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

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Abstract

The Achilles tendon (AT) is composed of three distinct in-series elastic subtendons, arising from different muscles in the triceps surae. Independent activation of any of these muscles is thought to induce sliding between the adjacent AT subtendons. We aimed to investigate displacement patterns during voluntary contraction (VOL) and selective transcutaneous stimulation of medial (MG_{stim}) and lateral (LG_{stim}) gastrocnemius between ruptured and healthy tendons, and to examine the representative areas of AT subtendons. Twenty-eight patients with unilateral AT rupture performed bilateral VOL at 30% of the maximal isometric un-injured plantarflexion torque. AT displacement was analysed from sagittal B-mode ultrasonography images during VOL, MG_{stim} and LG_{stim}. Three-way ANOVA revealed a significant two-way interaction of contraction type*location on the tendon displacement ($F(10\text{-}815)=3.72$, $p<0.001$). The subsequent two-way analysis revealed a significant contraction type*location interaction for tendon displacement ($F(10\text{-}410)=3.79$, $p<0.001$) in the un-injured limb only, where LG_{stim} displacement pattern was significantly different from MG_{stim} ($p=0.008$) and VOL ($p=0.005$). When comparing contraction types 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 minimum displacement between limbs. The displacement pattern was not different in non-surgically treated compared to un-injured tendons one-year post rupture. Our results suggest 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, leading to more displacement during electrically-induced contractions compared to the un-injured.

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.

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

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.

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 different muscular head of the triceps surae: soleus (SOL), medial gastrocnemius (MG), and 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 SOL fibres on the medial surface (5, 6). The degree of twist varies among individuals and can be classified into three types (5). This variation might lead to interindividual differences in the location of the MG, LG and SOL tendon fascicles along the length of the tendon (5). Due to this structure, AT is subjected to complex non-uniform loading that can cause heterogeneity of strain within the tendon (7).

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 non-uniformity 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

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 morphomecahnical 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

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.

Methods

Participants

Twenty-eight ATR patients (24 males, 4 females) treated at the Central Finland Health Care District agreed to participate (Table 1). ATR was diagnosed according to the American Academy of Orthopaedic Surgeons guidelines. Inclusion criteria were a minimum of 2 of the following 4 criteria: a positive Thompson test, decreased plantarflexion strength, presence of a palpable gap, and increased passive ankle dorsiflexion with gentle manipulation. Participants with re-occurring rupture were treated surgically and excluded from the sample, which contains only individuals with non-surgical treatment and early mobilization (22). This study was approved by the Ethics committee of Central Finland health care district (2U/2018). Participants signed an informed consent explaining the details of the study, possible risks, and

gave permission to use data for research purposes. Participants were invited to the laboratory 1-year ± 1.8 months after rupture.

Experimental procedure

B-mode ultrasound was used to examine tendon properties. Scans were done using a 3.6-cm linear probe (UST-5411, Aloka alpha10, Japan). First, the subtendon lengths of MG, LG and SOL were measured from a resting prone position with the subjects' feet over the edge of a table. The limb was scanned to find the most distal point of the muscle-tendon junction of each muscle head and the tendon insertion on the calcaneus, all of which were marked on the skin. The distance between the points was then measured with a measuring tape (23). The 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 intraclass correlation coefficient (ICC) was calculated (24). ICC was 0.99 (90% CI 0.97-

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

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

Data analysis

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

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 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.
208

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.

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 effect on tendon displacement ($F_{(10-492)} = 3.8$, $p<0.001$) at the UNJ limb, while the interaction effect was not significant for the INJ limb ($F_{(10-486)} = 1.11$, $p=0.353$). Simple pairwise comparisons were done between the contraction types for the UNJ with a Bonferroni adjustment applied. The analysis showed that the LG_{stim} displacement pattern was significantly different to MG_{stim} ($p=0.007$), and VOL ($p=0.003$) (Figure 1). Individual displacement patterns are shown in (Figure 2).

