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Title: Altered triceps surae muscle-tendon unit properties after 6 months of static stretching

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ABSTRACT

Introduction: This study examined the effects of 24 weeks of daily static stretching of the plantarflexors (unilateral 4x60 sec stretching while the contralateral leg served as a control, n=26) on joint range of motion (ROM), muscle-tendon unit morphological and mechanical properties, neural activation, and contractile function.

Methods: Torque-angle/velocity was obtained in passive and active conditions using isokinetic dynamometry, while muscle-tendon morphology and mechanical properties were examined using ultrasonography.

Results: Following the intervention, ROM increased (stretching +11±7°, control +4±8°), and passive torque (stretching -10±11 Nm, control -7±10 Nm) and normalized electromyographic amplitude (stretching -3±6%, control -3±4%) at a standardized dorsiflexion angle decreased. Increases were seen in passive tendon elongation at a standardized force (stretching +1.3±1.6 mm, control +1.4±2.1 mm) and in maximal passive muscle and tendon elongation. Angle of peak torque shifted towards dorsiflexion. No changes were seen in tendon stiffness, resting tendon length, or gastrocnemius medialis fascicle length. Conformable changes in ROM, passive dorsiflexion variables, tendon elongation, and angle of peak torque were observed in the non-stretched leg.

Conclusion: The present findings indicate that habitual stretching increases ROM and decreases passive torque, altering muscle-tendon behavior with the potential to modify contractile function.

Keywords:

Flexibility, passive resistance, length-tension, ultrasound, stretch tolerance

INTRODUCTION

Joint flexibility is an important component of human movement and while it is known that habitual stretching increases joint range of motion (ROM) (1), the physiological mechanisms behind increased ROM are not well understood (2). Potential mechanisms for an increase in ROM include neural properties and mechanical properties of the muscle-tendon units (MTUs) and passive joint structures. Since stretching-induced increases in ROM have been seen without changes in passive resistance, gains in ROM are in general attributed to increased tolerance to stretch or pain (1-5). However, the underlying specific neural mechanisms and the roles of pain threshold or sensitivity, peripheral reflex influence and neural activation are poorly understood and the findings remain equivocal: Reduced tonic reflex activity has been observed (6), contrasting unchanged electromyographic (EMG) amplitude (1, 4) and unchanged motoneuron excitability (7) following 3-6 weeks of stretching. Further to the tolerance theory, some stretching interventions have induced a rightward shift in the passive torque-angle curve (8, 9), a reduction in passive torque at standardized joint angles (10-12) or a reduction in passive joint stiffness (6, 8, 13). Changed passive torque-angle relationships may be a result of neural and/or structural changes. Animal studies have demonstrated that immobilization in lengthened positions may increase the number of serial sarcomeres and muscle fibre length (14), but the loads, intensities and volumes of such interventions are very different from what is done in functional human studies. Human stretching interventions with indirect measures of structural adaptations are inconsistent, with reports of increased fascicle length in some (15, 16) but not all studies (4, 12, 17, 18). To date, there seems to be no consensus on the feasibility of fascicle lengthening with stretching training in humans. Stretching for 3 weeks increased ROM and was accompanied by increased fascicle strain at standardized joint angle, authors suggesting a decrease in muscle stiffness without a concurrent change in tendon stiffness (4). Similarly, increased elongation of the tissue proximal to the MTJ was indicated after 4 weeks of stretching (12), and passive muscle/MTU stiffness was decreased following stretching for 4 (19) and 6 weeks (20), suggesting altered muscle behaviour with stretching training. Nonetheless, passive muscle-tendon mechanics remain highly complex due to serially coupled structures with different mechanical properties that undergo heterogeneous loading (21).

The effects of stretching training on tendon properties are similarly unclear, as intervention studies report unchanged tendon stiffness (3, 8, 11, 22), reduced stiffness (11, 23) and increased stiffness (20), and differences in stretching modality do not seem to fully

explain the discrepancies. Theoretically, ROM increases could relate to altered tendon morphology and mechanics including tendon stiffness, Young's modulus and/or tendon length, yet one recent study found no changes in tendon length or thickness after 6 weeks of stretching (16).

One advantage often attributed to stretching training is the potential influence on contractile function. Angle of peak torque may be changed due to the conditions for myofilament overlap (24), e.g. through the addition of serial sarcomeres or increased tendon elongation, or through an overall reduction in passive resistance. The addition of serial sarcomeres would furthermore enable limb rotation at the same angular velocity with reduced sarcomere shortening velocity, perhaps enabling greater torque production (25). Stretching for 6-8 weeks shifted angle of peak torque towards extended positions in a consistent manner in a few studies (26, 27), and only at some velocities in another study (28). The implications of stretching training on isokinetic work production are also not clear, with increased concentric work in one study (28), while this effect was only seen at certain velocities in another (26).

Taken together, previous studies are inconsistent with respect to the role of morphological, mechanical, and neural properties as mechanisms for increased ROM with stretching training. Studies applying more than 12 weeks of stretching are scarce and do not examine effects on mechanical properties of force bearing tissues or morphological adaptation (29, 30). Some studies speculate that early adaptations to stretching training occur at a sensory level (2) or are related to non-muscular structures which may be richly innervated but contribute marginally to passive torque (31), while adaptations altering passive resistance through the structural properties of the MTU may occur with greater stretching durations (2). Such a two-phasic pattern of adaptation is supported by some intervention studies, where maximal passive torque increased after 2-4 weeks, but returned to (10) or below (6) the initial level after 4-8 weeks. Increased passive torque at the new ROM obtained through stretching could result from increased pain tolerance or reduced sensory input due to adaptations in non-muscular structures. Nonetheless, stretching interventions of longer duration are required to examine the interplay between ROM and passive resistance to stretch (2).

The purpose of this study was therefore to examine the effects of 24 weeks of functional unilateral triceps surae stretching on ROM, on the morphological and mechanical properties of the MTU, on neural activation, and on contractile function. We hypothesized that ROM would increase by 8 weeks, while by 24 weeks, passive torque and corresponding EMG amplitude as well as tendon stiffness would be reduced. Maximal passive torque and

passive elongation of muscle and tendon were expected to increase. Muscle thickness, pennation angle, and fascicle length were hypothesized to remain unchanged. Active peak isokinetic torque was expected to occur at a more dorsiflexed joint angle.

METHODS

Subjects

A priori sample size calculations were done by estimating PRE-POST changes in passive torque of 3 Nm, with a standard deviation of 3 Nm, based on former stretching intervention studies (8, 11). Statistical power was set to 90 %. To accommodate an anticipated dropout of 25 %, 30 subjects were recruited among recreationally active university students. Exclusion criteria were lower limb injury in the last 6 months, musculoskeletal disease, conditions preventing stretching of the MTU (e.g., ankle impingement, talocrural joint tightness, neuromeningeal tightness, tingling due to sciatic nerve stretch), and/or a history of systematic stretching (>10 minutes/week). In accordance with the Declaration of Helsinki, the regional ethics committee approved the study, and each subject signed informed consent.

Experimental design

The study was designed as a within-subjects design with 24 weeks of unilateral stretching. The stretching leg was assigned by stratified randomization with a 50/50 distribution between dominant and non-dominant leg. The contralateral leg served as control. Two subjects had an initial side-to-side ROM difference of >10° and were assigned to stretch their least flexible leg. Leg testing order was randomized at the first test session and was replicated during subsequent sessions. Passive dorsiflexion, tendon stiffness, and contractile function tests were completed for one leg before proceeding to the second leg. All measurements and analyses were undertaken by the same investigators, who were always blinded to the leg assignment.

Each subject reported to the lab five times: A familiarization session, a pre-intervention session (PRE), a subset of tests after 8 and 16 weeks, and a post-intervention session (POST) 24-48 hours after the last bout of stretching. The subjects were instructed to refrain from training or stretching 24 hours prior to testing. In female subjects who did not take constant-dose oral contraceptives, all tests (including PRE, 8 weeks, 16 weeks, POST) were conducted within 14 days from the last menstruation, to avoid testing during the luteal phase (32). The subjects were instructed to maintain habitual activity level and diet, to refrain

from unaccustomed exercise, anti-inflammatory drugs, and nutritional supplements, and to report illness or injury during the experimental period.

Stretching intervention

Four repetitions of a 60-sec self-administered static ankle dorsiflexion stretch were performed daily for 24 weeks. The first 8 weeks, stretching was performed with straight knee joint (4 reps; Fig. 1A), while the remaining weeks were undertaken with the knee joint straight for 2 reps and flexed (Fig. 1B) for 2 reps, to ensure an effect on all compartments of the triceps surae. The subjects were instructed to place the stretching leg as far posteriorly as possible, while pushing the heel down to the ground, the forefoot pointing forward.

Prior to the intervention, written, verbal, and visual instructions were given. Proper stretching technique was verified at each test session. The subjects were instructed to produce the strongest sensation of stretch possible without being in direct pain. Pain perception during the stretching exercise was recorded on a 10 cm visual analogue scale (VAS score) at each session. The daily stretching was self-administered. Adherence throughout the intervention was monitored through daily written journals and phone calls every 3-4 weeks.

Experimental setup

Each test session started with all the resting measurements. Subsequently, warm-up, tendon stiffness, passive dorsiflexion, and tests of contractile function were all completed for one leg, before restarting with warm-up for the second leg. The order of the legs was randomized and was repeated identically at each test session. The experimental setup is illustrated in Fig. 1.

