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Title: Effects of muscle activation on shear between human soleus and gastrocnemius muscles

Year: 2017

Version:

Please cite the original version:

Finni Juutinen, T., Cronin, N., Mayfield, D., Lichtwark, G. A., & Cresswell, A. G. (2017). Effects of muscle activation on shear between human soleus and gastrocnemius muscles. *Scandinavian Journal of Medicine and Science in Sports*, 27(1), 26-34.
<https://doi.org/10.1111/sms.12615>

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1 **Author accepted manuscript published in:**

2 **Scandinavian Journal of Medicine and Science in Sports, 27 (1), 26-34.**

3 **doi:10.1111/sms.12615**

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5 **Effects of muscle activation on shear between human soleus and gastrocnemius**
6 **muscles**

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16 **Running title:** Activation alters shear between muscles

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Abstract

Lateral connections between muscles provide pathways for myofascial force transmission. To elucidate whether these pathways have functional roles *in vivo* we examined whether activation could alter the shear between the soleus (SOL) and lateral gastrocnemius (LG) muscles. We hypothesized that selective activation of LG would decrease the stretch-induced shear between LG and SOL. Eleven volunteers underwent a series of knee joint manipulations where plantar flexion force, LG and SOL muscle fascicle lengths and relative displacement of aponeuroses between the muscles were obtained. Data during a passive full range of motion was recorded, followed by 20° knee extension stretches in both passive conditions and with selective electrical stimulation of LG. During active stretch, plantar flexion force was 22% greater ($P < 0.05$) and relative displacement of aponeuroses was smaller than during passive stretch ($P < 0.05$). Soleus fascicle length changes did not differ between passive and active stretches but LG fascicles stretched less in the active than passive condition when the stretch began at angles of 70 and 90° of knee flexion ($P < 0.05$). The activity-induced decrease in the relative displacement of SOL and LG suggests stronger (stiffer) connectivity between the two muscles, at least at flexed knee joint angles, which may serve to facilitate myofascial force transmission.

Key words: myofascial force transmission, activation-dependent, shear strain, muscle stretch, muscle contraction, tendon, aponeurosis

Introduction

Common biomechanical models contain the assumption that the forces produced by individual muscles are transmitted serially to their tendons i.e. myotendinous force transmission (Delp et al., 1990; Hoang et al., 2005; Nordez et al., 2010). However, it has long been known that the forces produced by individual muscle fibres can be transmitted not only serially, but partially laterally to other muscles and tissues via connective structures at the cellular level (Street, 1983) and between the muscle bellies (for review see (Huijing, 1999; Maas and Sandercock, 2010; Purslow, 2010)). Lateral force transmission between the muscle bellies is commonly called myofascial force transmission (Huijing, 1999).

In the human lower limb, soleus (SOL) and gastrocnemii (GA) muscles share a common Achilles tendon. While the two heads of GA span the knee and ankle joints, SOL crosses only the ankle joint and might therefore be expected to be only influenced by movement of this joint. The adjacent aponeuroses of these muscles (SOL and GA) have been shown to displace differently upon activation, depending on the knee joint angle (Bojsen-Moller et al., 2004), which suggests that these muscles can act at least partially independently. However, since there are connective tissues connecting the aponeuroses of human SOL and GA muscles (Bojsen-Moller et al., 2004; Hodgson et al., 2006; Kinugasa et al., 2013), there is a possibility for myofascial force transmission to occur via these structures.

There is evidence to suggest that the GA and SOL muscles do not necessarily move totally independently of each other. Bojsen-Møller et al. (Bojsen-Møller et al., 2010) selectively stimulated the medial GA and used ultrasound (US) to measure the relative muscle displacement between SOL and medial GA *in vivo*. They reported that there was substantial movement in both muscles when GA was selectively stimulated and hence concluded that force is likely transmitted between SOL and GA.

