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**Title:** In vivo localized gastrocnemius subtendon representation within the healthy and ruptured human Achilles tendon

**Year:** 2022

**Version:** Accepted version (Final draft)

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**Please cite the original version:**

Khair, R. M., Stenroth, L., Cronin, N. J., Reito, A., Paloneva, J., & Finni, T. (2022). In vivo localized gastrocnemius subtendon representation within the healthy and ruptured human Achilles tendon. *Journal of Applied Physiology*, 133(1), 11-19.

<https://doi.org/10.1152/jappphysiol.00084.2022>

## Achilles MG and LG subtendon representation

### 1 **In vivo localised gastrocnemius subtendon representation within the healthy and** 2 **ruptured human Achilles tendon**

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23

## Achilles MG and LG subtendon representation

### 24 **Abstract**

25 The Achilles tendon (AT) is composed of three distinct in-series elastic subtendons, arising  
26 from different muscles in the triceps surae. Independent activation of any of these muscles is  
27 thought to induce sliding between the adjacent AT subtendons. We aimed to investigate  
28 displacement patterns during voluntary contraction (VOL) and selective transcutaneous  
29 stimulation of medial (MG<sub>stim</sub>) and lateral (LG<sub>stim</sub>) gastrocnemius between ruptured and  
30 healthy tendons, and to examine the representative areas of AT subtendons. Twenty-eight  
31 patients with unilateral AT rupture performed bilateral VOL at 30% of the maximal  
32 isometric un-injured plantarflexion torque. AT displacement was analysed from sagittal B-  
33 mode ultrasonography images during VOL, MG<sub>stim</sub> and LG<sub>stim</sub>. Three-way ANOVA  
34 revealed a significant two-way interaction of contraction type\*location on the tendon  
35 displacement ( $F(10-815)=3.72$ ,  $p<0.001$ ). The subsequent two-way analysis revealed a  
36 significant contraction type\*location interaction for tendon displacement ( $F(10-410)=3.79$ ,  
37  $p<0.001$ ) in the un-injured limb only, where LG<sub>stim</sub> displacement pattern was significantly  
38 different from MG<sub>stim</sub> ( $p=0.008$ ) and VOL ( $p=0.005$ ). When comparing contraction types  
39 between limbs there were no difference in the displacement patterns, but displacement  
40 amplitudes differed. There was no significant difference in the location of maximum or  
41 minimum displacement between limbs. The displacement pattern was not different in non-  
42 surgically treated compared to un-injured tendons one-year post rupture. Our results suggest  
43 that near the calcaneus, LG subtendon is located in the most anterior region adjacent to  
44 medial gastrocnemius. However, free tendon stiffness seems to be lower in the injured AT,  
45 leading to more displacement during electrically-induced contractions compared to the un-  
46 injured.

47

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### 48 **New & Noteworthy**

49 Using selective electrical stimulation, we report the distributions of medial and lateral  
50 gastrocnemius subtendon representations within the healthy and ruptured Achilles tendon. In  
51 the majority of our sample, lateral gastrocnemius subtendon was found in the most anterior  
52 region adjacent to medial gastrocnemius both in the healthy and ruptured, non-surgically  
53 treated tendon. The tendon internal displacement pattern does not seem to differ, but  
54 displacement amplitude and non-uniformity differed between healthy and ruptured tendons  
55 one-year post rupture.

56

57 **Key words:** Achilles tendon, architecture, geometry, anatomy, rupture, human.

58

### 59 **Introduction**

60 The Achilles tendon (AT) provides critical series elasticity to the triceps surae, amplifying  
61 power for activities such as walking and running (1) and playing a significant role in  
62 mechanical energy storage (2). Normal tendon function is disrupted by AT disorders that  
63 also cause pain and disability. Achilles tendon rupture (ATR) is prevalent in sport-related  
64 activities with an incidence of 31/100,000 individuals per year (3, 4). Understanding the  
65 normal and pathological biomechanical function of the AT is crucial to the diagnosis and  
66 management of AT-related maladies.

67 The AT has a complex hierarchical structure and is composed of distinct bundles of fascicles  
68 running continuously along the tendon, called subtendons. AT subtendons each arises from a  
69 different muscular head of the triceps surae: soleus (SOL), medial gastrocnemius (MG), and  
70 lateral gastrocnemius (LG) (5, 6). The tendon twists so that at the calcaneal tuberosity  
71 insertion, the MG fibres are located on the lateral surface, LG fibres more deeply, and the  
72 SOL fibres on the medial surface (5, 6). The degree of twist varies among individuals and can  
73 be classified into three types (5). This variation might lead to interindividual differences in  
74 the location of the MG, LG and SOL tendon fascicles along the length of the tendon (5). Due  
75 to this structure, AT is subjected to complex non-uniform loading that can cause  
76 heterogeneity of strain within the tendon (7).

77 *In vivo* studies have exploited advances in ultrasonic imaging and speckle tracking algorithms  
78 to reveal non-uniform motion within the AT (8, 9). The ability of subtendons to slide relative  
79 to each other is considered to be a function of a healthy tendon (9, 10). Healthy non-  
80 uniformity is characterised by smaller displacement of the superficial (posterior) tendon and  
81 larger displacement of the deep (anterior) tendon. Assuming that posteriorly the tendon  
82 consists of fascicles arising from both gastrocnemius muscles and that the anterior tendon  
83 consists of fascicles arising from soleus, researchers have tried to identify structure-function

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84 relationships (11, 12). However, the mechanism of non-uniform displacement and the  
85 representation of each subtendon within free AT in different individuals remains elusive due  
86 to potential differences in neural control strategies (7), the architecturally complex twisted  
87 tendon structure (5, 6), and the difficulty of visualizing individual subtendons using  
88 conventional imaging techniques (ultrasound or magnetic resonance imaging).

89 In recent studies, ruptured ATs have been found to display more uniform within-tendon  
90 displacement 1-year post-rupture (13, 14). In addition to an increase in length of the tendon,  
91 ATR leads to morphomechanical changes in the triceps surae muscles and subtendons (15,  
92 16). These changes seem to occur regardless of whether they were treated surgically or  
93 conservatively (13, 14), and might alter the force transmission mechanism in the muscle-  
94 tendon unit.

