

**RANGE OF MOTION, NEUROMECHANICAL AND ARCHITECTURAL
ADAPTATIONS TO PLANTAR FLEXOR STRETCH TRAINING IN HUMANS**

Blazevich, A.J.^{1,3}, Cannavan, D.², Waugh, C.M.³, Miller, S.C.^{3,4}, Thorlund, J.B.⁵, Aagaard, P.⁵ and Kay, A.D.⁶

Affiliations:

¹ Centre for Exercise and Sports Science Research, School of Exercise and Health Sciences, Edith Cowan University, Australia

² Department of Health and Human Performance, Seattle Pacific University, USA.

³ Centre for Sports Medicine and Human Performance, Brunel University, United Kingdom*

⁴ London Sport Institute, Middlesex University, United Kingdom

⁵ Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Denmark

⁶ Sport, Exercise and Life Sciences, The University of Northampton, United Kingdom

*Data collection was completed at Brunel University, UK.

Address for correspondence:

Anthony Blazevich, PhD
Centre for Exercise and Sports Science Research
School of Exercise and Health Sciences
Edith Cowan University
270 Joondalup Drive, Joondalup
Australia 6027

Phone: +61 8 6304 5472

Fax: +61 8 6304 5036

Email: a.blazevich@ecu.edu.au

Running Head: Neuromuscular and ROM adaptations with stretch training

ABSTRACT

The neuromuscular adaptations in response to muscle stretch training have not been clearly described. In the present study, changes in muscle (at fascicular and whole muscle levels) and tendon mechanics, muscle activity and spinal motoneuron excitability were examined during standardized plantar flexor stretches after 3 wk of twice-daily stretch training (4×30-s). No changes were observed in a non-exercising control group (N=9), however stretch training elicited a 19.9% increase in dorsiflexion range of motion (ROM) and 28% increase in passive joint moment at end ROM (N=12). Only a trend toward a decrease in passive plantar flexor moment during stretch (-9.9%, $p=0.15$) was observed and no changes in EMG amplitudes during or at end ROM were detected. Decreases in $H_{\max}:M_{\max}$ (tibial nerve stimulation) were observed at plantar flexed (gastrocnemius medialis and soleus) and neutral (soleus only) joint angles, but not with the ankle dorsiflexed. Muscle and fascicle strain increased (12 vs. 23%) along with a decrease in muscle stiffness (-18%) during stretch to a constant target joint angle. Muscle length at end ROM increased (13%) without a change in fascicle length, fascicle rotation, tendon elongation or tendon stiffness following training. A lack of change in MVC moment and RFD at any joint angle was taken to indicate a lack of change in series compliance of the muscle-tendon unit. Thus, increases in end ROM were underpinned by increases in maximum tolerable passive joint moment ('stretch tolerance') and both muscle and fascicle elongation rather than changes in volitional muscle activation or motoneuron pool excitability.

Keywords: muscle stiffness, tendon stiffness, stretch tolerance, flexibility, elasticity.

INTRODUCTION

The ability to move comfortably through large ranges of motion (ROM) is important because it underpins the successful performance of activities of daily living and athletic tasks (26, 41) and may influence soft tissue injury risk (4, 53). Flexibility training programs are commonly employed with the aim of improving ROM (6-8, 17, 23, 35, 42) and, sometimes, reducing the passive resistance to stretch (21, 42). In contrast, an increased stretch tolerance, rather than changes in the stiffness/viscoelastic properties of the muscle-tendon unit, has been proposed to explain the observed gain in joint ROM following stretch training (23, 35). However, there is a high inter-individual variability in the response to training and statistically significant improvements in joint ROM are not always reported after stretch training in healthy adults (36, 54) or in clinical populations (24). The major factor influencing our understanding of this variable response and, thus, the development of effective ROM-improving exercise interventions, is that the detailed mechanisms underpinning ROM improvements are not fully known and furthermore may differ between different muscles. It is therefore not possible to target these mechanisms with specific muscle stretch training (or muscle-loading, pharmacological or reflex- and afferent feedback-altering) interventions. This highlights an important gap in our understanding of exercise-related adaptations in the human muscle-tendon unit.

An increase in the maximum passive joint moment tolerated by subjects, i.e. an increase in stretch tolerance, is ubiquitously reported alongside improvements in maximum joint ROM in studies investigating the influence of longer periods (i.e. 3 – 8 weeks) of muscle stretch training (e.g. 8, 17, 23, 35). Logically, this change in stretch tolerance should be underpinned by a change in afferent feedback from peripheral receptors, contributing to a change in reflex- or cortically-derived muscular activity during muscle stretch. However, no change in

electromyographic activity (EMG) has been observed during a maximal hamstrings muscle stretch after 20 days of static stretch training (35) or during plantar flexor stretches after 6 weeks of training (21). Further, no changes in disynaptic reciprocal inhibition or presynaptic inhibition of spinal motoneurons were reported after 6 weeks of plantar flexor stretch training (25). In contrast, Guissard & Duchateau (21) reported decreased Hoffman (H-reflex) and tendon reflex responses in the plantar flexors, however the temporal responses across training (6 weeks) and detraining (30 days) were somewhat different from the concurrent change in maximum ROM and passive resistance during joint rotation. Notably, the changes in evoked spinal reflex activity were obtained using nerve stimulation procedures with the muscles positioned at relatively short lengths (i.e. with ankle in the neutral, anatomical position), so the results cannot be assumed to reflect those that might be obtained during a muscle stretch maneuver (18). Collectively, there is a lack of clarity as to the potential adaptive change in both reflex- and cortically-derived muscle activities during stretch after periods of stretch training, and it is not clear whether such changes during stretch might be related to improvements in ROM or are a source of the individual variability in the response to stretch training.

Both increases in maximum joint ROM and decreases in the resistance to passive muscle stretch should also be possible through changes in the mechanical properties of the muscle-tendon unit, or of the joint structures themselves. Arguments against a possible role of tissue mechanical adaptation persist (e.g. 52) because of the common finding that passive joint moment measured during joint rotation, considered to reflect the stiffness of the muscle-tendon unit acting across the joint, did not appear to change after stretch training despite significant increases in joint ROM being achieved (8, 17, 23, 35). Furthermore, tendon stiffness has been reported to remain unchanged after stretch training (32, 36). However, a

number of reports exist of decreases in passive joint resistance in response to stretch training, particularly at the ankle joint (21, 32, 36, 42). Also, increases in fascicle elongation during constant-angle plantar flexor muscle stretching were reported after stretch training by Nakamura et al. (42), even though they did not observe a concurrent increase in whole muscle lengthening through the same ROM. Importantly, no studies have monitored concurrent changes in muscle fascicle and whole muscle-tendon elongation during stretch following training, so it remains to be determined whether changes in intramuscular architectural properties might underpin changes in ROM after stretch training. Gaining a more detailed understanding of the response of tissue mechanical and architectural properties would appear to be an important step in expanding our understanding of the adaptive plasticity of the human muscle-tendon unit in response to stretch training.

The purpose of the present study was to study in detail the tissue mechanical, muscle architectural and neuromuscular responses to longitudinal stretch training. H-reflex measurements were used to assess whether changes in spinal motoneuron/interneuron excitability and/or pre-synaptic inhibition occurred within the plantar flexor muscles, and to examine if potential changes differed between stretched vs. non-stretched conditions, before and after 3 weeks of ankle stretching (dorsiflexion) training. The level of tonic muscle activity (EMG activity) was also recorded from the plantar flexors and the antagonist tibialis anterior during maximal stretches. Additionally, both rotation and elongation of gastrocnemius medialis (GM) muscle fascicles and elongation of the whole muscle were measured using ultrasound imaging methods, and elongation of the Achilles tendon was estimated from changes in GM length and ankle angle. ROM and H-reflex data from some of the present subjects have been incorporated into a previously-published analysis detailing changes in spinal reciprocal inhibition after stretch training (12).

METHODS

Subjects

Twenty-two healthy men volunteered to complete all study requirements (age = 18.6 ± 0.9 y; height = 1.81 ± 0.6 m; body mass = 73.9 ± 7.6 kg) and were thus randomly assigned to intervention (muscle stretching; N = 12) or non-stretching control (N = 10) groups, as described below. One control subject withdrew from the study for personal reasons, so 9 subjects were re-tested. Three additional subjects completed the stretch training and muscle architectural measurements in order to increase statistical power in these measurements (i.e. N = 15) because of the previously reported variability in between-subject changes in these measures (9); however they did not participate in any other tests. All subjects had previously performed muscle stretch training but had not performed specialist physical or muscle stretch training in the previous 12 months. The subjects reported no disability, musculoskeletal injury or neurological deficit that might affect their ability to stretch, and they refrained from stimulant/depressant ingestion (e.g. self-reported caffeine and alcohol consumption) for 6 h, and from intense physical activity within 72 h, of all testing. All subjects gave their written informed consent before participation. The study was approved by the Human Research Ethics Committee at the School of Sport and Education (Brunel University, UK) and all procedures were performed in accordance with the declaration of Helsinki.

