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4

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

7

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15

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

17

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25 **Abstract**

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

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

45

## 46 **Introduction**

47

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

57

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

68

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

75

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

87

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

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

100

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

111

## 112 **Methods**

113

### 114 *Subjects*

115

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

119

### 120 *Experimental set-up*

121

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

130

131 Data collection commenced with motor-driven passive knee flexion-extensions over a  
132  $90^\circ$  range of motion (from full extension to  $90^\circ$  of flexion) at  $10^\circ/\text{s}$ . Thereafter, passive  
133  $20^\circ$  knee extension stretches were elicited at  $20^\circ/\text{s}$ , starting from four different knee  
134 joint angles (30, 50, 70 and  $90^\circ$  of knee flexion) then returning to the start position after  
135 2.8 s. Eight seconds after the knee joint angle returned to its initial position, with subject  
136 relaxed, the LG muscle was selectively stimulated to produce a tetanic contraction, as  
137 described below, and 1.5 s after onset of the stimulation a  $20^\circ$  stretch was applied (Fig.  
138 3). Stimulation ceased 0.7 s after the end of the stretch. Two consecutive recordings  
139 were done at each of the four initial knee joint angles. To avoid thixotropic effects, 4-5

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

142

### 143 *Ultrasound imaging*

144

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

154

### 155 *Electrode placements and stimulation*

156

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

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

173

#### 174 *Signal acquisition*

175

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

183

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

188

189 *Data analysis*

190

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

208

209 For passive and active stretches, force measures and muscle parameters were calculated  
210 as mean values from the two trials. The timing of each analysis window is shown in

211 Figure 3. For passive conditions, mean values from a 500 ms window (window 1 in Fig.

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

220

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

226

### 227 *Statistics*

228

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

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

242

### 243 **Results**

244 Plantar flexion force was on average 22% greater during active than passive stretch ( $P$   
245  $<0.05$ ). Stretch starting from  $30^\circ$  resulted in greater ( $P <0.01$ ) force than for any other  
246 joint angle in both passive (55 N vs. 37 N at  $50^\circ$ , 28 N at  $70^\circ$  and 29 N at  $90^\circ$ ) and  
247 active conditions (64 N vs. 37 N, 35 N and 40 N).

248

249 The relative displacement of LG and SOL aponeurosis showed a significant main effect  
250 of joint angle and activation $\times$ joint angle interaction (both  $P <0.05$ ). The difference was  
251 localized to the knee joint angle of  $90^\circ$  where there was significantly less shear during  
252 active than passive stretch ( $P <0.05$ ) (Fig. 5B and 6). The slopes of the relative  
253 displacement of aponeuroses differed significantly between passive and active stretch  
254 (1.96 vs.  $0.29 \text{ mm} \times 10^{-2}/^\circ$ ,  $P <0.05$ ) (Fig 6).

255

256 When comparing the effects of passive vs. active stretch on muscle fascicle lengths, a  
257 two factor ANOVA revealed main effects of activation ( $P <0.001$ ) and joint angle ( $P$   
258  $<0.001$ ) on LG muscle stretch. The activation  $\times$  joint angle interaction was also  
259 significant ( $P <0.001$ ) for LG. Pairwise comparisons localized the effect to  $70^\circ$  and  $90^\circ$

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

265

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

271

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

280

281 **Discussion**

282

283 The main findings of this study were that the shear between LG and SOL was smaller in  
284 active than passive stretch but that the selective LG activation did not change SOL  
285 fascicle behaviour to stretch. The novel finding of activity induced decrease in  
286 interaponeurosis shear supports the hypothesis that activation reduces the capacity of  
287 the SOL and LG to move independently of each other compared to when passive (Fig.  
288 1). Theoretically, an increase in force due to activation can cause unfolding of  
289 tropocollagen molecules in a loading rate dependent manner (Gautieri et al., 2009) and  
290 at collagen fibril level there occurs unfolding of the crimp structure (Diamant et al.,  
291 1972) both of which have influence on connective tissue stiffness and potentially on the  
292 force transmission (Maas & Sandercock 2010) between the LG and SOL.

