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1	In vivo localised gastrocnemius subtendon representation within the healthy and
2	ruptured human Achilles tendon
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#### 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 displacement patterns during voluntary contraction (VOL) and selective transcutaneous 28 29 stimulation of medial (MGstim) and lateral (LGstim) gastrocnemius between ruptured and healthy tendons, and to examine the representative areas of AT subtendons. Twenty-eight 30 patients with unilateral AT rupture performed bilateral VOL at 30% of the maximal 31 isometric un-injured plantarflexion torque. AT displacement was analysed from sagittal B-32 33 mode ultrasonography images during VOL, MG<sub>stim</sub> and LG<sub>stim</sub>. Three-way ANOVA revealed a significant two-way interaction of contraction type\*location on the tendon 34 displacement (F(10-815)=3.72, p<0.001). The subsequent two-way analysis revealed a 35 significant contraction type\*location interaction for tendon displacement (F(10-410)=3.79, 36 37 p<0.001) in the un-injured limb only, where LGstim 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 the there were no difference in the displacement patterns, but displacement amplitudes differed. There was no significant difference in the location of maximum or 40 minimum displacement between limbs. The displacement pattern was not different in non-41 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 medial gastrocnemius. However, free tendon stiffness seems to be lower in the injured AT, 44 45 leading to more displacement during electrically-induced contractions compared to the uninjured. 46

#### 48 New & Noteworthy

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

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

#### 59 Introduction

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

67 The AT has a complex hierarchical structure and is composed of distinct bundles of fascicles running continuously along the tendon, called subtendons. AT subtendons each arises from a 68 different muscular head of the triceps surae: soleus (SOL), medial gastrocnemius (MG), and 69 70 lateral gastrocnemius (LG) (5, 6). The tendon twists so that at the calcaneal tuberosity insertion, the MG fibres are located on the lateral surface, LG fibres more deeply, and the 71 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 heterogeneity of strain within the tendon (7). 76

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

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tendon structure (5, 6), and the difficulty of visualizing individual subtendons using
conventional imaging techniques (ultrasound or magnetic resonance imaging).

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

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

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

#### 115 Methods

#### 116 **Participants**

Twenty-eight ATR patients (24 males, 4 females) treated at the Central Finland Health Care 117 118 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.

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#### **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 separate days. The subtendon lengths of the triceps surae muscles were measured and the 138 139 intraclass correlation coefficient (ICC) was calculated (24). ICC was 0.99 (90% CI 0.97-

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  $\sim 1$  cm inter-electrode distance. During measurements, participants sat in a custom-made ankle dynamometer (University of Jyväskylä, Finland) with the hip at  $120^{\circ}$ , knee at  $0^{\circ}$  (fully 145 146 extended), and the ankle and first metatarsophalangeal joints at  $90^{\circ}$  and  $0^{\circ}$  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  $\sim 2$  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 µs 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.

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

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

169 **Data analysis** 

Ultrasound B-mode image analysis of tendon displacement was done using a speckle tracking 170 171 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

190 electrically induced contractions. Peak torque was calculated for both voluntary and191 electrically induced contractions.

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#### **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 where 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.

#### 209 **Results**

Free tendon length below the SOL muscle insertion site was significantly longer in INJ compared to UNJ with a mean difference (95%CI) of 1.6 cm (0.6-2.6 cm; p=0.003). The INJ 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 cm; p<0.01) than in UNJ. There were no statistically significant differences in stimulation threshold or intensity between limb muscles or between limbs (Table 2).

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

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

224 To explore the differences in displacement patterns, the absolute values of the 6 locations were normalized to enable comparison between VOL and stimulation conditions (Figure 1). 225 226 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.

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

241 In UNJ, maximum displacement during MG<sub>stim</sub> occurred most frequently in the three most 242 anterior locations, while during LG<sub>stim</sub>, maximum displacement occurred most often in the most anterior (6<sup>th</sup>) location (Figure 4). This pattern was also found in INJ, where the most 243 244 frequent locations of maximum displacement during MGstim were in the anterior half of the 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 245 during LG<sub>stim</sub>, maximum displacement occurred in the 6<sup>th</sup> location in 48.2% of participants. 246 Minimum displacement was found in the most posterior location for the stimulation of both 247 muscles in both limbs. There was no statistically significant difference in maximum or 248 249 minimum displacement location between limbs.

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

Tendon non-uniformity was higher in UNJ compared with INJ with a mean difference (95%CI) of 0.11 mm (0.04 – 0.18mm, p=0.005) during MG<sub>stim</sub>, and 0.09 mm (0.03 – 1.42 mm, p<0.001) during LG<sub>stim</sub> (Figure 3). When non-uniformity was compared between stimulated muscles in the same limb, there was no statistically significant difference in either limb, with a mean difference (95%CI) of 0.016 mm (-0.06 – 0.09 mm) for UNJ and 0.003

mm (-0.05 - 0.05 mm) for INJ. One outlier was detected in LG<sub>stim</sub> mean displacement group 257 258 (Higher range:1.9mm, outlier:2.7mm), when the whole sample was used there was no significant difference in mean tendon displacement of the INJ between the contractions 259 260 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 significant difference (95%CI) of mean difference of 0.34 mm (0.007 – 0.61 mm, p=0.015). 262 263 In the UNJ, there was a significant difference in the mean displacement depending on the stimulated muscle, with a greater displacement when MG was stimulated (95%CI) of mean 264 265 difference of 0.22 mm (0.04 - 0.40 mm, p=0.016).

#### 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.

#### 292 Voluntary vs. stimulated contractions

293 Internal tendon displacement patterns were different during LG<sub>stim</sub> compared to VOL and 294 MG<sub>tim</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). Although lateral force transmission may occur (21), the main pathway of force is the stiffest 299 300 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 Pekala 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.

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

inferior field of view may not have been consistent across subjects relative to tendon length.
When comparing these observations to the anatomical maps provided by previous cadavers
studies, natural anatomical variation may explain the observed heterogeneity in peak
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 affected by lateral force transmission between different subtendons within the AT. Each 322 323 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

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.

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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.

#### 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 matrix adhesions caused by the rupture (10). Mean displacement in INJ was higher than in 371 372 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).

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

#### 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, which could lead to errors when estimating AT tissue displacement. The speckle tracking 393 394 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.

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

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#### 426 **Disclosure**

427 The authors declare that they have no competing interests.

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#### 551 Figure captions:

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

556

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

561

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

564

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

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

Participants (N=28)							
Age (years)	$42.4\pm9.3$						
Height (m)	$1.76\pm0.08$						
Body mass (Kg)	$82.5 \pm 12.2$						
Limb condition	Un-injured	Injured					
Free tendon length (cm)	$8.79\pm3.47$	$10.36\pm3.71$					
MG subtendon length (cm)	$18.90 \pm 1.92$	20.99 ±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.

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

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

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

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



# Injured





## MGstim









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

