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Effects of prolonged walking on neural and mechanical components of stretch responses in the human soleus muscle

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After repeated passive stretching, tendinous tissue compliance increases in the human soleus (SOL) muscle–tendon unit. During movement, such changes would have important consequences for neural and mechanical stretch responses. This study examined the existence of such effects in response to a 75 min walking intervention. Eleven healthy subjects walked on a treadmill at 4 km h^{-1} with a robotic stretch device attached to the left leg. Ultrasonography was used to measure SOL fascicle lengths, and surface EMG activity was recorded in the SOL and tibialis anterior (TA) muscles. Perturbations of 6 deg were imposed at three different measurement intervals: Pre (immediately before the walking intervention), Mid (after approximately 30 min of walking) and Post (immediately after the intervention). Between the Pre–Mid and Mid–Post intervals, subjects walked for 30 min at a gradient of 3%. After the intervention, the amplitude and velocity of fascicle stretch both decreased (by 46 and 59%, respectively; $P < 0.001$) in response to a constant external perturbation, as did short (33%; $P < 0.01$) and medium (25%; $P < 0.01$) latency stretch reflex amplitudes. A faster perturbation elicited at the end of the protocol resulted in a recovery of fascicle stretch velocities and short latency reflex amplitudes to the pre-exercise values. These findings suggest that repeated stretching and shortening of a muscle–tendon unit can induce short-term structural changes in the tendinous tissues during human walking. The data also highlight the effect of these changes on neural feedback from muscle sensory afferents.

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Abbreviations EMG, electromyography; GTO, Golgi tendon organ; MLR, medium latency stretch reflex; MTU, muscle–tendon unit; SLR, short latency stretch reflex; SOL, soleus muscle; TA, tibialis anterior muscle; TT, tendinous tissues.

During human walking, a complex sequence of events occurs in the lower limb, requiring constant interaction between muscular and tendinous tissues, which undergo repeated length changes. When a cyclic activity such as walking or running is repeated for a long duration and with sufficient intensity, reversible neural, structural and mechanical changes may occur, particularly in the lower limb. The severity and duration of these changes depend on the nature of the movement (Nicol *et al.* 2006). For example, previous studies have demonstrated an increase in muscle–tendon unit (MTU) compliance after repeated passive stretching (Avela *et al.* 2004). More specifically, the authors suggested that the increase in compliance occurred in the tendinous tissues (outer tendon and aponeuroses; TT), as has been reported to occur after repeated isometric contractions (Kubo *et al.* 2001; Maganaris *et al.* 2002).

In dynamic conditions, an increase in TT compliance would influence the pattern of force transfer between muscle fibres, tendons and bones, and could thus affect the afferent output from receptors located in these tissues, such as muscle spindles (and possibly Golgi tendon organs; GTO). Evidence of this effect on muscle spindles has been presented in passive conditions, whereby 1 h of repeated fast passive muscle stretching led to a reduction of short latency stretch reflex (SLR) amplitude of approximately 80% in the human soleus (SOL) muscle (Avela *et al.* 1999). This has been suggested to be due to a reduction in Ia afferent activity as a result of the increase in TT compliance

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(Avela *et al.* 2004). In human walking, stretch reflexes mediated by type Ia sensory afferents are believed to contribute significantly to corrective stumbling responses (e.g. Sinkjaer *et al.* 1996). Furthermore, in unconstrained walking, afferent feedback from GTO and muscle spindle group II afferents is believed to make an important contribution to the ongoing locomotor activity (Sinkjaer *et al.* 2000; Mazzaro *et al.* 2006; af Klint *et al.* 2008). Therefore, changes in TT compliance could affect the contribution of afferent feedback to unrestrained walking, as well as in response to rapid perturbations that elicit SLR responses.

During human walking, the triceps surae complex can be artificially stretched by applying a controlled rapid perturbation to the ankle joint (e.g. Sinkjaer *et al.* 1996). By combining this method with ultrasonography and electromyography (EMG), it is possible to examine changes in both mechanical and neural stretch responses throughout an exercise protocol. The primary aim of this study was to examine SOL muscle fascicle stretch responses and SLR amplitudes throughout a prolonged period of walking lasting approximately 75 min. Furthermore, as repeated passive stretching has been suggested to increase TT compliance (Avela *et al.* 2004), an additional aim was to indirectly examine the existence of such changes during locomotion. It was hypothesised that as the duration of walking increased, the amplitude and velocity of muscle fascicle stretch in response to controlled MTU perturbations would decrease, due to an increase in TT compliance. This would decrease afferent activity of the group Ia and II afferents, and consequently decrease the amplitudes of the short and medium latency stretch reflex responses. A reduction in muscle spindle afferent feedback was also expected to occur during unperturbed walking.