95 Voluntary contraction typically activates all synergistic muscles to a variable degree (17, 18)  
96 and leads to disproportionate tissue displacement within the tendon due to mechanical and  
97 structural differences between triceps surae muscles (19). During voluntary contractions,  
98 complex neuromuscular control of the triceps surae within and across healthy and injured  
99 individuals may confound interpretations of tissue displacement in adjacent subtendons. By  
100 removing the effects of neural control, one could potentially identify if changes in structure  
101 and material properties due to ATR modify the displacement pattern within the AT. Electrical  
102 transcutaneous stimulation can be used to stimulate a given muscle selectively (20, 21).  
103 Using this method, it can be assumed that selective activation of one of the triceps surae  
104 muscles induces serial force transmission that is observed as tendon displacement mainly in  
105 the area containing tendon fascicles arising from the activated muscle belly. Therefore, the  
106 stimulation method may also help to understand AT subtendon organization in vivo.

107 By using selective transcutaneous stimulation to medial and lateral gastrocnemius muscles  
108 we aimed to find out whether AT tissue displacement pattern differs in voluntary contraction

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109 and electrically evoked contractions between injured (INJ) and un-injured (UNJ) tendons.  
110 Examination of the displacement patterns during selective activation was expected to yield  
111 information about the representative areas of AT subtendons. We hypothesized that different  
112 contraction types would lead to different displacement patterns. Furthermore, it was  
113 hypothesized that INJ tendon would show less, and more uniform displacement compared to  
114 the UNJ tendon.

## Achilles MG and LG subtendon representation

### 115 **Methods**

#### 116 **Participants**

117 Twenty-eight ATR patients (24 males, 4 females) treated at the Central Finland Health Care  
118 District agreed to participate (Table 1). ATR was diagnosed according to the American  
119 Academy of Orthopaedic Surgeons guidelines. Inclusion criteria were a minimum of 2 of the  
120 following 4 criteria: a positive Thompson test, decreased plantarflexion strength, presence of  
121 a palpable gap, and increased passive ankle dorsiflexion with gentle manipulation.  
122 Participants with re-occurring rupture were treated surgically and excluded from the sample,  
123 which contains only individuals with non-surgical treatment and early mobilization (22). This  
124 study was approved by the Ethics committee of Central Finland health care district  
125 (2U/2018). Participants signed an informed consent explaining the details of the study,  
126 possible risks, and  
127 gave permission to use data for research purposes. Participants were invited to the laboratory  
128 1-year  $\pm$  1.8 months after rupture.

129

#### 130 **Experimental procedure**

131 B-mode ultrasound was used to examine tendon properties. Scans were done using a 3.6-cm  
132 linear probe (UST-5411, Aloka alpha10, Japan). First, the subtendon lengths of MG, LG and  
133 SOL were measured from a resting prone position with the subjects' feet over the edge of a  
134 table. The limb was scanned to find the most distal point of the muscle-tendon junction of  
135 each muscle head and the tendon insertion on the calcaneus, all of which were marked on the  
136 skin. The distance between the points was then measured with a measuring tape (23). The  
137 reliability of this method was tested, whereby four un-injured limbs was measured on two  
138 separate days. The subtendon lengths of the triceps surae muscles were measured and the  
139 intraclass correlation coefficient (ICC) was calculated (24). ICC was 0.99 (90% CI 0.97-



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140 0.99) with a coefficient of variation (CV) of 6.6%. Ultrasound imaging was then used to  
141 locate the thickest part of both gastrocnemius muscles, where the stimulating electrodes were  
142 placed. Participants' skin was shaved and cleaned with alcohol to ensure good conductivity.  
143 A pair of 32 mm diameter electrodes (Niva Medical Oy) was attached over each muscle with  
144 ~1 cm inter-electrode distance. During measurements, participants sat in a custom-made  
145 ankle dynamometer (University of Jyväskylä, Finland) with the hip at 120°, knee at 0° (fully  
146 extended), and the ankle and first metatarsophalangeal joints at 90° and 0° respectively. The  
147 foot was strapped to the dynamometer pedal and the thigh secured to the seat above the knee.  
148 To image tendon displacement, the ultrasound probe was attached longitudinally with the  
149 distal edge ~2 cm above the calcaneus.

150 A warm-up was done in the form of a series of standardized submaximal contractions.  
151 Starting with UNJ, unilateral maximal voluntary isometric contractions (MVCs) were  
152 performed followed by contractions corresponding to 30% of UNJ MVC. Then, with the  
153 participant relaxed, single stimulation pulses were elicited with increasing intensity using a  
154 constant current electrical stimulator (DS7AH; Digitimer, Hertfordshire, UK) until the motor  
155 threshold was exceeded, as confirmed by a visible muscle twitch (20, 21). If a corresponding  
156 displacement was not observed clearly in the US image of the AT, higher stimulation  
157 intensity was used. AT displacement was imaged 1 s before and throughout a tetanic pulse of  
158 1000  $\mu$ s at 100 Hz at the pre-determined stimulation intensity. MG and LG were stimulated in  
159 random order. The entire protocol was then repeated for INJ, starting with voluntary  
160 isometric contractions, followed by electrically induced contractions.

161 Force data were collected via a strain gauge transducer in the foot pedal of the ankle  
162 dynamometer. A potentiometer placed under the heel was used to detect heel lift during  
163 contractions. Data were sampled at 1 kHz via a 16-bit A/D board (Power 1401, Cambridge  
164 Electronic Design, Cambridge, UK) connected to the computer, and signals were recorded

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165 using Spike2 software (Cambridge Electronic Design, Cambridge, UK). To synchronize data,  
166 a TTL-pulse was sent manually via Spike2 to first trigger the data acquisition with the US  
167 device for 8 seconds and after 1 s to deliver the 0.7 s tetanus to either MG or LG. Ultrasound  
168 videos were sampled at 50 HZ and stored for further offline analysis.

### 169 **Data analysis**

170 Ultrasound B-mode image analysis of tendon displacement was done using a speckle tracking  
171 algorithm implemented in Matlab (R2020a, MathWorks Inc, Natick, MA, USA) according to  
172 the previously validated and published configuration of Slane and Thelen (9, 25). The region  
173 of interest location and size were defined for each subject manually to ensure that only  
174 tendon tissue was analysed. A grid of six nodes across the width of the tendon and eleven  
175 across the length of the tendon was generated (14). All tracking results were visually  
176 inspected to ensure that the nodes remained inside the tendon throughout the movement.  
177 Incremental displacements were fitted with a low-order polynomial (25). Displacements of  
178 nodes along each of the six antero-posterior rows were averaged and peak displacement of  
179 the average data were extracted for analysis. The six locations across the tendon starting from  
180 the posterior part to the anterior part are referred to as locations 1-6, respectively. The  
181 average peak displacement across the six locations was used to represent mean displacement.  
182 Locations of the maximum and minimum displacement were extracted. Tendon non-  
183 uniformity was expressed as the difference between minimal and maximal displacement in  
184 the tendon. To facilitate the comparison of displacement patterns between electrically  
185 induced contractions and volitional activation, the displacement data were normalized to a  
186 range between 0-1 where 0 is minimum displacement location and 1 is maximum  
187 displacement location. The relative displacement relation between 6 locations across the  
188 tendon is hereafter referred to as the displacement pattern. Displacement was normalized  
189 since voluntary contraction produced higher torque and overall AT displacement than

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190 electrically induced contractions. Peak torque was calculated for both voluntary and  
191 electrically induced contractions.