Overview

Subjects reported to the laboratory for 2 – 3 familiarization sessions (session 1: ROM and plantar flexion strength tests; session/s 2/3: tibial nerve stimulation familiarization and active joint moment targeting practice) and then for two testing sessions each before and after the 3-wk study period. On the first of two testing sessions, a 5-min cycle warm-up was performed

at 60 rpm with a 1-kg load (Monarch Exercise AB, Sweden) and then a series of four isometric plantar flexion contractions was performed whilst seated upright with the knee extended in an isokinetic dynamometer (Biodex, System 3, Biodex Medical Systems, Shirley, NY) at 50, 70, 90 and 100% of perceived maximum (4-s contraction, 20-s rest). After a 5-min rest, maximum tolerable stretch load (i.e. dorsiflexion end ROM) and maximum plantar flexor moment tests (MVC at 5 joint angles) were performed whilst ultrasound imaging was used to track concurrent changes in gastrocnemius medialis (GM) muscle length, fascicle length and fascicle angle; Achilles tendon length was later estimated from joint angle and muscle length data. In the second session, full H- and M-wave recruitment curves were obtained at rest by stimulating the tibial nerve with the ankle joint in 3 positions (plantar flexed, neutral and dorsiflexed, as described below). The subjects reported to the laboratory and completed the same warm-up as described above. Testing was performed at the same time of day (± 2 h) and with comparable diet and preceding physical activity patterns, which were recorded on the first test day and repeated on the second, for the 48 h before testing. All post-intervention testing was completed in the 2 – 4 day period after the final stretching session. Instructions during testing were provided by a researcher who was unaware of the group allocation of the subjects.

Stretch Training

The subjects were randomly allocated to either a stretch (STR) or no-stretch (CON; control) group by an assistant not connected with the study. STR subjects performed four 30-s ‘against-the-wall’ calf stretches (3) with 15-s inter-stretch recovery twice daily (between 7:00-10:00 and 18:00-21:00) for 3 weeks. The stretch was performed by placing two hands outstretched on a wall with one foot (non-stretched) approximately 30 cm from the wall and the other foot (stretched) behind the body in the sagittal plane. The non-stretched leg

remained bent at the knee while the stretched leg was held straight at the knee. Stretch was placed on the plantar flexor muscles (i.e. triceps surae) of the stretched leg; emphasis was placed on stretching the gastrocnemius muscles maximally but within the limit of pain. Total stretch duration for the 3-wk period was 4920 ± 144 s across 41.0 ± 1.2 sessions and self-reported training compliance was $93.2 \pm 2.7\%$. Stretch protocols of similar type, duration and frequency are known to rapidly induce increases in joint range of motion and muscle stretch tolerance, respectively (6, 19). The against-the-wall stretch was chosen (as opposed to, for example, the use of an isokinetic dynamometer) to reduce the time spent in the laboratory by the subjects (including weekends) in order to ensure a high training compliance. It would also increase the ecological validity of our findings since this stretch modality is commonly performed clinically as well as in sports.

Maximum Range of Motion (ROM) Assessment

On the first test day, the subject was secured in a seated position in the chair of the isokinetic dynamometer with the knee angle of the right leg at 0° (i.e. anatomical position). The subject was slightly reclined (hip angle at 75° ; 0° = full extension) in order to minimize the tension at the back of the knee, which can prevent maximal dorsiflexion (12, 15). The foot was firmly strapped to the ankle dorsi/plantar flexion footplate of the dynamometer and the lateral malleolus aligned with the dynamometer's axis of rotation. As described previously (11), the chair was positioned such that a small knee extension movement was required to straighten the knee, which caused some deformation of the chair and dynamometer shaft which then minimized heel lift from the footplate during ROM (and subsequent active joint moment) testing. Additional strapping across the foot, waist and chest was also used to minimize movement without impacting on dorsiflexion ROM. Passive ankle dorsiflexion rotations were then completed starting at 20° plantar flexion (0° = neutral position) and stopping when

subjects pressed a stop button, which immediately released the footplate and moved the ankle back to the start position. A slow joint angular speed of $2^{\circ}\cdot\text{s}^{-1}$ was used to ensure that variations in muscle-tendon viscosity (38, 49) and Ia-mediated mechanical responses did not influence the mechanical response to the stretch, and the subject's eyes were closed to prevent visual feedback of the stretch amplitude. Two trials were performed separated by a 1-min rest period, and the greatest dorsiflexion angle attained in either trial was taken as the maximum ROM.

Electromyogram (EMG), Joint Moment and Joint Position Recording

During ROM testing, EMG signals were continuously recorded from soleus (Sol), GM and tibialis anterior (TA). Self-adhesive surface EMG electrodes with a 10-mm recording surface (Tyco Healthcare, Neustadt, Germany) were placed in a bipolar configuration (20-mm inter-electrode distance) in the line of the fascicles observed using ultrasonography, according to SENIAM guidelines (27); the GM electrodes were sometimes moved slightly distally on the muscle to accommodate the ultrasound probe. The reference electrode was placed on the antero-superior aspect of the tibia. Electrodes were connected to a CED 1902 isolated differential amplifier (1000 \times ; input impedance = 10,000 M Ω ; CMRR > 100 dB) and band-pass filtered (10-500 Hz, Butterworth filter). EMG data were acquired simultaneously with joint moment and angle data (see below) at a 2000-Hz analog-digital conversion rate and smoothed with a symmetric moving root-mean-square (RMS) filter with a 100-ms time constant. Markings were applied to the skin with a permanent marker (and reapplied if necessary) to ensure identical electrode positioning before and after the 3-wk intervention period.

Ultrasonographic Recording

Movement of the Achilles tendon-GM muscle tendon junction (MTJ) was monitored during passive muscle-tendon stretch maneuvers using B-mode ultrasound (LOGIC Book XP, General Electric) with a 39-mm linear array probe scanning at 8 MHz (8L-RS, General Electric) (30, 31). Example images are shown in Figure 1. The ultrasound probe was secured to the skin using elastic strapping, and water-soluble transmission gel was applied to the probe surface to improve acoustic contact and allow for minimal pressure to be applied, reducing tissue compression. The probe was oriented so that the MTJ was clearly visible throughout the full ROM; some angulation of the probe in the longitudinal and transverse planes was required, which was variable between subjects. Ultrasound data acquisition was synchronized with passive plantar flexor moment and joint angle signal acquisitions using a 5-V TTL pulse directed to the ultrasound system. Ultrasound images were captured at a 29-Hz frame rate and transferred to a personal computer and MTJ position was later digitized manually using Peak Motus software (Peak Performance Technologies, Englewood, CO). Raw MTJ coordinate data were passed through a 3-Hz low-pass, 4th order, zero-lag Butterworth filter before calculating MTJ displacement. From these images, muscle and tendon length (and strain) were subsequently measured at each of 5 pre-designated joint angles, as described in detail below (see 'Data Analysis').

Five minutes after data were captured during the two maximum ROM efforts, a final (third) stretch was performed. Images of GM fascicles were obtained in real time using B-mode ultrasound (Esaote Biomedica, Italy) with a 45-mm linear array probe (10 MHz scanning frequency; Mega Esaote Biomedica) fixed to the skin at 50% of the distance from the popliteal crease to the distal insertion of GM onto the Achilles tendon. For these scans the probe was oriented in line with the fascicles such that longitudinal images were captured for

GM fascicle length and fascicle angle measurements, respectively. During this stretch maneuver the ankle joint was passively rotated from 30° plantar flexion to 30° dorsiflexion at 2°·s⁻¹, however 5-s pauses were imposed at each of 5 pre-designated joint angles (described below) to allow still images to be acquired. This protocol has been used previously by Abellaneda et al. (2; 20-s pause) and Blazevich et al. (11; 4-5 s pause), and allowed for the ultrasound probe to be oriented in line with the fascicles as they rotated during ankle rotation. This procedure enabled muscle fascicles to be clearly traced across the ultrasound images and the aponeurosis identified (9), thus allowing for a more accurate assessment of fascicle length and angle. For consistency, all images were obtained consistently at 4-5 s after a pause was initiated, which allowed sufficient time for probe realignment whilst minimizing stress relaxation in the muscle-tendon unit. Images were transferred to a personal computer for later offline analysis.

Maximal Active Joint Moment (MVC) and Rate of Force Development

An important aspect of the study was to separately examine the effects of stretch training on muscle and tendon tissues. However, it was also of interest to separate the effects of stretch training on the mechanical properties of series (SEC) and parallel (PEC) elastic components, distinct from its effects on muscle and tendon. Whilst some complex methodologies exist for this, an efficient method is to assess the effects of stretch training on both the active moment-angle relationship and the maximal iso-angular rate of rise in force (rate of force development: RFD) observed during maximal volitional muscle activation. Reductions in SEC stiffness would be expected to shift the moment-angle relation to the right (i.e. reduced passive force production at given muscle lengths (29)) and to reduce RFD in the initial contraction phase (0-50 ms) in the absence of increases in RFD during the later phase of force rise (100-200 ms) (16). Thus, after a 5-min rest, the subjects completed a second warm-up

sequence of four isometric plantar flexion contractions (50, 70, 90 and 100% of perceived maximum) at a 0° ankle angle with the hip inclined to 85° (this inclined position allowed for maximal contractions to be performed with minimal heel movement) before performing maximal contractions at each of five pre-designated joint angles (detailed hence). During pilot testing it was found that the passive joint moments developed throughout the ROM as well as the subjective ratings of stretch intensity differed substantially between subjects, leading to highly different abilities to generate active joint moments (as well as eliciting highly varying electrically-stimulated reflex responses). To account for this variability, the ankle joint angle at which 90% of peak passive tension (moment) occurred was obtained from the ROM test, then two equidistant angles were computed between this angle and 10° plantar flexion (i.e. where negligible passive joint moment was recorded in the subjects). A final angle was included that was more plantar flexed, but again equidistant, such that the selected iso-angular joint positions corresponded to -33, 0, 33, 66 and 100% of the angular distance between 10° plantar flexion and the joint angle at which 90% of peak passive tension occurred. These pre-designated joint positions are referred to as angles 1 through 5 and, on average, corresponded to: 1) $22.9 \pm 2.5^\circ$, 2) $10.0 \pm 0^\circ$, 3) $-2.9 \pm 2.5^\circ$, 4) $-15.7 \pm 4.9^\circ$ and 5) $-29.1 \pm 7.5^\circ$, where positive angles indicate plantar flexion. Iso-angular MVC and RFD testing was performed at these exact joint positions before and after the period of intervention.

