach maximum knee ROM. Visual feedback was not provided (i.e. screen display was not allowed) during the test in order to prevent participants from monitoring their knee ROM. Participants were instructed to leave the foot relaxed in order to reduce pain felt at the back of the knee and to ensure that the hamstring muscles were properly targeted. These procedures were performed in every training session to determine the knee ROM. Training intensity was set as the maximum knee ROM that could reached at the beginning of each training session (see Figure 2). Training progression was made though alteration in the knee joint angle only (i.e. knee in a more extended position) and no alteration was made in the hip flexion angle throughout the training sessions. No participant reached maximum knee extension in any training session.

For the stretch maneuvers, the dynamometer passively extended the knee at 5°/s until the pre-determined angle, and maximal knee ROM was maintained for 60 s. Eight 60-s repetitions were performed, totaling 480 s per session and a training volume of 5750 s over six weeks. A 30-s rest interval was allowed between repetitions. The same examiner supervised all training sessions and average temperature in the room during training sessions was controlled at 22°C.

Range of motion assessment

Maximum range of motion was assessed using a maximum passive straightleg raise test (PSLR). The participant was placed in the supine position with legs straight then instructed to remain relaxed and to not offer any resistance to the movement. The non-tested leg was stabilized with inelastic straps to avoid compensatory movements. One examiner passively and slowly lifted the participant's

leg into hip flexion until maximum tolerated amplitude, informed by the subject, was reached and a second examiner positioned the manual goniometer in the hip joint to measure hip ROM [5]. The axis of rotation of the goniometer was aligned in the center of the hip joint (great trochanter), the stationary arm was positioned along the lateral middle of the abdomen, using the pelvis as reference and the moving arm along the lateral middle of the femur. This test was performed once on each limb.

Passive torque assessment

Passive torque during stretch was measured in the isokinetic dynamometer (Cybex NORM; Ronkonkoma, NY, USA) in the sitting position. The participants were placed and stabilized, as previously described in the flexibility training session, and maximum knee ROM was set in each evaluation day according to the protocol used for the flexibility training. For the passive torque test, the dynamometer's lever arm passively extended the knee at 5°/s until the pre-determined angle was reached, then the leg was immediately returned to the starting position. The participants were instructed to remain relaxed and to not offer any resistance to the dynamometer's lever arm movement. Passive torque was analyzed at the same joint angle before and after training, so the maximal angle reached at pre- training was used to evaluate passive torque at post-training testing.

Muscle electrical activities of biceps femoris and semitendinosus were recorded during the passive torque test to ensure that activities were minimal (i.e. <5% of that recorded during a maximal voluntary contraction). Before electrode placement, the skin was shaved and cleaned with alcohol to reduce impedance. Bipolar surface electrodes (Hal, São Paulo, SP, Brazil) were placed with 20 mm interelectrode distance over biceps femoris and semitendinosus, according to

SENIAM [19] guidelines. Electrode position was carefully mapped using a transparent sheet to ensure replication of positioning at post-training testing. EMG signals were recorded using an 8-channel electromyographic system (Miotool 800 Wireless, Miotec Equipamentos Biomédicos, Porto Alegre, RS, Brazil), digitized at a sample frequency of 2000 Hz per channel, and stored on a personal computer. The EMG signals were filtered using a Butterworth digital filter with cut-off frequencies of 20 Hz (high pass) and 500 Hz (low pass). After filtering, EMG root mean square (RMS) amplitudes were obtained during three 1000-ms windows (at the beginning, the middle and at the end of the stretch) and the averages were used for analysis.

Exercise-induced muscle damage (EIMD) protocol

The participants were seated in the isokinetic dynamometer (Cybex Norm; Ronkonkoma, NY, USA) with trunk flexed at 85°. The knee joint of the exercised leg was aligned with the rotation axis of the dynamometer, the ankle of the leg was strapped to the dynamometer's lever arm, and the thigh, torso and pelvis were secured by straps. Participants performed six sets of 10 maximal voluntary eccentric hamstring contractions at an angular velocity of 60°/s with each leg on an isokinetic dynamometer. The participants were instructed to contract the knee flexors maximally to resist the knee-extending action of the dynamometer that moved the knee joint from a flexed (90°) to an extended (10°) position. Each set was separated by a 1-min rest period. The participants received strong verbal encouragement during exercise to generate maximal force and were given real-time visual feedback by means of a computerized visual display. All participants performed the eccentric exercise three to five days after the last training session and were carefully instructed to avoid alcohol and/or analgesic/anti-inflammatory drugs throughout the

days of muscle damage evaluations. Both legs (EL and CL) were tested one immediately after the other in a randomized order. Before and immediately, 24 h, 48 h and 72 h after the exercise protocol, tests of hip ROM, soreness, maximal torque and ultrasound imaging were performed as described below.