192

### 193 **Statistical analysis**

194 Statistical analysis was performed using JASP (JASP version 0.14.1, Amsterdam,  
195 Netherlands). The level of significance was set at  $p < 0.05$ . Three-way repeated-measures  
196 ANOVA was performed to investigate the effects of contraction type (VOL, MG, and LG  
197 stimulations), limb condition (INJ vs UNJ) and tendon location (across 6 locations) on the  
198 normalized displacement of the tendon. The main interest of the analysis is in three- and two-  
199 way interaction effects, indicating how the displacements are distributed between the tendon  
200 locations (i.e. are affecting the displacement pattern) in the different conditions and limbs. If  
201 significant three-way interactions were detected, two-way analysis was performed, followed  
202 by simple pairwise comparisons with Bonferroni-adjustment when a significant main effect  
203 was found. Greenhouse-Geisser adjustment was applied when the assumption of sphericity  
204 was violated. Skewness and kurtosis was checked to insure the normality of the data. If  
205 outliers were detected, the test was done with (i.e. the entire sample) and without the outlier.  
206 Limb differences (UNJ vs INJ) in AT non-uniformity, displacement amplitude, maximum  
207 and minimum displacement locations were compared using two-sided paired t-tests.

208

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### 209 **Results**

210 Free tendon length below the SOL muscle insertion site was significantly longer in INJ  
211 compared to UNJ with a mean difference (95%CI) of 1.6 cm (0.6-2.6 cm;  $p=0.003$ ). The INJ  
212 MG subtendon was also longer by 2.1 cm (1.5–2.7 cm;  $p<0.01$ ), and LG by 1.9 cm (1.2–2.6  
213 cm;  $p<0.01$ ) than in UNJ. There were no statistically significant differences in stimulation  
214 threshold or intensity between limb muscles or between limbs (Table 2).

215

216 Absolute displacement values and torque levels are reported in (Table 3). There was no  
217 statistically significant difference in stimulation evoked torque levels between limbs in  
218 response to stimulation of either muscle despite the stimulation inducing a significantly  
219 higher mean displacement in both INJ in muscles compared to UNJ. The mean (SD)  
220 magnitude of heel lift during electrically induced contractions was 0.04 mm (0.5) and 2.5 mm  
221 (4.0) during voluntary contractions.

222

### 223 **Voluntary and stimulation-induced displacement patterns**

224 To explore the differences in displacement patterns, the absolute values of the 6 locations  
225 were normalized to enable comparison between VOL and stimulation conditions (Figure 1).  
226 Three-way repeated-measures ANOVA was performed to evaluate the effects of contraction  
227 type, location and limb condition on tendon displacement. There was a significant two-way  
228 interaction of contraction type\*location on the tendon displacement ( $F(10-978) = 3.7$ ,  
229  $p<0.001$ ). Initial three-way analysis was followed by a two-way repeated-measures ANOVA  
230 for the effect of contraction type\*location on tendon displacement at the two levels of limb  
231 condition and the location\*limb condition on tendon displacement at each contraction type  
232 level.

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233 There was no significant location\*limb condition interaction effect on tendon displacement at  
234 each contraction type level. There was a significant contraction type\*location interaction  
235 effect on tendon displacement ( $F(10-492) = 3.8, p < 0.001$ ) at the UNJ limb, while the  
236 interaction effect was not significant for the INJ limb ( $F(10-486) = 1.11, p = 0.353$ ). Simple  
237 pairwise comparisons were done between the contraction types for the UNJ with a Bonferroni  
238 adjustment applied. The analysis showed that the  $LG_{stim}$  displacement pattern was  
239 significantly different to  $MG_{stim}$  ( $p = 0.007$ ), and VOL ( $p = 0.003$ ) (Figure 1). Individual  
240 displacement patterns are shown in (Figure 2).

241 In UNJ, maximum displacement during  $MG_{stim}$  occurred most frequently in the three most  
242 anterior locations, while during  $LG_{stim}$ , maximum displacement occurred most often in the  
243 most anterior (6<sup>th</sup>) location (Figure 4). This pattern was also found in INJ, where the most  
244 frequent locations of maximum displacement during  $MG_{stim}$  were in the anterior half of the  
245 tendon (frequency of maximal displacement: 4<sup>th</sup>: 21.4%, 5<sup>th</sup>: 32.2% and 6<sup>th</sup>: 32.7%), while  
246 during  $LG_{stim}$ , maximum displacement occurred in the 6<sup>th</sup> location in 48.2% of participants.  
247 Minimum displacement was found in the most posterior location for the stimulation of both  
248 muscles in both limbs. There was no statistically significant difference in maximum or  
249 minimum displacement location between limbs.

250

### 251 **Tendon non-uniformity and displacement amplitude during electrical stimulation**

252 Tendon non-uniformity was higher in UNJ compared with INJ with a mean difference  
253 (95%CI) of 0.11 mm (0.04 – 0.18mm,  $p = 0.005$ ) during  $MG_{stim}$ , and 0.09 mm (0.03 – 1.42  
254 mm,  $p < 0.001$ ) during  $LG_{stim}$  (Figure 3). When non-uniformity was compared between  
255 stimulated muscles in the same limb, there was no statistically significant difference in either  
256 limb, with a mean difference (95%CI) of 0.016 mm (-0.06 – 0.09 mm) for UNJ and 0.003

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257 mm (-0.05 – 0.05 mm) for INJ. One outlier was detected in LG<sub>stim</sub> mean displacement group  
258 (Higher range:1.9mm, outlier:2.7mm), when the whole sample was used there was no  
259 significant difference in mean tendon displacement of the INJ between the contractions  
260 induced when stimulating different muscles, with a mean difference (95%CI) of 0.28 mm (-  
261 0.004 – 0.56 mm, p=0.053), however when the test was done without the outlier there was a  
262 significant difference (95%CI) of mean difference of 0.34 mm (0.007 – 0.61 mm, p=0.015).  
263 In the UNJ, there was a significant difference in the mean displacement depending on the  
264 stimulated muscle, with a greater displacement when MG was stimulated (95%CI) of mean  
265 difference of 0.22 mm (0.04 – 0.40 mm, p=0.016).

