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**Biplanar ultrasound investigation of *in vivo* Achilles tendon displacement non-uniformity**

**Running head:** Achilles tendon displacement non-uniformity

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## Abstract

The Achilles tendon is a common tendon for the medial and lateral gastrocnemius and soleus muscles. Non-uniform Achilles tendon regional displacements have been observed *in vivo* which may result from non-uniform muscle loading and intra-tendinous shearing. However, prior observations are limited to the sagittal plane. This study investigated Achilles tendon tissue displacement patterns during isometric plantarflexor contractions in the coronal and sagittal planes. Fourteen subjects (5 female, 9 male,  $26 \pm 3$  yr) performed maximal isometric plantarflexor contractions with the knee in full extension and flexed to  $110^\circ$ . An ultrasound transducer positioned over the free Achilles tendon collected beam formed radio frequency (RF) data at 70 frames/s. Localized tissue displacements were analyzed using a speckle tracking algorithm. We observed non-uniform Achilles tendon tissue displacements in both imaging planes. Knee joint posture had no significant effect on tissue displacement patterns in either imaging plane. The non-uniform Achilles tendon tissue displacements during loading may arise from the anatomical organization of the sub-tendons associated with the three heads of the triceps surae. The biplanar investigation suggests that greatest displacements are localized to tissue likely to belong to soleus sub-tendon. This study adds novel information with possible implications for muscle coordination, function and muscle-tendon injury mechanisms.

**Keywords:** ultrasound, speckle tracking, triceps surae, sub-tendon

## Introduction

The Achilles tendon is one of the largest and strongest tendons in the human body and has an important functional role in locomotion, transmitting large triceps surae muscle forces to power functional activities like walking and running<sup>1,2</sup>. In addition, the series elasticity it provides to the triceps surae plays a significant role in mechanical energy storage and instantaneous power amplification at the level of muscle-tendon unit, thereby enhancing locomotor performance<sup>3,4</sup>. The Achilles tendon is also commonly injured causing pain and disability. Life-time incidence of Achilles tendon injuries are as high as 50% among competitive runners<sup>5</sup>. Understanding the biomechanical function of the Achilles tendon is a prerequisite for understanding its normal and pathological function.

The Achilles tendon has a complex anatomical structure. It is comprised of distinct bundles of fascicles, called sub-tendons, that arise and twist down from three heads of the triceps surae muscle<sup>6,7</sup>. The sub-tendons join together to form the free Achilles tendon – considered that portion distal to the soleus muscle-tendon junction. The biarticular medial and lateral gastrocnemius muscles differ in their physiological cross-sectional areas and thus force-generating capacities from those of the uniarticular soleus muscle<sup>8</sup>. Therefore, the Achilles tendon is likely subjected to complex non-uniform loading<sup>9</sup>.

Recent advances in dynamic ultrasound imaging and various speckle tracking algorithms permit the measurement of localized tendon tissue displacements within the Achilles tendon<sup>10</sup>. With the help of ultrasound speckle tracking, several recent *in vivo* studies have revealed non-uniform motion within the Achilles tendon during passive joint rotations, active muscle contractions and during locomotion<sup>11–15,29</sup>. These observations of non-uniform tendon motion, thus far isolated to sagittal plane measurements (i.e. antero-posterior differences), are consistent with sliding between adjacent sub-tendons. Indeed, the anterior aspect of the proximal free Achilles tendon is considered

to be covered by the soleus sub-tendon and the posterior part considered to be covered by sub-tendons of the gastrocnemius muscles<sup>7,16</sup>.

The non-uniform mechanical behavior of the Achilles tendon may play an important role in both normal function and the onset and progression of pathology. To date, *in vivo* imaging studies of displacement patterns in the human Achilles tendon have been focused exclusively on the sagittal plane – coronal plane imaging has yet to be performed. Therefore, considering the complex three-dimensional structure of the Achilles tendon<sup>16</sup>, there is currently a clear lack of knowledge regarding Achilles tendon tissue displacement non-uniformity. Improved understanding of Achilles tendon displacement patterns could help to elucidate factors important for normal function and for injury mechanisms of the triceps surae muscle and Achilles tendon.

The purpose of this study was to examine localized Achilles tendon tissue displacements during isometric plantarflexor muscle contractions using both sagittal (i.e., anterior-posterior differences) and coronal (i.e., medial-lateral differences) imaging planes. Knee joint angle alters force generation capacity of the gastrocnemius muscle due to its force-length characteristics and possibly due to muscle activation<sup>17</sup> and has been shown to affect relative motion between soleus and medial gastrocnemius during isometric contractions at the level of the muscle bellies<sup>18</sup>. Therefore, we repeated our measurements at extended and flexed knee joint postures, a difference that presumably affects load distribution between the triceps surface muscles. In the context of their anatomical organization<sup>7,16</sup>, previous studies suggest that the soleus sub-tendon exhibits greater tissue displacement compared to that within the gastrocnemius sub-tendons<sup>11–15</sup>. Therefore, we hypothesized that the Achilles tendon tissue displacements would be greatest in the anterior regions when imaged in the sagittal plane and in medial regions when imaged in the coronal plane - twist of the sub-tendons<sup>7</sup> may locate the soleus sub-tendon within the antero-medial aspect of the tendon. Additionally, we hypothesized that tendon tissue displacements would show greater non-uniformity with the flexed knee posture, which we would interpret in the context of greater loading non-uniformity compared to the knee extended posture due to reduced gastrocnemius force generation.