With the subject firmly strapped into the dynamometer and the joint angle set, two 4-s maximal plantar flexion contractions (MVC) were performed at all pre-designated joint angles with the intent to produce plantarflexor force 'as fast and hard as possible'. Testing was performed from plantar- (angle 1) to dorsiflexion (angle 5), and trials showing a drop in joint moment >5 Nm at the start of contraction were discarded (due to the active counter movement) and another trial was completed. At the completion of testing, a single MVC trial

was performed at the first (plantar flexed) joint angle to test for fatigue; and in all cases joint moment levels remained within $\pm 5\%$ for all subjects. Data were acquired at a 2000-Hz sampling frequency and filtered with a 14 Hz low-pass, 4th order zero-lag Butterworth filter and the peak MVC and RFD were obtained for analysis.

H-reflex and M-wave Measurements

On testing day 2, at the same time of day and after completing the same warm-up, Sol and GM H-reflex and M-wave amplitudes were measured at joint angles 2, 3 and 4. Angle 3 most closely corresponds to the angle tested in previous studies (-2.9° in the present study vs. 0° in other studies (21, 25)), whilst angles 2 and 4 were chosen to allow comparisons to be made with the ankle in plantar- (10°) and dorsiflexion (-15.7°). It was not possible to obtain reliable measurements at angle 5 (-29.1°), which could not be maintained comfortably by subjects for the duration of the test. Measurements were obtained in a quiet, temperature controlled room (23°C) and testing always progressed from plantar- to dorsiflexed angles.

The stimulation protocol used was similar to that described previously (11, 12). A 3.2-cm anode (fabric gel adhesive electrode; ValuTrode CF3200, Nidd Valley Medical, Harrogate, UK) was placed on the patella and a 1-cm diameter cathode was placed over the tibial nerve in the popliteal fossa. Both electrodes were then secured with zinc oxide tape. Using a small current, the cathode position was adjusted to elicit the greatest M-wave response as measured using surface EMG electrodes placed on Sol and GM (described below). Square-wave electrical stimulation pulses of 1-ms duration were delivered via a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK) at intervals of ~ 10 s once the subject had produced a stable, voluntary plantar flexion moment equal to 2% of the MVC measured at the specific joint angle. This procedure was used to improve measurement reliability (12, 40). The

stimulus intensity was increased gradually until a plateau in the M-wave amplitude ($\pm 3\%$ variability) was clearly observed (M_{\max}), allowing quantification of the full H/M recruitment curve using 18-30 stimuli (see example, Figure 2). Additional stimulations were sometimes delivered around the intensity at which the H-reflex amplitude appeared maximal in order to more accurately determine the maximum H-reflex (H_{\max}) amplitude.

Soleus and GM EMG activities during stimulation were recorded using self-adhesive electrodes placed in a pseudomonopolar configuration, with one electrode placed each on the medial aspect of Sol distal to GM and on the GM muscle belly. A second electrode in each pair was then placed ~ 10 cm proximal to the Achilles tendon-calcaneus junction, just above Kager's triangle (Figure 2), and a reference electrode was placed on the lateral malleolus. This electrode configuration has been used previously (12, 22, 45) and was found to provide larger H- and M- wave amplitudes and greater test-retest reliability in pilot testing. Markings were applied with a permanent marker (and reapplied if necessary) to ensure identical electrode placements after the 3-wk intervention period. The electrodes were connected to a differential preamplifier (Model 1902, Cambridge Electronic Design, Cambridge, UK) with common mode rejection >100 dB and input impedance of $10,000$ M Ω . Data were sampled at 2000 Hz using a Power1401 DAQ interface and stored on computer (Signal software, Cambridge Electronic Design). Peak H- and M-wave amplitudes were calculated automatically and plotted in real-time during the stimulations.

Data Analysis

EMG data analysis

Maximum RMS EMG amplitudes (for Sol, GM and TA) and EMG amplitudes at end ROM (i.e. at the point of maximal volitional stretch tolerance) were obtained from the filtered EMG

data recorded during the maximum ROM trial for each subject. Also, the joint angle at which EMG onset occurred in either Sol or GM was recorded, with EMG onset being defined as the point at which the RMS EMG amplitude exceeded baseline EMG by 0.003 mV (i.e. approximately three times the baseline EMG standard deviation) and did not return below this value for at least 100 ms. EMG amplitudes recorded during the stretch maneuvers were also normalized to the peak amplitude obtained during the maximal isometric contraction (at angle 3) to allow for comparisons between subjects.

Muscle-tendon unit length measurement

Triceps surae-Achilles tendon muscle-tendon unit (MTU) length was measured at rest in the neutral, anatomical position (0° ankle joint angle) using a flexible anthropometric tape from the medial femoral condyle to the Achilles-calcaneus junction (most posterior, superficial site). The equation reported by Grieve et al. (20) was then used to calculate changes in MTU length as the ankle joint was rotated. MTU length was thus estimated as the sum of measured MTU length in anatomical position and the change in length estimated for a given magnitude of joint rotation.

Muscle and tendon length measurement

The distance from the proximal end of the ultrasound probe to the medial condyle was measured by tape measure in triplicate (and the mean distance calculated) in each subject during the ROM tests. Later, ultrasound images acquired during this session were imported and digitized using Peak Motus software (Peak Performance Technologies). Points on the edge of the ultrasound image (at the skin) and on the MTJ were tracked manually to quantify MTJ displacement relative to the probe's edge. Muscle length was then determined by summing the distances from the medial condyle to the probe and the distance from the image

edge to the MTJ. Therefore, ‘muscle’ length was defined as the section of the MTU proximal to the MTJ (including the proximal tendinous attachment). Achilles tendon length was subsequently calculated by subtracting the muscle length from total MTU length, and thus represented the part of the MTU distal to the MTJ.

Muscle-tendon stiffness estimation

MTU, GM and Achilles tendon stiffness were estimated throughout the ROM as dF/dL , where F is the estimated force transferred through the tissue and L is its elongation between pre-designated joint angles. Force at each pre-designated joint angle was calculated as the quotient of joint torque and moment arm, with the moment arm being estimated from the equations reported by Grieve et al. (20) and assumed to be unchanging across the training period. Force was assumed to be homogeneous throughout the MTU, and thus forces in the muscle and tendon were considered to be equal. MTU, muscle and tendon stiffness were hence calculated as the change in force per change in tissue length between pre-designated joint angles: $k_{tiss} = (F_i - F_{i-1}) / (l_i - l_{i-1})$, where k_{tiss} is the tissue stiffness, F_i is the force at a given joint angle, F_{i-1} is force at the preceding angle, l_i is the tissue’s length at a given joint angle and l_{i-1} is its length at the preceding joint angle. Stiffness values were not computed between angles 1 and 2 because the very small tissue elongations in some subjects between these plantar flexed angles ensured that small errors in the measurement would cause large differences in tissue stiffness estimates.

Fascicle length and angle measurement

Ultrasound images were analyzed using Image J software (National Institutes of Health, Bethesda, MD). Points on the fascicles were digitized at ~2-3 mm intervals along the fascicle (following its curvature) from the fascicle-deep aponeurosis junction to either the superficial

aponeurosis or the edge of the image (for longer/elongated fascicles). When regions of the fascicles projected off the image, the invisible section length was estimated by extrapolation as outlined previously (9). Fascicle length was subsequently calculated as the sum of the visible and extrapolated portions of the fascicle. Two fascicles were measured in each image, and each was digitized three times; the mean fascicle length was taken as representative. Repeated measurements were performed with a CV of 2.0% (~1.4 mm).

Fascicle angle was defined as the angle of the fascicle relative to the deep aponeurosis. Fascicles tend to curve markedly at their insertion onto the deep aponeurosis (50) making them difficult to visualize in this region. Therefore one point on the fascicle was digitized ~3 mm from the deep insertion and a second point was digitized at 30% of the distance to the superficial aponeurosis (9). The CV for these measurements was 1.6% (~0.41°).

MVC and rate of force development (RFD)

Peak active plantarflexor joint moments (MVC strength) and RFD were calculated using methods similar to those described previously (1, 10). MVC was taken as the highest point of the moment-time curve obtained at each of the five angles. RFD was derived as the average gradient of the moment-time curve from the onset of contraction (point at which joint moment exceeded baseline by >5 Nm) to 50, 100, 200 and 400 ms (1), as well as the incremental gradient from 200 – 400 ms. According to data of Edman & Josephson (16) and others (10) the potential influence of training induced changes in SEC stiffness on RFD is expected to be greatest in the early force rise (i.e. 0 – 50 ms and 0 – 100 ms) while negligible in the later phase of force rise (i.e. 200 – 400 ms), which can be used to provide indirect evidence as to whether changes in SEC stiffness may have occurred over the training period.