(Insert Figure 1)

Anthropometry, resting muscle architecture, and morphological properties

Leg length was measured as the distance between the trochanter major and the floor while standing. Calf length was measured from the lateral femoral epicondyle to the calcaneal tuberosity. With subjects lying prone with the foot hanging freely off the examination bed, resting ankle joint angle was measured using a manual goniometer. B-mode ultrasonography was applied (HD11XE with a 50-mm linear array transducer L12-5, Philips, Bothell, WA, USA) to retrieve two image sets for the measurement of fascicle length, pennation angle, and muscle thickness of the gastrocnemius medialis (GM) and Achilles tendon length. The lower

half of the transducer frequency (5-12 MHz) was used with a built-in filter to optimize ultrasound penetration while preserving spatial resolution and contrast. All ultrasound still images were analysed using imaging software (Fiji ImageJ (33)). Muscle variables were obtained from sagittal images recorded at mid-length of the muscle belly, as detailed in our previous publication (34). Repositioning of the ultrasonography probe and EMG sensors at the same location on different test days was achieved by marking the positions and the subject's moles or other distinct marks on acetate sheets. The free Achilles tendon length (calcaneal insertion to soleus (SOL) MTJ) and whole Achilles tendon length (calcaneal insertion to GM MTJ) were measured along the tendon path from sequentially combined images, as detailed in our previous publication (34).

Electromyography

To ensure optimal skin impedance, shaving, gentle skin abrasion and cleaning with isopropanol was performed in accordance with SENIAM recommendations (35). EMG electrodes (Ambu, Blue Sensor N, Ballerup, Denmark) were placed on SOL, GM, and gastrocnemius lateralis (GL) with an inter-electrode distance of 20 mm, and a reference electrode was positioned on the tibial tuberosity. EMG signals were transmitted wirelessly (16-channel TeleMyo 2400 G2 Telemetry System, Noraxon Inc., Scottsdale, AZ, USA) to a receiver (Mini-receiver for TeleMyo G2, Noraxon Inc., Scottsdale, AZ, USA). EMG signals were filtered using a bidirectional zero-lag fourth-order Butterworth bandpass filter of 10-500 Hz, rectified, and integrated over 500 ms. EMG amplitudes were normalized to amplitudes recorded during maximal voluntary contractions (MVC).

Data sampling, synchronization, and post-processing

For passive dorsiflexion, tendon stiffness, and tests of contractile function, EMG, dynamometer data, and goniometer data were digitized and sampled at 1500 Hz (MyoResearch XP Master Edition 1.08.17, Noraxon Inc., Scottsdale, AZ, USA). A function generator (GwinStec, GFG-8215A, Good Will Instrument Co., Ltd, Tucheng City, Taiwan) and an electric trigger initiated sampling and enabled synchronization of data.

Post-processing was performed off-line using a software package (MATLAB and Statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Torque, dynamometer angle, and goniometer data were filtered using a bidirectional zero-lag fourth-order Butterworth low-pass filter of 10 Hz. Goniometer data were fitted to a

fourth-order polynomial equation. All passive dorsiflexion and tendon stiffness data were resampled to the ultrasound video frequency, while contractile function data were resampled to 200 Hz.

Tendon stiffness

Following a 5-minute bike ergometer warm-up (Monarch, 828E, Varburg, Sweden), the tensile stiffness of the free Achilles tendon and the whole Achilles tendon was examined through ultrasound videos (38-53 Hz) recorded during isometric ramp contractions. The ultrasound probe was placed sagittally over the distal MTJ of SOL and subsequently GM and was fixed to the leg using a custom-made rigid cast enabling stable positioning with minimal tissue compression. Echo-absorptive tape was applied to the skin to allow corrections for potential probe displacement relative to the skin.

Subjects were seated in a custom-made isometric ankle dynamometer (Gym2000, Geithus, Norway) instrumented with a load cell (U2A 500 Hottinger Baldwin Messtechnik, Darmstadt, Germany), with 90° hip flexion, with the knee straight and the foot strapped to the dynamometer at anatomical joint angle. The dynamometer settings were individually adjusted to align the axis of the ankle joint with that of the dynamometer. Unwanted joint movement was minimized by careful strapping of the limbs (34).

For the purpose of EMG normalization, the subjects performed two trials of plantar flexion MVC, separated by 60-sec rest. Subsequently, ramped plantar flexion contractions were carried out (constant rate of torque development (45 $Nm \cdot s^{-1}$) with real-time torque displayed to the subject, and with standardized verbal encouragement). Each trial was preceded by three brief sub-maximal contractions serving to pre-condition the tendon. Three trials were performed for SOL and for GM MTJ, separated by 120-sec rest. Additional trials were carried out if the subject did not follow the prescribed rate of torque development or if the ultrasonography plane or contact was lost during recording.

Plantar flexion torque was determined from load cell force and the perpendicular distance to the axis of joint rotation. Since ankle joint rotation during isometric plantar flexion was negligible (2.9±2.3° across groups), the recorded torques were not corrected for antagonist co-activation, as isometric contractions in the applied position do not involve antagonist fascicle shortening and that EMG recordings are primarily due to cross-talk (36). Tendon force was calculated from the plantar flexion torque and the instantaneous tendon moment arm as derived from ankle joint angle and leg length.

MTJ displacement was measured by semi-automated tracking (Tracker 4.11.0, Open Source Physics, Aptos, California, USA), interpolated at 50 N intervals, and then averaged from 3 valid trials. Tendon elongation was defined as proximal displacement of the MTJ, corrected for the influence of ankle joint rotation (37) by combining the instantaneous joint angles during ramped contractions with the linear relationship between MTJ displacement and joint angle obtained during a slow, passive ankle plantar flexion (0° to 3°).

Tendon force and tendon elongation data were cut off at 90 % of each individual's maximal force and fitted with a second-order polynomial (R^2=0.96-0.99) (34). Tendon stiffness was determined as the slope of the upper 20 % of the curve. Tendon elongation and strain are reported at common force (the greatest force achieved by all legs across time-points) and as individual maximum.

Slow, passive ankle dorsiflexion stretch

Passive torque, ankle joint angle, EMG (GM, GL, and SOL), and ultrasound videos (SOL MTJ, GM MTJ, GM fascicles) were obtained during passive dorsiflexion to maximal joint angle in an isokinetic dynamometer (HUMAC NORM 770, Computer Sports Medicine Inc., Stoughton, MA, USA). Subjects were seated with 65° hip flexion and extended knee. The mediolateral axis of the ankle joint was aligned with the dynamometer axis. Unwanted joint movement was minimized by strapping of the limbs (34). Subjects confirmed no sensation of stretch in the calf or hamstrings in the starting position. To prevent influence of visual perception, the tests were performed with eyes closed.

To determine ankle dorsiflexion ROM, an investigator manually rotated the foot at approximately $2°\cdot s^{-1}$ from a resting position to the maximally tolerated dorsiflexion. The dynamometer angle at this point was then recorded as the endpoint of ROM alongside with self-perceived pain (VAS score).

To assess passive resistance to stretch, the ankle joint was rotated by the dynamometer from 10° plantar flexion to maximal joint angle and back at $2°\cdot s^{-1}$ while subjects were fully relaxed. The procedure was repeated six times, separated by 120-sec rest, to secure six sets of EMG data along with two sagittal ultrasound videos (17-19 Hz) of the distal SOL MTJ, two of the distal GM MTJ and two of GM mid-belly fascicles. Echo-absorptive tape was applied to the skin to allow post-processing corrections for potential probe displacement relative to the skin. Additional trials were performed if torque or EMG signals indicated muscle

contraction (intermediate peak instead of a curvilinear torque development), or if the ultrasonography plane or contact was lost during recording.

Ankle joint angle was concurrently obtained with a 2D electro-goniometer (Noraxon Inc., Scottsdale, AZ, USA), secured to the medial part of the 1st metatarsal and distal tibia. For analyses of passive dorsiflexion, joint angles were obtained from the electro-goniometer, rather than from the dynamometer, to avoid the error induced by misalignment of the foot and dynamometer. Data are reported at anatomically neutral joint angle (0°), at standardized joint angle (defined as the maximal dorsiflexion angle that was common to each leg across time-points) and at maximal joint angle (defined as the maximal dorsiflexion angle achieved by each leg at the separate time-points).

GM fascicle lengths and pennation angles were measured by automatic tracking using optical flow algorithms (38). Displacement of the MTJ was measured by semi-automated tracking (Tracker 4.11.0, Open Source Physics, Aptos, California, USA), as detailed in our previous publication (34). Elongation and strain of fascicles are reported relative to resting length. MTU elongation was estimated from the average goniometer joint angles and calf length (39). Elongation of any structure proximal to the MTJ, represented by the displacement of the MTJ, is hereafter referred to as muscle elongation. Elongation occurring distally to the MTJ, calculated by subtracting distal MTJ displacement from MTU elongation, is hereafter referred to as tendon elongation. Muscle and tendon elongation are reported based on lengths measured at anatomical joint angle, as absolute values and as percent contribution to total MTU elongation. All data were interpolated at 0.05° intervals using spline functions and the valid trials were averaged.

Contractile function

Contractile function during isometric and isokinetic contractions was assessed using the same isokinetic dynamometer and position as for passive dorsiflexion. Isometric maximal plantar flexion torque was determined as peak torque over a 5-sec contraction. Two trials were performed at 10° plantar flexion and at 0°, 5°, 10° and 15° of dorsiflexion, all trials separated by 60-sec rest. Isokinetic concentric dorsi- and plantar flexion torque was determined between 10° of dorsiflexion and 30° of plantar flexion, at $30°\cdot s^{-1}$ (dorsi- and plantar flexion) and at 45, 60 and $90°\cdot s^{-1}$ (plantar flexion). Three trials were performed at each angular velocity separated by 60-sec rest. Warm-up consisting of three sub-maximal contractions at $30°\cdot s^{-1}$ preceded the tests. Standardized verbal encouragements and visual feedback of the

instantaneous torque were provided. The order of the trials was repeated identically PRE-POST, from least to greatest stretch and velocity, so that an eventual effect of stretch would not affect subsequent trials.

