



The effects of 6 weeks of constant-angle muscle stretching training on flexibility and muscle function in men with limited hamstrings' flexibility

Clarissa Müller Brusco^{1,2} · Anthony J. Blazevich² · Ronei S. Pinto¹

Received: 6 March 2019 / Accepted: 2 May 2019 / Published online: 10 May 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Purpose The aim of the present study was to evaluate the effects of 6 weeks of a constant-angle hamstring muscle flexibility training on muscle–tendon stiffness and the range of motion (ROM) in young men with limited hamstring ROM.

Methods 13 participants performed unilateral stretching training (EL), while the contralateral limb acted as control (CL). ROM, relative and peak passive torque, passive stiffness, dynamic knee flexion strength, and active optimum joint angle were assessed before and after the last training session. In addition, participants were tested during the first and last training sessions for first stretch sensation during the stretching procedure only in the EL.

Results Straight-leg raise and isokinetic knee ROM tests (both $p < 0.0001$; from 59.4 ± 8.1 to 70.3 ± 9.8 , from 28.3 ± 7.6 to 18.5 ± 5.2 , respectively) and peak passive torque ($p = 0.001$; from 53.1 ± 11.7 to 64.9 ± 12.3) increased only in EL and no changes in relative passive torque, passive stiffness, dynamic knee flexion strength, and active optimum joint angle ($p > 0.05$) were observed. At the point of first stretch sensation, significant increases in passive torque ($p = 0.004$) and angle ($p < 0.001$) were found from pre- to post-training.

Conclusion The flexibility training induced significant increases in ROM alongside increases in peak passive torque (stretch tolerance) and the ROM at which stretch was first perceived. However, this occurred without changes in muscle–tendon mechanical properties or transfer to the untrained limb (CL). These results suggest that limb-specific ROM increases were underpinned by neural adaptations.

Keywords Knee flexors · Passive torque · Passive muscle stiffness · Range of motion

Abbreviations

ANOVA	Analysis of variance
CL	Control condition
EMG	Electromyography
EL	Experimental condition
PSLR	Passive straight-leg raise

ROM	Range of motion
RMS	Root mean square

Introduction

Increases in range of motion (ROM) evoked by muscle flexibility (i.e., stretching) training allows for an increase in freedom of movement, which can be beneficial especially for less flexible individuals. Although the benefits obtained through flexibility training have been clearly shown, the mechanisms underpinning the increases in ROM are still unclear, with both morphological and neural adaptations being implicated (Akagi and Takahashi 2014; Blazevich et al. 2014; Guissard and Duchateau 2004; LaRoche and Connolly 2006; Nakamura et al. 2012). One suggested mechanism underpinning the chronic increase in ROM is a reduction in muscle stiffness (Akagi and Takahashi 2014; Guissard and Duchateau 2004). Nevertheless, many studies

Communicated by Olivier Seynnes.

✉ Clarissa Müller Brusco
clarissa.brusco@gmail.com

¹ Exercise Research Laboratory (LAPEX), School of Physical Education, Physiotherapy and Dance, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Felizardo, 750, Bairro Jardim Botânico, Porto Alegre, RS CEP 90690-200, Brazil

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

have reported negligible changes in muscle viscoelastic properties (Halbertsma and Goeken 1994; Magnusson et al. 1996b) or have found them not to be related to the improvement in ROM (Blazevich et al. 2014), suggesting that other mechanisms, such as changes in the nervous system, may be involved in ROM alterations (Freitas et al. 2017; Halbertsma and Goeken 1994; Magnusson 1998; Weppeler and Magnusson 2010).

Increases in the ability to tolerate stretching to greater passive joint moment, which has been referred as stretch tolerance, seem to be related to alterations in perceived stretch load during the stretch maneuver (Ben and Harvey 2010; Blazevich et al. 2014; Folpp et al. 2006; LaRoche and Connolly 2006; Magnusson et al. 1996b). Increased tolerance to higher levels of stretch torque has been reported after a flexibility-training program, allowing an individual to push the joint to a greater ROM for the same amount of perceived stretch intensity (Ben and Harvey 2010; Blazevich et al. 2014). In addition, an increase in the muscle length (joint angle) at which the first stretch sensation is felt has been reported, a phenomenon in which the participant informs the point at which the stretch of the muscle is first felt (Halbertsma and Goeken 1994; Ylinen et al. 2009). The first stretch sensation has been used as the endpoint of the ROM measurement in some studies (Bandy et al. 1997; Halbertsma and Goeken 1994); however, only a few studies have concomitantly evaluated the ROM and the torque level or muscle–tendon unit stiffness at that point (Halbertsma and Goeken 1994; Ylinen et al. 2009). Therefore, it is not clear whether changes in the first stretch sensation are related to changes in the perception of joint torque or tissue stiffness.

Pain is a possibly important sensation that could strongly impact on stretch perception, and thus maximum joint ROM. Nociceptive signals promote strong inhibition on muscle function and joint movement (Rossi and Decchi 1997) and may, therefore, be an important signal influencing maximum ROM. If this is the case, then one may speculate that the ROM reached in each training session might vary according to the personal daily pain perception. Few studies have recorded daily variations in ROM, as the participants are usually allowed to control the angle reached in the flexibility training programs themselves. Thus, controlling the ROM reached in each training session is necessary to better understand muscle behavior change to a chronically applied stretching stimulus. Performing the training sessions in an apparatus (e.g., isokinetic dynamometer) allows the precise control and measurement of the joint throughout the ROM during a flexibility training session and allows the measurement of the passive stiffness during the stretch maneuver.