Methods

Subjects

Eleven healthy subjects (9 males, 2 females; age 27 ± 3 years; height 175 ± 8 cm; weight 69 ± 9 kg) with no history of neuromuscular disorders volunteered to participate in this study. Prior to testing, subjects were fully informed of the procedures and risks, and each subject provided written informed consent. The study was approved by the local ethics committee (approval number N2008004), and was performed in accordance with the *Declaration of Helsinki*.

Apparatus and instrumentation

Subjects walked on a treadmill (Woodway; Waukesha, WI, USA) with the left leg attached to a portable robotic actuator capable of eliciting a stretch reflex by rapidly dorsiflexing the ankle joint (Fig. 1). Full details of the device are presented elsewhere (Andersen & Sinkjaer, 1995, 2003). Briefly, the device weighs approximately 900 g, and consists of a functional joint aligned with the ankle of the subject and attached to the foot and leg with a polypropylene plaster cast. The actuator is connected to an AC servomotor that applies torque to the functional joint through flexible Bowden cables. Ankle angle was measured with an optical encoder incorporated within the functional joint, and ankle velocity was determined by numerical differentiation of the ankle angular record.

An ultrasonographic device (Alpha-10; Aloka, Japan) was used to measure fascicle lengths in the SOL muscle during walking at a scanning frequency of 150 frames per second. The probe, which weighed approximately 130 g, was positioned 1–2 cm medial to the Achilles tendon,

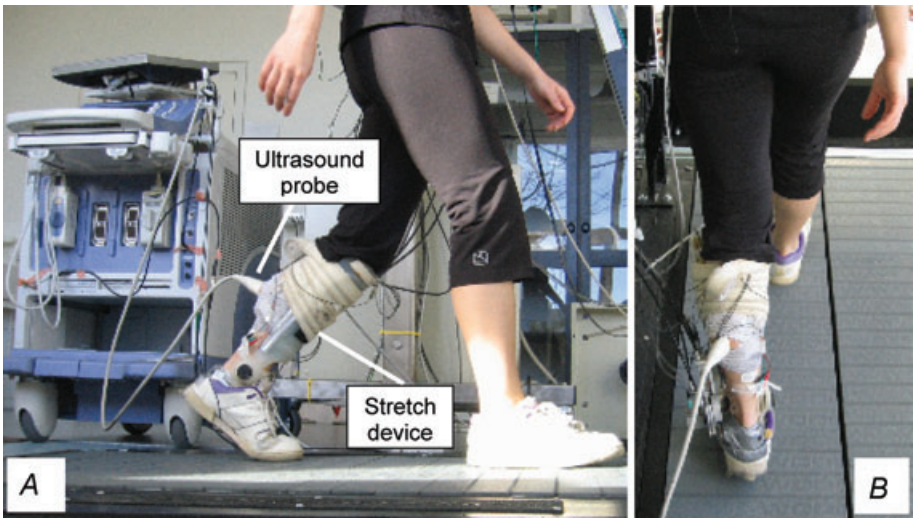

Figure 1. An example of a fully instrumented subject walking on the treadmill with the ankle stretch device and ultrasound probe secured to the left leg
A, side view; *B*, rear view. This figure was originally printed as Fig. 1 in Cronin *et al.* (2009).

immediately distal to the medial gastrocnemius muscle. The probe was secured over the skin surface with a custom-made support device to prevent movement of the probe relative to the muscle. The ultrasound settings were individually adjusted to optimise the contrast between muscle fascicles and connective tissues, which greatly aids the analysis process. The ultrasound device was positioned at the side of the treadmill during the walking experiments. The reliability of the ultrasound method of fascicle length calculation was determined by calculating the coefficient of variation between three different trials at each measurement interval and for each subject. The mean (± S.D.) coefficient of variation was 6 ± 4%, which is similar to values reported previously (e.g. Ishikawa *et al.* 2003; Cronin *et al.* 2008).

EMG activity was recorded in the SOL and tibialis anterior (TA) muscles of the left leg using bipolar surface electrodes (720; Ambu, Ballerup, Denmark) with an inter-electrode distance of 2 cm. The EMG signals were band-pass filtered (10 Hz–1 kHz). All signals were sampled at 2 kHz and stored for later analysis. A heel switch based on a force-sensitive resistor was placed in the insole of the left shoe to trigger the signal acquisition, and to synchronize the EMG, ankle trajectory and ultrasound data. To investigate the possibility of exercise-induced changes in muscle contractile properties, maximal M-waves were elicited in the SOL muscle before and immediately after the exercise protocol (see below).