The role and importance of force transmission in humans has also been examined by Tian et al. (Tian et al., 2012) who found that SOL muscle fascicles lengthened slightly during GA shortening induced by isolated knee flexion in a relaxed condition. They estimated that the magnitude of force that was transmitted between the passive muscles was only a few Newtons (<5 N) and suggested that much of the length change measured in SOL was due to shortening of the common tendon, as GA passive force fell during shortening. While in passive conditions this low level of force transmission is unlikely to have functional relevance, it is unclear whether this result might differ under active contraction conditions, where forces are typically higher, and hence muscle shortening may be greater, and aponeurosis strains may vary depending on the level of muscle activation (Finni et al., 2003).

Previously, muscle activation has been shown to increase aponeurosis stiffness in the frog semitendinosus *in vivo* (Lieber et al., 2000). Experiments on turkey lateral GA muscle have shown that the mechanism for increased longitudinal aponeurosis stiffness is likely to be transverse strain of the aponeurosis due to muscle bulging during activation (Azizi & Roberts, 2009). Activation-dependent aponeurosis behaviour has

also been modelled using nonlinear finite element models (Chi et al., 2010), which has provided a mechanical explanation for the human *in vivo* observations that aponeuroses can shorten upon contraction (Finni et al., 2003; Kinugasa et al., 2008). Given that inter-aponeurosis connections are likely to be influenced by strains in both the longitudinal and transverse directions, it might be expected that muscle activation may act to enhance myofascial force transmission although its contribution has been suggested to be small (Maas and Sandercock, 2010).

This study was designed to examine the effect of activation *per se* on myofascial force transmission in humans *in vivo*. While previous study by Bojsen-Møller et al. (2010) compared tissue displacements during active and passive conditions, we designed a protocol to isolate the activation effects by utilising a stretch protocol. Furthermore, in addition to examining the relative displacement between SOL and gastrocnemius aponeuroses we also examined muscle fascicle lengths and tested whether selective electrical activation of lateral GA (LG) muscle alters the behaviour of SOL and LG during stretch starting from four different knee joint angles (i.e. four different initial LG lengths). We hypothesised that activation would decrease the relative displacement between LG and SOL and also affect their fascicle behaviour (Fig. 1).

Methods

Subjects

Eleven healthy volunteers (aged 29 ± 6 yrs., body mass 79 ± 14 kg, height 180 ± 10 cm) participated after providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the local institutional ethics committee.

Experimental set-up

Subjects lay on their left side on a bench with their right foot securely strapped to a foot plate that was attached to a commercially available isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Shirley, NY). With an adjustable steel chain the ankle joint was set to an angle of $84 \pm 5^\circ$ between the sole of the foot and long axis of fibula, such that passive force from the calf muscles maintained fixed joint position. The centre of the knee joint was carefully aligned with the centre of rotation of the dynamometer. Anatomical angles of 30, 50, 70 and 90° of knee flexion from full extension (0°) were defined individually. A photograph of the setup is shown in Fig 2.

Data collection commenced with motor-driven passive knee flexion-extensions over a 90° range of motion (from full extension to 90° of flexion) at $10^\circ/\text{s}$. Thereafter, passive 20° knee extension stretches were elicited at $20^\circ/\text{s}$, starting from four different knee joint angles (30, 50, 70 and 90° of knee flexion) then returning to the start position after 2.8 s. Eight seconds after the knee joint angle returned to its initial position, with subject relaxed, the LG muscle was selectively stimulated to produce a tetanic contraction, as described below, and 1.5 s after onset of the stimulation a 20° stretch was applied (Fig. 3). Stimulation ceased 0.7 s after the end of the stretch. Two consecutive recordings were done at each of the four initial knee joint angles. To avoid thixotropic effects, 4-5

brief, low level voluntary contractions of the plantar flexors preceded each condition (Proske et al., 1993).

Ultrasound imaging

LG instead of medial gastrocnemius (MG) was chosen for imaging because the fascicles of SOL and LG can be visualized in a single US probe orientation more clearly than SOL and MG (Hodgson et al., 2006). The US transducer (LV 7.5 MHZ, 60 mm field of view, spatial resolution 0.086 mm, Telemed, Vilnius, Lithuania) was secured using an elastic bandage after its position was optimized for clear visibility of LG and SOL fascicles and aponeuroses which confirmed a perpendicular view through their plane, typically found in the distal half of LG. The entire protocol was repeated with the US transducer secured over the distal half of SOL for visualization of distal SOL fascicles alone.