The aim of the present study was to evaluate the effects of 6 weeks of controlled constant-angle hamstring muscle flexibility training on muscle–tendon stiffness and the maximum ROM assessed in tests that were specific vs. non-specific

to the training stretches in young men with limited hamstring range of motion. In addition, first stretch sensation during the stretch maneuver was monitored in the first and last training sessions in the experimental limb (EL) only. We hypothesized that maximal ROM would be increased in the experimental limb (EL) only and no alterations on muscle–tendon stiffness would be observed.

Materials and methods

Participants

Twenty-three healthy men volunteered to participate in the present study. Only individuals with limited hamstrings' flexibility were included, as they are an important target clinical population and were expected to benefit most from a stretch training program; however, they exhibit similar responses to a stretching training program as individuals without limited hamstrings' flexibility (Ayala et al. 2013). Each participant was expected to have a limited hamstring flexibility, defined as maximum of 80° hip flexion in the passive straight-leg raise test (PSLR) (Ayala et al. 2013). Based on this criterion, six participants were excluded from participation. A further three participants were excluded, because they were engaged in sports programs, and one participant dropped out prior to the first evaluation. Therefore, 13 healthy men (age 23.6 ± 3.9 years; body mass 77.6 ± 12.5 kg; height 177.1 ± 6.8 cm; body fat $16.0 \pm 8.2\%$) not engaged in strength or flexibility activities for at least 3 months prior to the study participated. All participants were free from functional limitations that could impair their performance, and they 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 due to study participation, and written informed consent was obtained from all participants. All procedures of the present study were approved by the University Institutional Review Board (965.097), and the study was conducted in accordance with the Declaration of Helsinki.

Experimental design

This study was designed to examine the effects of a constant-angle flexibility-training program in men with limited hamstring flexibility. For this purpose, the participant's right and left legs were allocated according to their leg dominance to experimental (EL) and control (CL) conditions (i.e., participants served as their own controls) (Akagi and Takahashi 2014; Folpp et al. 2006; Guissard and Duchateau

2004; Kubo et al. 2002), with some individuals having their dominant leg in the experimental group and others in the control group. Leg dominance was determined by asking the participant which leg is preferred to kick a ball. EL was exposed to constant-angle muscle stretching training for 6 weeks, as described below, and CL remained untrained. Seven participants were assigned their dominant limb and six were assigned their non-dominant limb as the experimental limb (EL).

In the first week of this study, participants attended two testing sessions with at least 48 h between, to be familiarized with the testing procedures and to determine test–retest reliability intraday. Data from the second testing day were used for pre- and post-training comparisons. Participants were tested pre- and post-training for: (1) maximum hip/knee range of motion (ROM); (2) passive torque; (3) passive stiffness; (4) dynamic knee flexion peak torque; and (5) active optimum joint angle. In addition, EL was tested during the first and last training sessions for: (6) first stretch sensation measured during the stretching procedure. From the second to the seventh week of the study, participants performed the flexibility training. Post-training assessments were completed 2–4 days after the last training session, although one participant was tested at 5 days. The same investigator conducted all the evaluations and the same instructions were given at all times.

Flexibility training

Participants completed two training sessions per week on non-consecutive days for 6 weeks (i.e., 12 training sessions). The knee flexor muscles were stretched using a passive, constant-angle stretch on an isokinetic dynamometer (Cybex Norm; Ronkonkoma, NY, USA) by rotating the knee from flexion ($\sim 90^\circ$) to extension ($\sim 0^\circ$) with the participants in a seated position. The thigh rested on a customized device that elevated it to 30° from horizontal (i.e., with hip angle at 30° flexion), similar to that used in a previous study (Brusco et al. 2018), thus moving the knee into extension and stretching the hamstring muscles (0 = full knee extension).

Before each training session, the participants warmed-up on a cycle ergometer (Movement Technology; SP, Brazil) for 5 min with the device's minimum load at a self-selected, comfortable and non-exhausting cadence. Prior to administration of the stretching procedure, ROM was determined with the participant seated on the dynamometer chair. The examiner passively and slowly moved the lower leg towards knee extension at a slow, constant speed ($\sim 5^\circ/\text{s}$; 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. During the flexibility training sessions, the participants were

instructed and encouraged to (1) reach their maximum tolerable stretch amplitude irrespective of the timing or magnitude of pain onset; (2) remain relaxed and not to offer any voluntary resistance to the movement; and (3) always reach maximum knee ROM. In each training session, participants were carefully instructed to leave the lower limb and the foot relaxed, to not offer any resistance to the movement. Visual feedback was not provided (i.e., screen display was not allowed) during the test to prevent participants from monitoring their ROM. These procedures were performed in every training session to determine the maximum ROM, and training intensity was then set as the maximum ROM that could be reached at the beginning of that training session (see Fig. 1).

For the stretch maneuvers, the dynamometer passively extended the knee at $5^\circ/\text{s}$ to the pre-determined angle (dynamic phase), and maximal ROM was maintained for 60 s (static phase). Eight 60-s repetitions were performed, totaling 480 s per session and a total training volume of 5760 s over 6 weeks. A 30-s rest interval was allowed between repetitions. The same examiner supervised all training sessions and the average temperature in the room during training sessions was controlled at 22°C .