Walking protocol

Subjects initially walked on the treadmill for 5 to 10 min at a gradient of 0% (level walking) and a speed of 4 km h^{-1} to become accustomed to the robotic actuator. At the end of this period, data were acquired from approximately 30 steps during normal unperturbed walking to generate a control profile of the ankle trajectory, and EMG activities of the SOL and TA. Average stance phase duration (mean ± S.D.) was then calculated from these steps as the time difference from heel contact to toe off in the left leg, as defined by the heel switch signals. Subjects rested for 10–15 min prior to the actual walking experiment in order to prevent any possible effects of the acclimatisation period on performance in the actual walking protocol. For the stretch reflex trials, recordings were made at three different measurement intervals; Pre (immediately before the walking intervention), Mid (after 30 min of walking) and Post (immediately after the intervention). To elicit stretch reflex responses, dorsiflexion perturbations were imposed at approximately the mid-stance phase (42%; see results section) at a rate of one perturbation every 5–10 strides. At each measurement interval, perturbed trials (approximately 200 deg s^{-1}) and control trials were recorded in a random order, until a minimum of 30 trials were obtained for each condition. This resulted in the collection of at least 60 trials per subject at each interval. At the Post interval, it was anticipated that SLR amplitudes would be lower than at the beginning of the exercise due to increased TT compliance. As muscle spindles contain velocity sensitive Ia afferents, an additional series of faster perturbations were performed at this interval at a velocity of approximately 270 deg s^{-1} (Post_F condition). Therefore, a minimum of 90 trials were collected in a random order at the Post interval. In all experimental conditions, the perturbation amplitude was 6 ± 1 deg. At each measurement interval, perturbed and control trials were recorded within a 5–7 min time period, and blood lactate samples were taken from the fingertip immediately afterwards. Only perturbed steps with a maximal deviation of ± 2% of the averaged control step duration were included in the analysis. This ensured that recordings were made for similar steps. Between the Pre–Mid and Mid–Post measurement intervals, subjects walked for 30 min at a gradient of 3% and a speed of 4 km h^{-1}. This activity was chosen in order to simulate a repetitive cyclic activity over a sustained period of time, whilst simultaneously minimising metabolic muscle activity and protocol time. The total walking time was approximately 75 min. As the total weight added to the left leg due to the stretch device and ultrasound probe was approximately 1 kg, a 1 kg weight was added to the right shank to minimize inter-limb asymmetry during the walking experiments. The addition of weight to the legs has been found to have a minimal effect on lower extremity kinematics during walking (Browning *et al.* 2007).

Data acquisition and analysis

EMG signals were rectified, low-pass filtered (40 Hz), and ensemble averaged (25–30 trials per experimental condition) to produce EMG profiles. To detect the onset latency of the SLR response, the averaged SOL EMG during control and perturbed steps were superimposed, and a window was defined from 20 to 60 ms after the stretch onset, which incorporates the physiological range for the onset of the SLR (e.g. Grey *et al.* 2004). Within this window, the onset of the SLR was determined by visual inspection. The amplitude of the SLR response was measured by calculating the peak SOL EMG within a 30 ms time period starting at the SLR onset, and then subtracting the mean non-perturbed EMG within the same 30 ms time window from the peak value. The amplitude of the medium latency component of the stretch reflex (MLR) was quantified in the same way, within a 30 ms window starting at the estimated MLR onset. However, the onset of the medium latency response cannot always be precisely defined, as the EMG does not always drop to the level of background EMG prior to the MLR onset (Grey *et al.* 2001). Consequently,

we did not attempt to measure the onset latency of the MLR precisely. Background EMG was quantified as the average rectified EMG between 20 and 100% of the stance phase. For the ultrasound analysis, an individual fascicle was identified in each image along its length between the superficial and deep aponeuroses, and was manually tracked using custom-made digitizing software. Fascicles were only chosen for analysis if they were visible throughout the entire stance phase. Fascicle velocity was calculated by numerically differentiating fascicle stretch amplitude with respect to time. For each subject, the ultrasound data of three steps were averaged for each condition and at each measurement interval, and all subjects' data were then pooled. To select the trials to be analysed in a given condition, the three trials where the ankle range of motion throughout the entire step was closest to that of the mean ankle range of motion were selected, to ensure that these trials were representative of the median response.