H-reflex and M-wave amplitude calculations

The three largest peak-to-peak, non-rectified H- and M-wave amplitudes were averaged in each condition and the $H_{\max}:M_{\max}$ ratio calculated. H-wave amplitudes elicited by repeated constant-current stimuli showed high reliability (CV = 2.4%).

Statistical Analysis

Statistical analysis was performed using SPSS v20 statistical software (SPSS, Chicago, IL). A two-way ANOVA with repeated measures (group \times time) was used to identify between-group differences in the changes in ROM during stretch. Separate three-way multivariate ANOVAs were used to examine changes in (i) fascicle length, fascicle strain and fascicle angle change; (ii) MTU, muscle and tendon lengths and elongation, and MTU, muscle and tendon stiffness during stretch; (iii) muscle activity during stretch, (iv) $H_{\max}:M_{\max}$; (v) and plantar flexor moment and RFD (group \times angle \times time). Bonferroni post-hoc tests and t-tests were implemented subsequent to the two- and three-way ANOVAs. Log transformation was performed on RFD data because of significant skewness (non-Gaussian data distribution). Statistical significance was accepted at an alpha level of 0.05, however p-values for near-significant results will also be presented where appropriate. Data are reported as mean \pm SD unless otherwise stated.

RESULTS

ROM And Passive Joint Moment

Stretch training (STR) resulted in a $19.9 \pm 10.8\%$ increase in maximum dorsiflexion ROM (i.e. peak dorsiflexion angle; $p < 0.001$) over the training period (Figure 3), while no change was observed in CON (mean change = $7.8 \pm 10.2\%$). The peak passive joint moment at

stretch termination (end ROM) increased in STR (85.5 ± 24.6 Nm to 109.5 ± 35.1 Nm, +28.0%) ($p < 0.05$) but did not change in CON (85.3 ± 29.0 Nm vs. 88.4 ± 24.7 Nm; 3.7%). There was no change in the passive mechanical resistance to stretch at any joint angle (1 – 5), even though the trained subjects were able to tolerate a greater passive joint moment after training. The apparent decrease in mean passive joint moment from angles 2 (10° plantar flexion) to 5 (-29.1°) in STR, as shown in Figure 4, was not statistically significant ($\Delta T_{0wk} = 42.3 \pm 11.9$ Nm, $\Delta T_{3wk} = 38.1 \pm 11.7$ Nm; 9.9%, $p = 0.15$).

EMG Activity in Maximum ROM Test

No pre- to post-training changes were observed in STR or CON in maximum EMG amplitude (Sol: 0.020 ± 0.011 vs. 0.023 ± 0.024 mV, GM: 0.017 ± 0.022 vs. 0.015 ± 0.024 mV, TA: 0.002 ± 0.002 vs. 0.003 ± 0.003 mV), EMG amplitude at end ROM (Sol: 0.014 ± 0.008 mV vs. 0.020 ± 0.021 mV, GM: 0.013 ± 0.017 mV vs. 0.013 ± 0.021 mV, TA: 0.002 ± 0.001 mV vs. 0.002 ± 0.003 mV) or joint angle at EMG onset (Sol: $27.0 \pm 9.4^\circ$ vs. $29.1 \pm 13.6^\circ$, GM: $22.1 \pm 13.0^\circ$ vs. $34.9 \pm 8.0^\circ$, TA: $39.0 \pm 15.7^\circ$ vs. $46.9 \pm 7.5^\circ$). When expressed as a percentage of EMG amplitudes measured during MVC (at angle 3), Sol maximum EMG amplitude and EMG at end ROM were 6.2% and 7.9% at wk 0 and 5.5% and 5.2% at wk 3, and GM maximum EMG amplitude and EMG at end ROM were 13.0% and 14.7% at wk 0 and 17.7% and 10.3% at wk 3.

Muscle and Tendon Elongation (Strain), Fascicle Length and Angle Changes During Stretch

Muscle strain measured from angles 2 – 5 (muscle strain from angles 1 – 2 was negligible and highly variable, and was therefore not included in the analysis) increased in STR from 0 to 3 wk ($9.8 \pm 1.6\%$ to $11.0 \pm 2.1\%$, $p < 0.05$) with no changes in CON ($9.7 \pm 1.6\%$ vs. $9.7 \pm$

1.8%). Muscle strain measured to end ROM also increased in STR ($10.8 \pm 1.9\%$ to $12.2 \pm 2.5\%$; $p < 0.05$), i.e. by 13%, but not in CON ($10.4 \pm 1.5\%$ vs. $10.7 \pm 1.6\%$). In contrast, tendon strain measured to joint angle 5 decreased in STR ($4.7 \pm 1.5\%$ to 3.8 ± 1.8 ; $p < 0.05\%$) but not in CON ($4.1 \pm 1.2\%$ vs. $4.1 \pm 1.5\%$), whilst tendon strain at end ROM did not change ($STR_{0wk} = 7.2 \pm 2.3\%$ vs. $STR_{3wk} = 7.7 \pm 2.9\%$; $CON_{0wk} = 6.7 \pm 1.3\%$ vs. $CON_{3wk} = 7.3 \pm 1.7\%$).

As shown in Figure 5, fascicle strain measured from joint angles 1 to 5 was greater after training in STR than before training ($STR_{0wk} = 39.3 \pm 14.0\%$, $STR_{3wk} = 48.2 \pm 16.2\%$) and different to CON ($CON_{0wk} = 36.2 \pm 6.1\%$, $CON_{3wk} = 37.3 \pm 9.0\%$; $p < 0.05$). Thus, fascicles elongated more during dorsiflexion through a set relative ROM after the stretch training. However, there were no differences in fascicle length between the groups at any joint angle, indicating that there was no overall change in resting fascicle length with stretch training. For example, fascicle lengths at angles 1 and 5 in STR ($STR_{0wk} = 47.8 \pm 10.6$ mm and 66.6 ± 9.2 mm, respectively; $STR_{3wk} = 45.6 \pm 11.2$ mm and 67.6 ± 8.8 mm) were similar to CON ($CON_{0wk} = 49.2 \pm 5.8$ mm and 67.0 ± 5.9 mm; $CON_{3wk} = 49.0 \pm 5.8$ mm and 67.3 ± 5.3 mm).

MTU, Muscle and Tendon Stiffness

MTU stiffness calculated between angles 2-3, 3-4 and 4-5 (no joint moment change occurred between angles 1 and 2) increased with joint angle change into dorsiflexion. Congruent with the finding that the pre- to post-training reduction in the change in joint moment between angles 2 – 5 in STR did not reach significance (see above), the reduction in MTU stiffness measured over the same joint range also did not reach significance in STR although a tendency for decreased MTU stiffness was observed (0 wk = 20.6 ± 5.1 N·mm⁻¹, 3 wk = 18.3

$\pm 5.1 \text{ N}\cdot\text{mm}^{-1}$; $p = 0.075$). Also, no changes were observed in CON and there was no change in stiffness measured from angle 5 to end ROM in either group.

Passive muscle stiffness measured from angles 2 - 5 decreased in STR ($30.1 \pm 11.8 \text{ N}\cdot\text{mm}^{-1}$ to $24.5 \pm 10.8 \text{ N}\cdot\text{mm}^{-1}$; mean change = $-18.0 \pm 18.3\%$; $p < 0.01$) whereas no change was observed in CON ($24.5 \pm 9.3 \text{ N}\cdot\text{mm}^{-1}$ vs. $23.8 \pm 10.8 \text{ N}\cdot\text{mm}^{-1}$; mean change = $-1.8 \pm 38.6\%$) (Figure 6). In contrast, tendon stiffness remained unchanged in STR ($56.8 \pm 27.9 \text{ N}\cdot\text{mm}^{-1}$ vs. $68.3 \pm 35.7 \text{ N}\cdot\text{mm}^{-1}$; mean change = $+7.9 \pm 40.2\%$) and in CON ($48.6 \pm 18.3 \text{ N}\cdot\text{mm}^{-1}$ vs. $48.5 \pm 17.6 \text{ N}\cdot\text{mm}^{-1}$; mean change = $+4.4 \pm 33.7\%$).

Peak Active Joint Moment (MVC) and RFD

No changes were found in maximum active plantarflexor moment (MVC strength) after the training period in STR or CON at any joint angle (Figure 7). Furthermore, no changes were found in RFD measured from the onset of force to 50, 100, 200 or 400 ms, or in the interval 200 - 400 ms.

H- and M-wave Amplitudes

Sol and GM $H_{\max}:M_{\max}$ ratios were depressed when obtained in stretched conditions (more dorsiflexed joint angle positions) compared to more neutral or plantar flexed positions ($p < 0.01$). No pre- to post-training changes in the maximal M-wave amplitude were observed in STR or CON in either Sol or GM. However, H-wave amplitudes, and thus $H_{\max}:M_{\max}$ ratios (see Table 1), decreased in both muscles after training in STR when obtained in a plantar flexed (angle 2: 10°) and neutral position (Sol at angle 3: -2.9°), but no pre- to post-training changes were observed in stretched conditions (angle 4: -15.7°). No changes were observed in CON.