Active peak torque and optimum angle assessments

Isometric and dynamic knee flexion peak torques were measured on the isokinetic dynamometer, and the optimum angle (i.e. peak torque angle) was captured during a dynamic knee flexion strength test. The participants were seated with their hips flexed at 85° (0° = anatomical position) and the dynamometer's axis of rotation was aligned with the lateral femoral condyle of the tested limb while straps were secured across the thigh, torso and pelvis to restrict compensatory movements. After a warm-up of ten submaximal dynamic contractions at 60° /s, the participants performed two attempts of 3-s maximal isometric knee flexion contractions at 40° of knee flexion (0° = full knee extension), with a 3-min recovery period between each attempt. The highest peak torque value was used for analysis. Dynamic peak torque and the optimum angle were assessed through five consecutive concentric contractions at 60° /s. The highest peak torque values and the joint angle at which the maximum peak torque occurred were used for analysis. Participants received verbal encouragement to perform maximal force in both tests, and visual feedback was provided on a monitor.

Muscle thickness and echo intensity assessments

Biceps femoris and semitendinosus images were obtained by B-mode ultrasonography (Toshiba, VMI, Japan) using a high-resolution linear probe of 8.0 MHz (38 mm probe width). Images were obtained at 50% of the distance between the greater trochanter of the femur and the lateral knee joint line, according to previously suggested methods [20]. All measurements were obtained while the participants lay in the prone position with the muscle in a relaxed state and after resting in a supine position for at least 15 min to allow fluid shifts to stabilize. A water-based gel was used in an amount enough to promote acoustic contact and have a clear image of the superficial and the depth muscle interfaces without causing excessive probe pressure on the skin and the transducer was positioned perpendicular to the evaluated muscles. Probe position was carefully mapped using a transparent sheet and marked on the skin with a demographic pen to ensure replication position at all testing days. For each muscle, three images were obtained and the same images were used for analyses of muscle thickness and echo intensity (i.e. echogenicity). The muscle thicknesses were determined as the distance between the interfaces of the superficial and deep aponeuroses, and ultrasound echo intensities determined using the maximal muscle area, avoiding bone and subcutaneous fat tissue, based on a histogram of gray scale (0 = black, 255 = white). Ultrasound echo intensity is a quantitative estimation made through a grey scale histogram analysis that reflects contractile and non-contractile tissue ([21,22]. All image analyses were performed using Image J software (version1.37, National Institutes of Health, Washington, D.C., USA). In the first week of the study, participants attended two familiarization sessions and reliability of these measures was tested. Test-retest ICCs for muscle thickness of the two hamstring muscles

analyzed in this study were 0.94-0.99, and CVs were 1.3-3.1%; for echo intensity, test-retest ICCs were 0.67-0.92 and CVs were 6.2-14.1%.

Muscle soreness assessment

A 100-mm visual analog scale (100-mm continuous line, where 0-mm represented "no pain" and 100-mm represented "extreme pain") was used to quantify muscle soreness. The participants were asked to mark their level of muscle soreness on the scale after a full range of motion movement of knee flexion/extension [12].

Statistical Analysis

Normality of data was tested using the Shapiro Wilk test, and Levene's test was used to verify homogeneity. After determining that the sample was normally distributed, student's t-tests for independent data were used to compare pre-training data between legs (i.e. between 'groups'). In order to examine the adaptations to flexibility training, two-way analyses of variance (ANOVA) with repeated measures (2x2 - group [EL versus CL] x time [pre-training versus post-training]) were used. When a significant group x time interaction was found, Bonferroni post-hoc tests were used to examine between-group (i.e. between-leg) and between-time differences. For data analysis, within-group comparisons were used, while change scores were used for between-group comparisons. In addition, the effect sizes for the effects of the flexibility training measured variables were calculated according to Cohen (1992).

In order to examine the effect of the flexibility training on muscle damage responses, a two-way ANOVA (2x5-group [EL versus CL] x time [pre, 0, 24, 48, 72 h]) for repeated measures was used. When the ANOVA showed a significant main effect, Bonferroni post-hoc tests were used to further examine the differences. Significance level was set at α <0.05. All analyses were performed with SPSS software 17.0 (IBM, Somers, NY, USA), and results are reported as mean ± SD.

Results

Adaptations to flexibility training

Before the flexibility training, no significant difference (p>0.05) was observed between EL and CL for any outcome (Table 1). The ANOVA showed a significant main effect for time (p<0.001) and a group × time interaction (p=0.003) for hip ROM (18.1 \pm 11.2% for EL vs. 2.5 \pm 8.2% for CL). Only EL significantly increased hip ROM (p<0.001) from pre- to post-training. Passive torque (p=0.95), peak isometric torque (p=0.09), active optimum angle (p=0.74) and both biceps femoris (p=0.47) and semitendinosus (p=0.23) muscle thickness were unchanged in both groups. Significant main effects for time (p=0.008) were observed, but no group (leg) × time interaction (p=0.82), for dynamic peak torque (-7.4 \pm 10.9% for EL vs. -9.3 \pm 14.4% for CL).