Fig. 1 Stretching maneuver, showing the **a** start and **b** end position

Pre- and post-training evaluations

Range of motion assessment

Maximum range of motion was assessed by two different tests using a maximum passive straight-leg raise test (PSLR) and a passive knee extension in the isokinetic dynamometer. Both tests followed the same procedures, were performed once on each limb, and the examiner passively moved the limb until the maximum ROM was informed by the participant regardless of the timing or magnitude of pain onset; participants were stabilized and were instructed to remain relaxed and to not offer any voluntary resistance to the movement.

For the PSLR, the participant was placed in a supine position with legs straight and the non-tested leg stabilized with Velcro® straps positioned right above the knee and ankle. One examiner passively and slowly lifted the participant's leg into hip flexion and a second examiner positioned the manual goniometer on the hip joint to measure ROM (Ayala et al. 2013). For the knee extension test, participants were positioned in the isokinetic dynamometer according to the position described for the flexibility training. The examiner passively and slowly lifted the participant's leg into maximum knee extension, and the angle displayed by the isokinetic dynamometer was used as the maximum ROM. Visual feedback was not provided (i.e., screen display was not allowed) during the tests to prevent participants from monitoring their ROM.

Passive torque assessment

Passive torque during stretch was measured in the isokinetic dynamometer in the sitting position and the dynamometer's torque and angle data were recorded at a frequency of 2000 Hz per channel and exported to a personal computer. The participants were placed and stabilized according to the position described for the flexibility training, and ROM was set as the maximum knee extension reached in the ROM test. For the passive torque test, the dynamometer's lever arm passively extended the knee at 5°/s to the pre-determined angle achieved in the ROM assessment (described above), then the leg was immediately returned to the starting position. Participants were instructed to remain relaxed and not offer any voluntary resistance to the dynamometer's lever arm movement. Passive torque was obtained at two points: (1) relative passive torque was obtained at the same maximum joint angle reached before training to examine changes in passive torque after training and (2) maximum ROM that was obtained both before and after the training.

Muscle electrical activity was recorded during the maximal isometric voluntary contraction and the passive torque tests to ensure that during the passive torque test

the electrical activity was minimal (i.e., $\leq 5\%$) (Blazeovich et al. 2014). To normalize the electromyography (EMG) signal during the passive test, two attempts of 3-s maximal isometric knee flexion contractions at 40° of knee flexion (0° = full knee extension) were performed with a 3-min recovery between attempts with the participant in the sitting position. Skin preparation and electrode placement for the biceps femoris and semitendinosus followed the SENIAM guidelines. Electrode position was carefully mapped using a transparent sheet to ensure replication of positioning at post-training testing (Brusco et al. 2018). EMG signals were recorded using an eight-channel electromyographic system (Miotool 800 Wireless, Miotec Equipamentos Biomédicos, Porto Alegre, RS, Brazil) and digitized at sample frequency of 2000 Hz per channel. The EMG signals were Butterworth filtered using cut-off frequencies of 20 Hz (high pass) and 500 Hz (low pass). After filtering, EMG root mean square (RMS) was obtained in three 1000-ms time windows, at the beginning, middle and end of the movement (which was defined as the first 1 s of the passive movement, the middle of the torque–time data, and the last 1 s of the passive movement), and the average was expressed as a ratio to the maximal EMG activity measured in the maximal voluntary isometric contraction.

Passive stiffness assessment

Passive stiffness was calculated using passive torque and angle data from pre- and post-training passive torque evaluation. The passive torque–angle curve was divided into thirds and was used for analyses the change in passive torque divided by change in position of the last third of the curve (Cabido et al. 2014; Magnusson et al. 1996a). Passive stiffness was calculated in two different ways: (1) with the angles relative to the maximum ROM reached before and after training (maximum passive stiffness) and (2) the same angle reached before training were used to calculate the passive stiffness after training (relative passive stiffness).

Active peak torque and active optimum angle assessments

To determine whether the active optimum angle changed after the stretching training program, dynamic hamstring knee flexion torque was measured on the isokinetic dynamometer and the active optimum angle (i.e., peak torque angle) was captured during the test. Before testing, the system was calibrated and participants were seated with their hips flexed at 85° (0° = anatomical position), the dynamometer's axis of rotation was aligned with the lateral femoral condyle of the tested limb, and straps were secured across the thigh, torso, and pelvis to restrict compensatory movements. A warm-up of 10 submaximal dynamic contractions at 60°/s was performed. Maximal dynamic torque and

active optimum angle were then 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 the tests and visual feedback was provided on a monitor.

First and last training session evaluations

First stretch sensation

The first stretch sensation was evaluated in the first and last training sessions during the stretching movement in EL only. As the CL did not perform any flexibility training, this evaluation was not measured in the control leg. Before the testing, the participants were carefully instructed that their leg would be passively moved by the dynamometers' lever arm and a button should be pressed at the moment they first felt stretching in the hamstrings. To facilitate instruction comprehension, comparison was made with the stretching performed in the PSLR and with the determination of maximum ROM tests, where the limb was passively moved, and at some point, the sensation of stretch was first perceived. The participants then determine whether it coincided with their maximum ROM. The measurement was only performed after the participant was confident with the instructions. To measure the first stretch sensation, the participants were placed and stabilized and were given a button to be pressed at the moment they first felt stretching in the hamstrings as the limb was passively moved by the dynamometers' lever arm.