293

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

301

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

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

313

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

329

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

342

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

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

359

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

376

377 In conclusion, this study showed, for the first time *in vivo* in humans, that muscle  
378 activation can reduce relative displacement between SOL and gastrocnemius muscles at  
379 flexed knee position. The activity-induced synergistic movement of SOL and LG may  
380 suggest an increased likelihood for myofascial force transmission but it may also  
381 magnify myotendinous force transmission by increasing synergistic muscle behaviour  
382 that has been shown to enhance displacement of myotendinous junction (Kinugasa et al.  
383 2013). Understanding the functionality of myofascial force transmission helps to shed  
384 light on the mechanical complexity of force transmission, which cannot be captured  
385 precisely by simple models.

386

### 387 **Perspectives**

388 Force transmission between SOL and GA can have functional implications. Since both  
389 muscles are attached to the Achilles tendon, differential forces between muscle  
390 compartments may induce considerable heterogeneity in the stresses within the tendon  
391 cross-section, since there is distinct regional representation of tendon fibre bundles from  
392 different compartments of the triceps surae (Szaro et al., 2009). Because there is a  
393 negligible amount of force transmission between tendon fascicles (Haraldsson et al.,  
394 2008) there can be considerable shear strains within the tendon which appeared in  
395 previous experiments when optic fiber inserted through Achilles tendon was bent after  
396 high loads of triceps surae (T Finni personal observations). Consequently, force  
397 transmission between the muscles may serve to decrease non-uniform loads in the  
398 Achilles tendon. While the connectivity at the muscle level may be considered to reduce  
399 non-uniformities within Achilles tendon, a degree of relative movement of superficial  
400 and deep portions of the tendon (Franz et al., 2015) has also been observed.

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

409

410

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- 505  
506

507 **Competing interests**

508 None.

509

510 **Author contributions**

511 Conception and design of experiments: T.F., N.J.C., G.A.L. and A.G.C. Collection,

512 analysis and interpretation of data: T.F., N.J.C., D.M., G.A.L. and A.G.C. Drafting the

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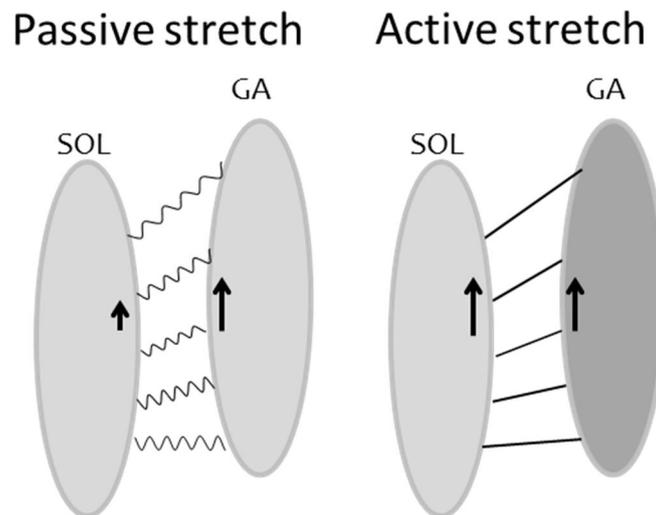
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523 **Figures and legends**