Exercise induced muscle damage (EIMD)

The work produced in all eccentric exercise repetitions were summed in order to define the total work performed, and no significant difference (p>0.05) was found between EL (5723 ± 1271 J) and CL (5389 ± 1264 J). The two-way ANOVA showed a significant main effect for time, where decreases in isometric (p<0.001) and

dynamic peak torques (p<0.001) (Figure 3), optimal angle (p=0.002) and hip ROM (p=0.003) were observed (Figure 4). Additionally, increases in muscle soreness (p<0.001) (Figure 4), biceps femoris (p<0.001) and semitendinosus (p<0.001) muscle thickness (Figure 5E and F), and biceps femoris (p=0.04) and semitendinosus (p=0.001) echo intensities (Figure 5G and H) were observed. However, no group x time interaction was found (p>0.05).

Isometric and dynamic peak torques in EL and CL were significantly lower (p<0.05) at all time points after the eccentric exercise when compared to preexercise values, with no difference between legs (Figure 3). For CL, isometric torque decreased 27 - 39% after eccentric exercise (0 h = -27 ± 15%, 24 h = -32 ± 19%, 48 h = -39 ± 26%, 72 h = -35 ± 22%), and dynamic strength decreased 21 - 32% (0 h = -24 ± 17%, 24 h = -21 ± 23%, 48 h = -32 ± 26%, 72 h = -25 ± 30%). For EL, isometric torque decreased 24 - 33% (0 h = -24 ± 13%, 24 h = -26 ± 26%, 48 h = -33 ± 37%, 72 h = -25 ± 27%) and dynamic peak torque decreased 23 - 38% (0 h = -28 ± 13%, 24 h = -23 ± 22%, 48 h = -38 ± 27%, 72 h = -29 ± 20%).

The active optimum angle was significantly decreased (p<0.05) (i.e. toward extension) from before to immediately, 48 h and 72 h after the eccentric exercise in EL and CL, with no differences between legs (Figure 4A). For EL, optimum angle decreased 14 - 24% (0 h = -19 \pm 20%, 48 h = -14 \pm 22%, 72 h = -24 \pm 22%), and for CL it decreased 21 - 26% (0 h = -20 \pm 14%, 48 h = -21 \pm 15%, 72 h = -26 \pm 24%).

Significant decreases in maximum passive hip ROM were found (p<0.05) from before to 48 h and 72 h after eccentric exercise for both EL and CL, with no differences between legs (Figure 4B). For EL, hip ROM decreased 13 \pm 15% and 12 \pm 13% at 48 h and 72 h after eccentric exercise, respectively, and for CL the hip

ROM decreased 15 \pm 14% and 16 \pm 12% at 48 h and 72 h after eccentric exercise, respectively.

Muscle soreness increased significantly (p<0.05) from before exercise to all time points after eccentric exercise in EL and CL, with no differences between legs (Figure 4C).

Biceps femoris muscle thickness increased significantly (p<0.05) at all time points after the eccentric exercise, with no differences between legs (Figure 5E and F). For EL there were increases of 7 - 13% (0 h = 8 ± 4%, 24 h = 7 ± 5%, 48 h = 11 ± 7%, 72 h = 13 ± 8%) while for CL the increases were 4 - 8% (0 h = 4 ± 4%, 24 h = 4 ± 3%, 48 h = 8 ± 6%, 72 h = 8 ± 11%). Semitendinosus muscle thickness increased significantly (p<0.05) for EL (18 ± 9%) and CL (14 ± 12%) at 72 h after exercise, with no differences between legs.

A main effect of time was observed for ultrasound echo intensity changes; whilst no significant differences at any time point were detected after the eccentric exercise for the biceps femoris (Figure 5G), a significant increase (p<0.05) was observed from pre-exercise to 72 h after exercise for EL (50 \pm 56%) and CL (41 \pm 39%) in semitendinosus, with no differences between legs (Figure 5H).