Electrode placements and stimulation

Activation of LG was evoked by delivering individual or trains of 500 μ s square-wave pulses generated by a constant-current stimulator (DS7A, Digitimer Ltd, Hertfordshire, UK). Bipolar electromyography (EMG) electrodes (Ag/AgCl, $\varnothing$ 24 mm, Arbo ECG electrodes, Tyco Healthcare, Neustadt, Germany) were placed over lateral and medial parts of SOL to confirm the absence of any M-waves during LG stimulations and monitor possible SOL activity during passive knee joint rotations. SOL was confirmed to be silent in all conditions. To stimulate LG, an anode was placed over the lateral

border of the LG muscle (distal to popliteal fossa). The optimal stimulation position that generated a maximum response was determined by using a low current intensity and moving the cathode about the proximo-lateral region of LG. After the optimal place for the stimulating electrodes was found, stimulation intensity for selective LG activation was determined by recording single plantar flexor twitches while decreasing the current intensity until the SOL M-wave disappeared but a LG twitch response was still present (Fig. 4). Brief stimulus trains (~1 s at 50 Hz) were used to further check that the selected stimulus intensity produced sufficient plantar flexor force by LG to be detectable by the force transducer.

Signal acquisition

Knee joint angle was measured using the Biodex system and plantar flexion force measured using an S-shaped load cell (STC-250, Scale Components, Brisbane, Australia). EMG signals were amplified (x1000) (NL884 Pre-amplifier, Digitimer Ltd, Hertfordshire, UK) and band-pass filtered (10 Hz-500 Hz) (NL820 Isolator, Neurolog, Digitimer Ltd, Hertfordshire, UK). Analogue signals were sampled using a 16-bit analogue-to-digital converter at 1 kHz (Power 1401, Cambridge Electronic Design, UK) and recorded using Spike2 software (Spike2 v 6.10, Cambridge Electronic Design, UK).

Ultrasound images from SOL and LG muscles were collected using PC based software (Echowave II, Telemed, Vilnius, Lithuania) at a sampling frequency of 40 Hz. A digital output signal from the ultrasound system was recorded with Spike2 to synchronise the US images with the other data.

Data analysis

Muscle fascicle lengths were determined using an automated fascicle tracking algorithm validated previously (Cronin et al., 2011; Gillett et al., 2013). The software uses image information from a user-defined region to predict fascicle behaviour. For all measurements (distal SOL, proximal SOL, LG) the region of the visible cross-section of muscle was defined and an optical flow algorithm calculated movement of muscle across the region based on a least square fit of an affine transformation (full details available in Cronin et al., 2011) calculated between consecutive frames. The transformation at each frame could then be applied to end-points of fascicles defined in the first frame to provide an indication of fascicle length changes across the trial. The movement of the end-points of the fascicles, therefore, represent the net movement of tissue in that region of the muscle. The coordinates of the origin and insertion of the fascicles were exported for the purpose of examining the relative displacement between the superficial SOL aponeurosis (indicated by the movement of the distal end of the SOL fascicle, Fig. 5) and the deep LG aponeurosis (indicated by the distal end of the LG fascicle, Fig. 5). The relative displacement, which reflects the inter-aponeurosis shear strain, was calculated by subtracting the superior-inferior displacement of the LG fascicle insertion from the SOL fascicle insertion (Fig. 5).

For passive and active stretches, force measures and muscle parameters were calculated as mean values from the two trials. The timing of each analysis window is shown in Figure 3. For passive conditions, mean values from a 500 ms window (window 1 in Fig.