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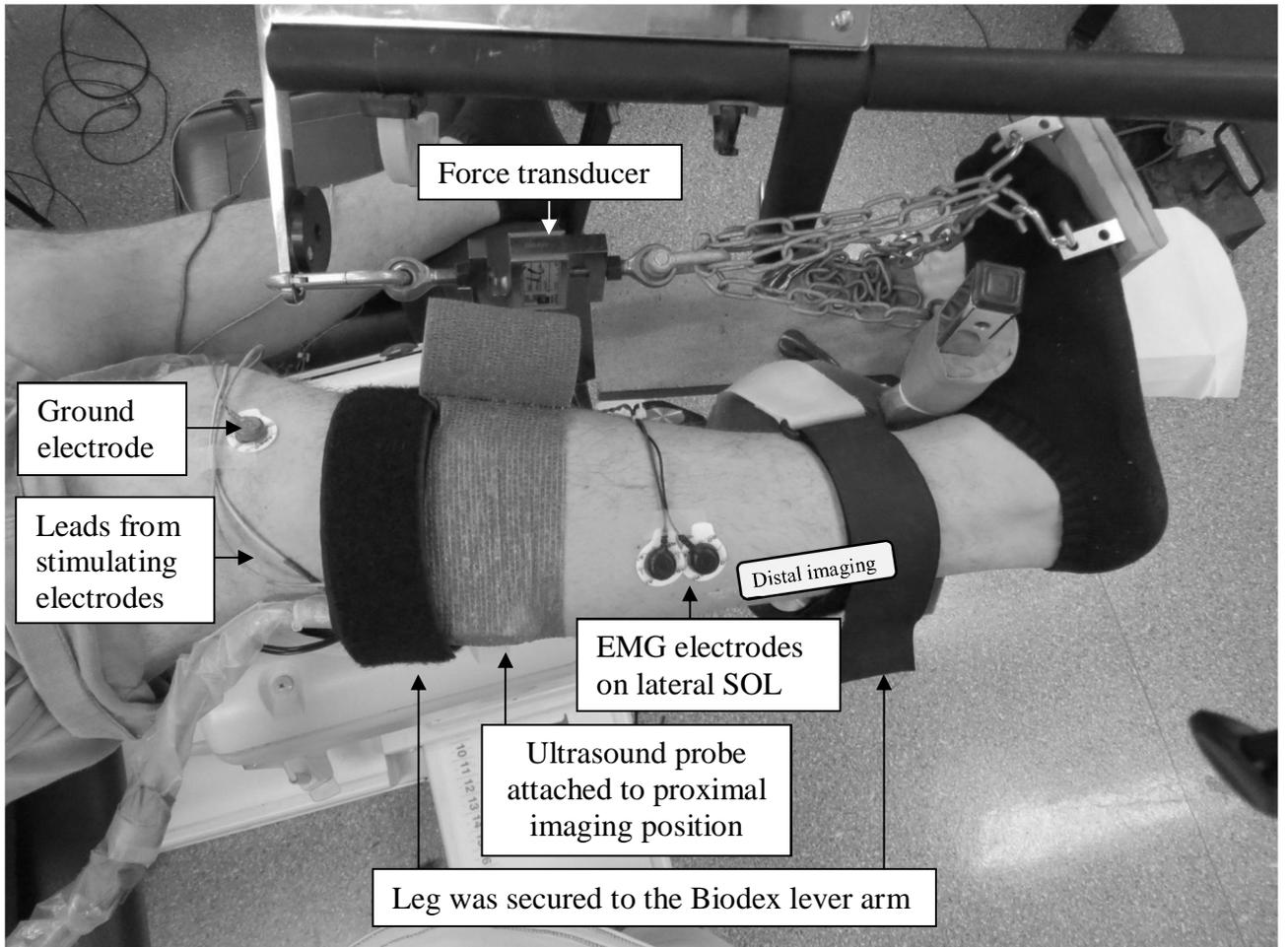
525 Figure 1. Schematic representation of the effects of gastrocnemius muscle (GA) stretch  
526 with (right) and without (left) GA activity on soleus (SOL) displacement (arrows).  
527 LEFT: With muscles relaxed the connective tissue linkages are more compliant and the  
528 effect of myofascial force 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.



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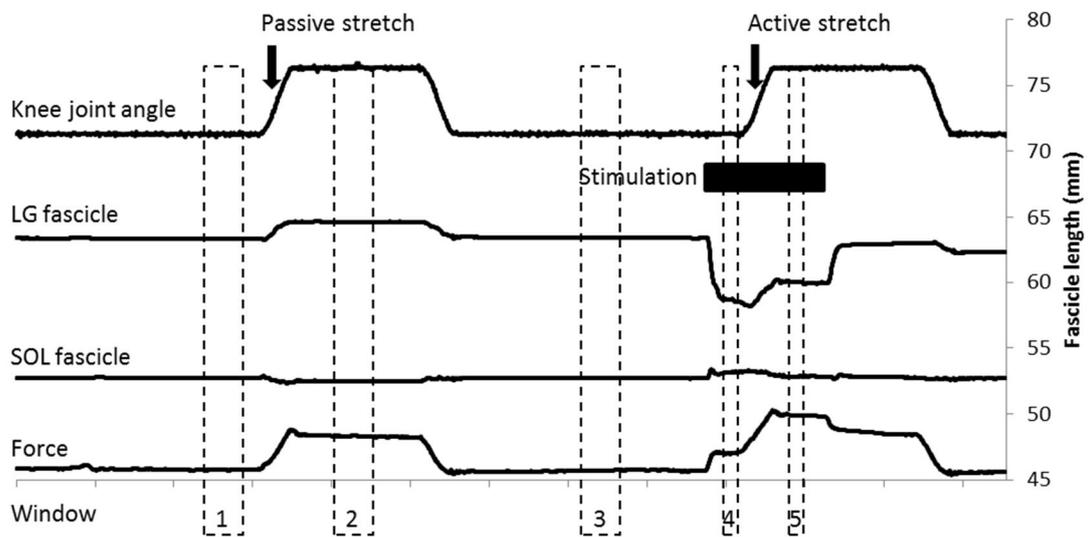