The manual trigger button was connected to a computer that was also connected to the isokinetic dynamometer, and passive torque and position data were digitized at a frequency of 2000 Hz per channel and recorded synchronously on a personal computer. The moment the trigger button was pressed, a mark was made in the passive torque and angle position curves. Passive torque and angle position data of the first repetition of the stretch maneuver in the first and the last training sessions were used for analysis.

Statistical analysis

To compare pre-training data between groups, student's *t* tests for independent data were used. To verify the adaptations to the flexibility training, two-way analysis of variance (ANOVA) with repeated measures (group [EL and CL] × time [pre-training and post-training]) was used. When a time × group interaction was found, *t* tests were used to locate differences. The effect sizes for the effects of the flexibility training on measured variables were calculated according to Cohen (Cohen 1988).

For analysis of the first stretch sensation, a paired-samples *t* test was used. To compare ROM reached throughout all training sessions, an analysis of variance (ANOVA) with repeated measures was used. Significance level was set at $\alpha < 0.05$. All analyses were performed with the software SPSS 17.0 (IBM, Somers, NY, USA), and results are reported as mean (SD).

Results

Participants

Adherence to the training program was 99.4%, with only one participant missing one training session.

Adaptations to flexibility training (pre- and post-training evaluations)

Before the flexibility training, no significant difference ($p > 0.05$) was found between EL and CL for any outcome (Table 1). The ranges of motion reached in each training session are displayed in Fig. 2. The ANOVA revealed a significant main effect for time ($p < 0.0001$) and group × time interaction ($p < 0.0001$) for PSLR. Only EL significantly increased PSLR ROM ($p < 0.0001$) from pre- to post-training. Maximum knee extension ROM showed a significant main effect for time ($p = 0.001$) and a group × time interaction ($p < 0.001$). Only EL significantly increased maximum knee extension ROM ($p < 0.0001$) from pre- to post-training. However, relative passive torque, maximum, and relative passive stiffness showed no significant main effect for time ($p = 0.95$, $p = 0.55$, and $p = 0.29$, respectively) or group × time interaction ($p = 0.32$, $p = 0.33$, and $p = 0.55$, respectively). Peak passive torque showed a significant main effect for time ($p = 0.01$) and a group × time interaction ($p = 0.001$), with only EL significantly increasing ($p = 0.002$) from pre- to post-training. No significant main effect for time ($p = 0.07$ and $p = 0.58$, respectively) or group × time interaction ($p = 0.57$ and $p = 0.58$, respectively) was observed for dynamic torque or active optimum angle.

First and last training session evaluations

First stretch sensation

For the first stretch sensation (Fig. 3), paired-samples *t* tests showed significant ($p = 0.004$) increases (26.3%) in passive torque from pre- (47.7 ± 10.8 Nm) to post-training (58.4 ± 9.4 Nm) and a significant ($p < 0.001$) increase (34.6%) in angle from pre- ($34.9^\circ \pm 11.9$) to post-training ($22.3^\circ \pm 9.7$, i.e., a more stretched position).

Table 1 Absolute values [(means (SD))] of before and after flexibility training for passive straight-leg rise (PSLR ROM) and knee extension range of motion (knee extension ROM), relative and peak pas-

sive torque, passive stiffness, dynamic peak torques, active optimum angle, and rate of torque development

	Experimental leg (EL)			Control leg (CL)			Effect size
	Pre-training	Post-training	$\Delta\%$	Pre-training	Post-training	$\Delta\%$	
PSLR ROM ($^{\circ}$)	59.4 \pm 8.1	70.3 \pm 9.8* [†]	19.0 \pm 13.2	63.2 \pm 6.8	64.1 \pm 8.2	1.5 \pm 7.5	0.68
Knee extension ROM ($^{\circ}$)	28.3 \pm 7.6	18.5 \pm 5.2* [†]	-32.2 \pm 24.5	26.7 \pm 8.7	26.5 \pm 6.2	-5.9 \pm 34.7	1.39
Relative passive torque (Nm)	53.1 \pm 11.7	55.0 \pm 9.2	6.3 \pm 20.9	54.7 \pm 8.9	54.7 \pm 9.6	-1.8 \pm 21.1	0.03
Peak passive torque (Nm)	53.1 \pm 11.7	64.9 \pm 12.3* [†]	25.1 \pm 24.7	54.7 \pm 8.9	52.6 \pm 6.0	-2.1 \pm 17.2	1.27
Maximum passive stiffness (Nm/ $^{\circ}$)	0.83 \pm 0.40	0.86 \pm 0.28	24.1 \pm 88.4	0.79 \pm 0.32	0.69 \pm 0.25	2.8 \pm 42.3	0.78
Relative passive stiffness (Nm/ $^{\circ}$)	0.83 \pm 0.40	0.80 \pm 0.25	12.9 \pm 65.7	0.79 \pm 0.32	0.68 \pm 0.21	-3.3 \pm 40.1	0.39
Dynamic torque (Nm)	110.2 \pm 19.4	106.3 \pm 18.1	-2.7 \pm 14.0	107.2 \pm 11.4	100.1 \pm 20.6	-7.2 \pm 13.9	0.31
Active optimum angle ($^{\circ}$)	39.7 \pm 11	37.2 \pm 7.5	-0.3 \pm 32.1	40.5 \pm 8.4	40.5 \pm 10.1	3.7 \pm 30.6	0.37