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

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_{stim}, and 0.09 mm (0.03 – 1.42 mm, $p<0.001$) during LG_{stim} (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_{stim} mean displacement group (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 induced when stimulating different muscles, with a mean difference (95%CI) of 0.28 mm (-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). 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 difference of 0.22 mm (0.04 – 0.40 mm, p=0.016).

Discussion

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

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

292 **Voluntary vs. stimulated contractions**

293 Internal tendon displacement patterns were different during LG_{stim} compared to VOL and
294 MG_{tim} 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_{stim} 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_{stim} displacement patterns (Figure 1).
313 However, during MG_{stim} 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_{stim}, there was more individual variation in the location of peak
316 displacement in MG_{stim}. Due to individual differences in free tendon length, the superior-

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_{stim} (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

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

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

Limitations

There are several limitations of this study. First, the nature of two-dimensional imaging may 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 algorithm uses a low order polynomial fit to regularize displacement (25). This may reduce variation in displacement between the six locations across the tendon. However, filtering has been deemed necessary to reduce noise and erroneous estimates (40), and was applied here in the same manner as in previous studies (32). Furthermore, it should be noted that LG muscle has different compartments that are innervated by two main nerves and numerous sub-branches (41), so stimulation might activate different branches of the muscle causing more variability to the displacement pattern. Furthermore, selective activation of LG might stiffen the connective tissue between SOL and LG, facilitating force transmission (29). Thus, the representation of LG or MG subtendon that we observed within the AT may have been influenced by lateral force transmission at the level of the muscle or tendon. However, this effect was likely minimal since it has been shown that lateral force sharing within the human Achilles tendon is small at low forces (42).

Conclusion

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

The authors declare that they have no competing interests.

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Figure captions:

Figure 1. Mean normalized displacement patterns ± 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 ($p < 0.05$).

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 ($p < 0.05$).

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 ± SD.

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 ± SD).

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

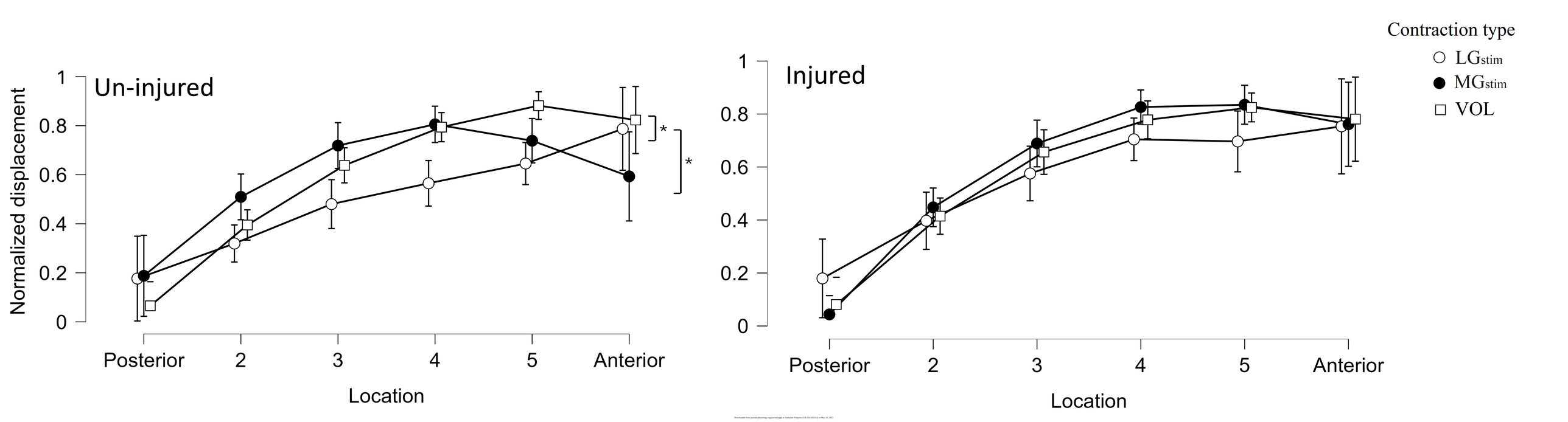
Table 2. Descriptive data of motor thresholds, selective electrical stimulation-induced contractions intensities of medial (MG) and lateral (LG) gastrocnemius muscles in the un-injured and injured limbs, and comparisons between limbs and muscles.