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543 Figure 3. Example of raw recordings of plantar flexion force, knee joint angle and  
 544 lateral gastrocnemius (LG) and soleus (SOL) muscle fascicle lengths from proximal  
 545 probe position. In this example the stretches were elicited from 30° knee joint angle.  
 546 The timing of the LG stimulation is shown with a black box. Grey windows below the  
 547 x-axis indicate the time points where force and ultrasound data were analysed. The  
 548 effect of passive stretch was calculated by subtracting mean of window 1 from mean of  
 549 window 2, and the effect of active stretch was calculated by subtracting mean of  
 550 window 4 from mean of window 5. For the effect of contraction the mean of window 4  
 551 was subtracted from mean of window 3. The y-axis on right is fascicle length (mm).  
 552 Other values are shown in arbitrary units. X-axis time scale: 2 s between tick marks.  
 553

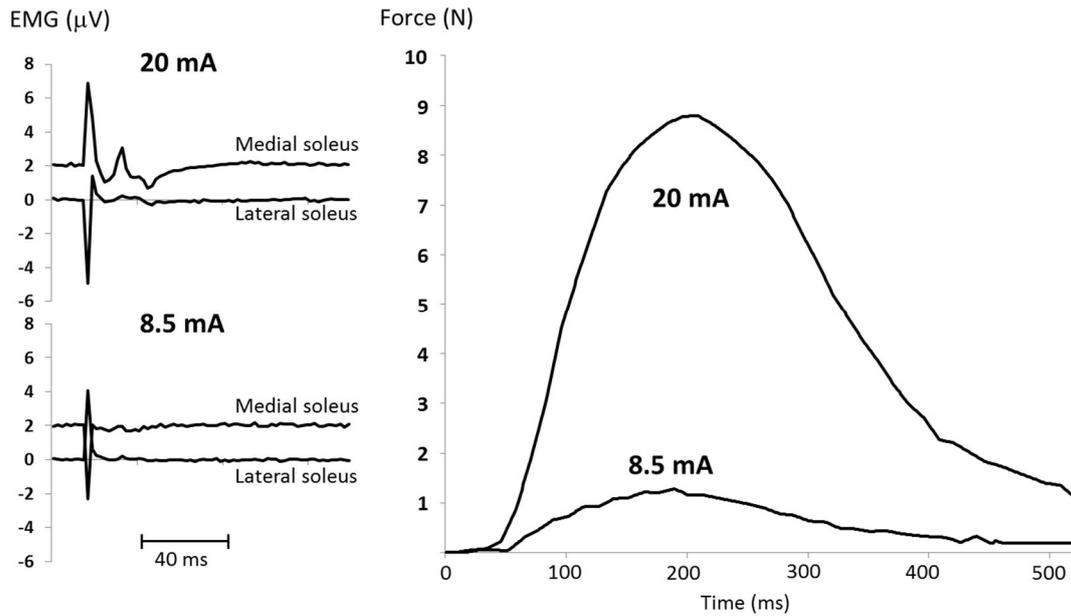


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557 Figure 4. Optimal stimulation intensity producing plantar flexion force but without  
558 electrical activity in soleus muscle was searched by decreasing the current until the  
559 soleus M-wave disappeared. The figure shows EMG (left) and force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