At each measurement interval, capillary blood samples were taken from the fingertip to determine blood lactate concentration. The samples were analysed using a commercially available lactate meter (Lactate Pro LT-1710; Arkray Inc., Kyoto, Japan). Before and after the exercise protocol, maximal M-waves were evoked using an electrical stimulator (NoxiTest, IES 230; Aalborg, Denmark). Subjects were seated with the left foot firmly attached to a pedal capable of measuring the torque response. A 2 cm-diameter spherical cathode was placed in the popliteal fossa, and a rectangular anode was positioned just superior to the patella of the test leg. Pulses of 1 ms duration were elicited, and stimulation location was determined based on the appearance of a maximal M-wave concurrent with an absence of activity in the antagonistic TA muscle. The stimulus intensity was then set to approximately 150% of the maximal M-wave to ensure maximal motor unit activation in each trial. Three trials were performed immediately before and after the walking protocol with 10–15 s between trials. From the resulting torque curves, peak torque, time to peak torque and total twitch duration were determined. The recorded signals were sampled at 10 kHz, filtered (10 Hz–5 kHz) and stored for later analysis. The post-exercise M-wave measurements were performed within 10–12 min of exercise cessation.

Statistics

Prior to analysis, the data were tested for normality. When a normal distribution was found, repeated measures analysis of variance (factors: Pre, Mid, Post_S and Post_F) was used to test for significance. For all ANOVAs, Geisser–Greenhouse adjustments were made if the covariance matrix sphericity assumption was violated. In situations where the normality assumptions could not be met, Wilcoxon signed rank tests were used. When significant differences were observed, Bonferroni's *post hoc* test was used to identify the location of the differences. Pearson's product–moment correlation was used to correlate the changes in SLR amplitude and fascicle velocity, and Student's t-test for paired data was used to examine changes in stimulation-evoked parameters. For all statistical tests, significant differences were determined based on a level of significance of $P < 0.05$. Results are presented as means ± S.D.

Results

The mean stance phase duration in these experiments was 672 ± 35 ms, and showed no significant changes throughout the walking protocol (P values between 0.681 and 0.803). As variations in the timing of a perturbation within the stance phase can modulate SLR responses (e.g. Sinkjaer *et al.* 1996), the onset of the perturbation was determined relative to the total stance phase duration at each measurement interval. This analysis revealed that the perturbation onset was consistent between intervals, occurring at 42 ± 2, 42 ± 2 and 42 ± 3% of the stance phase at the Pre, Mid and Post intervals, respectively. Data from one subject are presented in Fig. 2.

The mean perturbation velocities induced at the ankle joint for the normal perturbations (all measurement intervals: Pre, Mid and Post_S) and faster perturbations (only in the Post_F condition) were 194 ± 21 and 270 ± 46 deg s^{-1}, respectively. To ensure that the perturbation at the ankle joint was constant at the Pre, Mid and Post_S intervals, the slopes of the ankle angular displacement records were analysed, and no significant differences were observed between measurement intervals (P values between 0.107 and 0.986), confirming that a constant perturbation was induced at the ankle joint at all measurement intervals.

In response to a constant external perturbation, the amplitude and velocity of muscle fascicle stretch clearly decreased as the duration of walking increased (Figs 2 and 3). Bonferroni *post hoc* tests revealed that between the Pre and Post_S intervals, fascicle stretch amplitude decreased by $46 \pm 13\%$ ($F_{1,10} = 14.001$, $P < 0.001$), and fascicle stretch velocity decreased by $59 \pm 13\%$ ($F_{1,10} = 16.358$, $P < 0.001$). Concurrently, SLR amplitude decreased by $33 \pm 19\%$ ($F_{1,10} = 15.423$, $P < 0.01$) and MLR amplitude decreased by $25 \pm 20\%$ ($F_{1,10} = 4.604$, $P < 0.01$). In response to a faster perturbation at the Post interval (Post_F), fascicle stretch amplitude remained $42 \pm 20\%$ ($P < 0.01$) lower than the Pre value, whereas fascicle stretch velocity recovered, and did not differ from the Pre value ($1 \pm 8\%$ higher; $P = 0.563$). SLR amplitude also recovered at the Post_F interval, and did not differ from the Pre value ($6 \pm 9\%$ higher; $P = 0.721$), whereas MLR amplitude was unaffected by the faster perturbation ($P = 0.918$), and remained $20 \pm 33\%$ lower than the Pre

value ($P < 0.05$). Between the Pre and Post intervals, SLR latency was unchanged (40 ± 3 to 43 ± 5 ms; $P = 0.059$), although onset latency was visually determined and may thus be prone to minor interpretation errors. Pre-stretch fascicle length shortened from 3.64 ± 0.84 cm at Pre to 3.35 ± 0.70 cm ($F_{1,10} = 18.220$, $P = 0.01$) at Post, and fascicle length at the point of ground contact shortened from 3.55 ± 0.94 cm to 3.23 ± 0.84 cm ($F_{1,10} = 15.656$, $P < 0.001$).