	Injured		**Un-injured**		***P*-values comparing stimulations**			
	MG	**LG**	**MG**	**LG**	between limbs		between muscles	
					MG	**LG**	**INJ**	**UNJ**
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.

	Injured			**Un-injured**		
	MG	**LG**	**VOL**	**MG**	**LG**	**VOL**
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)



MGstim

LGstim

VOL

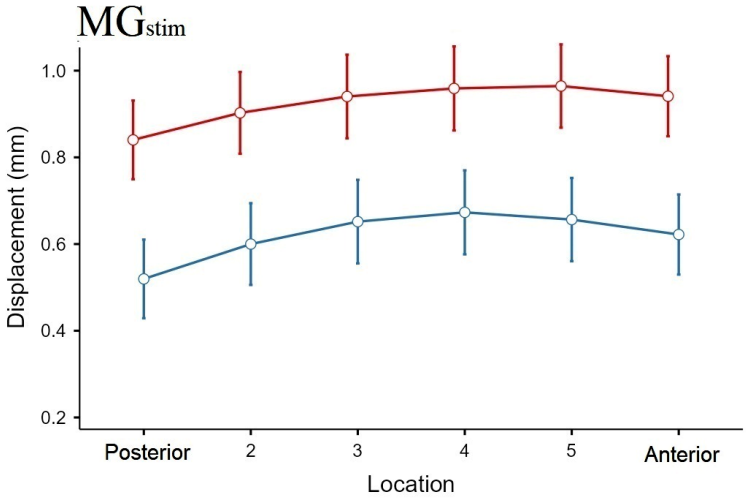
Normalized displacement

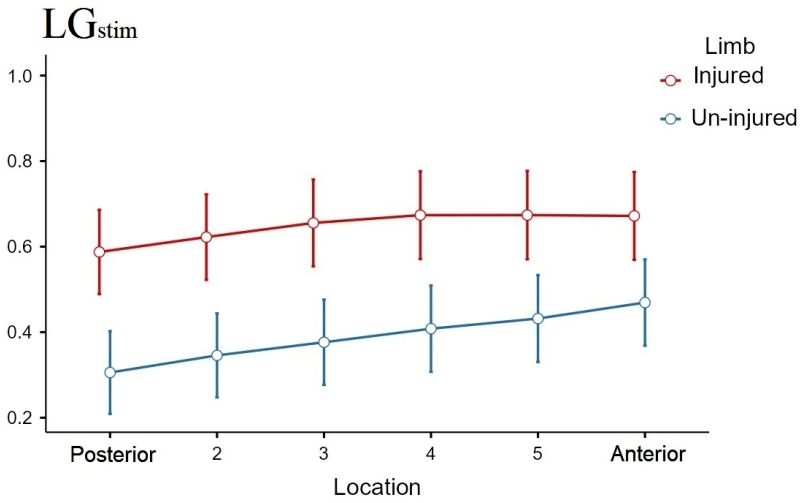
1.0
0.5
0.0

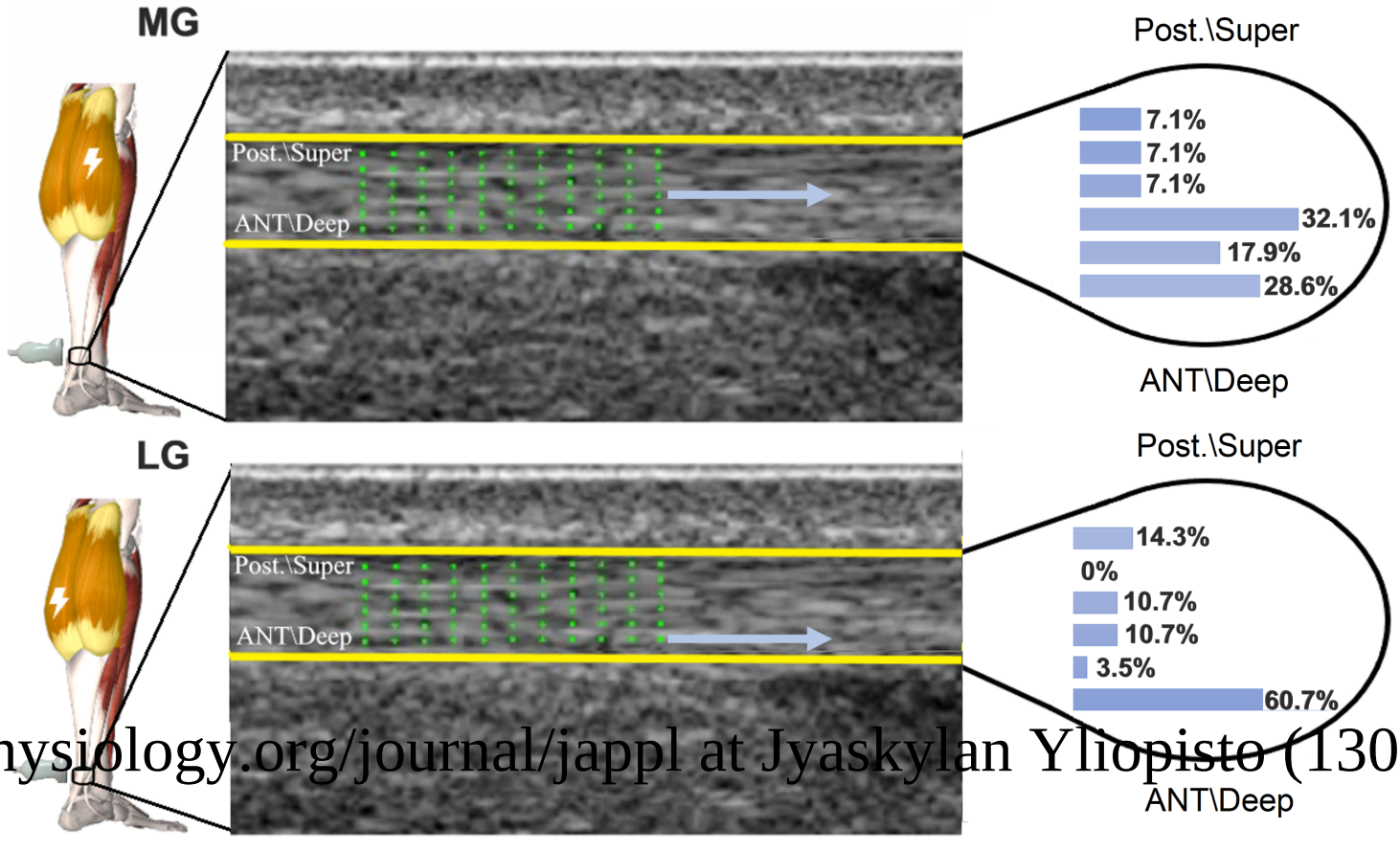
Posterior
2
3
4
5
Anterior

Location

*
*





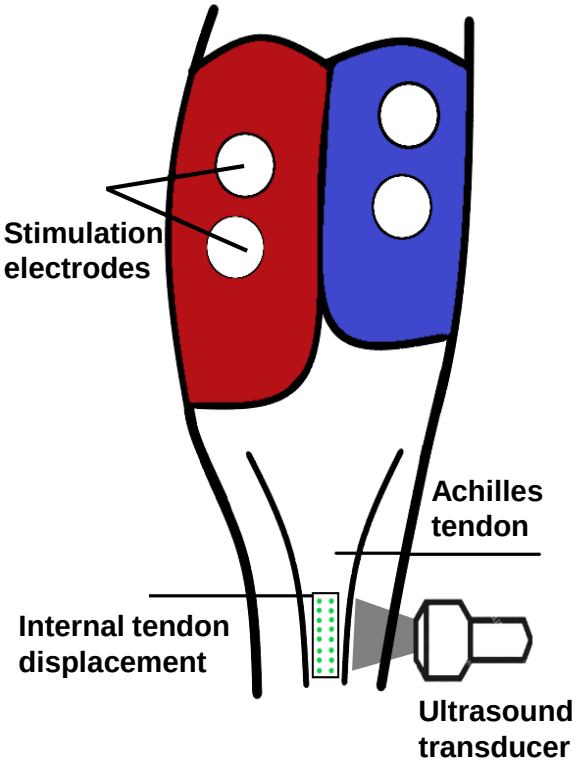