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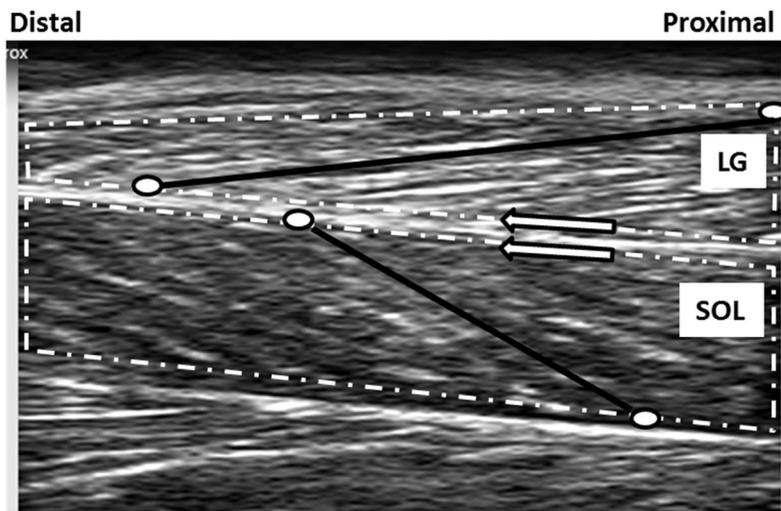
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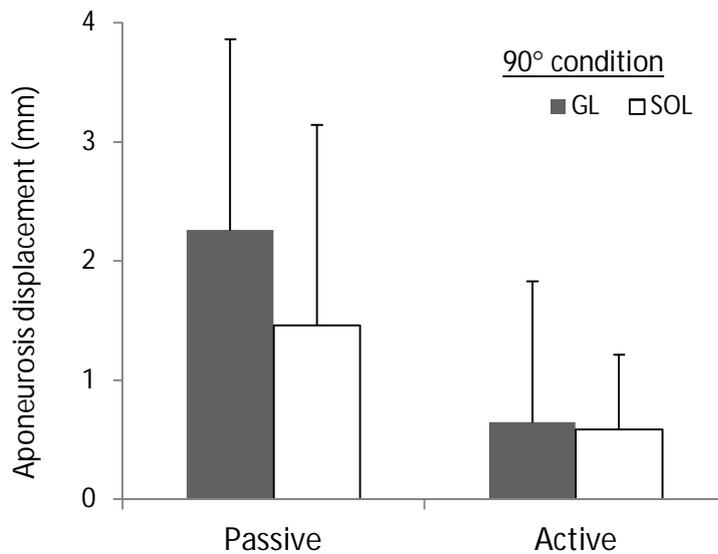
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567 Figure 5. A) Ultrasound image from proximal position. A region of interest (dashed  
568 white rectangles) and a fascicle were first defined from both soleus (SOL) and lateral  
569 gastrocnemius (LG) muscles before the automated tracking algorithm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