The group Ia mediated SLR is predominantly velocity sensitive and the group II afferents contributing to the MLR (Grey *et al.* 2001) are predominantly length sensitive. To verify that the stretch reflex responses reported in this study were consistent with these basic characteristics, changes in stretch reflex amplitudes were correlated with changes in muscle fascicle stretch amplitude and velocity, respectively. This analysis showed that SLR amplitude was well correlated with fascicle stretch velocity (Pearson's correlation: $r = 0.788$; $P < 0.001$) but not with fascicle stretch amplitude ($r = -0.002$; $P = 0.992$), and MLR amplitude was correlated with fascicle stretch amplitude ($r = 0.687$; $P < 0.001$) but not with fascicle stretch velocity ($r = 0.054$; $P = 0.763$). Mean group correlations for all subjects are shown in Fig. 4.

During unperturbed walking, Bonferroni *post hoc* tests showed that the mean background SOL EMG decreased by $22 \pm 12\%$ ($F_{1,10} = 4.235$, $P < 0.001$) between the Pre and Post intervals. Peak fascicle lengthening throughout the stance phase also decreased from 0.41 ± 0.15 cm at Pre to 0.26 ± 0.11 cm ($F_{1,10} = 7.236$, $P < 0.05$) at Post, showing that the muscle fascicles were stretched less during the stance phase at the end of the exercise protocol. Background TA EMG did not change significantly between the Pre and Post intervals (decreased by 5%; $P = 0.933$).

Between the Pre and Post measurement intervals, no significant changes were observed in maximal M-wave amplitude (t-test: decreased by $6.0 \pm 24\%$; $P = 0.694$), stimulation-evoked peak twitch force (increased by $1.9 \pm 11\%$; $P = 0.377$), time to peak torque (decreased by $6 \pm 10\%$; $P = 0.092$) or total twitch duration (increased by $3 \pm 16\%$; $P = 0.638$). Blood lactate exhibited a non-significant decrease from 1.5 ± 0.9 to 1.1 ± 0.6 mmol l^{-1} between Pre and Post.

Discussion

The main findings of this study were that walking for 75 min decreased the amplitude and velocity of fascicle stretch (by 46 and 59%, respectively), as well as SLR and MLR amplitudes (by 33% and 25%, respectively) in response to a constant external perturbation. A faster perturbation elicited at the end of the protocol led to a recovery of fascicle stretch velocities and SLR amplitudes to approximately the pre-exercise values. In unperturbed walking, a general decrease in ongoing locomotor EMG was observed. These findings, in addition to a lack of change in electrically evoked muscle twitch

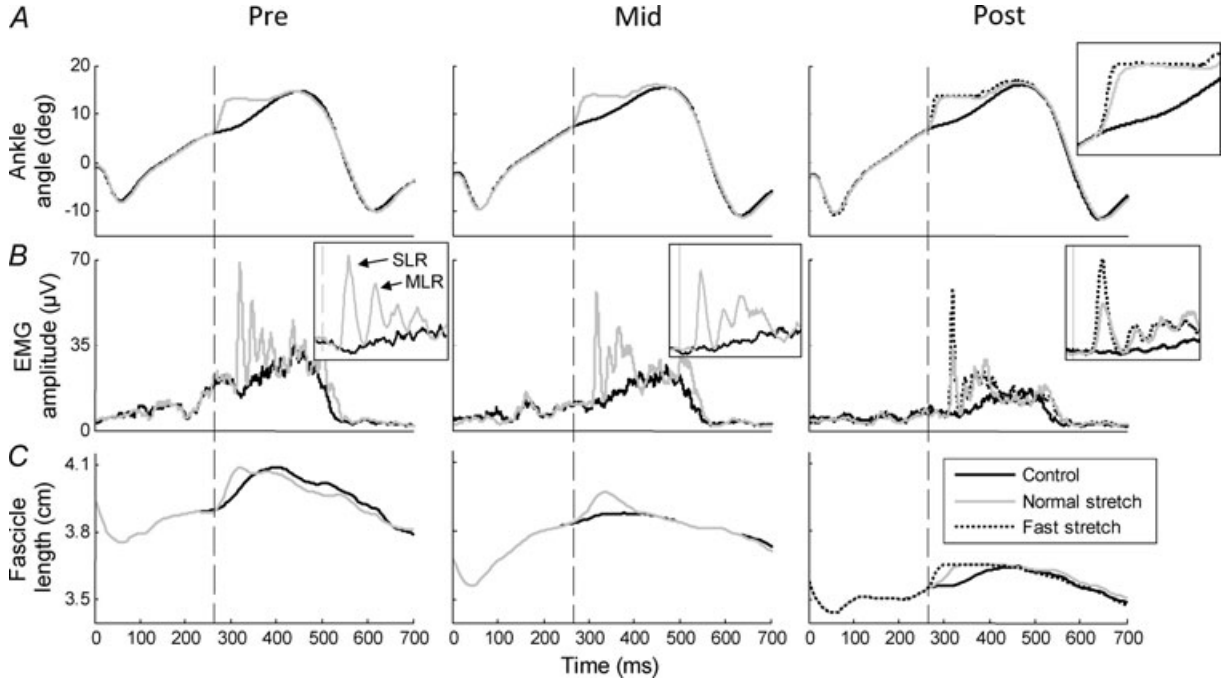

Figure 2. Neural and mechanical stretch responses from one representative subject
A, mean ankle trajectory ($n = 28$); *B*, SOL EMG activity ($n = 28$); *C*, fascicle length ($n = 3$). Dashed vertical lines represent the stretch onset.