Isokinetic peak torque, angle of peak torque and work were determined from the trial with the greatest peak torque. Positive work was calculated as the area below the torque-angle curve after interpolating torque at 0.1° intervals.

Reliability

The muscle/tendon ultrasonography measurements at rest had excellent internal consistency (Cronbach's alpha > 0.98-0.99). For the remaining variables, reliability was established from the literature, and original reliability data were not collected during the study. The methods have been used in previous work at our and others' labs. Their satisfactory reliability has consistently been demonstrated, with r=0.98-0.99, CV=6-9% for the passive stretch methods (40) and with ICC>0.90 for ROM, passive stretch, EMG, tendon stiffness, and muscle architecture (41).

Statistical analysis

Resting length of the free Achilles tendon, EMG amplitudes and VAS scores during passive dorsiflexion tests were not normally distributed (D'Agostino & Pearson normality test) and were hence log-transformed to normality. Baseline characteristics were analysed using paired, two-tailed Student's t-test. One-way ANOVA for repeated measures was used to identify changes in VAS scores during stretching exercise. Two-way (leg x time) ANOVA for repeated measures was used to identify main effects and interactions, in which case post hoc, Sidak's multiple comparisons tests were performed, using multiplicity adjusted *P* values. The level of significance was set to α=0.05. All data are presented as mean±standard deviation. Cohen's *d* effect sizes (ES) were calculated to estimate the magnitude of the treatment effects, with the magnitude of effects considered either small (0.20-0.49), medium (0.50-0.79), and large (>0.80) (42).

RESULTS

Four subjects withdrew from the study due to unrelated injury or personal reasons. The final data set comprised 26 subjects; 17 women and 9 men (age 22.0±1.6 years, height and mass women: 169±7 cm, 61±11 kg, men: 184±5 cm, 80±12 kg), reporting a weekly training volume

of 2±2 hours of endurance activities and 1±1 hours of strength training during the intervention period.

For technical reasons, one subject was excluded from passive dorsiflexion analyses, one from the passive dorsiflexion EMG analyses, and one from the passive dorsiflexion ultrasound analyses. Due to insufficient ultrasound video quality or goniometer malfunctioning, five subjects were excluded from free Achilles tendon stiffness analyses and three from whole Achilles tendon stiffness analyses. Thus, the sample size for passive dorsiflexion was n=25, for passive dorsiflexion EMG and ultrasound n=24, for free and whole Achilles tendon stiffness n=21 and 23, respectively. For isometric strength, the sample size was n=19 and 8 at 10° and 15° dorsiflexion, respectively, due to restricted ROM in the remaining subjects. Adherence to the training was 89±10 %, resulting in 10.9±1.5 hours or approximately 40,000±5,000 sec of static stretching exercise.

ROM and pain during passive stretching

Stretching resulted in a bilateral increase in dorsiflexion ROM (interaction $P<0.005$, side $P=0.66$, time $P<0.001$, ES=1.12, Fig. 2). Perceived pain during the passive ROM test was unchanged (interaction $P=0.58$, side $P=0.81$, time $P=0.06$, ES=0.15, stretching PRE 4.2±2.1 cm, POST 4.0±2.9 cm, control PRE 4.2±2.2 cm, POST 4.3±2.7 cm). Self-perceived pain intensity during stretching exercise was reduced from baseline to subsequent time-points (intervention $P<0.001$, ES=0.95, PRE 4.6±2.6 cm, 8 weeks 2.2±2.2 cm, 16 weeks 2.6±2.0 cm, POST 2.0±1.7 cm).

(Insert Figure 2)

Resting properties of the MTU

Resting ankle joint angle, Achilles tendon morphological properties and GM fascicle length were unchanged, while GM thickness and pennation angle increased with time in both legs (Table 1). Fascicle length and tendon lengths normalized to resting MTU length showed the same statistical outcome as absolute values and are not reported.

(Insert Table 1)

Mechanical properties and EMG amplitude during passive dorsiflexion

Passive torque (Fig. 3A, B) decreased with time at anatomical joint angle in the stretching leg (interaction P=0.29, side P=0.48, time P<0.005, ES=0.31, Fig. 3E) and at standardized joint angle in both legs (interaction P=0.40, side P=0.34, time P<0.001, ES=0.24, Fig. 3F), while passive torque at maximal joint angle increased in the stretching leg (interaction P<0.01, side P=0.55, time P<0.001, ES=0.81, Fig. 3G).

EMG amplitudes of GM, GL, and SOL (Fig. 3C, D) at standardized joint angle decreased with time in both legs (interaction P=0.43-0.80, side P<0.07-0.30, time P<0.001, ES=0.07-0.23, Fig. 3H). GM pennation angle change during passive dorsiflexion was negligible (CON PRE 14.4±3.5° at 0°, 14.9±3.8° at maximal joint angle, STR POST 13.2±3.5° at 0°, 13.4±3.4° at maximal joint angle) and was not altered by the intervention (interaction P=0.35-0.57, side P=0.30-0.35, time P=0.09-0.65, ES=0.20-0.27).

(Insert Figure 3)

No interaction effect was seen for GM fascicle elongation, but there was a time effect in the control leg at standardized joint angle and in both legs at maximal joint angle (Table 2). Elongation of the SOL and GM MTU at maximal joint angle (Fig. 4B, D) was changed in both legs, with increased elongation of tendon in both legs, increased elongation of muscle only in the stretching leg, and with increased contribution from tendon elongation (except control leg GM, where P=0.08 for contribution at maximal joint angle). At standardized joint angle (Fig. 4A, C), the control leg SOL MTU showed decreased elongation of muscle and increased elongation and contribution of tendon, while the GM MTU displayed no changes. Elongations normalized to resting MTU length give the same statistical outcomes as absolute values and are not reported.

(Insert Table 2)
(Insert Figure 4)

Tendon mechanical properties

Neither free Achilles tendon stiffness (interaction P=0.22, side P=0.52, time P=0.20, ES=0.39, stretching PRE 554±218 $N \cdot mm^{-1}$, POST 552±190 $N \cdot mm^{-1}$, control PRE 547±184 $N \cdot mm^{-1}$, POST 488±144 $N \cdot mm^{-1}$, Fig. 5A) nor whole Achilles tendon stiffness (interaction P=0.54, side P=0.89, time P=0.42, ES=0.19, stretching PRE 349±84 $N \cdot mm^{-1}$, POST

347±100 $N \cdot mm^{-1}$, control PRE 361±84 $N \cdot mm^{-1}$, POST 341±76 $N \cdot mm^{-1}$, Fig. 5B) changed after the intervention. Tendon elongation and strain at common force level and at maximal elongation increased with time, in both legs for the whole Achilles tendon (elongation interaction P=0.49-0.88, side P=0.23-0.37, time P<0.001, strain interaction P=0.63-0.98, side P=0.23-0.24, time P<0.001, ES=0.00-0.21, Fig. 5B), and in the control leg for the free Achilles tendon (elongation interaction P=0.22-0.30, side P=0.76-0.87, time P<0.01, strain interaction P=0.36-0.47, side P=0.75-0.99, time P<0.005, ES=0.23-0.40, Fig. 5A).

(Insert Figure 5)

Contractile function

Maximal isometric plantar flexor torque was unchanged at all joint angles (interaction P=0.06-0.64, side P=0.72-0.83, time P=0.32-0.84, ES=0.13-0.56, Fig. 6A). Concentric plantar flexor peak torque was unchanged across angular velocities (interaction P=0.32-0.64, side P=0.75-0.99, time P=0.14-0.74, ES=0.18-0.29, Fig. 6B), while a time effect was seen for concentric dorsiflexion peak torque (interaction P=0.67, side P=0.97, time P<0.001, ES=0.13, stretching PRE 16.1±5.1 Nm, POST 17.6±5.8 Nm, P=0.26, control PRE 16.2±5.7 Nm, POST 17.4±5.8 Nm). The angle of peak torque of the plantar flexor muscles shifted towards more dorsiflexed position in both legs (Fig. 6C), at 30, 45 and $60° \cdot s^{-1}$ (interaction P=0.62-0.84, side P=0.69-0.91, time P<0.005-0.05, ES=0.06-0.15), but not at $90° \cdot s^{-1}$ (interaction P=0.56, side P=0.88, time P=0.11, ES=0.17). Work at $30° \cdot s^{-1}$ was unchanged (interaction P=0.41, side P=0.80, time P=0.31, ES=0.24, stretching PRE 156±45 J, POST 160±8 J, control PRE 155±35 J, POST 156±38 J).

(Insert Figure 6)

DISCUSSION

The purpose of this study was to examine the effects of 24 weeks of functional unilateral triceps surae stretching on ROM, on the morphological and mechanical properties of the MTU, on neural activation, and on contractile function. Comparing the intervention and control leg, ROM, passive torque at maximal joint angle, and passive MTU elongation at maximal joint angle increased, supporting our hypotheses. Also as hypothesised, no indications of altered morphological properties were observed. Further to our hypotheses,

significant changes were seen in the non-stretching control leg, suggesting a cross-education effect of habitual unilateral stretching.