266

267 **Discussion**

268 In this study, we examined internal AT displacement patterns during voluntary and selective  
269 transcutaneous stimulation of medial and lateral gastrocnemius to investigate differences  
270 within the AT tissue displacement between INJ and UNJ limbs of patients after AT rupture  
271 and to inspect the representative areas of subtendons. The lowest stimulation intensity that  
272 induced a visible contraction was used to ensure selective activation of only the targeted  
273 muscle. As hypothesized, displacement patterns during voluntary and electrically induced  
274 contractions were different; the displacement pattern was significantly different during LG<sub>stim</sub>  
275 compared to VOL and MG<sub>stim</sub> in both limbs. There was no statistically significant difference  
276 when the displacement patterns were compared for each stimulated contraction between  
277 limbs. Thus, with the assumption that the stimulation-induced force is primarily serially  
278 transmitted to tendon fascicles, the subtendon organization does not seem to be altered in the  
279 non-surgically treated limb of ATR patients. In UNJ, peak tendon displacement during  
280 MG<sub>stim</sub> tended to occur more posteriorly compared to VOL. Overall, the anterior half of the  
281 AT underwent larger displacement than the superficial posterior part in all contraction  
282 conditions.

283 Despite higher mean displacement in INJ, displacement was more uniform when compared to  
284 UNJ during contractions induced by stimulating MG and LG. Tendon stiffness also seemed  
285 to be lower in INJ, since ankle joint torque was similar during muscle stimulations in both  
286 limbs, but the displacement was larger in INJ than in UNJ. However, this was not observed  
287 for voluntary contractions in which tendon mean tendon displacement did not differ between  
288 the limbs. Marked inter-individual differences were observed in internal tendon motion.  
289 Thus, when investigating AT anatomical organization and internal force sharing, an  
290 individualized approach might help to understand AT force sharing mechanisms and tendon  
291 recovery from injury.

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### 292 **Voluntary vs. stimulated contractions**

293 Internal tendon displacement patterns were different during LG<sub>stim</sub> compared to VOL and  
294 MG<sub>stim</sub> in UNJ. In VOL, peak displacement was typically found in the two most anterior  
295 locations. Voluntary contraction leads to disparate tissue displacement within the tendon due  
296 to disproportionate activation of synergistic muscles and mechanical structural differences  
297 between triceps surae muscles (7, 19). On the other hand, the low, stimulation-induced force  
298 can be assumed to be mainly transmitted serially to the targeted muscle's subtendon (26).  
299 Although lateral force transmission may occur (21), the main pathway of force is the stiffest  
300 structure. Hence, the location of peak displacement in response to stimulation can be  
301 considered to reveal the location of tendon fascicles within the cross-section of AT.

302 Displacement during LG<sub>stim</sub> peaked in the anterior tendon, implying that the most anterior area  
303 could be occupied by tendon fascicles arising from LG subtendon. In anatomical studies,  
304 Pękala et al. (2017) and Edama et al. (2015) found that SOL occupied the anterior portion and  
305 LG the lateral portion of the tendon at the level of the SOL muscle-tendon junction.  
306 However, due to high torsion within AT, LG tendon fascicles are likely located anteriorly in  
307 the more distal tendon (5, 6). Furthermore, in a recent study, three tendons were dissected,  
308 and 3D computer aided models were constructed based on these tendons. In the model that  
309 twisted the most, LG subtendon was found to completely occupy the anterior portion of the  
310 distal AT (27). Therefore, anatomical studies are consistent with the present observations  
311 regarding the location of the LG subtendon.

312 There was no difference between VOL and MG<sub>stim</sub> displacement patterns (Figure 1).  
313 However, during MG<sub>stim</sub> displacement peaked around the 4th and 5th locations in UNJ,  
314 indicating that fascicles originating from MG could be present in the mid-to-anterior part of  
315 the tendon. Unlike in LG<sub>stim</sub>, there was more individual variation in the location of peak  
316 displacement in MG<sub>stim</sub>. Due to individual differences in free tendon length, the superior-



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317 inferior field of view may not have been consistent across subjects relative to tendon length.  
318 When comparing these observations to the anatomical maps provided by previous cadavers  
319 studies, natural anatomical variation may explain the observed heterogeneity in peak  
320 displacement in response to MG<sub>stim</sub> (5, 6, 27).

321 In addition to the anatomical origin, the observed peak displacement locations may have been  
322 affected by lateral force transmission between different subtendons within the AT. Each  
323 subtendon transmits the force from a single muscle belly but not fully independently, and  
324 force could be laterally transmitted between triceps surae muscle bellies or even subtendons  
325 (28, 29). AT force and subsequent displacement might be distributed unevenly with a bias  
326 toward the SOL subtendon since SOL subtendon fascicles have been found previously to be  
327 compliant in rats (29) and in human cadavers (30) although contradictory results have also  
328 been reported (27). This raises questions about the forces transmitted through connective  
329 tissue or inter-fascicular matrix, which could be crucial for force transmission mechanisms  
330 and inter-fascicular gliding within the tendon (10, 31).

331 In summary, during VOL and electrically induced contractions of MG and LG, minimum  
332 displacement always occurred in the posterior tendon and maximum displacement in the mid-  
333 to-anterior tendon. The same observation was made in a recent study where SOL and MG  
334 were electrically stimulated (32), and the tendon was split into two halves for analysis  
335 purposes; the anterior half always displaced the most in response to MG and SOL  
336 stimulations in different ankle positions. This is consistent with the observations made in this  
337 study as we found that the mid-to-deep part of the tendon displaced most when MG was  
338 stimulated and the deep part when LG was stimulated. The difference between the two  
339 studies is in the interpretation of the data in regard to which subtendon are presented in the  
340 deep part of the tendon. In Lehr et al. the tendon was split in consideration to the function-  
341 structure relationship (11, 12), and the authors interpreted a larger non-uniformity and

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342 displacement in the representative part of the stimulated muscle tendon when SOL was  
343 stimulated compared to MG as an evidence of consistency with the anatomical function-  
344 structure consideration (32). We relied on the principle that the main pathway of force is the  
345 stiffest structure. Thus, when a muscle is selectively stimulated the arising regional tendon  
346 displacement can inform us about regions of the tendon corresponding to fascicles arising  
347 from different triceps surae muscles. Based on the beforementioned we found that the  
348 gastrocnemius subtendons are located in the mid-to-anterior part of the tendon, and that LG is  
349 probably located most anteriorly.