DISCUSSION

The purpose of the present study was to describe the biomechanical, muscle architectural and neuromuscular adaptations resulting from a 3-wk period of static plantar flexor muscle stretch training. The plantarflexor muscle group was chosen for study as it is amenable to both the neurophysiological and ultrasound-based testing methods required and because of its importance in the performance of vital activities of daily living (i.e. walking, jogging, stair climbing and postural balance tasks) and in many types of athletic performance. The training elicited an increase in maximal dorsiflexor ROM (19.9%), although the mean decrease in passive resistance to stretch (passive joint moment at pre-designated joint angles) was not statistically altered. Concomitant with this, there was no evidence of a change in reflex-mediated or volitional muscle activity (EMG amplitude or onset) during muscle stretch, and there was no detectible change in spinal motoneuron excitability (as estimated by the $H_{\max}:M_{\max}$ ratio) when measured in the soleus and gastrocnemius muscles on stretch despite significant decreases in $H_{\max}:M_{\max}$ were observed when the muscles were not on stretch (angle 4 vs. 3 in Table 1). Thus, there was no apparent change in neuromuscular activity at elongated muscle lengths that could have contributed to the increased ROM after the training period. In contrast, an increase in both fascicle and whole muscle lengthening during stretch was observed after the training, simultaneous with a reduction in tendon elongation, and an increase in muscle length (13%) at stretch termination (end ROM). These changes appeared to result from a decrease in muscle stiffness without a concurrent change in tendon stiffness, which in integrated terms was translated into a trend ($p=0.07$) for decreased MTU stiffness. Collectively, these data strongly suggest that changes in the passive elastic properties of human skeletal muscle can be induced by stretch training, but also indicate that the mechanical properties of the whole MTU may appear to remain unaltered despite significant

changes being elicited in the mechanical properties of the muscle or tendon separately. These are the first data to demonstrate differential tissue-dependent responses to stretch training and indicate an important mechanism by which the integrated musculo-tendinous system can respond to prolonged stretch training in humans.

Changes in Muscle Activity and Reflex Gain

A ubiquitous finding of previous research is that stretch training elicits an increase in the passive joint moment tolerated by subjects during a stretch, i.e. there is an increase in stretch tolerance (8, 17, 23, 35), and this was also observed in the present study (Figures 3 & 4). Theoretically, this change in tolerance should be underpinned by a change in either the afferent feedback from peripheral receptors or a change in the perception of, and thus volitional response to, passive muscle stretch. Nonetheless, no changes in the maximum EMG amplitude, EMG amplitude at stretch termination or joint angle at EMG onset were observed in response to the stretch training. These results are in agreement with those of Guissard & Duchateau (21) and Magnusson et al. (35) and further indicate that changes in muscle activity do not occur after stretch training and could not be associated with the subjective decision to terminate the stretch.

Previous research has indicated, however, that changes in resting spinal motoneuron excitability may occur concomitant to both acute (5) and chronic (12, 21) stretch interventions, providing evidence for at least some neurally-mediated physiological adaptation that could influence stretch tolerance (end ROM). Nonetheless, changes in spinal motoneuron excitability, assessed by monitoring changes in H-reflex amplitude, were measured with the muscle at a non-stretched length (i.e. with the ankle in anatomical position) in those studies, which might not provide insight into the level of spinal excitability

in more marked muscle stretch conditions (i.e. closer to end ROM). The results of the present study are in agreement with the results of Guissard & Duchateau (21) in that training-induced decreases in H-reflex amplitude ($H_{\max}:M_{\max}$) were found with the ankle in plantar flexion (10°) and near-neutral (-2.9°) positions, however our results show that H-reflex excitability remained unchanged when measured with the ankle dorsiflexed (-15.7°) and thus with the muscle on stretch. These new data suggest that stretch training does not elicit change in Ia afferent spindle reflex gain with the muscle at a long (stretched) length. These results are consistent with the findings of a lack of change in muscle activity during stretch as observed by us and others (21, 35) and a lack of change in disynaptic reciprocal inhibition or presynaptic inhibition with stretch training reported by Hayes et al. (25). It is also of interest that 3-wk plantar flexor stretch training has been shown to increase reciprocal inhibition (of TA onto spinal plantar flexor motoneurons) with the ankle held in dorsiflexion (12). However, the subjects in the present study were asked to remain relaxed (no TA EMG activity was detected during stretch in eight STR subjects at 0 wk and nine subjects at 3 wk) and there was no change in TA activity during stretch test maneuvers across the training period. Thus, changes in reciprocal inhibition are unlikely to have influenced end ROM in the present study. Collectively, these data suggest that any neuromuscular changes in response to stretch training are more likely to involve a change in the perception of and volitional response to the stretch (i.e. higher-order cortical processing), in parallel with the observed changes in the passive mechanical properties of the muscle tissue itself.

Changes in Muscle-tendon Properties

Some researchers have hypothesized that mechanical adaptations in the muscle-tendon unit are likely to be minimal (e.g. 52), possibly because passive joint moment measured during joint rotation, considered to reflect the stiffness of the muscle-tendon units acting across the

joint, is not always shown to change after stretch training despite significant increases in joint ROM being achieved (8, 17, 23, 35). The present data are in agreement with this hypothesis because statistically significant changes in passive joint moment were not observed (i.e. pre- to post-training difference in the change in joint moment = +9.9%, $p = 0.15$) when measured at the same submaximal angles (see Figure 5). Our findings contrast those of others (21, 32, 36, 42) who found significant reductions in passive joint moment during ankle rotation (plantar flexor stretch) after several weeks of training. Interestingly, the pre- to post-training change in passive joint moment approached statistical significance in the present study and the change was highly variable (e.g. for angle 5, $SD_{\Delta\text{moment}}/\text{mean}_{\Delta\text{moment}} = 182\%$). The possibility exists that the high degree of inter-individual variability might have contributed to the disparate findings in previous studies. However, factors such as total stretch volume, intensity of stretch, number of stretch repetitions might also influence the likelihood of achieving a decrease in passive MTU resistance to stretch. Because of the important functional implications of a decrease in resistance, the relative significance of the above factors should be examined explicitly in future research.

Two important and novel findings of the present study, however, were that after 3 wk stretch training (i) increases in both whole muscle (12.2%; see Figure 1) and fascicle (22.6%; see Figure 5) elongation, but not fascicle rotation, were observed during a maximal tolerable stretch along with a decrease in tendon strain (-19.1%), and (ii) the muscle elongation at stretch termination increased by 13.0% whereas no change was detected in tendon elongation. To our best knowledge, only one previous study has examined the possibility of changes in fascicle and muscle extensibility with stretch training, with Nakamura et al. (42) reporting an increase in GM fascicle elongation during a maximal stretch maneuver and yet no change in whole muscle elongation. The present results are important because they show that

differential changes in muscle vs. tendon mechanical properties can be achieved through stretch training, which may have implications for muscle function and movement performance. The present observations also conform with findings of Magid & Law (33), who found that passive tension within sarcomeres of mechanically skinned skeletal muscle fibres was exponentially related to whole muscle tension, suggesting that resting tension arose within myofibers rather than in PEC connective tissues. In the present study the training-induced changes in fascicle and muscle elongation occurred with a simultaneous decrease in tendon elongation when measured at a given joint angle, and a similar magnitude of tendon strain at stretch termination pre- and post-training. These findings can be reconciled with the observed decrease in muscle stiffness (-18.0%) yet lack of change in tendon stiffness observed after the training period (cf. Figure 6). Notably, the lack of change in tendon stiffness with prolonged stretch training is a consistent finding (32, 36). The present results are of substantial importance not only because they show that muscle offers a greater adaptive plasticity in response to stretch training than tendon, but also because they show that changes in muscle stiffness and elongation can occur even when no changes in passive joint moment during stretch are observed. Thus, the assumption that a lack of change in passive joint moment during stretch is indicative of a lack of change in muscle or tendon mechanical properties may be incorrect; some reconsideration of conclusions drawn from previous studies may thus be warranted as the passive joint moment test may not be sensitive to small changes in individual tissues.

Given that a decrease in muscle stiffness was observed after the training, it is interesting to speculate as to the possible mechanisms underpinning the change. Data from the present and other (32, 36) studies are suggestive that stretch training is not sufficient to elicit changes in tendon properties, at least in young healthy individuals. However, alterations may potentially

have occurred in intra-fibrillar, myofilament, cross-bridge or titin stiffness (37, 43, 46). Another possibility is that there were changes within the parallel elastic component (PEC). The endo-, peri- and epimysia are thought to substantially influence passive stretch resistance (19) and the perimysium in particular contributes a significant volume to the PEC and is considered a major contributor to the passive resistance to stretch (14, 47). While it is not possible to directly measure training-induced changes in intramuscular elastic components in humans, possible changes in passive intra-fibrillar SEC stiffness (e.g. myofilaments, cross-bridges) are predicted to shift the active joint moment-joint angle relationship towards dorsiflexion (i.e. longer muscle lengths; ref. 29) and/or reduce the rate at which force is developed (RFD) during the early rise of force in a maximal isometric contraction (16), the latter when operating on the ascending limb of the moment-angle curve. In the present study, maximal active joint moment and RFD were both measured at five joint angles before and after training and no changes were detected (see Figure 7). This finding is consistent with Guissard & Duchateau (21) who also reported no change in iso-angular peak force or RFD after plantarflexor stretch training. Although more rigorous experiments should be done in the future, the present results (i.e. a lack of change in the moment-angle relation, RFD or tendon stiffness) provide some evidence that changes in intramuscular SEC stiffness were not elicited.