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

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

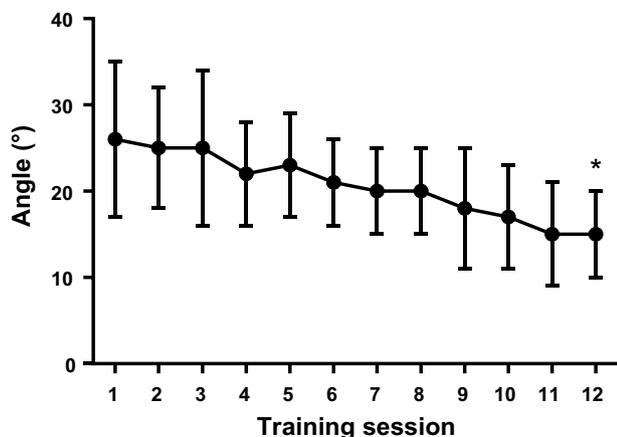


Fig. 2 Mean range of motion reached in each training session. *Significantly different ($p < 0.05$) from training sessions 1, 2, 4, 5, 7, and 8. The range of motion reached increased throughout the 12 training sessions, but significant increases were found only in training session 12 when compared to training sessions 1, 2, 4, 5, 7, and 8

Discussion

The main finding of the present study was that 6 weeks of progressive constant-angle flexibility training induced significant increases in ROM in both specific (isokinetic) and non-specific (straight-leg raise) tests (~19–32%), which were accompanied by increases in peak passive torque in the experimental leg (EL) only; however, no alterations were found in relative passive torque, passive stiffness, dynamic torque, and in optimum angle. Furthermore, it was found that subjects' perception of stretch sensation occurred at a greater ROM and with greater passive torque in the stretch applied in the last training session. These findings suggest that the increases in ROM were

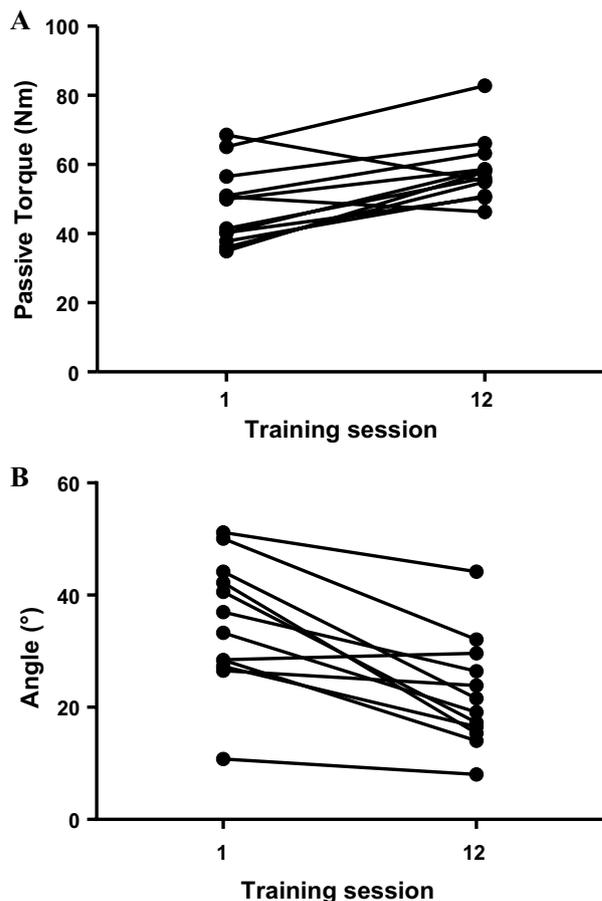


Fig. 3 Individual passive torque (a) and position (b) of the first stretch sensation measured in the first (session 1) and in the last (session 12) training session. Significant increases were found for a passive torque and b for the angle of the first stretch sensation ($p = 0.004$ and $p < 0.001$, respectively)

underpinned by neural mechanisms rather than mechanical alterations and reinforce the sensory theory that has been suggested by Halbertsma and Göeken (Halbertsma and Goeken 1994) and others (Freitas et al. 2017; Magnusson et al. 1996b; Weppeler and Magnusson 2010).