350

351 Gastrocnemius and soleus have different functional roles, despite having a common distal  
352 tendon and working synergistically as ankle plantar flexors (33, 34). It has been suggested  
353 that in order to perform their differing functional roles, these muscles rely on the ability of  
354 the subtendons to displace relative to each other (35, 36). It is of interest to investigate if  
355 normal subtendon organization can be restored after ATR as this most likely is a prerequisite  
356 for restoring normal Achilles tendon and triceps surae function including the functional  
357 independence of the muscles. If tendon fascicles that were originally part of different  
358 subtendons would merge during the healing process this could result in reduced capacity for  
359 relative movement between subtendons and disruption of the normal function of the Achilles  
360 tendon. In fact, ATR followed by surgical reconstruction has been shown to reduce non-  
361 uniform tendon motion observed using speckle tracking (8, 37). Our tendon displacement  
362 data (Figure 2) and our previous report (14) suggest that there are considerable individual  
363 variations in the subtendon organization in both ruptured and un-injured tendons. This  
364 signifies the importance of an individualized assessment and interpretation of the subtendon  
365 organization and function after ATR.

366

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### 367 **Tendon non-uniformity and displacement amplitude during electrical stimulation**

368 Consistent with previous studies, we found a more uniform displacement pattern in INJ  
369 compared to the contralateral tendon 1-year post rupture (13, 14), suggesting impaired sliding  
370 within the injured tendon. Limited inter-fascicular sliding might be a result of interfascicular  
371 matrix adhesions caused by the rupture (10). Mean displacement in INJ was higher than in  
372 UNJ. As the same amount of torque was produced during stimulation, this result suggests  
373 lower stiffness in INJ. However, this differs from our previous results, where we reported that  
374 stiffness of the entire MG tendon during isometric voluntary contraction was similar between  
375 injured and un-injured tendons 1-year post rupture (38). This would suggest that in the free  
376 distal AT, mechanical properties (stiffness) may be altered locally and manifest themselves at  
377 low force levels, while globally stiffness seems to be similar between limbs. This discrepancy  
378 between our observations could indicate an extension of the toe region, or slackness of the  
379 tendon in the INJ limb while the linear region of the force-displacement curve would be  
380 similar between the limbs. A similar phenomenon of an extended range of tendon strain at  
381 low stresses has been reported previously after 4 weeks of limb unloading by suspension  
382 (39).

383

384 We found no differences between limbs (UNJ vs INJ) in the locations of maximum or  
385 minimum displacement when stimulating either MG or LG, consistent with our previous  
386 findings during voluntary contractions (14). Furthermore, in the three contraction types the  
387 displacement patterns were similar when compared between limbs. Thus, the anatomical  
388 subtendon organization does not seem to be altered after a rupture in non-surgically treated  
389 tendons.

## Achilles MG and LG subtendon representation

### 390 **Limitations**

391 There are several limitations of this study. First, the nature of two-dimensional imaging may  
392 not fully capture the complex three-dimensional behaviour of the triceps surae subtendons,  
393 which could lead to errors when estimating AT tissue displacement. The speckle tracking  
394 algorithm uses a low order polynomial fit to regularize displacement (25). This may reduce  
395 variation in displacement between the six locations across the tendon. However, filtering has  
396 been deemed necessary to reduce noise and erroneous estimates (40), and was applied here in  
397 the same manner as in previous studies (32). Furthermore, it should be noted that LG muscle  
398 has different compartments that are innervated by two main nerves and numerous sub-  
399 branches (41), so stimulation might activate different branches of the muscle causing more  
400 variability to the displacement pattern. Furthermore, selective activation of LG might stiffen  
401 the connective tissue between SOL and LG, facilitating force transmission (29). Thus, the  
402 representation of LG or MG subtendon that we observed within the AT may have been  
403 influenced by lateral force transmission at the level of the muscle or tendon. However, this  
404 effect was likely minimal since it has been shown that lateral force sharing within the human  
405 Achilles tendon is small at low forces (42).

### 406 **Conclusion**

407 To conclude, Achilles tendon displacement patterns were different in response to selective  
408 stimulation of LG compared to MG stimulation or voluntary contraction. Our results suggest  
409 that when imaged from a mid-sagittal view, the gastrocnemius subtendons are located in the  
410 mid-to-anterior part of the tendon, and that LG is probably located most anteriorly. Previous  
411 anatomical studies support these results, but more investigations are needed since results in  
412 the literature are inconsistent. The stimulation method could allow for a more individualized  
413 approach for investigation of tendon organization, that might help to better understand the  
414 complex mechanics and triceps surae subtendon representations within the Achilles tendon.

### **Achilles MG and LG subtendon representation**

415 We found no evidence that non-surgical treatment of ATR alters the displacement pattern  
416 within the tendon suggesting that non-surgical treatment may preserve the normal subtendon  
417 organization. However, differences in displacement amplitude and non-uniformity of the  
418 tendon displacement were present between the limbs in electrically stimulated conditions.  
419 These findings suggest an extended toe region of the tendon force-displacement curve after  
420 ATR and potential adhesions preventing non-uniform displacements.

## Achilles MG and LG subtendon representation

### 421 **Grants**

422 This study was funded by Academy of Finland grant #323168, UNderstanding REStoration  
423 of Achilles Tendon function after rupture (UNRESAT), and in part by Academy of Finland  
424 grant #332915.

425

### 426 **Disclosure**

427 The authors declare that they have no competing interests.

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549

550

## Achilles MG and LG subtendon representation

551 **Figure captions:**

552 Figure 1. Mean normalized displacement patterns  $\pm$  SD during voluntary and selective electrically  
553 induced contractions of the medial (MG) and lateral (LG) gastrocnemius muscles in the un-injured  
554 (left) and injured limb (right). Graphs represent group means Individual patterns in the un-injured  
555 limb are shown in Figure 2. \* Difference between the contraction types ( $p < 0.05$ ).

556

557 Figure 2. Normalized displacement patterns in the un-injured limb. Left: Raw data points for each  
558 participant during voluntary and selective electrical stimulation across the 6 locations of the Achilles  
559 tendon. Right: Box plots of means and SD for each location (1-6 respectively).  
560 \* Difference between the contraction types ( $p < 0.05$ ).

561

562 Figure 3. Tendon displacement (mm) of the whole sample during gastrocnemius muscle stimulation at  
563 each of the six locations across the tendon width. The values are expressed as mean  $\pm$  SD.

564

565 Figure 4. Distribution of peak displacement locations across the tendon in the sagittal view when  
566 medial gastrocnemius (MG, upper) or lateral gastrocnemius (LG, lower) was selectively stimulated in  
567 the un-injured limb.

568

Table 1. Patient characteristics, free Achilles tendon length, and medial and lateral gastrocnemius subtendon lengths (mean  $\pm$  SD).