properties, support the hypothesis that repeated stretching and shortening of a muscle–tendon unit induces a short-term increase in TT compliance (Avela *et al.* 2004). Although this hypothesis was based on responses to passive stretching, the present study suggests that the same phenomenon occurs during low intensity human walking. The finding of a decrease in locomotor EMG also suggests that the increased compliance may have decreased the ongoing sensory feedback from muscle spindles during unrestrained walking (Sinkjaer *et al.* 2000; af Klint *et al.* 2008, 2009). As well as peripheral changes, neural changes in the spinal and supraspinal integration of sensory input may also have occurred. However, this aspect was not investigated in this study.

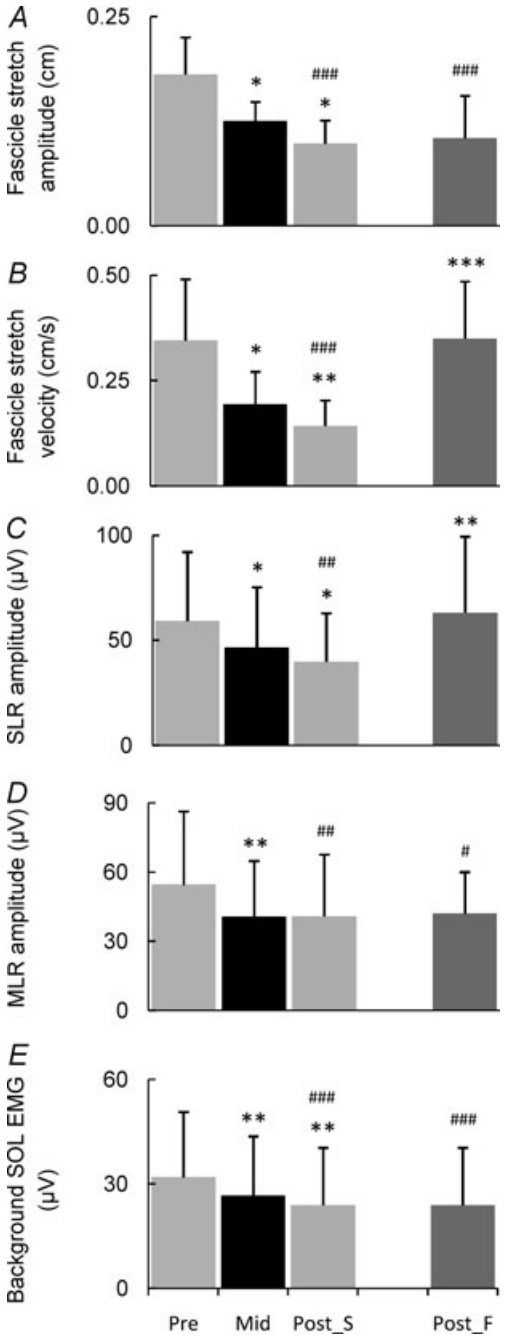

Figure 3. Group mean changes in fascicle and EMG related parameters throughout the intervention
A, fascicle stretch amplitude; *B*, fascicle stretch velocity; *C*, SLR amplitude; *D*, MLR amplitude; *E*, background SOL EMG. In all cases, $n = 11$. *, ** and *** denote a significant difference from the previous measurement interval at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively. $^{\#,\#\#}$ and $^{\#\#\#}$ denote a significant difference from the Pre measurement interval at the same significance levels.

Before discussing the findings of this study, some methodological issues should be addressed. As knee angles were not measured during this study, it was not possible to accurately determine the potential effects of changes in gait mechanics on changes in SLR responses. However, ankle trajectories and stance phase durations were unaffected by the exercise protocol (e.g. Fig. 2). Consequently, any changes in lower limb gait mechanics are likely to have been minimal. To ensure that the rotation of the functional joint was transmitted to the ankle joint of the subject, a very tight fitting was made between the calf and the foot of the subject and the functional joint. However, due to the high torque generation of the ankle extensors during walking (Winter, 1987), it is likely that part of the stretch was absorbed by the tissues and fixtures surrounding the ankle joint, as well as the compliance of the functional joint itself. Therefore, the stretch of the ankle extensors may have been less than that of the functional joint. Nonetheless, the stretch device has been designed with the aim of minimizing this error, and examination of the ankle displacement records confirmed that the perturbation delivered to the ankle joint was the same at all measurement intervals.