When comparing studies, it must be noted that the intervention stimuli may be highly different, both with respect to study duration and stretching volume – time under stretch. In the present study, time under stretch was approximately 40,000 sec for a duration of 24 weeks, while for example Magnusson *et al.* used 9,000 sec over 3 weeks (1), and Ben & Harvey used 56,000 sec over 6 weeks (5). The present study aimed to provide conclusions applicable to sports and exercise, by applying doses that can realistically be maintained on a daily basis in sports and exercise settings, over a duration that approaches habitual stretching.

ROM and cross-education

Following 24 weeks of stretching, ankle dorsiflexion ROM increased by 11° in the stretching leg and by 4° in the control leg. ROM continued increasing between 8 and 24 weeks (Fig. 2), although the perceived pain during stretching exercise was significantly reduced after 8 weeks. These data indicate that although stretching may seem less intense after the initial weeks, ROM may still be affected. ROM did not change significantly between 16 and 24 weeks. Possibly, the most flexible subjects were at this point approaching a level of ROM where the anatomical constraints of the ankle joint begin opposing further motion.

To the best of our knowledge, a bilateral increase in ROM after unilateral stretching training has not previously been reported. Although reports exist of acute bilateral gains in ROM following unilateral stretching (43, 44), previous unilateral stretching training studies found unchanged control leg ROM (5, 6, 8, 45, 46) and unchanged control leg passive torque (8, 45). Previous studies were limited to 3-10 weeks of stretching, while in the present study, increases in control leg ROM did not reach significance until 16 weeks. The present findings may be explained physiologically or biomechanically. Considering the proposed role of tolerance and sensory factors for increasing ROM (1, 2), the bilateral responses to stretching training may likely be ascribed to adaptations in the neural pathways, somewhat similar to the cross-education effects reported with strength training (47). An alternative possible explanation is cross-education effects involving adaptations in the motor areas (48).

EMG amplitude and pain during passive dorsiflexion

When passively stretched to a standardized joint angle the EMG amplitude of GM, GL and SOL decreased after long term stretching with no difference between legs (Fig. 3 C, D),

which is in contrast to previous reports of unchanged EMG amplitude following 3 weeks of stretching (1). It is therefore possible that such neural adaptation may require greater durations or volumes of stretching. However, decreased EMG amplitude seems consistent with the reduced tonic reflex reported following 6 weeks of stretching (6). Combined with the unchanged EMG amplitude and pain at the increased maximal joint angle, these findings support the notion that reflex activity and pain are important mechanisms for ROM.

Torque-angle properties during passive dorsiflexion

Passive torque and joint stiffness were reduced at comparable dorsiflexion angles after the present intervention (Fig. 3 A, B). Previous intervention studies are inconsistent, with no change (1, 3, 5), or reduced torque at standardized joint angles (10-12) or reduced passive joint stiffness (6, 8, 13) following stretching. The discrepancies may be related to methodological differences, including intervention duration and/or stretch volume. In the present study, the reduced passive torque in both legs at standardized joint angle corresponds with the reduced EMG amplitude. While the magnitude of the potential influence from reduced EMG amplitude cannot presently be assessed, these data support an association between neural activation and active muscle stiffness and passive resistance to stretch. However, reduced passive torque at anatomical joint angle (Fig. 3E), where almost no EMG was recorded, suggests that changes in neural activation do not fully explain the observed changes in passive torque. Since structural connective tissue properties contribute to passive resistance (49), this finding may indicate that stretching has induced a change in structural properties of connective tissue, although the present dataset cannot ascertain whether such change occurred.

The present 35 % increase in passive torque at maximal joint angle in the stretching leg is relatively similar to previous studies with short interventions (e.g., 28 % after 3 weeks (4)), despite large differences in ROM gains. This suggests that sensory adaptations enabling the subject to tolerate a greater maximal joint angle and torque may occur in the first weeks of stretching, while ROM is subsequently increased by factors that also reduce passive resistance, such as neural activation or structural adaptations.

Morphological properties

Unexpectedly, a small but consistent increase in GM thickness and resting pennation angle was observed bilaterally after the stretching intervention (Table 1). Limited explanation can

be offered, other than perhaps seasonal variation due to PRE data being collected after summer and POST data being collected during spring. However, the self-reported activity level during the intervention period did not vary between subjects. On the other hand, no change was seen in muscle strength and thus any functional relevance is likely negligible. GM fascicle length and Achilles tendon length remained unchanged. A few human intervention studies have reported increased fascicle length after stretching (15, 16) and longer GM fascicles have been observed in professional ballet dancers compared to non-stretching controls (34). To standardize tension in the present study, resting fascicle length was acquired with the foot hanging freely and no difference was seen in resting ankle angle. Furthermore, fascicle length at 0° was 4-6 mm greater than at resting length and increased with time. The susceptibility of fascicle length measurements to low levels of tension demonstrated here may explain the discrepancy between the present and previous studies regarding fascicle length at 0°. For more direct measures of the effect of stretching training on muscle adaptations, invasive methods and/or more advanced imaging techniques, e.g., second harmonic generation microendoscopy (50) could be applied. Given the current methods, no stretching-induced changes in muscle or tendon length were observed.

Tendon mechanical properties

The unchanged tendon stiffness observed in the present study is in line with previous studies applying static stretching training (3, 8, 11, 22). However, reduced tendon stiffness has been reported following proprioceptive neuromuscular facilitation (23) or ballistic (11) stretching training, and in a cross-sectional study, ballet dancers had less Achilles tendon stiffness compared to controls (34). It is thus possible that potential tendon adaptations require stimuli of greater amplitude, for example as achieved with strength training (51), than was attained by the present stretching protocol.

The increased tendon elongation and strain at common and maximal force levels of ramped isometric contractions (Fig. 5) remain compatible with unchanged tendon stiffness, as an increase in toe-region strain would allow greater tissue elongation at low force levels without altering the force-elongation slope (stiffness) near maximal force. Thus, a potential effect of stretching training on tendon material properties and toe limit strain should be examined in further studies. Increased tendon elongation in the control leg is surprising since tendon tissue is not expected to respond to central neural adaptations, however, this finding is consistent with observations in the control leg during passive dorsiflexion.

Passive MTU elongation

At standardized joint angle, elongation of the GM muscle and whole Achilles tendon was unchanged in both legs. At the maximal joint angle, which was increased after training, the whole Achilles tendon elongation increased in both legs while GM muscle elongation increased only in the stretching leg. In the stretching leg, the tendon contribution to total MTU elongation was increased at maximal joint angle; the relative contribution of tendon (35 %) versus muscle (65 %) changed to 51 % and 49 % from PRE to POST. In the control leg, the change in contribution from 39 % and 61 % PRE to 47 % and 53 % POST was not significant (P=0.08). These values compare well to a recent report of 50/50 contribution between muscle and tendon during submaximal acute stretches (52). GM fascicle elongation and strain increased in the control leg at standardized joint angle and increased similarly in both legs at maximal joint angle (Fig. 4). The differences between the methods of calculating muscle and fascicle elongation (MTJ displacement versus mid-belly scans) do not allow for direct comparisons between the two variables. Furthermore, muscle elongation may be slightly underestimated due to stretching of the skin when approaching large joint angles. These factors may explain the different contribution ratios reported in a previous cross-sectional study where GM fascicles accounted for 72 % and tendon 28 % at 30° dorsiflexion (53).

In the present study, the SOL MTU responded similarly to the GM MTU, but with increased contribution from tendon in both legs at maximal joint angle, and with decreased muscle elongation and increased tendon elongation in the control leg at standardized joint angle. The lack of change in the stretching leg at standardized joint angle contrasts with another intervention study that showed increased muscle and fascicle strain and decreased tendon strain following 3 weeks of stretching (4). Differences between studies may be related to study duration and the unchanged passive torque at standardized joint angle in the study by Blazevich *et al.* (4). In the present study, the increased elongation of the free Achilles tendon in the control leg is surprising. However, the finding of increased tendon elongation during passive trials does match the findings of increased tendon elongation during ramped contraction, both at maximal elongation and at common force. Furthermore, a matching tendency was seen for both legs and muscles at standardized joint angle of passive dorsiflexion. Increases in tendon toe limit strain, as speculated above, could potentially facilitate the increased tendon elongation and contribution at lower torque levels.

The present data suggest that when maximal MTU elongation increased following stretching training, elongation of SOL and GM muscle bellies contributed only in the stretching leg, indicating that stretching may have modified muscle stiffness. However, control leg ROM increased less than stretching leg ROM, constituting a smaller increase in total MTU elongation, which perhaps owing to the sensitivity of muscle elongation measures, may explain why muscle elongation did not increase significantly in the control leg. Greater muscle elongation with greater ROM is in line with cross-sectional studies showing greater maximal muscle or fascicle elongation in flexible subjects (17, 34, 53). On the other hand, after the present intervention, tendon contribution to total MTU elongation increased. These findings match our former observation that both muscle and the series elastic element contributes to the greater MTU elongation in flexible ballet dancers (34). Potentially, at low tension, muscle/fascicle compliance may be large relative to tendon compliance (52), while at greater tension, muscle/fascicle compliance may be similar or lower than tendon compliance, letting tendon take up an increased portion of the elongation. The relationship between tension, toe strain limit, muscle compliance, and tendon compliance entails closer examination in future studies.