<b>Participants (N=28)</b>		
Age (years)	42.4 $\pm$ 9.3	
Height (m)	1.76 $\pm$ 0.08	
Body mass (Kg)	82.5 $\pm$ 12.2	
<b>Limb condition</b>	<b>Un-injured</b>	<b>Injured</b>
Free tendon length (cm)	8.79 $\pm$ 3.47	10.36 $\pm$ 3.71
MG subtendon length (cm)	18.90 $\pm$ 1.92	20.99 $\pm$ 2.20
LG subtendon length (cm)	21.59 $\pm$ 1.60	23.51 $\pm$ 1.99

Table 2. Descriptive data of motor thresholds, selective electrical stimulation-induced contractions intensities of medial (MG) and lateral (LG) gastrocnemius muscles in the un-injured and injured limbs, and comparisons between limbs and muscles.

	<b>Injured</b>		<b>Un-injured</b>		<b>P-values comparing stimulations</b>			
	<b>MG</b>	<b>LG</b>	<b>MG</b>	<b>LG</b>	between limbs		between muscles	
					<b>MG</b>	<b>LG</b>	<b>INJ</b>	<b>UNJ</b>
Stimulation intensity mA (SD)	20.36 (9.26)	18.48 (5.63)	17.75 (9.26)	16.07 (7.37)	0.164	0.155	0.413	0.097
Threshold mA (SD)	15.50 (9.75)	15.25 (4.76)	14.07 (7.98)	19.75 (7.71)	0.513	0.408	0.634	0.210

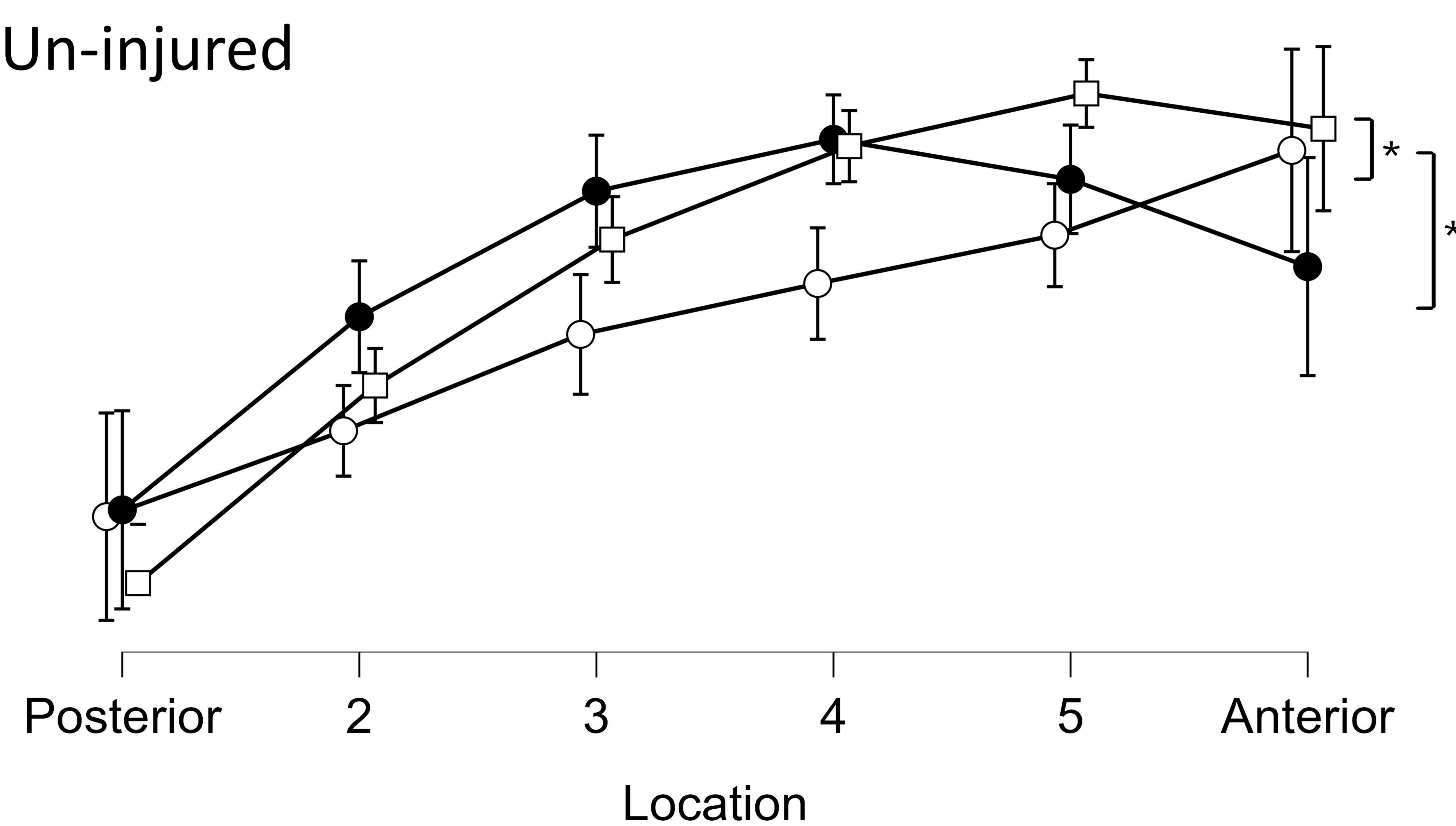
*P*-values using un-adjusted pairwise t-test.

Table 3. Descriptive data of mean displacement, non-uniformity, and absolute torque of electrically induced and voluntary contractions.