In vivo localised gastrocnemius subtendon representation within the healthy and ruptured human Achilles tendon

N=28

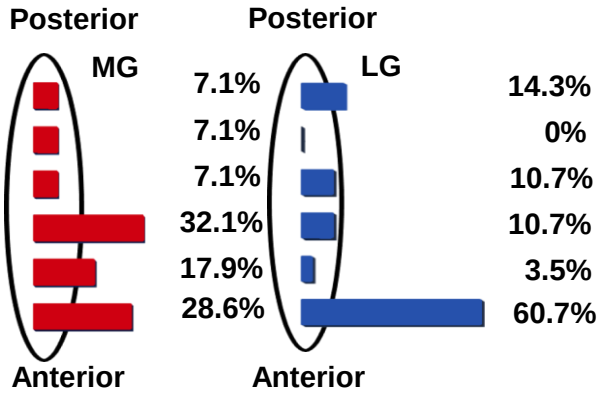
METHODS

Selective electrical stimulation & Voluntary plantarflexion



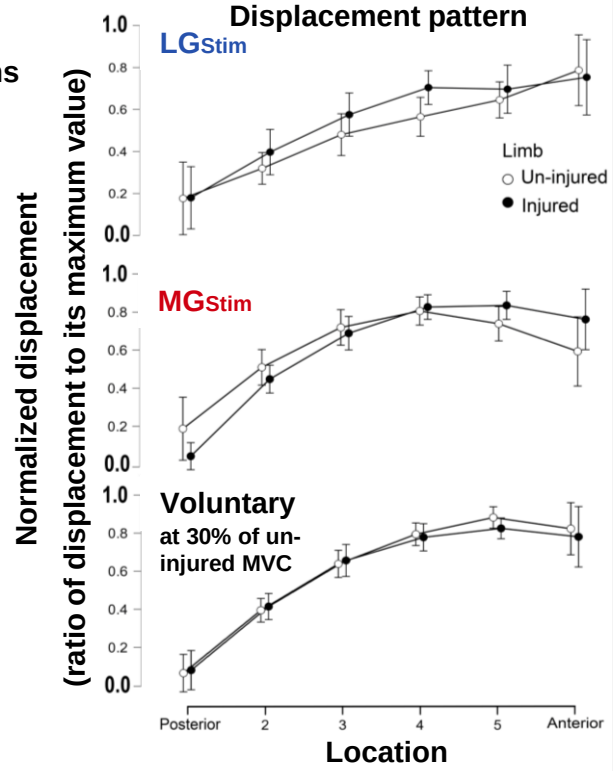
RESULTS

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.