3) reflecting baseline before stretch, were subtracted from the mean values from a 500 ms window (window 2) taken 1 s after the stretch during a period of stable force. Effects of active stretch were calculated by subtracting the mean over a 50 ms window just before active stretch (window 4) from the mean over a 50 ms window (window 5) during a period of stable force before the stimulation ended. Fascicle length changes and relative displacement of aponeuroses were then calculated both for the passive and active stretch conditions. To examine the effect of stimulation per se on fascicle length changes, the mean of window 4 was subtracted from mean of window 3.

For passive 90° range of motion knee extension trials, plantar flexion force, knee angle and fascicle lengths were determined in 10° increments for both knee flexion and extension. Passive knee rotation trials from three subjects were lost due to data buffering issues in these long trials, so data are reported from eight subjects for this condition.

Statistics

Means and standard deviations (SD) of each outcome measure were calculated. Distribution of the data was checked for normality using a Shapiro-Wilk test. Repeated measures ANOVA was used to test the effects of different joint angles on force. A two factor ANOVA (activation × joint angle or muscle × joint angle) for repeated measures was used to evaluate potential differences in the relative displacement of aponeuroses and fascicle length across these factors. Where significant main effects were observed, Bonferroni corrected pairwise comparisons were used to identify the location of

differences. A linear regression was used to examine the effect of knee joint angle on fascicle behaviour during passive knee extension-flexion tasks and the relative displacement of aponeuroses in passive versus active stretch conditions. The slopes were calculated for each individual and the differences in slopes were tested using paired T-test or Wilcoxon Signed Rank Test. Significance was accepted at the $P \leq 0.05$ level.

Results

Plantar flexion force was on average 22% greater during active than passive stretch ($P < 0.05$). Stretch starting from 30° resulted in greater ($P < 0.01$) force than for any other joint angle in both passive (55 N vs. 37 N at 50° , 28 N at 70° and 29 N at 90°) and active conditions (64 N vs. 37 N, 35 N and 40 N).

The relative displacement of LG and SOL aponeurosis showed a significant main effect of joint angle and activation $\times$ joint angle interaction (both $P < 0.05$). The difference was localized to the knee joint angle of 90° where there was significantly less shear during active than passive stretch ($P < 0.05$) (Fig. 5B and 6). The slopes of the relative displacement of aponeuroses differed significantly between passive and active stretch (1.96 vs. $0.29 \text{ mm} \times 10^{-2}/^\circ$, $P < 0.05$) (Fig 6).

When comparing the effects of passive vs. active stretch on muscle fascicle lengths, a two factor ANOVA revealed main effects of activation ($P < 0.001$) and joint angle ($P < 0.001$) on LG muscle stretch. The activation $\times$ joint angle interaction was also significant ($P < 0.001$) for LG. Pairwise comparisons localized the effect to 70° and 90°

angles, where LG fascicles resisted stretch better when activated (Fig. 7A). Mean lengthening for LG fascicles was 1.8 ± 1.3 mm in passive and 1.6 ± 1.8 mm during active stretch, while SOL fascicle length changes were very small and non-significant (passive stretch: distal SOL 0.4 ± 1.1 mm, proximal SOL 0.1 ± 0.9 mm; active stretch: distal SOL 0.5 ± 1.0 mm, proximal SOL 0.1 ± 1.0 mm).

Selective LG stimulation induced before the stretches caused differential shortening of fascicles in the two muscles (three regions) (main effect, $P < 0.01$) that was not affected statistically by the knee joint angle. The mean fascicle length changes across all joint angles showed LG shortening by 8.3 ± 3.1 mm, proximal SOL shortening by 4.0 ± 3.6 mm, while the mean change in distal SOL was 0.1 ± 2.5 mm (Fig. 7B).

Passive knee joint flexion throughout the 90° range of motion caused a mean LG fascicle shortening of 0.10 mm/ $^\circ$ while the shortening of proximal SOL fascicle was small in comparison (0.007 mm/ $^\circ$). During passive knee extensions over the same range of motion, LG fascicles lengthened more than SOL (0.11 mm/ $^\circ$ vs. 0.006 mm/ $^\circ$), the behaviour between LG and SOL fascicles being significantly different ($P < 0.01$). Distal SOL fascicle showed a different behaviour to those of proximal SOL with an increase in length of 0.03 mm/ $^\circ$ during knee flexion, but the differences in net fascicle length changes between the two locations were not significant (Fig. 8).