Previous studies have demonstrated large decreases in SLR amplitudes in response to repeated passive stretching in seated conditions (Avela *et al.* 1999, 2004). Avela *et al.* (1999) proposed that this could be attributed to a reduction in excitatory drive from the Ia afferents onto the α-motoneurones, which was in turn caused by an increase in TT compliance (see also Wilson *et al.* 1997; Avela *et al.* 2004). The latter suggestion was partly based on a shortening of the passive fascicle length after the repeated stretching protocol. In the present study, fascicle lengths at the point of ground contact and immediately prior to stretch both shortened between the Pre and Post_S intervals. In addition, muscle fascicle stretch amplitude and velocity both decreased dramatically, as did SLR and MLR amplitudes. Changes in fascicle stretch parameters were also well correlated with changes in SLR and MLR amplitudes. These findings, in addition to a lack of change in blood lactate concentration, and a recovery of SLR amplitudes when a series of faster (constant amplitude) perturbations were performed, suggest that the exercise protocol caused an increase in TT compliance during walking. Furthermore, as electrically evoked contractile

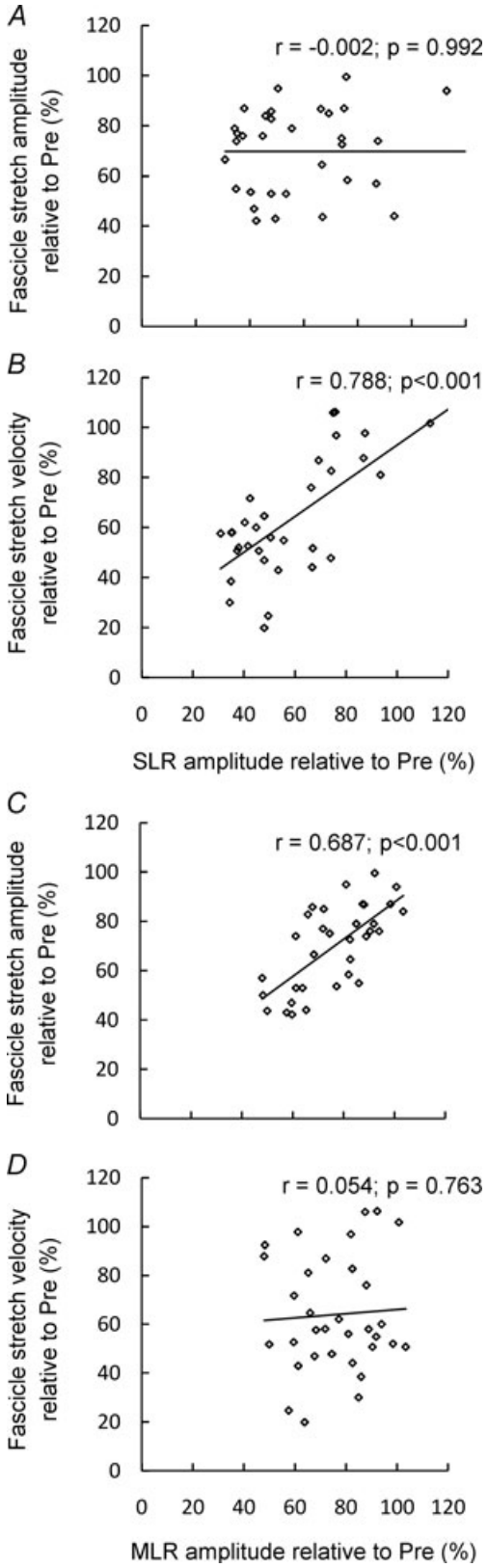

Figure 4. Correlations between changes in SLR and MLR amplitudes and changes in fascicle stretch parameters in response to external perturbations

All values from the Mid, Post_S and Post_F intervals are expressed relative to the Pre condition, and data from all subjects are shown (11 data points and 3 measurement intervals: $n = 33$). *A*, SLR amplitude and fascicle stretch amplitude; *B*, SLR amplitude and fascicle stretch velocity; *C*, MLR amplitude and fascicle stretch amplitude; *D*, MLR amplitude and fascicle stretch velocity.