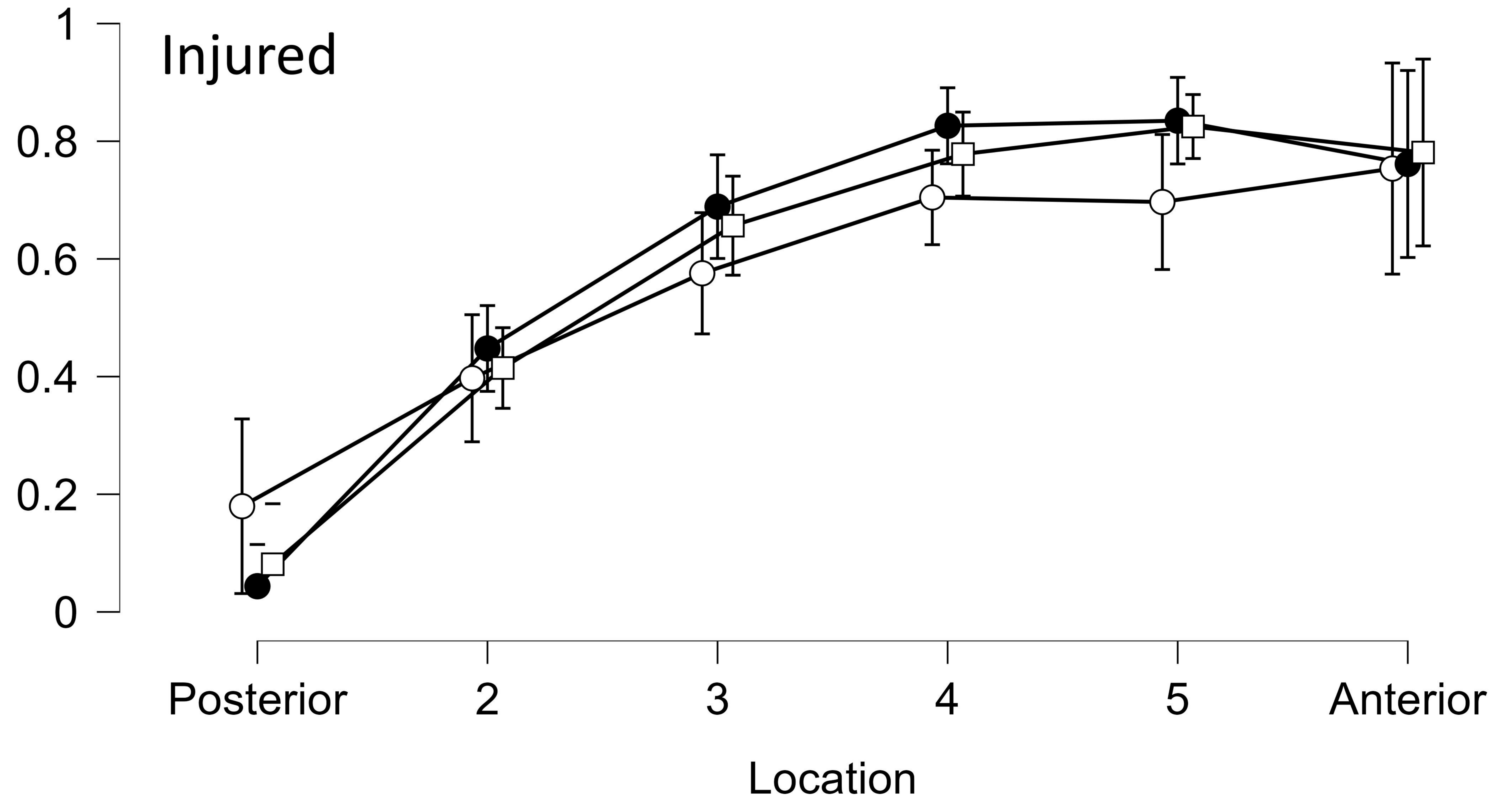
	<b>Injured</b>			<b>Un-injured</b>		
	<b>MG</b>	<b>LG</b>	<b>VOL</b>	<b>MG</b>	<b>LG</b>	<b>VOL</b>
Mean displacement mm (SD)	0.93 (0.65)	0.65 (0.57)	3.52 (1.71)	0.61 (0.48)	0.39 (0.27)	3.63 (1.18)
Tendon non-uniformity mm (SD)	0.14 (0.11)	0.15 (0.12)	0.85 (0.79)	0.25 (0.23)	0.24 (0.17)	1.48 (1.04)
Torque Nm (SD)	5.18 (2.98)	3.24 (2.72)	57.98 (16.30)	5.56 (4.05)	3.67 (2.53)	57.78 (16.23)