Discussion

The main findings of this study were that the shear between LG and SOL was smaller in active than passive stretch but that the selective LG activation did not change SOL fascicle behaviour to stretch. The novel finding of activity induced decrease in interaponeurosis shear supports the hypothesis that activation reduces the capacity of the SOL and LG to move independently of each other compared to when passive (Fig. 1). Theoretically, an increase in force due to activation can cause unfolding of tropocollagen molecules in a loading rate dependent manner (Gautieri et al., 2009) and at collagen fibril level there occurs unfolding of the crimp structure (Diamant et al., 1972) both of which have influence on connective tissue stiffness and potentially on the force transmission (Maas & Sandercock 2010) between the LG and SOL.

The results showed that SOL and LG moved almost in unison during active LG knee extension, indicated by zero shear in the active condition for all knee joint angles tested. We believe that LG activation may have induced transverse bulging of the muscle belly and that this could potentially also stiffen the connective tissue structures between SOL and LG, particularly the inter-aponeurosis connections. This could in turn facilitate the synchronous movement of the muscle tissue during LG contraction and potentially, via increased stiffness, enhance myofascial force transmission between these muscles.

Several previous studies have elucidated the presence of structures enabling myofascial force transmission, which include connective tissues and neurovascular tract (Bojsen-Moller et al., 2004; Huijing, 2009). In humans, myofascia has been shown to transmit forces between the latissimus dorsi and gluteal muscles (Carvalhais et al., 2013), between the flexor carpi ulnaris and other wrist flexors (de Bruin et al., 2011), between

the semitendinosus and gracilis muscles (Snoeck et al., 2014) and between GA and SOL (Bojsen-Møller et al., 2010; Huijing et al., 2011). Despite existing lateral connections (Bojsen-Møller et al., 2004; Hodgson et al., 2006), SOL and GA, which have different muscle lengths and a different number of articulations, experience relative movement during maximal voluntary contractions (Bojsen-Møller et al., 2004) and normal locomotion conditions during running (personal observations).

Previously Bojsen-Møller et al. (Bojsen-Møller et al., 2010) applied selective medial GA stimulation during which the superficial aponeurosis of SOL and the deep aponeurosis of medial GA underwent the same magnitude of displacement. Their protocol tested the effect of activation-induced contraction on an adjacent relaxed muscle whereas the present study compared both aponeurosis displacement and fascicle lengths in response to stretch in active and passive conditions. In general, results of both studies showed that the relative displacement of aponeuroses is minimal when one of the adjacent muscles is selectively activated. One explanation for this observation may be that the active shortening of GA elongates the Achilles tendon (Tian et al., 2012) and pulls the SOL aponeurosis of insertion proximally, thereby passively shortening SOL fascicles. However, the present results of distal SOL fascicle lengths (0.1 mm displacement upon LG stimulation) and the results from Bojsen-Møller et al. (2010), who found very small movement in the distal SOL (upon medial GA activation it displaced only 0.1 or 0.6 mm in knee flexed or extended position, respectively), suggest that there may also be mechanical interaction between SOL and GA more proximally.

The present result that fascicle behaviour of SOL was similar between active and passive stretch suggests that the activity induced reduction in interaponeurosis shear is a local phenomenon and not reflected more globally to the muscle. However, the selective stimulation of LG applied before the stretch shortened its fascicles by approximately 8 mm, whereas the adjacent SOL shortened by 4 mm (Fig. 7B). For comparison, these muscles shorten about 18 and 9 mm, respectively, during a maximal isometric voluntary plantar flexion (Finni, 2006). The effect of active LG contraction on the SOL fascicles was only seen in the proximal SOL, while the effect of LG contraction on distal SOL fascicle lengths was close to zero (Fig 7B). However, it is difficult to differentiate how much of the 4 mm shortening of the proximal SOL is due to myofascial force transmission and how much is due to common tendon lengthening without tracking the movements along the entire length of the aponeurosis.