Contractile function

Despite a small increase in GM thickness, the present intervention did not increase isometric or isokinetic strength, matching most (3, 4, 6, 8, 13, 45) but not all (27, 28) stretching intervention studies. Plantar flexion work was also unchanged, matching again some previous data (26) but not all (28). Following the intervention, the angle of peak torque shifted towards a more dorsiflexed position at the lower angular velocities, with no difference between legs (Fig. 6), matching some (26, 27) but not all studies (28). Taken together, unchanged torque and work production along with the architecture data suggest no addition of serial sarcomeres with the present stretching intervention.

Limitations

Although the present 24-week within-subjects design has advantages, there are also limitations to such a design. Muscle or tendon tissue properties may change because of external factors such as dietary status or seasonal changes in activity, affecting both legs similarly. It cannot be excluded that the changes seen in the control leg may be related hereto. Potential cross-over effects may influence control-intervention comparisons, thus challenging

the control design of the study. The lack of original reliability data for some variables limits the interpretation of the present findings. For logistic reasons we did not collect a complete set of reliability data. The reliability of the methods is established in the literature and the study statistical power was deemed sufficient a priori. However, the changes in the control group were not anticipated and with hindsight, a complete set of reliability data specific to this study would have helped mitigating the risk of type I error. Additional studies are therefore required to ascertain that the present changes in control leg ROM as well as passive torque, elongation, and EMG amplitude during passive trials originate from physiological factors.

Learning effects in e.g. tests of contractile function can potentially bias results, however, the tests sessions were separated by 8-16 weeks, so such effects seem unlikely. Investigator bias cannot be ruled out, but during passive tests, investigators and subjects had no active role, and during post processing, investigators were blinded. It seems that for the automated torque-angle analyses, the changes in passive torque and EMG likely reflect the intervention. The potential changes to be observed by ultrasonography are small, and some measurements may be approaching current resolution limits. Finally, while the triceps surae muscles are well suited for ultrasonography and investigations of passive resistance, there may be joint constraints (e.g., ligamentous constraints or bony contact) that restrict ROM from approaching extreme joint angles. The most flexible subjects may have approached such angles within the 24 weeks, leading to an underestimation of the effects of stretching.

Conclusions

Twenty-four weeks of functional unilateral static stretching increased dorsiflexion ROM, maximal passive dorsiflexion torque, and MTU elongation at maximal joint angles in the stretching leg compared to the control leg, affirming the role of central neural factors. Passive dorsiflexion torque and EMG amplitude at standardized joint angles decreased, suggesting a role of peripheral factors. By 16 weeks, ROM also increased in the control leg. The significant and conforming PRE-POST changes both in the training leg and the control leg are taken to indicate a cross-over effect of habitual stretching.

The greater passive torque tolerated by the stretching leg indicates that pain threshold or pain sensitivity was affected, which may have influenced muscle stiffness through reduced sensory input. This corresponds with reduced passive torque in both legs at comparable PRE-POST joint angles. Reflex activity may contribute to passive torque via its contribution to muscle stiffness. This is supported by the corresponding reductions in EMG amplitude and

passive torque at standardized joint angle. However, reduced passive torque at anatomical joint angle, where almost no EMG amplitude was recorded, suggests that neural activation does not entirely explain the change in passive torque.

In conclusion, stretching training increases ROM through central neural adaptations and possibly structural adaptations within the MTU or within passive tissues that span the joint, and has the potential to modify passive and active torque-angle relations.

Further research is required to understand the relations between pain, reflexes, neural activation, resistance of passive structures, passive torque, and ROM with stretching training. Additional insight may be gained e.g. by investigating central responses, connective tissue composition, or by applying more advanced imaging techniques during passive motion.

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CONFLICTS OF INTEREST AND SOURCE OF FUNDING

The authors declare no conflicts of interest and no external funding. The results of the present study do not constitute endorsement by ACSM. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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APPENDICES

Supplemental Digital Content 1.docx (table)

Supplemental Digital Content 2.docx (table)

FIGURE CAPTIONS

Figure 1. Experimental setup including timeline on the vertical axis, methods and order of testing on the horizontal axis, and stretching exercises with straight (A) and bent (B) knee on the right side. The methods pictograms represent anthropometry, resting electromyographic measurements, warm-up, and tests of tendon stiffness, passive dorsiflexion, and contractile function. The expected outcomes are presented in the bottom row, where ↓ indicates a reduction, ↑ indicates an increase and == indicates no change to the variable.

Figure 2. Ankle dorsiflexion range of motion (ROM) in the stretching and control leg before (PRE), during and after (POST) 24 weeks of stretching. † indicates an interaction effect (P<0.005), * indicates a difference from PRE (P<0.001), § indicates a difference from 8 weeks (P<0.001).

Figure 3. A, B) Passive torque and C, D) normalized (maximal voluntary contraction) EMG amplitude during passive dorsiflexion for the stretching leg (A, C) and the control leg (B, D) at anatomical, standardized, and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. In the stretching leg, standardized joint angle=PRE maximal joint angle. Error bars are left out for legibility. EMG, electromyography; SOL, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis. E-G) Statistical analyses of passive torque and H) SOL EMG amplitude: † indicates an interaction effect (P<0.001-0.01), ‡ indicates a time effect (P<0.001-0.005), * indicates significant post hoc tests (P<0.001-0.05). Statistical analyses of GM and GL EMG are identical to SOL and are not shown. For absolute values and detailed statistics, see Supplemental Digital Content 1 (table).

Figure 4. Muscle and tendon elongation during passive dorsiflexion. A-B) Gastrocnemius medialis (GM) and the whole Achilles tendon (AT), C-D) soleus (SOL) and the free Achilles tendon, in the stretching leg (STR) and control leg (CON) at standardized and maximal joint angle, before (PRE) and after (POST) 24 weeks of stretching. Numbers on the bars represent

percent contribution to MTU elongation. † indicates an interaction effect for elongation (P<0.001-0.01), ‡ indicates a time effect for elongation and contribution (P<0.001-0.05), ↓↑ indicate significant post hoc tests for contribution (P<0.001-0.05). For absolute values and detailed statistics, see Supplemental Digital Content 2 (table).

Figure 5. Force-elongation relations during isometric plantar flexion. A) Free Achilles tendon (AT), B) whole Achilles tendon, in the stretching leg (STR) and control leg (CON) before (PRE) and after (POST) 24 weeks of stretching. Continuous lines represent group mean up to common force level, symbols represent individual maximal force and elongation. ‡ indicates a time effect for tendon elongation (P<0.001-0.01).

Figure 6. Torque-angle and torque-velocity relations. A) Isometric plantar flexion torque-angle relation, B) isokinetic plantar flexion torque-velocity relation, C) isokinetic torque-angle of peak torque relation, in the stretching leg (STR) and control leg (CON) before (PRE) and after (POST) 24 weeks of stretching. Note that the Y axes differ and are broken.
‡ indicates a time effect (P<0.005-0.05).

Experimental setup:

1 → 2 → 3

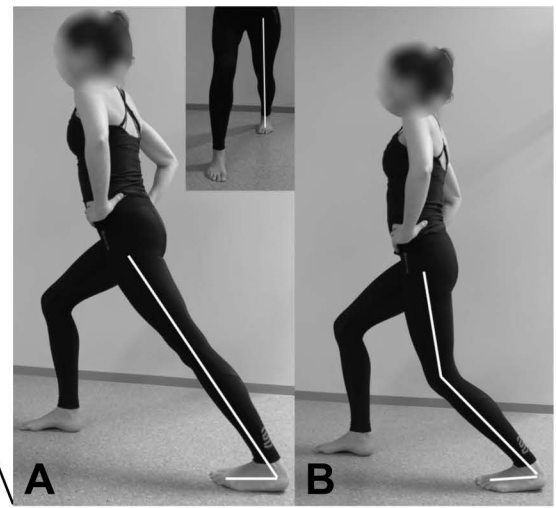
Methods:

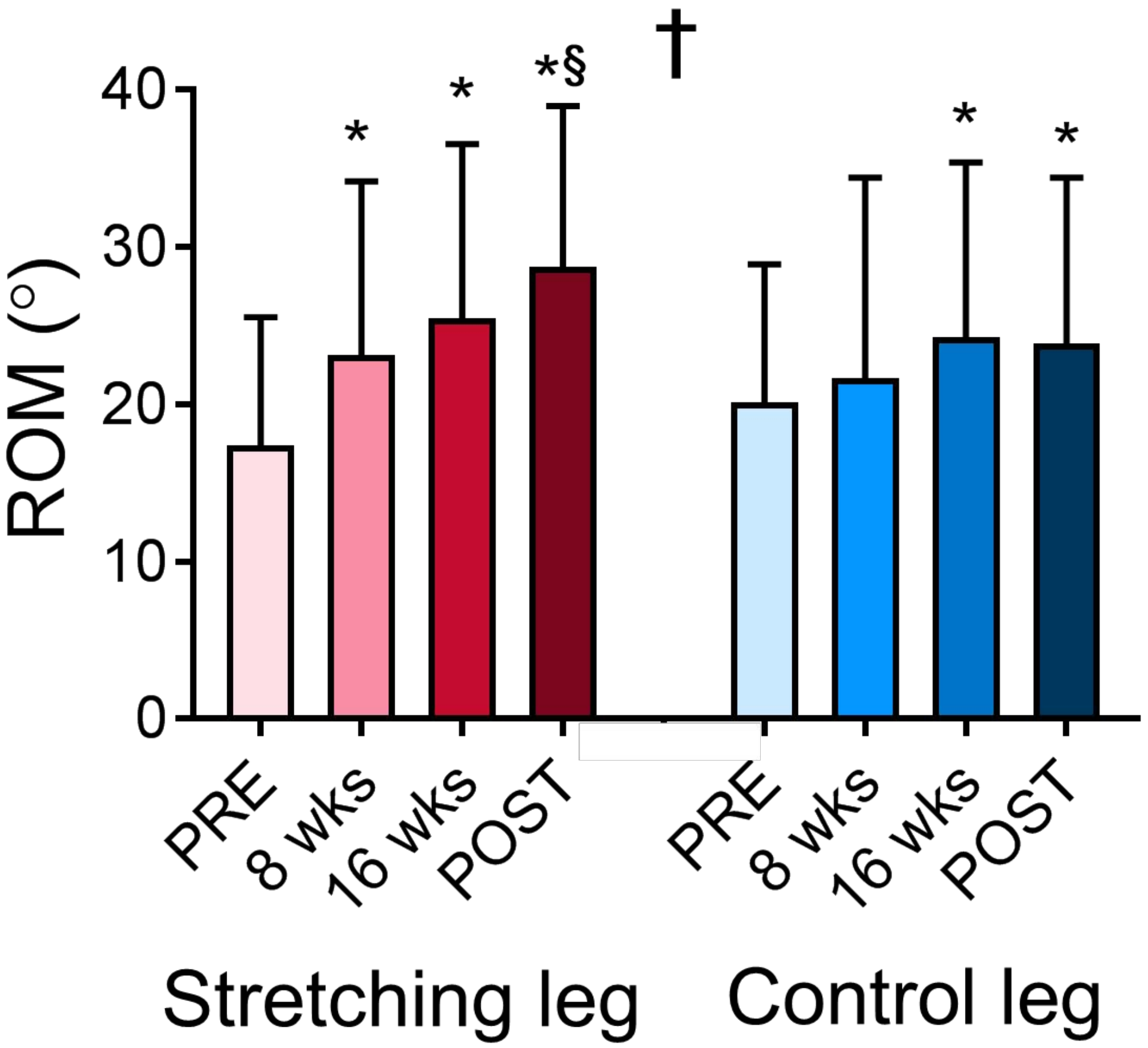
Time-line:

	1	2	3	4	5	6
Familiarization	Baseline	Baseline	Warm-up	Familiarization	Familiarization	Familiarization
PRE	-	-	Warm-up	Baseline	Baseline	Baseline
8 weeks	-	-	Warm-up	-	Intermediary	-
16 weeks	-	Intermediary	Warm-up	Intermediary	Intermediary	-
POST	POST	POST	Warm-up	POST	POST	POST
Hypotheses	Height == Mass == Leg length ==	**Muscle:** Fascicle len == Pennation == Thickness == **Tendon:** Length ==	-	**Tendon:** Stiffness ↓	**Passive:** ROM ↑ Torque @ std. angle ↓ Torque @ max. angle ↑ Elongation ↑ EMG ↓	**Contractile function:** Max torque == Angle of peak torque ↑

Stretching intervention:

One leg: 60 s x 4 reps, daily




†
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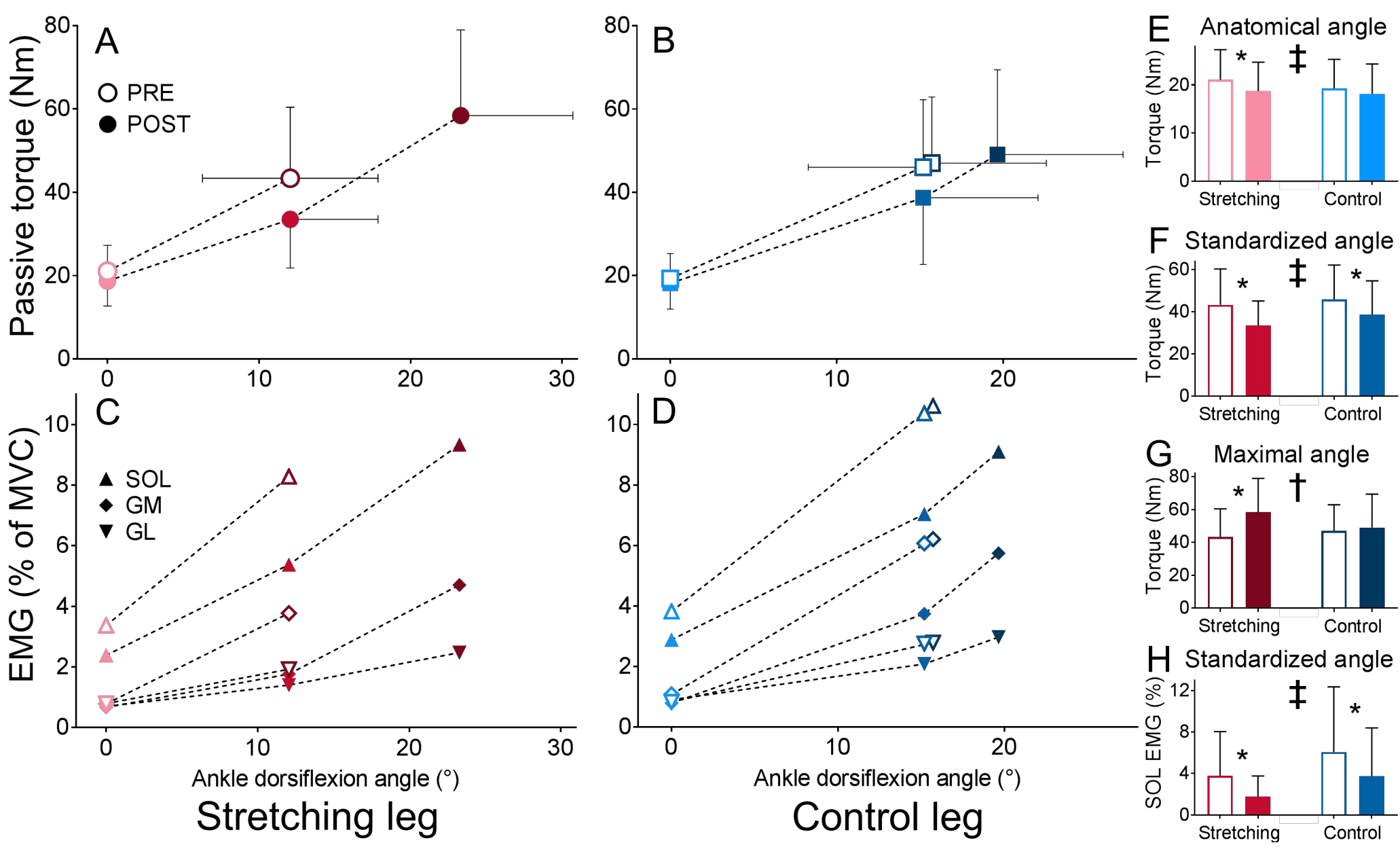
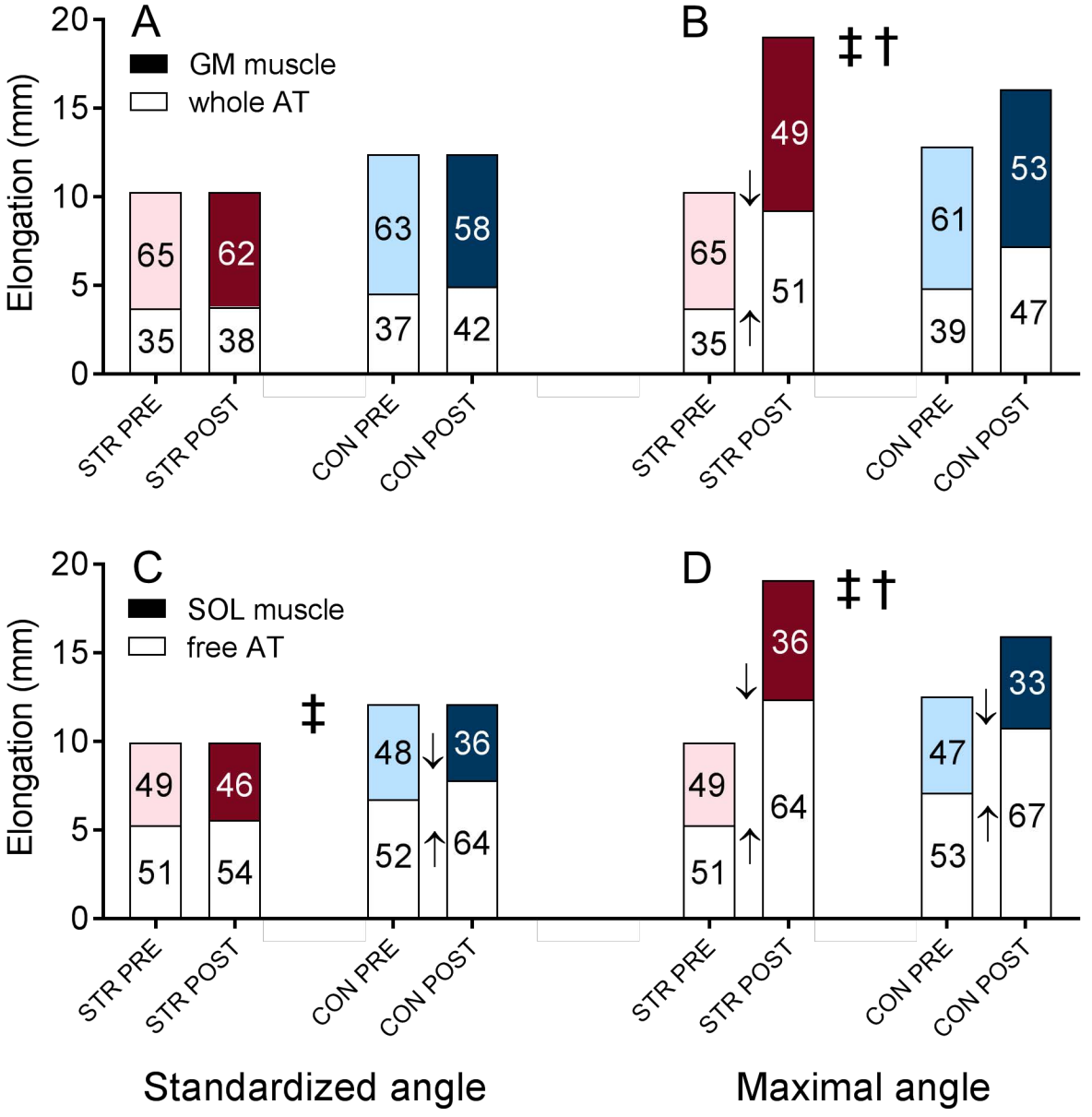
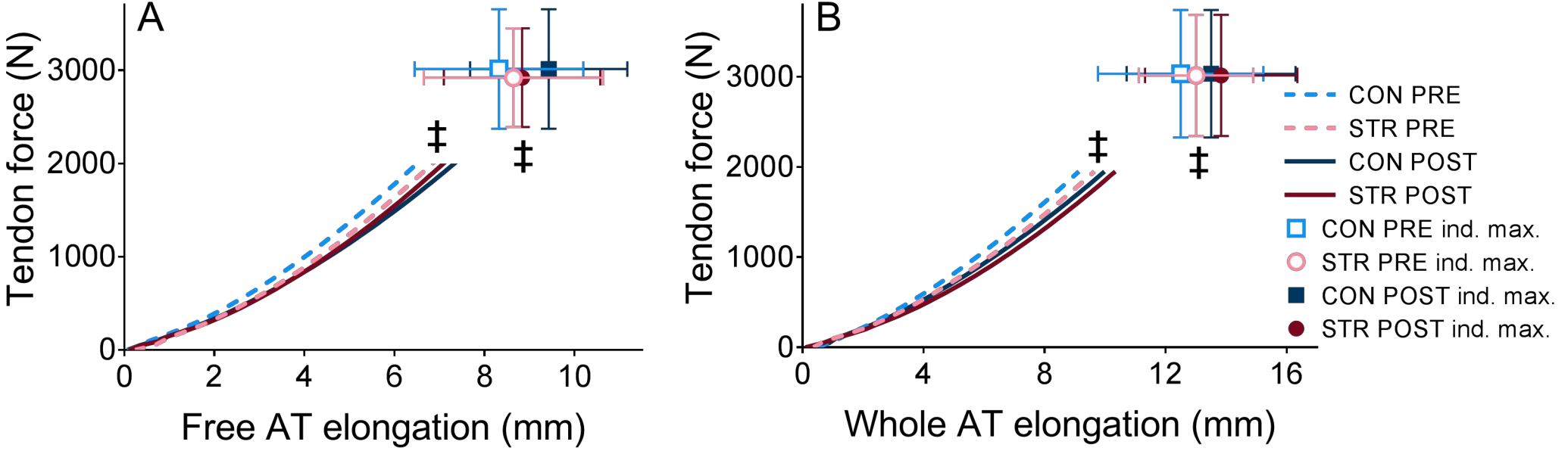
*
*
*
*
40
30
20
10
0
ROM (°)
PRE
8 wks
16 wks
POST
PRE
8 wks
16 wks
POST
Stretching leg
Control leg