Normalized displacement

Un-injured



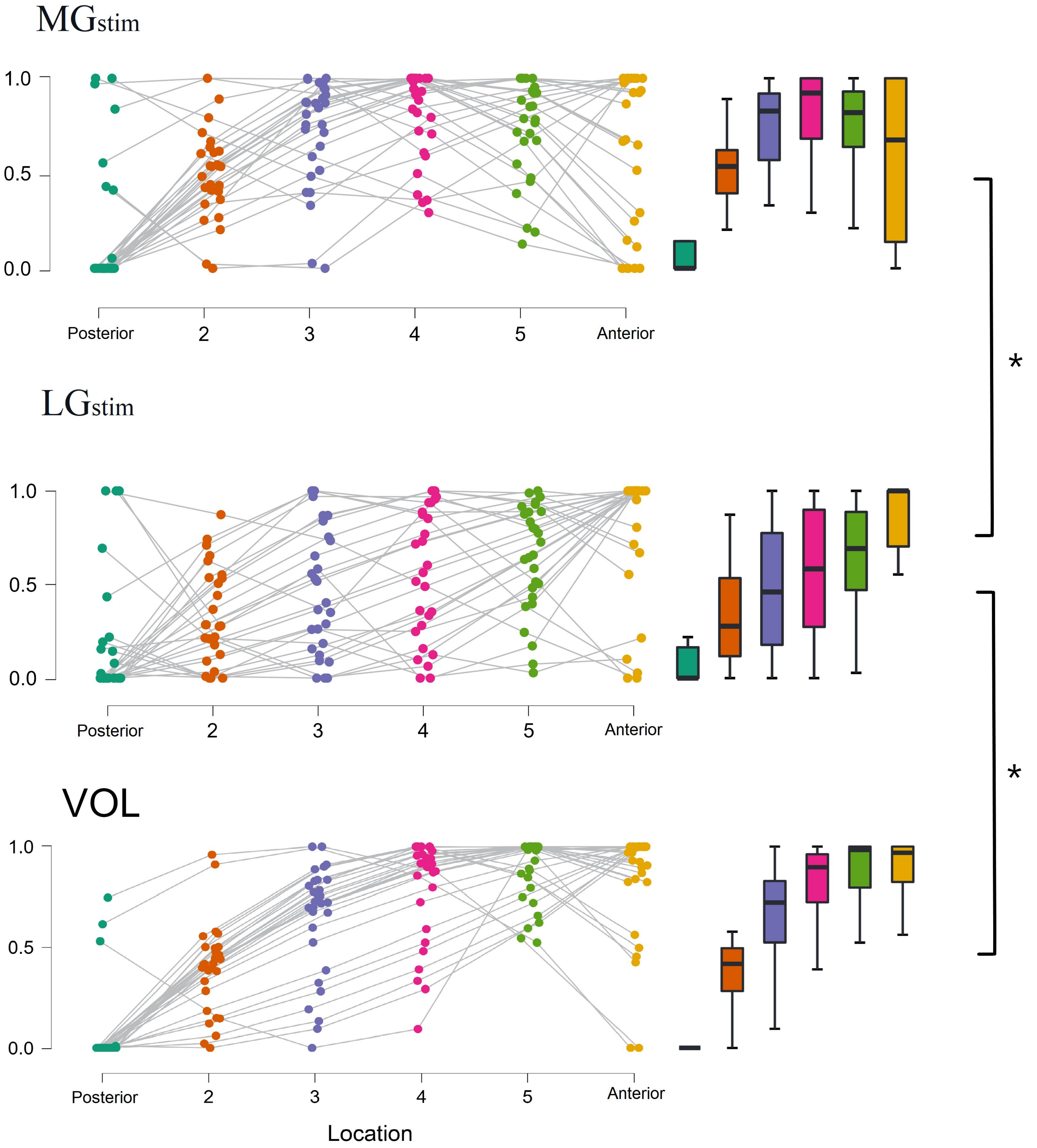
Injured



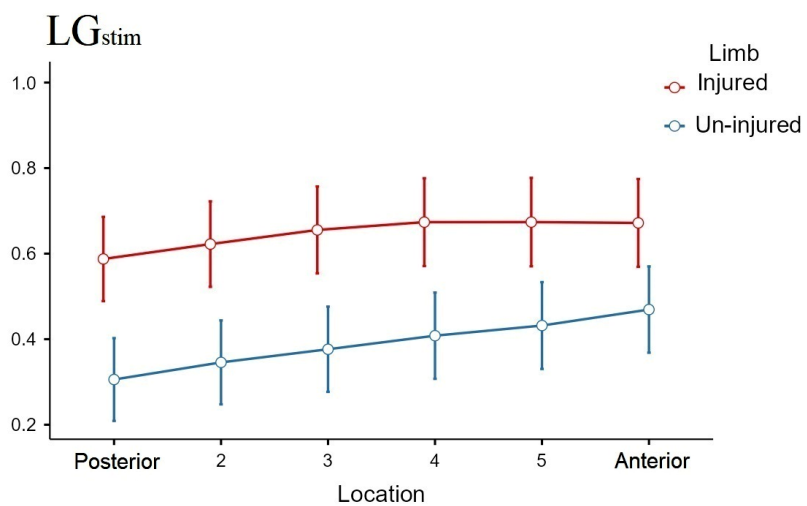
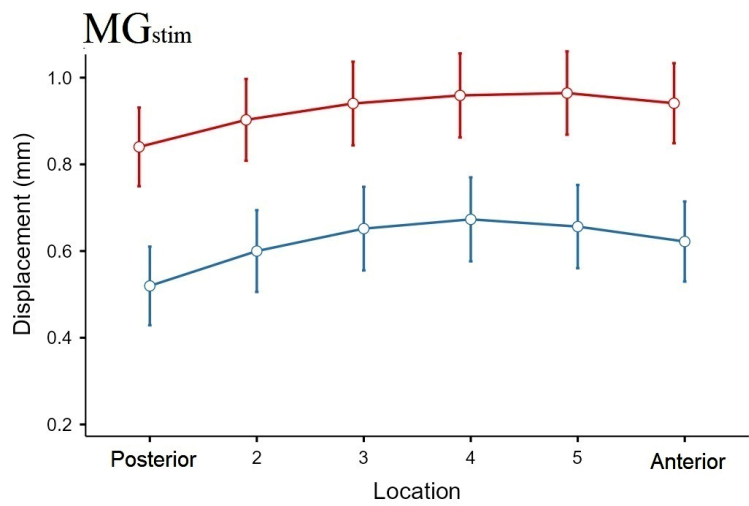
Contraction type

- LG<sub>stim</sub>
- MG<sub>stim</sub>
- VOL

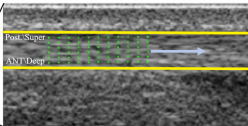
Normalized displacement



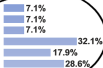




**MG**

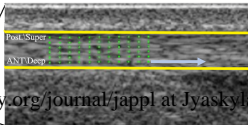


Post./Super



ANT./Deep

**LG**



Post./Super



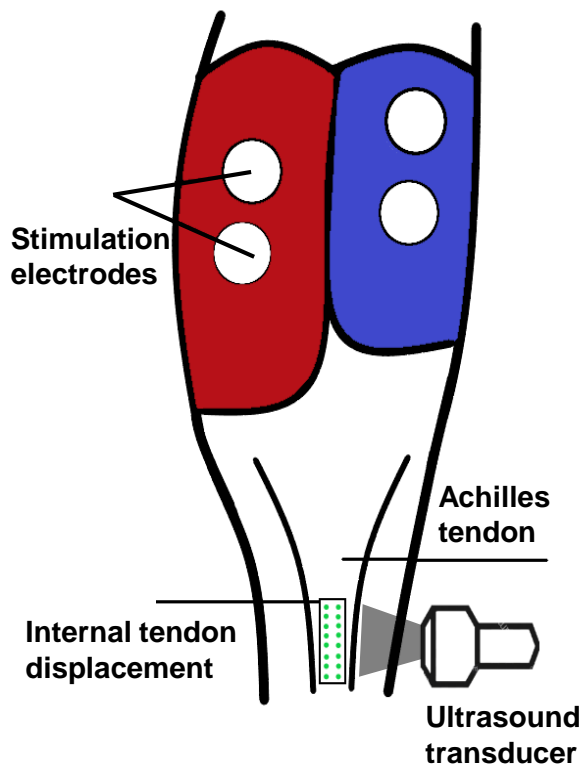
ANT./Deep

# In vivo localised gastrocnemius subtendon representation within the healthy and ruptured human Achilles tendon

N=28

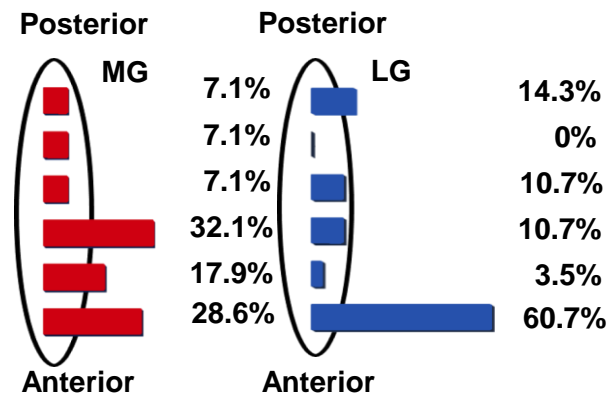
## METHODS

Selective electrical stimulation & Voluntary plantarflexion



## RESULTS

Frequency of maximal displacement locations during selective electrical stimulations



**MG** subtendon → anterior mid region  
**LG** subtendon → most anterior region

## CONCLUSION

When comparing healthy and ruptured tendons one-year post rupture, tendon internal displacement patterns were not different, but absolute displacement amplitude and non-uniformity did differ. **LG** subtendon was found in the most anterior region adjacent to **MG** in both healthy and non-surgically treated tendons.