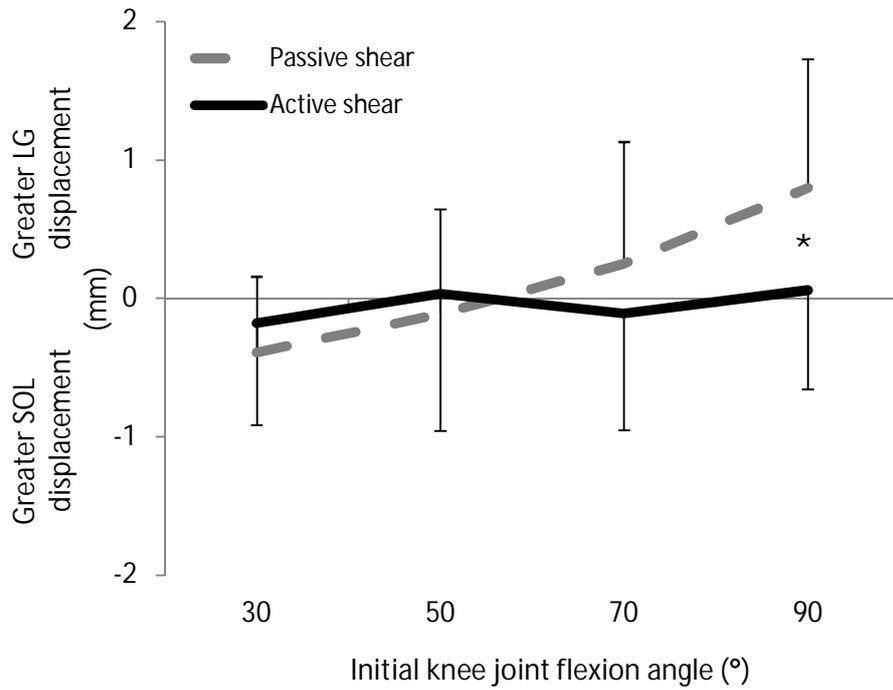
574 A



582 B



585 Figure 6: Effect of selective gastrocnemius stimulation during stretch on inter-  
586 aponeurosis shear between lateral gastrocnemius (LG) and soleus (SOL) muscles.  
587 Values at 90° knee joint angle ( $P < 0.05$ ) and the slopes were significantly different  
588 between passive and active stretches ( $P < 0.05$ ). Error bars represent standard deviations.  
589