Two main theories have been suggested to be involved in ROM increases after stretch training: the mechanical (Guissard and Duchateau 2004; Kubo et al. 2002; Nakamura et al. 2012) and the sensory theories (Ben and Harvey 2010; Chan et al. 2001; Halbertsma and Goeken 1994; Magnusson et al. 1996b; Weppeler and Magnusson 2010). Regarding the mechanical theory, alterations in the muscle–tendon unit mechanical properties after a stretch training program, with decreased passive stiffness (Guissard and Duchateau 2004), decreased MTU stiffness (Kubo et al. 2002) and decreased passive torque at a given length (Nakamura et al. 2012) being reported. It is suggested that these alterations might be related to adaptations in non-contractile muscle proteins and extracellular connective tissue structures (Gajdosik 2001; Nakamura et al. 2012). Although no alterations in relative passive torque and muscle stiffness were found in the present study, it cannot be excluded that some modification of connective tissue or of extracellular matrix properties occurred. The passive torque test may not be a sensitive test to detect small changes in individual tissues (Blazevich et al. 2014), as it represents the global resistance developed by the neuromusculoskeletal complex (Nordez et al. 2017); therefore, more specific tests (e.g., ultrasound elastography) should be performed to determine whether some tissues are specifically affected by the stretch training. It is important to note that the aforementioned studies reporting mechanical alterations evaluated a different muscle group than in the present study (i.e., plantar flexors) and also that only one experimental study assessing the hamstring muscles has reported an increase in resting muscle fascicle length (Freitas and Mil-Homens 2015). In addition, those previous studies also used a higher training frequency (i.e., more than three training sessions per week) and intensity (i.e., constant-torque method was used) than in the present study (i.e., twice a week using the constant-angle method). Nonetheless, such intensities and frequencies of stretch are not requisite for ROM adaptations to be evoked, as shown in the current and previous studies. Similarly, no significant changes in the active optimum angle or dynamic peak torque were found in the present study. The lack of shift in active optimum angle in particular may be illustrative of a lack of change in muscle characteristics such as sarcomere number, since change in sarcomere number have been associated with changes in active force–length properties even when no changes in passive force–length properties are observed in animal models (Goldspink et al. 1974; Tabary et al. 1972). In this regard, the current results are consistent with findings from the previous stretch training studies (Aquino et al.

2010; Blazevich et al. 2014). However, the results are not consistent with other studies in which a higher total training volume and weekly training frequency were used and a shift in the optimum angle towards a longer hamstring muscle length was reported (Chen et al. 2011; Ferreira et al. 2007). Regardless, the lack of change in the active components of the muscle further support the possibility that the increase found in ROM was underpinned by neural rather than muscular adaptations.

In the present study, ROM was measured using two different techniques; the PSLR was used to allow comparison to other studies, because it is commonly used in rehabilitation settings, and the isokinetic knee extension test was performed to evaluate the effects of training using a test identical to that used in training. Significant increases in both ROM tests (PSLR: 10.9° and isokinetic: 9.8°) were observed that are similar to those reported in studies using a comparable training period (Ben and Harvey 2010; Ferreira et al. 2007; Reid and McNair 2004) as well as longer training periods (Ayala et al. 2013; Chan et al. 2001; e Lima et al. 2015) in which increases in the peak passive torque (similar to our findings for the first stretch sensation) were observed. It is thus believed that the increases found in ROM in the present study were predominantly underpinned by neural adaptations. This assumption is based on the lack of alteration in the mechanical properties observed in the present study (i.e., relative passive torque and muscle stiffness) and also the findings of an altered first stretch sensation, which occurred at a greater ROM and with higher passive torque values, indicating that the participants increased their stretch tolerance after training. The alteration of stretch perception seems to be a determining factor in stretching exercises, and it has been shown to be correlated with the joint angle increase (Freitas et al. 2015b). It may, therefore, be suggested that stretching performed at higher intensities could induce greater acute joint ROM gains than submaximal intensities (Freitas et al. 2015b).

The intensity of the stretch protocol used in the present study was maximal in every training session. Care was taken to stimulate the participants to always reach their maximum ROM; however, variations in the angle reached during the training sessions (i.e., not always towards longer muscle lengths) occurred along the flexibility training program, probably due to daily variations in the stretch perception. It should be noted that the pathways that modulate the stretch perception are still not clear. The determination of the maximal ROM is dependent upon not only the muscular tissues but also on non-muscular structures (e.g., tendons, ligaments, joint capsule, skin, fascia, nerves, vessels, etc.) and the nervous system, and fascia are believed to play an important role in the stretch perception and in the limitation of the maximal range of motion (Nordez et al. 2017). It is possible that the stretch perception in these structures

varies day to day and this, therefore, mediates the maximum ROM reached in each training session. As the provision of ROM information by direct measurement did not seem to be determining for the increments observed, and if the participants' pain perceptions modulate the maximum ROM reached, then it might be suggested that individuals should be encouraged to always reach maximum ROM without the necessity to measure the joint amplitude reached when performing stretches exercises.

The stretch protocol necessary for the purposes of the present study was the constant-angle method; therefore, the stretch was performed to maximum ROM only in the first stretch repetition and (as the same range of motion was maintained) intensity was probably submaximal for the subsequent repetitions. Studies investigating responses to a single stretch session have indicated that the effects of stretching on joint and muscle depend on its intensity (Freitas et al. 2015a), where higher stretching intensities elicit greater gains in maximum ROM (Freitas et al. 2015b). Nevertheless, to the best of the authors' knowledge, only one recent study (Muanjai et al. 2017) has examined the chronic adaptations induced by constant-torque stretching at different intensities and the researchers reported no differences in the adaptations induced by groups that stretched to the point of pain or to the point of discomfort without pain. There is a lack of studies comparing the chronic adaptations induced by different modalities of stretch training (i.e., constant angle, constant torque, ballistic, and stretch in active muscles). Since constant-torque stretching allows a greater intensity of stretch than constant-angle stretching, and the production of a greater negative work by muscle–tendon unit and, subsequently, greater acute alterations (Herda et al. 2011; Konrad et al. 2017), flexibility training programs using the constant-torque method are likely able to induce greater chronic adaptations.