Nonetheless, given that fascicle elongation increased, intra- and inter-fascicular structures such as the titin filaments (33, 37, 46, 51), fibre-based connective tissues (46), intracellular amorphous material (43), inter-muscular myofascial connectivity (28) or the number of serially-arranged sarcomeres within fibres (48) might be considered candidates for change; muscle fibre type, which may also influence muscle extensibility (43), is unlikely to have been altered by the present regime of training. Of these, a change in serial sarcomere number

is often speculated to underpin changes in muscle or fascicle extensibility (e.g. 19, 52) and, in fact, increases in sarcomere number have been observed after prolonged, intense muscle strain was imposed by tibial lengthening in humans (13). Nonetheless, the present data are not consistent with this hypothesis because there was no pre- to post-training change in absolute fascicle length measured at any of the five joint angles (even though muscle strain increased after training). These results are consistent with those recently obtained in rat lateral gastrocnemius muscle after 8 weeks of muscle stretch training resembling that used by humans (10×60 -s stretch, $3.\text{wk}^{-1}$), where no changes in serial sarcomere number were observed (44). In the absence of firm evidence in the present and other studies, it may be concluded that increases in serial sarcomere number are probably not a major factor influencing muscle extensibility after short-term stretch training in healthy individuals.

Factors Influencing ROM: differential findings between longitudinal and cross-sectional studies

An important aim of stretch-related research is to understand the factors that most influence ROM, with a view to developing exercise, pharmaceutical and other strategies to influence those factors. Two ways to achieve this are to: (i) quantify differences in neuromuscular function during muscle stretch in ‘flexible’ vs. ‘inflexible’ individuals (i.e. cross-sectional analysis), and (ii) to describe the neuromuscular changes that occur with interventions (such as stretch training) that improve ROM (i.e. longitudinal analysis). Based on the results of the present study, and others (8, 17, 23, 35), it appears as though stretch training elicits a clear increase in the tolerance to stretch. It is possible that this modification results from a change in the interpretation and response of the central nervous system to peripheral (afferent) feedback, although this possibility has not been explicitly tested. It is also possible that it at least partly results from adaptive modifications (reductions) in muscle (and fascicle) stiffness,

allowing a greater elongation of the muscle during stretch and at stretch termination. Nonetheless, the change appears not to be associated with changes in spinal excitability (ref. 25, and current study) or the quantity of muscle activity (or lack hereof) present during stretch (ref. 35, and current study). Nor is it associated with changes in tendon stiffness (current study and refs 32, 36), and thus elongation, or changes in fascicle rotation during stretch (current study). The results of cross-sectional studies also consistently point to stretch tolerance being an important factor (11, 34, 39), however there is also evidence that a later onset of reflex (or perhaps volitional) muscle activity, increased fascicular rotation during muscle lengthening and a greater elongation of the tendon during stretch might be important (11). This comparison is interesting because it highlights the fact that both approaches lead to different conclusions as to the most important factors, with the only consistent finding being that stretch tolerance is a key factor influencing ROM. The logical conclusion, therefore, is that while some musculo-tendinous factors might influence ROM, stretch tolerance is the major influencing factor. Thus, a primary aim of future research is to more completely study supraspinal responses to stretch in flexible individuals and after periods of stretch training in order to pinpoint those regions in the central nervous system and related peripheral nervous system (Ia and Ib afferent inputs, group III and IV afferents) potentially associated with the control of stretch tolerance.

REFERENCES

1. **Aagaard P, Simonsen EB, Andersen JL, Magnusson P, and Dyhre-Poulsen P.** Increased rate of force development and neural drive of human skeletal muscle following resistance training. *J Appl Physiol* 93: 1318-1326, 2002.
2. **Abellaneda S, Guissard N, and Duchateau J.** The relative lengthening of the myotendinous structures in the medial gastrocnemius during passive stretching differs among individuals. *J Appl Physiol* 106: 169-177, 2009.
3. **Alter MJ.** *Science of Flexibility*. Illinois: Human Kinetics, 2004.
4. **Arnason A, Sigurdsson SB, Gudmundsson A, Holme I, Engebretsen L, and Bahr R.** Risk factors for injuries in football. *Am J Sports Med* 32: 5S-16S, 2004.
5. **Avela J, Kyrolainen H, and Komi PV.** Altered reflex sensitivity after repeated and prolonged passive muscle stretching. *J Appl Physiol* 86: 1283-1291, 1999.
6. **Bandy WD, and Irion JM.** The effect of time of static stretch on the flexibility of the hamstring muscles. *Phys Ther* 74: 845-850, 1994.
7. **Bandy WD, Irion JM, and Briggler M.** The effect of time and frequency of static stretching on flexibility of the hamstring muscles. *Phys Ther* 77: 1090-1096, 1997.
8. **Ben M, and Harvey LA.** Regular stretch does not increase muscle extensibility: a randomized controlled trial. *Scand J Med Sci Sports* 20: 136-144, 2010.
9. **Blazevich AJ, Cannavan D, Coleman DR, and Horne S.** Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. *J Appl Physiol* 103: 1565-1575, 2007.
10. **Blazevich AJ, Cannavan D, Horne S, Coleman DR, and Aagaard P.** Changes in muscle force-length properties affect the early rise of force in vivo. *Muscle Nerve* 39: 512-520, 2009.
11. **Blazevich AJ, Cannavan D, Waugh CM, Fath F, Miller SC, and Kay AD.** Neuromuscular factors influencing the maximum stretch limit of the human plantar flexors. *J Appl Physiol* 113: 1446-1455, 2012.
12. **Blazevich AJ, Kay AD, Waugh C, Fath F, Miller S, and Cannavan D.** Plantarflexor stretch training increases reciprocal inhibition measured during voluntary dorsiflexion. *J Neurophysiol* 107: 250-256, 2012.
13. **Boakes JL, Foran J, Ward SR, and Lieber RL.** Muscle adaptation by serial sarcomere addition 1 year after femoral lengthening. *Clin Orthop Relat Res* 456: 250-253, 2007.
14. **Borg TK, and Caulfield JB.** Morphology of connective tissue in skeletal muscle. *Tissue Cell* 12: 197-207, 1980.
15. **Cannavan D, Coleman DR, and Blazevich AJ.** Lack of effect of moderate-duration static stretching on plantar flexor force production and series compliance. *Clin Biomech* 27: 306-312, 2012.
16. **Edman KA, and Josephson RK.** Determinants of force rise time during isometric contraction of frog muscle fibres. *J Physiol* 580: 1007-1019, 2007.
17. **Folpp H, Deall S, Harvey LA, and Gwinn T.** Can apparent increases in muscle extensibility with regular stretch be explained by changes in tolerance to stretch? *Aust J Physiother* 52: 45-50, 2006.
18. **Frigon A, Carroll TJ, Jones KE, Zehr EP, and Collins DF.** Ankle position and voluntary contraction alter maximal M waves in soleus and tibialis anterior. *Muscle Nerve* 35: 756-766, 2007.
19. **Gajdosik RL.** Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clin Biomech* 16: 87-101, 2001.
20. **Grieve DW, Pheasant S, and Cavanagh PR.** Prediction of gastrocnemius length from knee and ankle joint posture. In: *Biomechanics VI-A*, edited by Assmussen E, and Jorgensen K. Baltimore: University Park Press, 1978, p. 405-412.
21. **Guissard N, and Duchateau J.** Effect of static stretch training on neural and mechanical properties of the human plantar-flexor muscles. *Muscle Nerve* 29: 248-255, 2004.