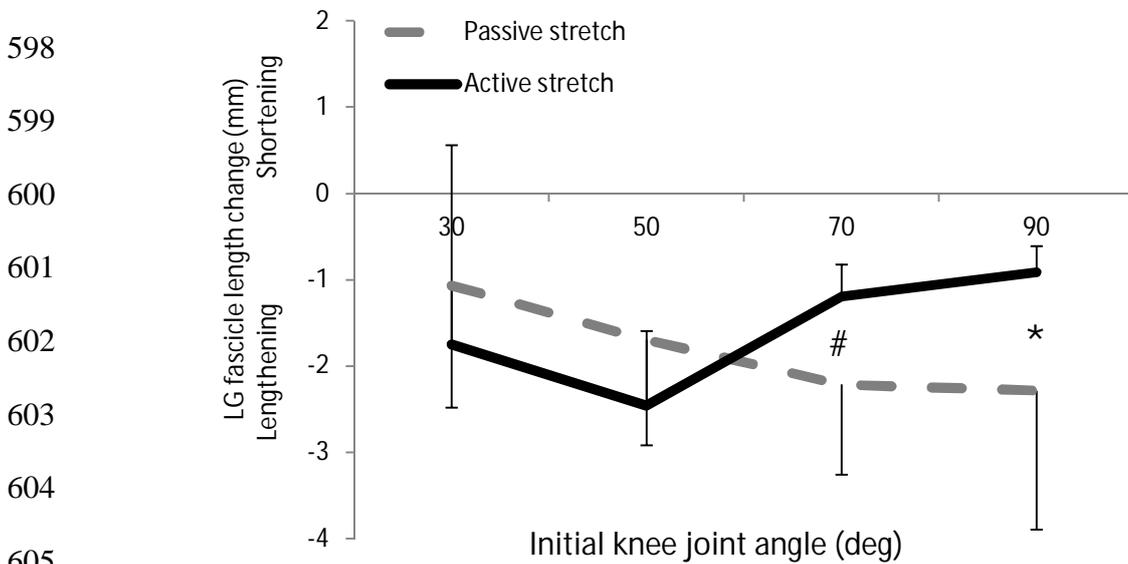


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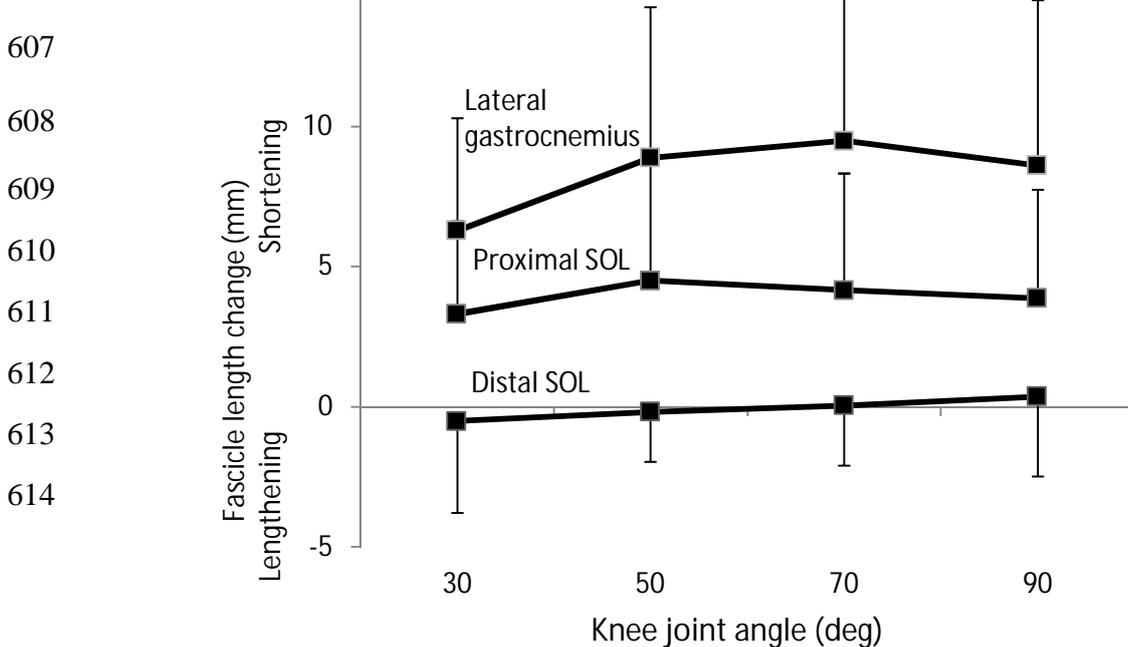
591 Figure 7. A) Effects of active and passive stretches on lateral gastrocnemius muscle  
 592 fascicle length change. B) Effect of lateral gastrocnemius stimulation (induced prior to  
 593 stretch) on fascicle length change of lateral gastrocnemius, and soleus at the proximal  
 594 and distal imaging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

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

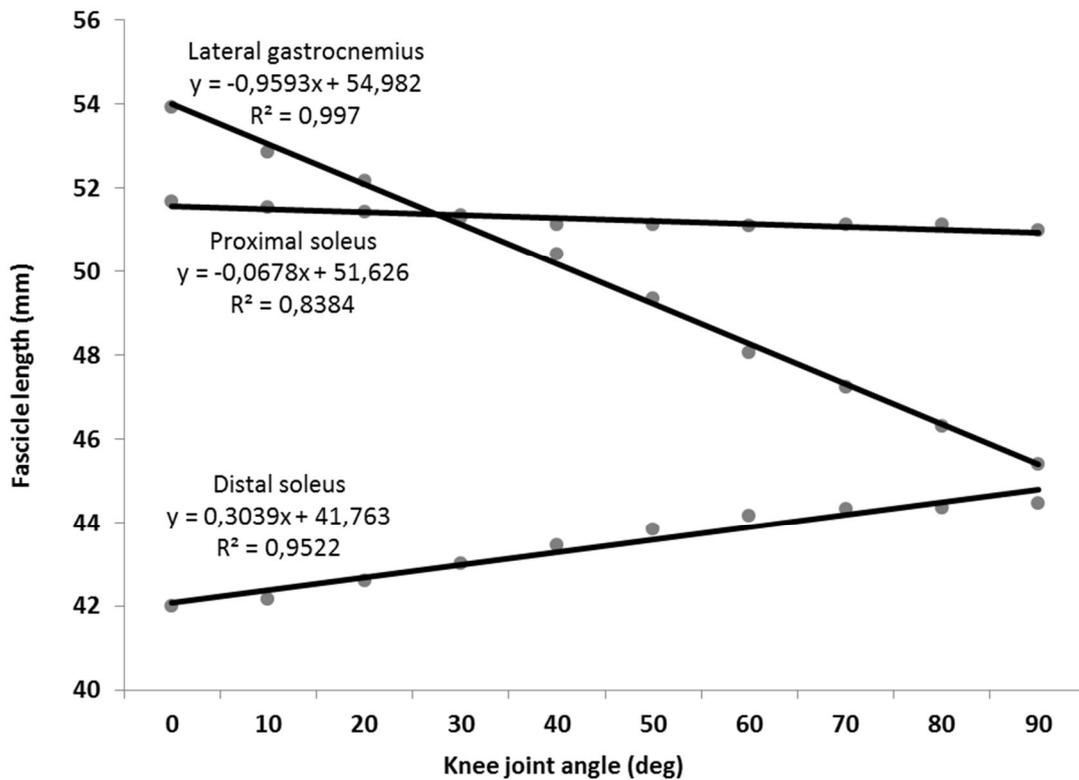
597 A



606 B



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



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