responses were unaffected 10–12 min after cessation of exercise, this effect is presumably short lasting, as has been reported in passive conditions, whereby stretch resisting force recovered to pre-exercise values within 15 min of the end of the exercise (Avela *et al.* 2001). As previous studies have shown that Achilles outer tendon stiffness is generally unaffected by long-term cyclic loading (de Zee *et al.* 2000) and static stretching (Kay & Blazevich, 2009), the increased soleus TT compliance observed in this study may have primarily occurred in the aponeuroses. Muscle spindles are predominantly velocity- and length-sensitive receptors (Cooper, 1961). Any reduction in fascicle stretch velocity due to changes in TT compliance would ultimately reduce muscle spindle group Ia afferent feedback (Rack & Westbury, 1984; Cronin *et al.* 2008), and thus decrease the SLR amplitude in response to a perturbation. Similarly, a reduction in fascicle stretch amplitude would be expected to decrease the firing rates of group II afferents, and thereby decrease the MLR amplitude in response to a perturbation (Grey *et al.* 2001).

During unperturbed walking in the present study, background SOL EMG decreased as the exercise progressed. This may be due to a decreased afferent input to the motoneurone pool during the stance phase. For example, an increase in TT compliance may reduce the facilitatory input from GTO to the locomotor neural drive (Faist *et al.* 2006), although the lack of changes in ankle kinematics (and presumably Achilles tendon force) observed in this study suggest that this is unlikely. Alternatively, the reduced lengthening of the soleus muscle fascicles in the stance phase could reduce afferent input from muscle spindles to the motoneurones. As feedback from muscle spindle (and GTO) afferents has been suggested to contribute importantly to the ongoing SOL activity during walking (Sinkjaer *et al.* 2000; Mazzaro *et al.* 2006; af Klint *et al.* 2008), these changes may explain the reduction of locomotor background EMG observed in this study. A decrease in background EMG could alter motoneurone recruitment gain (Kernell & Hultborn, 1990; Nielsen & Kagamihara, 1993), which could influence SLR and MLR responses. Therefore, this mechanism may also have contributed to the observed changes in perturbation responses.

Despite the changes in background SOL EMG, walking speed and ankle kinematics were unchanged throughout the exercise, suggesting that length changes at the MTU

level were similar throughout the protocol. As length changes of the MTU are distributed throughout the muscle and tendinous tissues, the shortening of SOL fascicle length due to the exercise intervention, combined with the smaller fascicle lengthening during the stance phase, suggests that a larger proportion of MTU stretch occurred in TT at the end of the exercise. The increase in TT compliance would enable the muscle fibres to contract relatively slowly (e.g. Lichtwark & Wilson, 2008), whilst the larger TT stretch would facilitate the storage of elastic energy in the tendinous tissues. This could potentially increase the efficiency of the SOL MTU, which may partly explain how background EMG and fascicle lengthening decreased while ankle kinematics and walking speed were unchanged in this study. It is also possible that other mechanisms, such as increased activation of synergistic muscles to compensate for decreased SOL activation, may account for the apparent paradox of lower SOL muscle activation and unchanged ankle kinematics. This kind of compensation has been reported after higher intensity activities such as fatiguing exercise (e.g. Akima *et al.* 2002).

In this study, the magnitudes of changes in muscle fascicle and EMG responses were generally quite similar between the Pre–Mid and Mid–Post measurement intervals, although this was not the case for MLR amplitude, where no Mid–Post changes were observed. For all measured variables (Fig. 3), significant differences were observed between the Pre and Mid intervals, suggesting that TT compliance may have been altered after just 30 min of walking. This suggestion is consistent with previous data obtained after repeated passive stretching, whereby SOL SLR amplitude clearly decreased after just 15 min (Avela *et al.* 1999).

In conclusion, the findings of the present study suggest that prolonged walking can lead to a short-term increase in tendinous tissue compliance in the human soleus MTU, which results in a reduction in the amplitude and velocity of stretch that is transmitted to the muscle fascicles. This causes a decrease in the amplitude of SLR and MLR responses to perturbations, which could influence the ability to recover from a balance disturbance during gait. A decrease in the stretch transmitted to the muscle fascicles may also decrease afferent feedback from muscle spindles during unperturbed walking. As muscle spindle afferents are thought to make an important contribution to background locomotor EMG (Sinkjaer *et al.* 2000; Mazzaro *et al.* 2006; af Klint *et al.* 2008), a decrease in their firing rates may influence motor control during walking. After the exercise protocol, ankle kinematics were unchanged but the muscle fascicles were stretched less during the stance phase. Thus, it seems that a greater proportion of MTU length change occurred in TT after exercise. This may have increased elastic energy storage, thus potentially increasing the efficiency of the SOL MTU during walking.

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Author contributions

All of the listed authors have made a significant contribution to this study in terms of the study design and/or data analysis, writing and revision of the manuscript, and providing final approval of the submitted material. All of the experiments described in this manuscript were performed at Aalborg University in Denmark.

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