Regarding the small magnitude of muscle motion observed in the present conditions, it is important to note that with the US technique as used presently, the results reflect movements in the direction of the imaging plane. While the measured magnitudes of fascicle length changes and tissue displacements were small, significant differences observed between the passive and active conditions confirm that US has sufficient resolution to investigate this paradigm. However, the technique cannot capture possible out of plane rotations that have been shown to occur with voluntary contraction forces as low as 20% of maximum (Hodgson et al., 2006). Furthermore, in cadaveric dissections the connective tissue bands running between the aponeuroses of SOL and GA have various orientations (Bojsen-Moller et al., 2004), possibly influencing the relative movement of muscles in a given condition in an orientation-dependent manner.

In the present setup the placement of the US probe was chosen to give clear visibility of fascicles and the aponeuroses, confirming the perpendicular orientation of the probe during the entire trial. Furthermore, the low contraction intensities used should limit the out of plane rotations and motion of the muscle, however we cannot quantify this in our current setup.

Passive range of motion. The present results regarding fascicle behaviour during the passive knee range of motion are generally consistent with the earlier findings of Tian et al. (Tian et al., 2012) showing that the GA fascicles shorten during passive knee flexion and lengthen during knee extension by about 0.1 mm/°, while SOL length changes were much smaller. In relaxed muscles, Tian et al. (Tian et al., 2012) estimated that passive myofascial force transmission between SOL and GA was less than 5 N. This calculated force represented soleus force as a result of elongation of its fascicles during passive knee joint rotation. Because we did not observe significant elongation of soleus fascicles during active as compared to passive stretch it is not rational to do similar calculations. Instead, we show that the effect of activation at this low level of force (only 20% greater than passive stretch) caused only local effects at the muscle interface (significantly decreased shear between muscles at flexed knee joint). While the low force level limits generalizability, it may be speculated that greater forces accompanied by smaller tissue displacements during voluntary plantar flexor contractions in normal daily activities would further enhance myofascial force transmission via lateral pathways.

In conclusion, this study showed, for the first time *in vivo* in humans, that muscle activation can reduce relative displacement between SOL and gastrocnemius muscles at flexed knee position. The activity-induced synergistic movement of SOL and LG may suggest an increased likelihood for myofascial force transmission but it may also magnify myotendinous force transmission by increasing synergistic muscle behaviour that has been shown to enhance displacement of myotendinous junction (Kinugasa et al. 2013). Understanding the functionality of myofascial force transmission helps to shed light on the mechanical complexity of force transmission, which cannot be captured precisely by simple models.

Perspectives

Force transmission between SOL and GA can have functional implications. Since both muscles are attached to the Achilles tendon, differential forces between muscle compartments may induce considerable heterogeneity in the stresses within the tendon cross-section, since there is distinct regional representation of tendon fibre bundles from different compartments of the triceps surae (Szaro et al., 2009). Because there is a negligible amount of force transmission between tendon fascicles (Haraldsson et al., 2008) there can be considerable shear strains within the tendon which appeared in previous experiments when optic fiber inserted through Achilles tendon was bent after high loads of triceps surae (T Finni personal observations). Consequently, force transmission between the muscles may serve to decrease non-uniform loads in the Achilles tendon. While the connectivity at the muscle level may be considered to reduce non-uniformities within Achilles tendon, a degree of relative movement of superficial and deep portions of the tendon (Franz et al., 2015) has also been observed.

Consequently, both compliance (allowing a degree of independency) and connectivity (allowing a degree of force transmission) at multiple levels of the system provides flexibility to the system that may be necessary for the function of these synergistic plantarflexor muscles. In addition to its relevance in healthy populations, a large number of force transmitting pathways is likely to be important in pathological conditions. Because of the multiple force transmitting pathways along the muscle-tendon system damage or microtrauma to a part of muscle or tendon may not have such a detrimental effect (Huijing et al., 2003; Snoeck et al., 2014).

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Competing interests

None.