22. **Hadoush H, Tobimatsu Y, Nagatomi A, Kimura H, Ito Y, and Maejima H.** Monopolar surface electromyography: a better tool to assess motoneuron excitability upon passive muscle stretching. *J Physiol Sci* 59: 243-247, 2009.
23. **Halbertsma JP, and Goeken LN.** Stretching exercises: effect on passive extensibility and stiffness in short hamstrings of healthy subjects. *Arch Phys Med Rehabil* 75: 976-981, 1994.
24. **Harvey LA, Byak AJ, Ostrovskaya M, Glinsky J, Katte L, and Herbert R.** Randomised trial of the effects of four weeks of daily stretch on extensibility of hamstring muscles in people with spinal cord injuries. *Aust J Physiother* 49: 176-181, 2003.
25. **Hayes BT, Harter RA, Widrick JJ, Williams DP, Hoffman MA, and Hicks-Little CA.** Lack of neuromuscular origins of adaptation after a long-term stretching program. *J Sport Rehabil* 21: 99-106, 2012.
26. **Hemmerich A, Brown H, Smith S, Marthandam SS, and Wyss UP.** Hip, knee, and ankle kinematics of high range of motion activities of daily living. *J Orthop Res* 24: 770-781, 2006.
27. **Hermens HJ, Freriks B, Disselhorst-Klug C, and Rau G.** Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 10: 361-374, 2000.
28. **Huijing PA, van de Langenberg RW, Meesters JJ, and Baan GC.** Extramuscular myofascial force transmission also occurs between synergistic muscles and antagonistic muscles. *J Electromyogr Kinesiol* 17: 680-689, 2007.
29. **Kawakami Y, and Lieber RL.** Interaction between series compliance and sarcomere kinetics determines internal sarcomere shortening during fixed-end contraction. *J Biomech* 33: 1249-1255, 2000.
30. **Kay AD, and Blazevich AJ.** Isometric contractions reduce plantar flexor moment, Achilles tendon stiffness, and neuromuscular activity but remove the subsequent effects of stretch. *J Appl Physiol* 107: 1181-1189, 2009.
31. **Kay AD, and Blazevich AJ.** Moderate-duration static stretch reduces active and passive plantar flexor moment but not Achilles tendon stiffness or active muscle length. *J Appl Physiol* 106: 1249-1256, 2009.
32. **Kubo K, Kanehisa H, and Fukunaga T.** Effect of stretching training on the viscoelastic properties of human tendon structures in vivo. *J Appl Physiol* 92: 595-601, 2002.
33. **Magid A, and Law DJ.** Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* 230: 1280-1282, 1985.
34. **Magnusson SP, Simonsen EB, Aagaard P, Boesen J, Johannsen F, and Kjaer M.** Determinants of musculoskeletal flexibility: viscoelastic properties, cross-sectional area, EMG and stretch tolerance. *Scand J Med Sci Sports* 7: 195-202, 1997.
35. **Magnusson SP, Simonsen EB, Aagaard P, Sorensen H, and Kjaer M.** A mechanism for altered flexibility in human skeletal muscle. *J Physiol* 497 (Pt 1): 291-298, 1996.
36. **Mahieu NN, McNair P, De Muynck M, Stevens V, Blanckaert I, Smits N, and Witvrouw E.** Effect of static and ballistic stretching on the muscle-tendon tissue properties. *Med Sci Sports Exerc* 39: 494-501, 2007.
37. **Maruyama K, Kimura S, Yoshidomi H, Sawada H, and Kikuchi M.** Molecular size and shape of beta-connectin, an elastic protein of striated muscle. *J Biochem* 95: 1423-1433, 1984.
38. **Matthews PB.** The dependence of tension upon extension in the stretch reflex of the soleus muscle of the decerebrate cat. *J Physiol* 147: 521-546, 1959.
39. **McHugh MP, Kremenic IJ, Fox MB, and Gleim GW.** The role of mechanical and neural restraints to joint range of motion during passive stretch. *Med Sci Sports Exerc* 30: 928-932, 1998.
40. **Meunier S, Pol S, Houeto JL, and Vidailhet M.** Abnormal reciprocal inhibition between antagonist muscles in Parkinson's disease. *Brain* 123 (Pt 5): 1017-1026, 2000.
41. **Mulholland SJ, and Wyss UP.** Activities of daily living in non-Western cultures: range of motion requirements for hip and knee joint implants. *Int J Rehabil Res* 24: 191-198, 2001.

42. **Nakamura M, Ikezoe T, Takeno Y, and Ichihashi N.** 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, 2012.
43. **Olsson MC, Kruger M, Meyer LH, Ahnlund L, Gransberg L, Linke WA, and Larsson L.** Fibre type-specific increase in passive muscle tension in spinal cord-injured subjects with spasticity. *J Physiol* 577: 339-352, 2006.
44. **Peixinho CC, Martins NS, de Oliveira LF, and Machado JC.** Structural adaptations of rat lateral gastrocnemius muscle-tendon complex to a chronic stretching program and their quantification based on ultrasound biomicroscopy and optical microscopic images. *Clin Biomech* 29: 57-62, 2014.
45. **Pinniger GJ, Nordlund M, Steele JR, and Cresswell AG.** H-reflex modulation during passive lengthening and shortening of the human triceps surae. *J Physiol* 534: 913-923, 2001.
46. **Prado LG, Makarenko I, Andresen C, Kruger M, Opitz CA, and Linke WA.** Isoform diversity of giant proteins in relation to passive and active contractile properties of rabbit skeletal muscles. *J Gen Physiol* 126: 461-480, 2005.
47. **Purslow PP.** Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *J Biomech* 22: 21-31, 1989.
48. **Tabary JC, Tardieu C, Tardieu G, and Tabary C.** Experimental rapid sarcomere loss with concomitant hypoextensibility. *Muscle Nerve* 4: 198-203, 1981.
49. **Tian M, Hoang PD, Gandevia SC, Herbert RD, and Bilston LE.** Viscous elements have little impact on measured passive length-tension properties of human gastrocnemius muscle-tendon units in vivo. *J Biomech* 44: 1334-1339, 2011.
50. **Van Leeuwen JL, and Spoor CW.** Modelling mechanically stable muscle architectures. *Phil Trans Royal Soc Lond B Biol Sci* 336: 275-292, 1992.
51. **Wang K, McCarter R, Wright J, Beverly J, and Ramirez-Mitchell R.** Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophys J* 64: 1161-1177, 1993.
52. **Weppler CH, and Magnusson SP.** Increasing Muscle Extensibility: A Matter of Increasing Length or Modifying Sensation? *Phys Ther* 90: 438-449, 2010.
53. **Witvrouw E, Danneels L, Asselman P, D'Have T, and Cambier D.** Muscle flexibility as a risk factor for developing muscle injuries in male professional soccer players. A prospective study. *Am J Sports Med* 31: 41-46, 2003.
54. **Worrell TW, Smith TL, and Winegardner J.** Effect of Hamstring Stretching on Hamstring Muscle Performance. *J Orthop Sports Phys Ther* 20: 154-159, 1994.

Table 1. Mean (SD) $H_{\max}:M_{\max}$ values recorded from soleus (Sol) and gastrocnemius medialis (GM) in three pre-designated joint positions at 0 and 3 wk. Pre- to post-training decreases in $H_{\max}:M_{\max}$ were observed at joint angles 2 ($+10 \pm 0^\circ$) and 3 (Sol only; $-2.9 \pm 2.5^\circ$), but not at angle 4 ($-15.7 \pm 7.5^\circ$). * $p < 0.05$. $H_{\max}:M_{\max}$ decreased from angle 2 to angle 3 (†) and 4 (††).

| Angle | Sol | | | | GM | | | |
|------------|---------------|----------------|------------|---------|---------------|---------------|------------|---------|
| | 0 wk | 3 wk | Change (%) | P-value | 0 wk | 3 wk | Change (%) | P-value |
| CON | | | | | | | | |
| 2 | 0.54 (0.16) | 0.55 (0.17) | 3.6 | 0.37 | 0.47 (0.16) | 0.49 (0.16) | 3.9 | 0.54 |
| 3 | 0.49 (0.18)† | 0.49 (0.18) | -0.9 | 0.83 | 0.45 (0.20) | 0.47 (0.22) | 3.6 | 0.48 |
| 4 | 0.39 (0.19)†† | 0.42 (0.20)†† | 5.7 | 0.22 | 0.30 (0.14)†† | 0.32 (0.15) | 7.0 | 0.16 |
| STR | | | | | | | | |
| 2 | 0.60 (0.18) | 0.50 (0.17) | -16.0* | 0.00 | 0.53 (0.20) | 0.47 (0.18) | -11.4* | 0.03 |
| 3 | 0.58 (0.17) | 0.51 (0.20) | -12.6* | 0.01 | 0.49 (0.20) | 0.45 (0.20) | -7.6 | 0.19 |
| 4 | 0.47 (0.21)†† | 0.45 (0.20) †† | -3.1 | 0.63 | 0.40 (0.25)†† | 0.41 (0.21)†† | 2.6 | 0.76 |

Figure captions

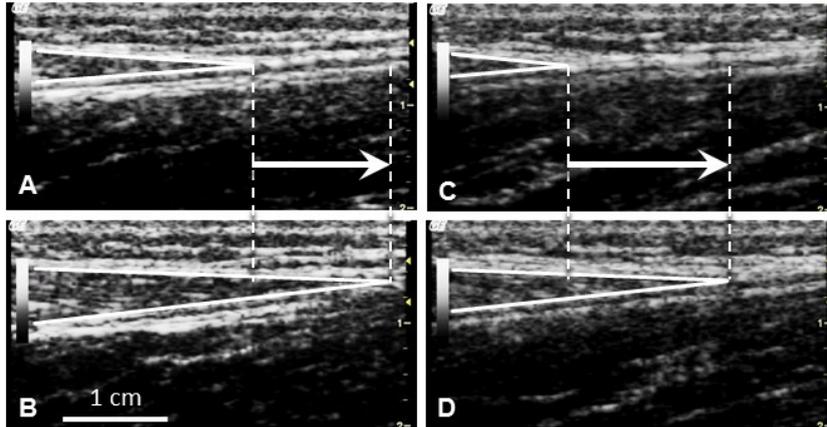


Figure 1. Example ultrasound images (cropped) from one subject showing muscle-tendon junction (MTJ) displacement (large arrows) during ankle rotation from angle 3 to 5 before (images A and B) and after (images C and D) training. A greater MTJ displacement, indicating a greater muscle lengthening, occurred after training in the experimental (STR) group.