Discussion

Adaptations to flexibility training

Six weeks of flexibility training resulted in a 10.6° (± 6.3°) hip ROM, which is similar to the improvements reported in other studies (~ 10°) that have investigated hamstring flexibility training over a comparable period of time (~6 wk) [6,23,24]. Furthermore, these results are similar to the gains reported in other studies (9.5° to

14°) using a longer training duration (8 - 12 wk) [5,25-27], as well as those using 3 -7 training sessions per week of 8.9° to 12.6° [25,6,23,24]. The similarities in the increases in ROM between the present study and others using longer training durations and/or greater weekly frequencies might be attributed to the high weekly volume training used in the present study, which was higher than most previous studies [28,23,24]. In present study, a total training volume of 5760 s was accumulated, while other researchers imposed 900 s to 5400 s of stretch [28,23,24], although a few have used higher total volumes of 5040 s to 7220 s [29,6,30]. Of these studies using longer durations of stretch, only Cipriani et al. [29] found a much greater improvement in ROM than observed in the present study, with increases of 24.2° to 28.0° in hamstring ROM after six weeks of training performed twice daily.

In the present study, the increase in ROM occurred without concomitant changes in the passive torque required to stretch the muscle, which suggests that there were no alterations in hamstring muscle-tendon unit viscoelastic properties [6,10,8]. Since an alteration in passive torque was not found to occur with the increased ROM, it is possible that an increase in stretch tolerance, which may be associated with reduction in perception of pain or discomfort [16,31], may have at least partly underpinned the increased ROM; this conclusion has been previously indicated as an important factor related to increases in ROM [6,16,7].

The resistance to passive stretch, however, may be influenced by the amount of contractile and non-contractile muscle proteins and extracellular connective tissue, and the endomysium, perimysium, and epimysium are believed to greatly influence on passive stretch resistance [32]. It has additionally been suggested that flexibility training might be able to induce alterations in intrafibrillar, myofilament, cross-bridge, or titin stiffness, and it is also possible that adaptations might occur within the parallel

elastic components [7]. However, as passive torque measured during stretch was unchanged in the present study, it appears that the training program may not have evoked a significant modification of connective tissue or extracellular matrix properties. Nonetheless, a lack of change in passive torque during stretch cannot be taken as proof of a lack of alteration in muscle or tendon mechanical properties because the passive torque test may not be sensitive to small changes in individual tissues [7]. Regardless, it is possible that stretching programs of different duration, frequency and intensity characteristics may influence muscle mechanical properties differently.

No significant changes were detected either in the angle of peak voluntary knee flexion torque or optimum angle after training. These results are consistent with those of Aquino et al. [33], who performed static stretching training three times a week for eight weeks, but contrast those of Chen et al. [12] and Ferreira et al. [23], who observed a shift in the optimum angle towards a longer hamstring muscle length after imposing flexibility training protocols with a higher total training volume and weekly training frequency, respectively. Such changes might speculatively occur if sarcomeres were added in series, increases in the compliance of series elastic structures were induced, or alterations in other connective tissues, including the extracellular matrix, occurred. Our findings are similar to those of previous studies [e.g. Blazevich, et al. [7]] and do not indicate that sarcomerogenesis or changes in connective tissue properties occurred (or were not sufficient to be detected). The lack of change in optimum angle may thus be speculatively explained by different adaptations being induced by the current training program as compared to those in the study of Chen et al. [12].

Muscle damage responses

Although McHugh et al. [9] proposed that symptoms of EIMD vary according to muscle compliance and muscle flexibility, our findings did not confirm that the adaptations induced by monitored flexibility training necessarily confer a protective effect (i.e. reduced force loss or soreness) to the hamstring muscle group after acute bout of an eccentric exercise. This is despite individuals with limited hamstring flexibility being selected from a larger cohort of study volunteers.

A prominent functional impairment caused by muscle damage in humans is a prolonged reduction in strength [2]. In the present study, EL and CL showed similar reductions in isometric and dynamic concentric strength measured at all time points after the eccentric exercise. Similarly, Eston et al. [11] did not report an effect of PNF stretching training of the hamstrings on the recovery of isometric strength after eccentric exercise, although the authors reported a tendency towards a faster recovery of muscle strength at long muscle lengths. Nonetheless, Chen et al. [34] reported faster strength recovery in the groups that performed eight weeks of static and PNF flexibility training than a non-stretching control group, and full recovery was reported five days after eccentric exercise, which is a longer time period than the evaluated in the present study (i.e. three days). However, based on these results it might be concluded that stretching using different duration, frequency and intensity characteristics might be required to induce a protective effect.

Changes in the optimum angle for torque production have been observed after a protocol of eccentric exercise, which has been attributed to sarcomere disruption [34] although other factors are also likely to influence it. In the present study, a similar shift in optimum angle towards longer muscle lengths was observed in both legs after the eccentric exercise; this result indicates that sarcomere

disruption (or other related factors) might have been similar between groups. Previous authors have suggested that an intense flexibility training program might induce alterations in serial sarcomere number [35], although there is no evidence for this in humans after traditional stretching programs. Any such change might theoretically shift the optimum active angle to longer muscle length and potentially induce a protective effect on EIMD by reducing the tension along the muscle fibers [9,12]. However, the lack of alteration in the optimum angle after the flexibility training program in the present study indicates (among other things) a lack of any protection from potential sarcomere disruption. On the other hand, it is very likely that the optimal angle shift is not a sensitive marker of sarcomere remodeling, and it cannot fully explain the protective effect [36] because the shift in the optimal angle does not last four weeks, which is a time frame over which the repeated bout effect is clearly evident [34].