It is also important to note that the changes found after the stretch training program were observed in the stretched leg (EL), whereas no alteration was found in the contralateral control limb (CL) for both PSLR and knee extension ROM tests, and also for peak passive torque, in agreement with the previous research (Guissard and Duchateau 2004; Kubo et al. 2002). This indicates that the central (i.e., neural) adaptations were not sufficiently broad as to alter ROM in an untrained limb. Nonetheless, in a recent review, Nordez et al. (Nordez et al. 2017) suggested that a flexibility training might increase an individuals' overall stretch tolerance which could increase ROM in non-stretched muscle (Behm et al. 2016; Chaouachi et al. 2017). Little is known about the possible adaptations induced in non-stretched limbs (i.e., contralateral effect), as it has been rarely examined. Therefore, the reasons for the discrepancies between the current findings and previous (Behm et al. 2016; Chaouachi et al. 2017) are not clear. As no alterations were found in CL in

the present study, we believe that the cross-transfer effect was negligible and it is possible to attribute the adaptations found in the EL to the training program only.

In summary, the 6-week flexibility training used in the present study induced significant increases in ROM in tests that were both specific (isokinetic knee extension test) and non-specific (passive straight-leg raise test) to the training exercise without inducing alterations in muscle–tendon or joint mechanical properties in men with limited hamstrings' flexibility. These increases occurred simultaneously with increases in both 'stretch tolerance' (i.e., peak passive torque during stretch) and the ROM at which stretch was first perceived by the participants (i.e., stretch perception) and without detectable alterations in the active force–length and velocity properties of the muscle, which clearly points to a neural basis for the increase in ROM.

Limitations

A possible limitation of the present study is that the first stretch sensation was only measured in EL. As it was measured in the first and in the last training sessions and no stretch training stimuli were imposed on CL, the present study design allowed the measurement of EL only. Future studies might check this assumption by including the measurement of the first stretch sensation in the non-trained limb to better understand possible cross-transfer effects. However, it is important to note that no increases in ROM or peak passive torque were detected in CL, and it is possible that if any alteration might have occurred in the first stretch sensation, it was not sufficient to result in an increase in maximal ROM; thus, any adaptation must have been negligible.

Perspectives

The constant-angle flexibility training program used in the present study induced significant increase in ROM that are believed to be related to neural adaptations; however, the mechanism that allow to increase ROM without alteration in the muscle mechanical components are still not clear. Future studies should explore the possible neural mechanism underpinning the increase in ROM and the alteration in stretch sensation after a stretching training program. In addition, the comparison of different types of stretching training using different training volumes, intensities (i.e., constant torque vs. constant angle) and weekly frequency should be further investigated to test the possible different neural and mechanical adaptations induced by different training programs and also its effects on the possible cross-transfer effect in the non-trained limb.

Acknowledgements The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) -Brazil

and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Brazil for financial support and all volunteers for their participation in this project.