A
PRE
POST
Passive torque (Nm)
B
C
SOL
GM
GL
EMG (% of MVC)
Ankle dorsiflexion angle (°)
Stretching leg
D
Ankle dorsiflexion angle (°)
Control leg
E
Anatomical angle
Torque (Nm)
Stretching
Control
F
Standardized angle
Torque (Nm)
Stretching
Control
G
Maximal angle
Torque (Nm)
Stretching
Control
H
Standardized angle
SOL EMG (%)
Stretching
Control


A
GM muscle
whole AT
Elongation (mm)
20
15
10
5
0
65
35
62
38
63
37
58
42
STR PRE
STR POST
CON PRE
CON POST
B
‡ †
65
35
49
51
61
39
53
47
C
SOL muscle
free AT
‡
49
51
46
54
48
52
36
64
D
‡ †
49
51
36
64
47
53
33
67
Standardized angle
Maximal angle


A
B
Tendon force (N)
3000
2000
1000
0
0
2
4
6
8
10
12
16
Free AT elongation (mm)
Whole AT elongation (mm)
‡
CON PRE
STR PRE
CON POST
STR POST
CON PRE ind. max.
STR PRE ind. max.
CON POST ind. max.
STR POST ind. max.

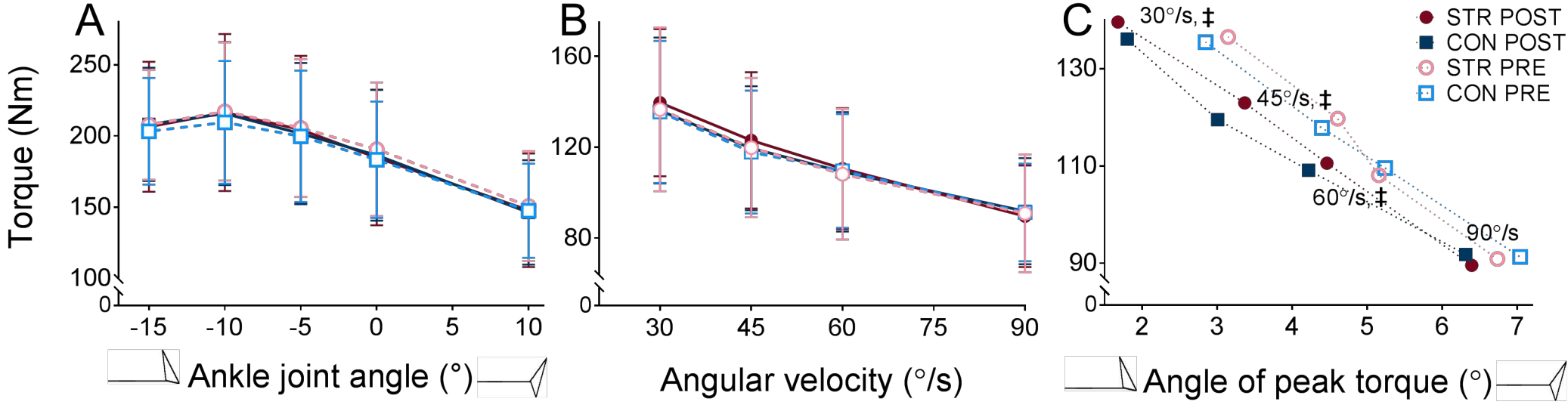

A
B
C
Torque (Nm)
250
200
150
100
0
-15
-10
-5
0
5
10
Ankle joint angle (°)
160
120
80
0
30
45
60
75
90
Angular velocity (°/s)
130
110
90
0
2
3
4
5
6
7
Angle of peak torque (°)
30°/s, ‡
45°/s, ‡
60°/s, ‡
90°/s
STR POST
CON POST
STR PRE
CON PRE

Table 1. Resting properties of the ankle joint, gastrocnemius medialis, and the Achilles tendon, before (PRE) and after (POST) 24 weeks of stretching. ES signifies effect size.

		PRE	POST	*P* inter.	*P* side	*P* time	Post hoc *P*	ES
Resting ankle angle (°)	Stretching	21.5 ± 3.5	23.1 ± 4.2	0.09	0.92	0.26	0.09	0.48
	Control	22.6 ± 4.4	22.2 ± 3.6				0.89	
Gastrocnemius medialis								
Thickness (mm)	Stretching	19.7 ± 2.1	21.2 ± 2.0	0.44	0.56	< 0.001	< 0.001	0.02
	Control	19.8 ± 2.3	21.7 ± 2.7				< 0.001	
Pennation angle (°)	Stretching	22.0 ± 2.9	23.9 ± 3.5	0.99	0.88	< 0.001	< 0.005	0.00
	Control	21.8 ± 3.3	23.8 ± 3.5				< 0.001	
Fascicle length (mm)	Stretching	53.8 ± 6.1	54.5 ± 5.8	0.52	0.89	0.48	0.57	0.18
	Control	53.9 ± 7.5	53.9 ± 7.2				1.00	
Achilles tendon								
Free AT length (mm)	Stretching	55.3 ± 20.2	57.6 ± 20.7	0.34	0.59	0.47	0.42	0.27
	Control	53.5 ± 19.3	53.8 ± 20.8				0.98	
Whole AT length (mm)	Stretching	178.8 ± 22.1	184.0 ± 22.4	0.19	0.98	0.18	0.13	0.36
	Control	181.5 ± 21.7	181.6 ± 22.2				1.00	

Table 2. Length, elongation, and strain of gastrocnemius medialis (GM) fascicles during passive dorsiflexion, before (PRE) and after (POST) 24 weeks of stretching. Elongation and strain are reported based on fascicle resting length, as measured with subjects resting in prone position. ES signifies effect size.