Alternatively, one factor that seems to influence muscle damage is the participation of the connective tissue and extracellular matrix. An important function of the extracellular matrix is to protect muscle from injury by decreasing excessive muscle strain by increasing passive tension [e.g. see Hyldal et al. [36]]. Also extracellular matrix cells such as fibroblasts, macrophages and endothelial cells have been shown to contribute to myofiber degeneration [37]. Based on the importance of the non-contractile tissue, an intervention that promotes adaptation in these tissues would be expected to induce a protective effect. However, in the present study no significant changes in muscle-tendon unit mechanical properties or active torque-angle relation, which are influenced by parallel and series elastic components and by changes in series compliance, respectively, or protection against EIMD were observed. When considered within this framework, it might be speculated

that flexibility training programs that sufficiently influence the connective tissues (including the extracellular matrix) might be more expected to reduce muscle damage; flexibility training programs with longer durations or higher frequencies or intensities may be needed to evoke such changes and should therefore be trialed in future experiments.

Another functional impairment caused by muscle damage is a reduced ROM, attributed to edema in the affected tissues and particularly in the myotendinious junction [38]. In the present study, hip ROM was similarly reduced at 48 h and 72 h post-exercise for EL and CL. LaRoche and Connolly [10] reported no changes in ROM after an acute bout of eccentric exercise performed after stretch training. These differences might be due to the use of a submaximal eccentric exercise protocol in their study, whereas maximum eccentric contractions were performed in the present study. It is well known that the magnitude of EIMD is related to the intensity of the exercise, and that maximal contractions such as those performed on an isokinetic dynamometer usually induce major changes in markers of muscle damage and a longer recovery time is needed [39]. Nonetheless, Chen et al. [12] reported a smaller ROM reduction after maximal eccentric exercise after eight weeks of static or PNF flexibility training, and ROM was fully recovered five days after maximal eccentric contractions. Therefore, characteristics of the program used by Chen et al. [12] may have influenced the magnitude of EIMD. For example, training volume and frequency were higher, which resulted in greater improvements in hip ROM (24.0°) than in the present study (10.6°) and, since the participants recruited by Chen et al. [12] were more flexible at pre-training (ROM was 96.1° vs. <60°), the hip ROM reached after training was much higher than the ROM reached by the participants in the present study. It is possible that the protective effect in ROM after

EIMD might be dependent on the participants' ability to reach a greater ROM than that reported in the present study. Thus, individuals, including those with poor flexibility such as the participants used in the current study, might benefit from a more intense training program (e.g. constant torque rather than constant-angle stretching) [40], which might then reduce passive stiffness and induce a greater ROM improvement, and hence confer some protective effect on EIMD.

Muscle thickness (MT) and echo intensity (EI) assessed by ultrasound have been widely used to evaluate swelling and edema as part of the EIMD phenomenon. Most studies have evaluated upper limb muscles (e.g. biceps brachii) [2,41], yet few have evaluated lower limb muscles (e.g. the hamstrings). In the present study, biceps femoris MT was significantly increased at all time points after eccentric exercise, and semitendinosus MT was significantly greater 72 h post-exercise. For El, biceps femoris was not significantly altered while semitendinosus was significantly greater 72 h after exercise. It may be assumed that the flexibility training did not induce structural alterations in the hamstring that were sufficient to reduce swelling and edema. In contrast with the present results, Chen et al. [41] observed increases in biceps femoris echo intensity after eccentric exercise, although these authors used a constant-area region of interest of 2 cm² rather than using the maximum muscle area as in the present study. When evaluating a larger muscle area, it is possible to analyze different parts of the muscle that could have been affected by EIMD, which might account for the differences found [42]. In a comparison study between upper and lower limb responses to EIMD, Chen et al. [41] reported that the upper limb muscles were more susceptible to muscle damage than the lower limb. Taking into account such differences, it is possible that a higher

exercise intensity is required to induce significant changes in echo intensity after eccentric exercise in the hamstrings.

In summary, the results of the present study showed that six weeks of constant-angle flexibility training using eight sets of 60 s duration at maximal tolerable angle evoked a significant increase in hamstring hip ROM in inflexible individuals. However, the program was not sufficient to confer a protective effect against eccentric exercise-induced muscle damage, as indicated by the lack of differences between stretched and non-stretched legs for markers of muscle damage and soreness. The program did not alter passive stiffness or active peak torque angle, and may not therefore have induced adaptations in the muscle fibers or connective tissues that were sufficient to confer a protective effect.