Author contributions CMB: conceived and designed the research, conducted experiments, analyzed data, and wrote the manuscript. AJB: analyzed data and wrote the manuscript. RSP: conceived and designed the research, analyzed data, and wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Akagi R, Takahashi H (2014) Effect of a 5-week static stretching program on hardness of the gastrocnemius muscle. *Scand J Med Sci Sports* 24:950–957. <https://doi.org/10.1111/sms.12111>
- 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 Ther* 15:26–31. <https://doi.org/10.1016/j.math.2009.05.006>
- 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. *Phys Ther Sport* 14:98–104. <https://doi.org/10.1016/j.ptsp.2012.03.013>
- Bandy WD, Irion JM, Briggler M (1997) The effect of time and frequency of static stretching on flexibility of the hamstring muscles. *Phys Ther* 77:1090–1096
- Behm DG, Cavanaugh T, Quigley P, Reid JC, Nardi PS, Marchetti PH (2016) Acute bouts of upper and lower body static and dynamic stretching increase non-local joint range of motion. *Eur J Appl Physiol* 116:241–249. <https://doi.org/10.1007/s00421-015-3270-1>
- Ben M, Harvey LA (2010) Regular stretch does not increase muscle extensibility: a randomized controlled trial. *Scand J Med Sci Sports* 20:136–144. <https://doi.org/10.1111/j.1600-0838.2009.00926.x>
- Brusco CM et al (2018) The effects of flexibility training on exercise-induced muscle damage in young men with limited hamstrings flexibility. *Scand J Med Sci Sports*. <https://doi.org/10.1111/sms.13068>
- 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. *J Appl Physiol* 117:452–462. <https://doi.org/10.1152/jappphysiol.00204.2014>
- Cabido CE, Bergamini JC, Andrade AG, Lima FV, Menzel HJ, Chagas MH (2014) Acute effect of constant torque and angle stretching on range of motion, muscle passive properties, and stretch discomfort perception. *J Strength Condition Res Natl Strength Condition Assoc* 28:1050–1057. <https://doi.org/10.1519/jsc.000000000000241>
- Chan SP, Hong Y, Robinson PD (2001) Flexibility and passive resistance of the hamstrings of young adults using two different static stretching protocols. *Scand J Med Sci Sports* 11:81–86
- 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. *Clin Physiol Funct Imaging* 37:23–29. <https://doi.org/10.1111/cpf.12263>
- Chen CH, Nosaka K, Chen HL, Lin MJ, Tseng KW, Chen TC (2011) Effects of flexibility training on eccentric exercise-induced muscle damage. *Med Sci Sports Exerc* 43:491–500. <https://doi.org/10.1249/MSS.0b013e3181f315ad>
- Cohen J (1988) *Statistical power analysis for the behavioral sciences*, 2nd edn. Erlbaum Associates, New York, NY
- Ferreira GN, Teixeira-Salmela LF, Guimaraes CQ (2007) Gains in flexibility related to measures of muscular performance: impact of flexibility on muscular performance. *Clin J Sport Med* 17:276–281. <https://doi.org/10.1097/JSM.0b013e3180f60b26>
- 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? *Aust J Physiother* 52:45–50
- Freitas SR, Mil-Homens P (2015) Effect of 8-week high-intensity stretching training on biceps femoris architecture. *J Strength Condition Res Natl Strength Condition Assoc* 29:1737–1740. <https://doi.org/10.1519/jsc.0000000000000800>
- Freitas SR, Andrade RJ, Larcoupaille L, Mil-homens P, Nordez A (2015a) Muscle and joint responses during and after static stretching performed at different intensities. *Eur J Appl Physiol* 115:1263–1272. <https://doi.org/10.1007/s00421-015-3104-1>
- Freitas SR, Vilarinho D, Rocha Vaz J, Bruno PM, Costa PB, Mil-homens P (2015b) Responses to static stretching are dependent on stretch intensity and duration. *Clin Physiol Funct Imaging* 35:478–484. <https://doi.org/10.1111/cpf.12186>
- 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? *Scand J Med Sci Sports*. <https://doi.org/10.1111/sms.12957>
- Gajdosik RL (2001) Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clin Biomech* 16:87–101
- Goldspink G, Tabary C, Tabary JC, Tardieu C, Tardieu G (1974) Effect of denervation on the adaptation of sarcomere number and muscle extensibility to the functional length of the muscle. *J Physiol* 236:733–742
- Guissard N, Duchateau J (2004) Effect of static stretch training on neural and mechanical properties of the human plantar-flexor muscles. *Muscle Nerve* 29:248–255. <https://doi.org/10.1002/mus.10549>
- Halbertsma JP, Goeken LN (1994) Stretching exercises: effect on passive extensibility and stiffness in short hamstrings of healthy subjects. *Arch Phys Med Rehabil* 75:976–981
- Herda TJ et al (2011) Effects of two modes of static stretching on muscle strength and stiffness. *Med Sci Sports Exerc* 43:1777–1784. <https://doi.org/10.1249/MSS.0b013e318215cda9>
- Konrad A, Budini F, Tilp M (2017) Acute effects of constant torque and constant angle stretching on the muscle and tendon tissue properties. *Eur J Appl Physiol* 117:1649–1656. <https://doi.org/10.1007/s00421-017-3654-5>
- Kubo K, Kanehisa H, Fukunaga T (2002) Effect of stretching training on the viscoelastic properties of human tendon structures in vivo. *J Appl Physiol* 92:595–601. <https://doi.org/10.1152/jappphysiol.00658.2001>
- LaRoche DP, Connolly DA (2006) Effects of stretching on passive muscle tension and response to eccentric exercise. *Am J Sports Med* 34:1000–1007. <https://doi.org/10.1177/0363546505284238>
- 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. *Clin J Sport Med* 25:55–60. <https://doi.org/10.1097/jsm.0000000000000069>
- Magnusson SP (1998) Passive properties of human skeletal muscle during stretch maneuvers: a review. *Scand J Med Sci Sports* 8:65–77
- Magnusson SP, Simonsen EB, Aagaard P, Kjaer M (1996a) Biomechanical responses to repeated stretches in human hamstring muscle in vivo. *Am J Sports Med* 24:622–628
- Magnusson SP, Simonsen EB, Aagaard P, Sorensen H, Kjaer M (1996b) A mechanism for altered flexibility in human skeletal muscle. *J Physiol* 497(Pt 1):291–298

- Muanjai P et al (2017) The effects of 4 weeks stretching training to the point of pain on flexibility and muscle tendon unit properties. *Eur J Appl Physiol* 117:1713–1725. <https://doi.org/10.1007/s00421-017-3666-1>
- Nakamura M, Ikezoe T, Takeno Y, Ichihashi N (2012) Effects of a 4-week static stretch training program on passive stiffness of human gastrocnemius muscle-tendon unit in vivo. *Eur J Appl Physiol* 112:2749–2755. <https://doi.org/10.1007/s00421-011-2250-3>
- Nordez A et al (2017) Non-muscular structures can limit the maximal joint range of motion during stretching. *Sports Med* 47:1925–1929. <https://doi.org/10.1007/s40279-017-0703-5>
- Reid DA, McNair PJ (2004) Passive force, angle, and stiffness changes after stretching of hamstring muscles. *Med Sci Sports Exerc* 36:1944–1948
- Rossi A, Decchi B (1997) Changes in Ib heteronymous inhibition to soleus motoneurons during cutaneous and muscle nociceptive stimulation in humans. *Brain Res* 774:55–61
- Tabary JC, Tabary C, Tardieu C, Tardieu G, Goldspink G (1972) Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J Physiol* 224:231–244
- Weppler CH, Magnusson SP (2010) Increasing muscle extensibility: a matter of increasing length or modifying sensation? *Phys Ther* 90:438–449. <https://doi.org/10.2522/ptj.20090012>
- Ylinen J, Kankainen T, Kautiainen H, Rezasoltani A, Kuukkanen T, Hakkinen A (2009) Effect of stretching on hamstring muscle compliance. *J Rehabil Med* 41:80–84. <https://doi.org/10.2340/16501977-0283>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.