GM fascicle length (mm)		**PRE**	**POST**	***P* inter.**	***P* side**	***P* time**	**Post hoc *P***	**ES**
At anatomical angle	Stretching	58.4 ± 8.3	59.9 ± 8.6	0.46	0.69	< 0.005	0.15	0.21
	Control	57.0 ± 8.0	59.4 ± 7.7				< 0.05	
At standardized angle	Stretching	61.7 ± 8.7	64.4 ± 9.5	0.15	0.88	< 0.001	< 0.05	0.41
	Control	60.3 ± 7.3	65.1 ± 9.1				< 0.001	
At maximal angle	Stretching	61.7 ± 8.7	67.4 ± 8.7	0.76	0.65	< 0.001	< 0.001	0.09
	Control	60.4 ± 7.2	66.5 ± 9.7				< 0.001	
GM fascicle elongation (mm)								
At anatomical angle	Stretching	4.4 ± 7.2	5.6 ± 7.2	0.40	0.95	< 0.05	0.48	0.25
	Control	3.7 ± 5.9	6.1 ± 5.4				0.06	
At standardized angle	Stretching	7.8 ± 7.5	10.1 ± 8.1	0.15	0.81	< 0.001	0.13	0.43
	Control	6.9 ± 5.6	11.8 ± 6.3				< 0.001	
At maximal angle	Stretching	7.8 ± 7.5	13.1 ± 7.5	0.56	0.89	< 0.001	< 0.001	0.17
	Control	7.0 ± 5.6	13.3 ± 6.9				< 0.001	
GM fascicle strain (%)								
At anatomical angle	Stretching	8.7 ± 13.7	10.7 ± 13.2	0.40	0.97	< 0.05	0.55	0.24
	Control	7.3 ± 11.4	11.8 ± 10.0				0.07	
At standardized angle	Stretching	14.9 ± 14.3	19.0 ± 14.9	0.17	0.78	< 0.001	0.16	0.41
	Control	13.5 ± 10.9	22.3 ± 10.8				< 0.005	
At maximal angle	Stretching	14.9 ± 14.3	24.6 ± 14.3	0.62	0.92	< 0.001	< 0.001	0.15
	Control	13.7 ± 10.8	25.1 ± 12.1				< 0.001	

Supplemental Digital Content 1. Joint angle, passive torque, and electromyographic (EMG) amplitude of gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SOL) during passive dorsiflexion, before (PRE) and after (POST) 24 weeks of stretching. ES signifies effect size.

Ankle dorsiflexion angle (°)		**PRE**	**POST**	***P* inter.**	***P* side**	***P* time**	**Post hoc *P***	**ES**
At maximal angle	Stretching	12 ± 6	23 ± 7	< 0.001	0.99	< 0.001	< 0.001	1.26
	Control	16 ± 7	20 ± 8				< 0.005	
At standardized torque	Stretching	11 ± 6	17 ± 8	0.09	0.52	< 0.001	< 0.001	0.49
	Control	14 ± 7	17 ± 7				< 0.005	
Passive torque (Nm)								
At anatomical angle	Stretching	21 ± 6	19 ± 6	0.29	0.48	< 0.005	< 0.01	0.31
	Control	19 ± 6	18 ± 6				0.29	
At standardized angle	Stretching	43 ± 17	34 ± 12	0.40	0.34	< 0.001	< 0.001	0.24
	Control	46 ± 16	39 ± 16				< 0.005	
At maximal angle	Stretching	43 ± 17	58 ± 21	< 0.01	0.55	< 0.001	< 0.001	0.81
	Control	47 ± 16	49 ± 20				0.77	
EMG SOL (% of MVC)								
At anatomical angle	Stretching	3.4 ± 2.8	2.4 ± 1.9	0.38	0.52	0.15	0.89	0.26
	Control	3.8 ± 3.7	2.9 ± 3.0				0.20	
At standardized angle	Stretching	8.3 ± 6.7	5.4 ± 4.5	0.99	0.38	< 0.001	< 0.005	0.00
	Control	10.4 ± 8.6	7.0 ± 5.9				< 0.01	
At maximal angle	Stretching	8.3 ± 6.7	9.3 ± 6.8	0.42	0.54	0.94	0.84	0.24
	Control	10.6 ± 8.6	9.1 ± 7.0				0.78	

EMG GM (% of MVC)

At anatomical angle	Stretching	0.8 ± 0.5	0.7 ± 0.6	0.50	0.51	0.53	0.59	0.23
	Control	1.1 ± 1.4	0.8 ± 0.5				1.00	
At standardized angle	Stretching	3.8 ± 4.3	1.8 ± 2.0	0.43	0.12	< 0.001	< 0.001	0.40
	Control	6.1 ± 6.3	3.7 ± 4.7				< 0.01	
At maximal angle	Stretching	3.8 ± 4.3	4.7 ± 5.0	0.18	0.40	0.87	0.49	0.14
	Control	6.2 ± 6.2	5.7 ± 6.4				0.65	

EMG GL (% of MVC)

At anatomical angle	Stretching	0.8 ± 0.6	0.7 ± 0.6	0.64	0.81	0.64	0.75	0.14
	Control	0.8 ± 0.7	0.9 ± 1.0				1.00	
At standardized angle	Stretching	1.9 ± 1.8	1.4 ± 1.9	0.80	0.18	< 0.001	< 0.01	0.07
	Control	2.8 ± 2.5	2.1 ± 2.2				< 0.05	
At maximal angle	Stretching	1.9 ± 1.8	2.5 ± 2.7	0.34	0.33	0.57	0.48	0.28
	Control	2.8 ± 2.5	3.0 ± 2.8				0.96	

Supplemental Digital Content 2. Elongation of the gastrocnemius medialis (GM) and soleus (SOL) muscles and the Achilles tendon, and percent contribution to total muscle-tendon unit (MTU) elongation, during passive dorsiflexion, before (PRE) and after (POST) 24 weeks of stretching. Muscle and tendon elongation are reported based on lengths measured at anatomical joint angle, as absolute values and as percent contribution to total MTU elongation. ES signifies effect size.

GM muscle elongation (mm)		**PRE**	**POST**	***P* inter.**	***P* side**	***P* time**	**Post hoc *P***	**ES**
At standardized angle	Stretching	6.6 ± 2.4	6.5 ± 2.7	0.57	0.16	0.38	0.97	0.16
	Control	7.8 ± 3.3	7.5 ± 2.9				0.52	
At maximal angle	Stretching	6.6 ± 2.4	9.8 ± 2.6	< 0.001	0.74	< 0.001	< 0.001	1.02
	Control	8.0 ± 3.3	8.9 ± 3.0				0.16	
Whole Achilles tendon elongation (mm)								
At standardized angle	Stretching	3.7 ± 3.9	3.8 ± 2.7	0.57	0.28	0.38	0.97	0.16
	Control	4.6 ± 3.6	4.9 ± 3.0				0.52	
At maximal angle	Stretching	3.7 ± 3.9	9.2 ± 4.6	< 0.01	0.67	< 0.001	< 0.001	1.02
	Control	4.8 ± 3.5	7.2 ± 4.1				< 0.01	
GM muscle elongation (% contribution to MTU elongation)								
At standardized angle	Stretching	65 ± 21	62 ± 11	0.89	0.51	0.12	0.53	0.04
	Control	63 ± 24	58 ± 15				0.41	
At maximal angle	Stretching	65 ± 21	49 ± 13	0.16	0.98	< 0.001	< 0.001	0.41
	Control	61 ± 24	53 ± 15				0.08	
Whole Achilles tendon elongation (% contribution to MTU elongation)								
At standardized angle	Stretching	35 ± 21	38 ± 11	0.89	0.51	0.12	0.53	0.04
	Control	37 ± 24	42 ± 15				0.41	
At maximal angle	Stretching	35 ± 21	51 ± 13	0.16	0.98	< 0.001	< 0.001	0.41
	Control	39 ± 24	47 ± 15				0.08	

SOL muscle elongation (mm)

At standardized angle	Stretching	4.7 ± 2.4	4.4 ± 2.2				0.60			
				0.11	0.67	< 0.01		0.64		
	Control	5.4 ± 3.0	4.3 ± 2.8				< 0.005			
At maximal angle	Stretching	4.7 ± 2.4	6.7 ± 3.0				< 0.001			
				< 0.001	0.61	< 0.05		1.02		
	Control	5.4 ± 3.0	5.2 ± 3.1				0.79			

Free Achilles tendon elongation (mm)

At standardized angle	Stretching	5.3 ± 3.6	5.6 ± 3.7				0.60			
				0.11	< 0.05	< 0.01		0.34		
	Control	6.7 ± 4.2	7.8 ± 3.7				< 0.005			
At maximal angle	Stretching	5.3 ± 3.6	12.4 ± 5.0				< 0.001			
				< 0.01	0.70	< 0.001		1.21		
	Control	7.1 ± 4.3	10.8 ± 4.8				< 0.001			

SOL muscle elongation (% contribution to MTU elongation)

At standardized angle	Stretching	49 ± 19	46 ± 15				0.69			
				0.13	0.27	< 0.05		0.44		
	Control	48 ± 27	36 ± 17				< 0.05			
At maximal angle	Stretching	49 ± 19	36 ± 13				< 0.001			
				0.77	0.56	< 0.001		0.09		
	Control	47 ± 28	33 ± 16				< 0.005			

Free Achilles tendon elongation (% contribution to MTU elongation)

At standardized angle	Stretching	51 ± 19	54 ± 15				0.69			
				0.13	0.27	< 0.05		0.44		
	Control	52 ± 27	64 ± 17				< 0.05			
At maximal angle	Stretching	51 ± 19	64 ± 13				< 0.001			
				0.77	0.56	< 0.001		0.09		
	Control	53 ± 28	67 ± 16				< 0.005			