Limitations

A possible limitation of the present study was that the evaluators were nonblinded to the angle reached during the range of motions tests and to the participants' legs allocation. Other possible limitation was the use of the participants' contralateral limb acting as their own control. This method have some advantages (as previously stated) but it may be a possible limitation due to the potential of crosstransfer adaptation, and if this was the case, the CL could also be protected in a certain degree. However, it is not clear whether a flexibility training program may induce ROM increases in the contralateral non-trained limb as previous studies have reported contradictory findings [43,44], and in the present study no ROM alteration was found in the contralateral limb, which suggests that cross-transfer may not be always present after flexibility training programs. In the present study, CL did not show any benefit from possible cross-transfer effect in any variable evaluated,

therefore we believe that the possible protection induced by cross transfer to CL was minimal.

Perspectives

The flexibility training program used in the present study was not sufficient to confer a protective effect on exercise-induced muscle damage (EIMD), even though significant increase in ROM was found in previously inflexible participants. In future studies, training programs with higher stretch intensities (e.g. constant- torque) or with greater weekly frequency (e.g. three to five session per week) might be tested for their abilities to elicit adaptations in the connective tissue (including the extracellular matrix) and muscle fibers, and subsequently for their ability to influence EIMD. Based on the current data, low weekly frequency flexibility programs may not influence the risk of muscle damage in response to unaccustomed (especially eccentric) exercise, even in inflexible individuals, although the possibility exists that effective programs might be identified in the future.

Acknowledgements

The authors would like to thank CAPES-Brazil and CNPq-Brazil for financial support, and all volunteers for their participation in this project.

References

1. Friden J, Lieber RL (2001) Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. Acta physiologica Scandinavica 171 (3):321-326. doi:10.1046/j.1365-201x.2001.00834.x

2. Radaelli R, Bottaro M, Wilhelm EN, Wagner DR, Pinto RS (2012) Time course of strength and echo intensity recovery after resistance exercise in women. Journal of strength and conditioning research / National Strength & Conditioning Association 26 (9):2577-2584.

doi:10.1519/JSC.0b013e31823dae96

3. Howatson G, van Someren KA (2008) The prevention and treatment of exercise-induced muscle damage. Sports medicine 38 (6):483-503

4. Feland JB, Myrer JW, Schulthies SS, Fellingham GW, Measom GW (2001) The effect of duration of stretching of the hamstring muscle group for increasing range of motion in people aged 65 years or older. Physical therapy 81 (5):1110-1117

5. Ayala F, Sainz de Baranda P, De Ste Croix M, Santonja F (2013) Comparison of active stretching technique in males with normal and limited hamstring flexibility. Physical therapy in sport : official journal of the Association of Chartered Physiotherapists in Sports Medicine 14 (2):98-104. doi:10.1016/j.ptsp.2012.03.013

6. Ben M, Harvey LA (2010) Regular stretch does not increase muscle extensibility: a randomized controlled trial. Scandinavian journal of medicine & science in sports 20 (1):136-144. doi:10.1111/j.1600-0838.2009.00926.x

7. Blazevich AJ, Cannavan D, Waugh CM, Miller SC, Thorlund JB, Aagaard P, Kay AD (2014) Range of motion, neuromechanical, and architectural adaptations to plantar flexor stretch training in humans. Journal of applied physiology 117 (5):452-462. doi:10.1152/japplphysiol.00204.2014

8. Freitas SR, Mendes B, Le Sant G, Andrade RJ, Nordez A, Milanovic Z (2017) Can chronic stretching change the muscle-tendon mechanical properties? A review. Scandinavian journal of medicine & science in sports. doi:10.1111/sms.12957

9. McHugh MP, Connolly DA, Eston RG, Kremenic IJ, Nicholas SJ, Gleim GW (1999) The role of passive muscle stiffness in symptoms of exercise-induced muscle damage. The American journal of sports medicine 27 (5):594-599

10. LaRoche DP, Connolly DA (2006) Effects of stretching on passive muscle tension and response to eccentric exercise. The American journal of sports medicine 34 (6):1000-1007. doi:10.1177/0363546505284238

11. Eston RG, Rowlands AV, Coulton D, McKinney J, Gleeson NP (2007) Effect of flexibility training on symptoms of exercise-induced muscle damage: A preliminary study. J Exerc Sci Fit 5 (1):33-39

12. Chen CH, Nosaka K, Chen HL, Lin MJ, Tseng KW, Chen TC (2011) Effects of flexibility training on eccentric exercise-induced muscle damage. Medicine and science in sports and exercise 43 (3):491-500. doi:10.1249/MSS.0b013e3181f315ad