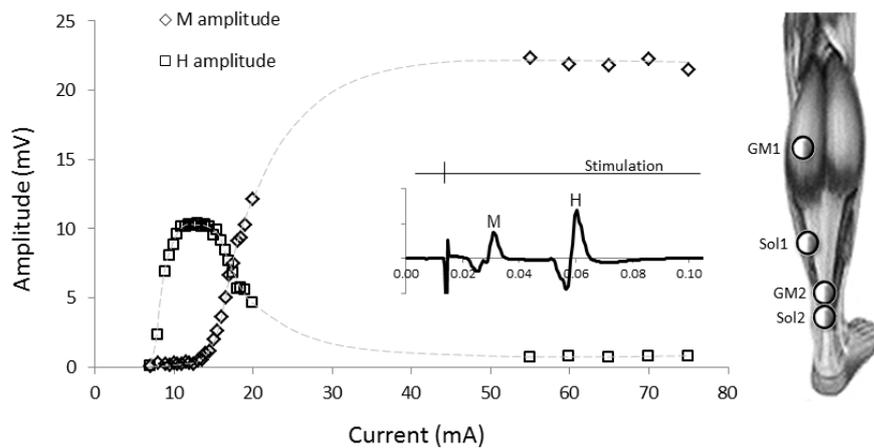


Figure 2. H- and M-wave measurement. H- and M-wave recruitment curves were obtained from soleus (electrodes Sol1 and Sol2 on right) and gastrocnemius medialis (electrodes GM1 and GM2) by gradually increasing electrical stimulus intensity to the tibial nerve to elicit a Ia afferent reflex volley (H-wave) as well as a direct motor response (M wave) (see inset).

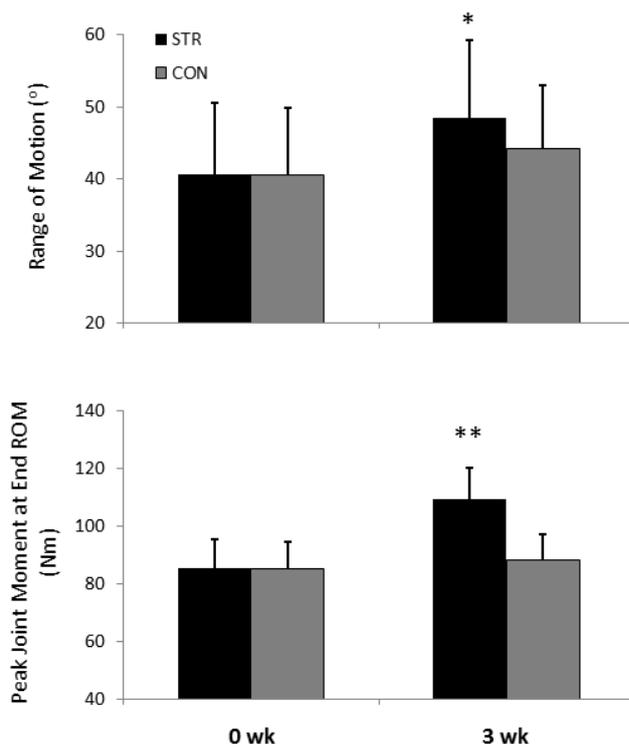


Figure 3. Range of motion (ROM) and peak passive joint moment measured at end ROM before (0 wk) and after (3 wk) stretch training (STR) or no training (control; CON). ROM and peak passive moment recorded at end ROM (i.e. stretch tolerance) increased in STR only
 * $p < 0.05$; ** $p < 0.01$.

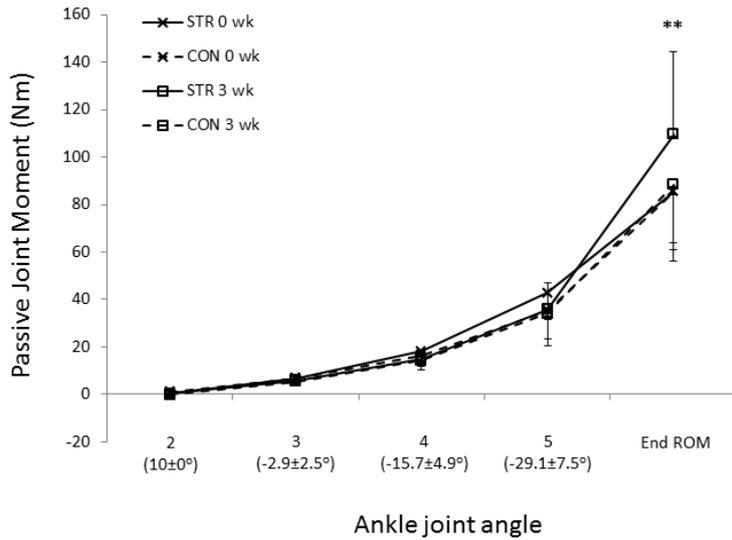


Figure 4. Passive plantarflexor joint moment resisting dorsal joint rotation obtained at pre-designated joint angles (2 – 5; normalized to subject’s maximum ROM, as described in the text). There was a continuous increase in passive moment as the ankle was rotated into dorsiflexion (negative joint angles 2 -5), and passive moment increased at end range of motion after the intervention in STR only $**p<0.01$.

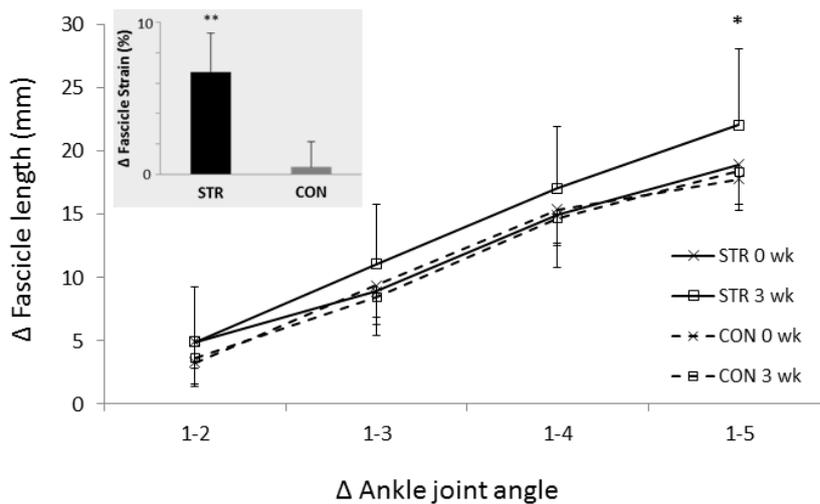


Figure 5. Change in GM fascicle length (mm) as the ankle was rotated from angle 1 ($22.9 \pm 2.5^\circ$) to angle 5 ($-29.1 \pm 7.5^\circ$). GM fascicles lengthened more in STR after the training ($*p<0.05$), and the pre- to post-training change (Δ) in fascicle strain reached statistical difference in STR only (inset, $** p<0.01$).

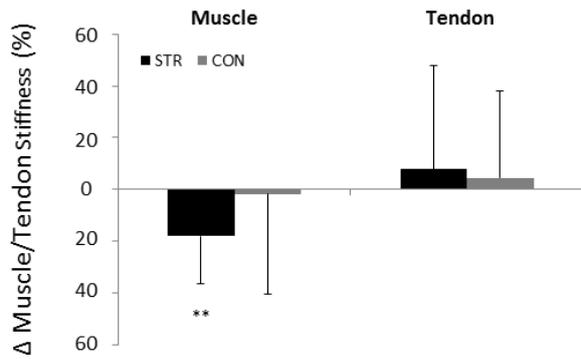


Figure 6. Change (Δ) in muscle-tendon (MTU) and tendon stiffness (measured from angles 2 – 5) after 3 wk stretch training (STR) compared to non-exercising controls (CON). Pre- to post-training difference: * $p < 0.05$.

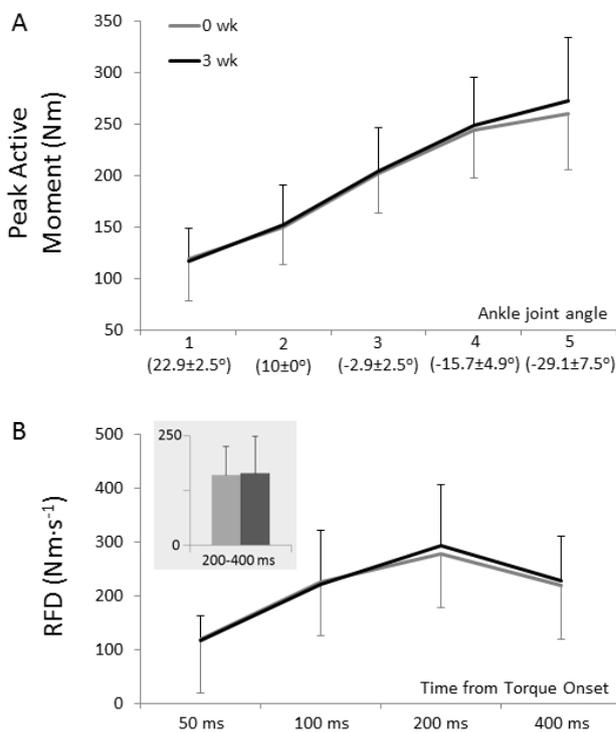


Figure 7. Maximum active joint moment (MVC) and rate of force development (RFD) obtained before (0 wk) and after 3 wk stretch training in STR. There were no changes in joint-specific MVC in STR (A) or CON (data not shown). There were also no changes in RFD (B) measured from the onset of force to 50, 100, 200 or 400 ms, respectively, or in incremental RFD measured from 200-400 ms (inset).