Author contributions

Conception and design of experiments: T.F., N.J.C., G.A.L. and A.G.C. Collection, analysis and interpretation of data: T.F., N.J.C., D.M., G.A.L. and A.G.C. Drafting the article or revising it critically for important intellectual content: T.F., N.J.C., G.A.L. and A.G.C. All authors approved the final version of the manuscript. The experiments were performed at the Centre for Sensorimotor Neuroscience, School of Human Movement Studies, The University of Queensland, Australia.

Funding

T. Finni and N.J. Cronin were supported by a travel grant by the University of Jyväskylä.

523 Figures and legends

524

525 Figure 1. Schematic representation of the effects of gastrocnemius muscle (GA) stretch
526 with (right) and without (left) GA activity on soleus (SOL) displacement (arrows).
527 LEFT: With muscles relaxed the connective tissue linkages are more compliant and the
528 effect of myofascial force transmission and the consequent displacement of SOL is
529 small. RIGHT: Activation of GA induces structural changes on the aponeurosis and
530 intermuscular connective tissues that enhances force transmission and enables greater
531 displacement of the passive soleus. This has previously been illustrated by Maas &
532 Sandercock (2010). Arrows denote the hypothesized magnitude of displacement.

Figure 2. Experimental setup. Subject's leg attached to the Biodex system with a custom-made plantar flexion force measurement device. Stimulation electrodes are not visible but covered by straps. Approximate location for distal imaging site distal to myotendinous junction of the gastrocnemius is shown.

Figure 3. Example of raw recordings of plantar flexion force, knee joint angle and lateral gastrocnemius (LG) and soleus (SOL) muscle fascicle lengths from proximal probe position. In this example the stretches were elicited from 30° knee joint angle. The timing of the LG stimulation is shown with a black box. Grey windows below the x-axis indicate the time points where force and ultrasound data were analysed. The effect of passive stretch was calculated by subtracting mean of window 1 from mean of window 2, and the effect of active stretch was calculated by subtracting mean of window 4 from mean of window 5. For the effect of contraction the mean of window 4 was subtracted from mean of window 3. The y-axis on right is fascicle length (mm). Other values are shown in arbitrary units. X-axis time scale: 2 s between tick marks.

Figure 4. Optimal stimulation intensity producing plantar flexion force but without electrical activity in soleus muscle was searched by decreasing the current until the soleus M-wave disappeared. The figure shows EMG (left) and force responses (right) at two different currents, 20 mA (upper traces) and 8.5 mA (lower traces).

Figure 5. A) Ultrasound image from proximal position. A region of interest (dashed white rectangles) and a fascicle were first defined from both soleus (SOL) and lateral gastrocnemius (LG) muscles before the automated tracking algorithm was run. Arrows show that relative displacement of the aponeuroses was quantified just adjacent to the aponeuroses. B) Example showing distal displacement of aponeurosis when the stretch was initiated from 90 degree in passive or active conditions. Error bars represent standard deviations.

A

B

Figure 6: Effect of selective gastrocnemius stimulation during stretch on inter-aponeurosis shear between lateral gastrocnemius (LG) and soleus (SOL) muscles. Values at 90° knee joint angle ($P < 0.05$) and the slopes were significantly different between passive and active stretches ($P < 0.05$). Error bars represent standard deviations.

Figure 7. A) Effects of active and passive stretches on lateral gastrocnemius muscle fascicle length change. B) Effect of lateral gastrocnemius stimulation (induced prior to stretch) on fascicle length change of lateral gastrocnemius, and soleus at the proximal and distal imaging positions at the different knee joint configurations. Error bars represent standard deviations.

$P < 0.01$, * $P < 0.05$ between active and passive stretches.

A

B

Figure 8. Length changes for lateral gastrocnemius and soleus as a function of knee joint angle during passive knee joint rotations (N=8). Ankle angle was fixed at $84 \pm 5^\circ$. Grey circles represent measured mean values and solid lines represent linear regression lines. Standard deviations of about 1 cm (range 8-12 mm) are due to differences in initial fascicle lengths and are omitted for clarity.