13. Blazevich AJ, Cannavan D, Waugh CM, Fath F, Miller SC, Kay AD (2012) Neuromuscular factors influencing the maximum stretch limit of the human plantar flexors. Journal of applied physiology 113 (9):1446-1455. doi:10.1152/japplphysiol.00882.2012

14. Chen YW, Hubal MJ, Hoffman EP, Thompson PD, Clarkson PM (2003) Molecular responses of human muscle to eccentric exercise. Journal of applied physiology 95 (6):2485-2494. doi:10.1152/japplphysiol.01161.2002

15. Chapman D, Newton M, Sacco P, Nosaka K (2006) Greater muscle damage induced by fast versus slow velocity eccentric exercise. International journal of sports medicine 27 (8):591-598. doi:10.1055/s-2005-865920

16. Folpp H, Deall S, Harvey LA, Gwinn T (2006) Can apparent increases in muscle extensibility with regular stretch be explained by changes in tolerance to stretch? The Australian journal of physiotherapy 52 (1):45-50

17. Nosaka K, Sakamoto K, Newton M, Sacco P (2001) How long does the protective effect on eccentric exercise-induced muscle damage last? Medicine and science in sports and exercise 33 (9):1490-1495

18. Magnusson SP, Simonsen EB, Aagaard P, Kjaer M (1996) Biomechanical responses to repeated stretches in human hamstring muscle in vivo. The American journal of sports medicine 24 (5):622-628

19. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology 10 (5):361-374

20. Blackburn JT, Pamukoff DN (2014) Geometric and architectural contributions to hamstring musculotendinous stiffness. Clinical biomechanics 29 (1):105-110. doi:10.1016/j.clinbiomech.2013.10.011

21. Pillen S, Tak RO, Zwarts MJ, Lammens MM, Verrijp KN, Arts IM, van der Laak JA, Hoogerbrugge PM, van Engelen BG, Verrips A (2009) Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. Ultrasound in medicine & biology 35 (3):443-446. doi:10.1016/j.ultrasmedbio.2008.09.016

22. Arts IM, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ (2010) Normal values for quantitative muscle ultrasonography in adults. Muscle & nerve 41 (1):32-41. doi:10.1002/mus.21458

23. Ferreira GN, Teixeira-Salmela LF, Guimaraes CQ (2007) Gains in flexibility related to measures of muscular performance: impact of flexibility on muscular performance. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 17 (4):276-281. doi:10.1097/JSM.0b013e3180f60b26

24. Reid DA, McNair PJ (2004) Passive force, angle, and stiffness changes after stretching of hamstring muscles. Medicine and science in sports and exercise 36 (11):1944-1948

25. Chan SP, Hong Y, Robinson PD (2001) Flexibility and passive resistance of the hamstrings of young adults using two different static stretching protocols. Scandinavian journal of medicine & science in sports 11 (2):81-86

26. Freitas SR, Mil-Homens P (2015) Effect of 8-week high-intensity stretching training on biceps femoris architecture. Journal of strength and conditioning research / National Strength & Conditioning Association 29 (6):1737-1740. doi:10.1519/JSC.00000000000800

27. e Lima KM, Carneiro SP, Alves Dde S, Peixinho CC, de Oliveira LF (2015) Assessment of muscle architecture of the biceps femoris and vastus lateralis by ultrasound after a chronic stretching program. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 25 (1):55-60. doi:10.1097/JSM.00000000000000069

28. Bandy WD, Irion JM, Briggler M (1998) The effect of static stretch and dynamic range of motion training on the flexibility of the hamstring muscles. The Journal of orthopaedic and sports physical therapy 27 (4):295-300. doi:10.2519/jospt.1998.27.4.295

29. Cipriani D, Abel B, Pirrwitz D (2003) A comparison of two stretching protocols on hip range of motion: implications for total daily stretch duration. Journal of strength and conditioning research / National Strength & Conditioning Association 17 (2):274-278

30. Marshall PW, Cashman A, Cheema BS (2011) A randomized controlled trial for the effect of passive stretching on measures of hamstring extensibility, passive stiffness, strength, and stretch tolerance. Journal of science and medicine in sport / Sports Medicine Australia 14 (6):535-540. doi:10.1016/j.jsams.2011.05.003

31. Weppler CH, Magnusson SP (2010) Increasing muscle extensibility: a matter of increasing length or modifying sensation? Physical therapy 90 (3):438-449. doi:10.2522/ptj.20090012

32. Gajdosik RL (2001) Passive extensibility of skeletal muscle: review of the literature with clinical implications. Clinical biomechanics 16 (2):87-101

33. Aquino CF, Fonseca ST, Goncalves GG, Silva PL, Ocarino JM, Mancini MC (2010) Stretching versus strength training in lengthened position in subjects with tight hamstring muscles: a randomized controlled trial. Manual therapy 15 (1):26-31. doi:10.1016/j.math.2009.05.006

34. Chen TC, Nosaka K, Sacco P (2007) Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. Journal of applied physiology 102 (3):992-999. doi:10.1152/japplphysiol.00425.2006

35. Proske U, Morgan DL (2001) Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. The Journal of physiology 537 (Pt 2):333-345

36. Hyldahl RD, Chen TC, Nosaka K (2017) Mechanisms and Mediators of the Skeletal Muscle Repeated Bout Effect. Exercise and sport sciences reviews 45 (1):24-33. doi:10.1249/JES.0000000000000095

37. Mackey AL, Kjaer M (2016) Connective tissue regeneration in skeletal muscle after eccentric contraction-induced injury. Journal of applied physiology:jap 00577 02016. doi:10.1152/japplphysiol.00577.2016

38. Cheung K, Hume P, Maxwell L (2003) Delayed onset muscle soreness : treatment strategies and performance factors. Sports medicine 33 (2):145-164

39. Nosaka K, Newton M (2002) Difference in the magnitude of muscle damage between maximal and submaximal eccentric loading. Journal of strength and conditioning research / National Strength & Conditioning Association 16 (2):202-208

40. Freitas SR, Vaz JR, Bruno PM, Andrade R, Mil-Homens P (2016) Stretching Effects: High-intensity & Moderate-duration vs. Low-intensity & Long-duration. International journal of sports medicine 37 (3):239-244. doi:10.1055/s-0035-1548946

41. Chen TC, Lin KY, Chen HL, Lin MJ, Nosaka K (2011) Comparison in eccentric exercise-induced muscle damage among four limb muscles. European journal of applied physiology 111 (2):211-223. doi:10.1007/s00421-010-1648-7

42. Caresio C, Molinari F, Emanuel G, Minetto MA (2015) Muscle echo intensity: reliability and conditioning factors. Clinical physiology and functional imaging 35 (5):393-403. doi:10.1111/cpf.12175

43. Akagi R, Takahashi H (2014) Effect of a 5-week static stretching program on hardness of the gastrocnemius muscle. Scandinavian journal of medicine & science in sports 24 (6):950-957. doi:10.1111/sms.12111

44. Chaouachi A, Padulo J, Kasmi S, Othmen AB, Chatra M, Behm DG (2017) Unilateral static and dynamic hamstrings stretching increases contralateral hip flexion range of motion. Clinical physiology and functional imaging 37 (1):23-29. doi:10.1111/cpf.12263

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/sms.13068 This article is protected by copyright. All rights reserved.

Tables

Table 1. Absolute values (means \pm SD) before (pre) and after (post) flexibility training for range of motion (ROM), passive torque, isometric and dynamic peak torques, optimum angle, biceps femoris (MT_{BF}) and semitendinosus (MT_{ST}) muscle thickness (respectively).

Experimental leg (EL)				Control leg (CL)			
	Pre-training	Post-training	Δ%	Pre-training	Post-training	Δ%	Effect size
ROM (°)	59.6 ± 7.9	70.2 ± 10.4* [†]	18.1 ± 11.2	63.8 ± 6.2	65.4 ± 8.1	2.5 ± 8.2	0.51
Passive torque (Nm)	51.6 ± 10.9	53.5 ± 9.3	6.7 ± 23.2	53.8 ± 9.7	51.5 ± 8.6	-2.2 ± 19.1	0.22
Isometric torque (Nm)	132.3 ± 22.6	122.1 ± 18.8	-7.3 ± 7.9	126.5 ± 22.9	124.2 ± 30.7	-1.9 ± 14.6	0.08
Dynamic torque (Nm)	113.7 ± 20.2	104.6 ± 17.3	-7.4 ± 10.9	108.0 ± 12.4	99.0 ± 23.6	-9.3 ± 14.4	0.27
Optimum angle (°)	37.1 ± 10.8	36.8 ± 7.2	-6.0 ± 34.1	37.7 ± 7.2	39.7 ± 9.1	8.8 ± 30.3	0.35
MT _{BF} (mm)	23.4 ± 2.9	22.9 ± 3.1	-2.2 ± 3.8	24.7 ± 2.4	24.9 ± 2.2	1.1 ± 3.6	0.74
MT _{ST} (mm)	29.0 ± 5.4	29.2 ± 5.6	0.5 ± 2.4	27.6 ± 5.2	27.8 ± 4.8	1.0 ± 2.6	0.26

*p<0.05 (significantly different from pre-training)

[†] p<0.05 (significantly different from control leg)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/sms.13068

Figures

Figure 1.



Echo intensity, muscle thickness, ROM, DOMS, passive torque, isometric and dynamic peak torque, and optimum angle

Figure 2.

torque, isometric and

dynamic peak torque,

and optimum angle



Figure 3.



This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/sms.13068