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## Materials and methods

### *Subjects and experimental protocol*

Fourteen healthy young adults (5 female, 9 male, age  $26 \pm 3$  yr, height  $1.80 \pm 0.08$  m and body mass  $75.3 \pm 10.2$  kg) volunteered for the study. The subjects were free from past or current Achilles tendon disorders and did not report any previous Achilles tendon pain. All participants gave written informed consent before participating in the study as per the University of Wisconsin – Madison Institutional Review Board.

The Achilles tendon displacements were measured during isometric plantarflexor contractions in extended and flexed knee postures (Fig. 1). Subjects were first familiarized to conduct the isometric contractions, after which they performed warm-up by walking on a treadmill for six minutes at a comfortable speed. The warm-up also served to precondition the Achilles tendon<sup>19</sup>. After this, subjects were seated, and their lower leg was positioned for the isometric plantarflexor contractions in randomly assigned extended or flexed knee postures (knee in  $110^\circ$  flexion). Subjects then performed at least two maximal voluntary isometric contractions (MVIC). If the peak force obtained during the second contraction differed more than 10% from the first, subjects performed a third contraction. The contractions lasted about three seconds with visual confirmation of plateauing of the force generation. After the MVIC measurements, subjects performed ramped isometric plantarflexor contractions that followed a symmetric increase and decrease in force generation over a total contraction duration of three seconds. Force signal was displayed for the subjects on a computer screen and a metronome provided audio cue to for timing the contractions. Subjects were instructed to produce a smooth rise and fall of the force signal and reach maximal effort at the peak of the ramp. The smooth force signal profile was required to minimize large tendon tissue displacements between ultrasound frames and hence to facilitate tracking of tendon displacement (see below for details of the tracking). An ultrasound probe was attached over the free Achilles tendon during the contractions in either the sagittal and coronal

planes. The order in which ultrasound imaging was done in sagittal and coronal planes was randomized. After conducting five successful ramped isometric contractions for both imaging planes, MVIC measurements and ramped isometric contractions were repeated for the other leg posture (knee extended or flexed).

#### *Data collection*

Force was recorded during the MVIC and ramped contractions as the sum of forces recorded by two load cells (LCM300 Futek, Irvine, CA, sampling rate 1000 Hz) mounted in series with chains that together with an aluminum bar positioned under the metatarsal heads of the foot held the ankle at a fixed position of 90°. Therefore, the force measured by the load cells represented plantarflexor force generation.

During the isometric ramped contractions, a 10 MHz, 38 mm linear array ultrasound transducer (L14-5 W/38, Ultrasonix Corporation, Richmond, BC, USA) fixed over the free Achilles tendon (distal to soleus muscle-tendon junction) imaged the tendon in sagittal and coronal planes. For sagittal plane imaging, the probe was positioned over the posterior aspect of the tendon and fixed using a custom orthotic<sup>13</sup>. For coronal plane imaging, the probe was positioned over the lateral aspect of the tendon, posterior to lateral malleolus, and secured using elastic Velcro straps. In both imaging planes, the probe position was fine-tuned to produce an image where the tendon extended throughout the whole image and striated echo pattern of the tendon was clearly visible. In coronal plane imaging, the probe was also supported by hand to preserve image quality of the tendon as described above during the isometric contractions. During each contraction, ultrasound radio frequency (RF) data was collected at 70 frames/s synchronously with force data.

### *Data reduction and analysis*

Force measured by each load cell was summed and filtered using a fourth order zero-lag 15 Hz Butterworth low pass filter. Peak force from MVIC and ramped contractions was extracted and used for further analysis.

Localized Achilles tendon tissue displacements during the ramped isometric contractions were analyzed from the ultrasound data using a previously reported and validated speckle tracking algorithm<sup>10,20</sup>. The algorithm has been previously used to track Achilles tendon tissue motion during passive and eccentric ankle joint rotations<sup>14</sup> and walking<sup>13</sup>. Start and end of the contractions were detected from the force data with a threshold level of 5% of the peak force obtained during the contraction. Ultrasound radiofrequency (RF) data collected between the start and end of each contraction were used in the analysis. The RF data was up-sampled by a factor of four in longitudinal and transverse directions. A grid of nodes (16x6) was placed within Achilles tendon boundaries by manually identifying tendon borders in a B-mode image created from the RF data at the first frame of the image sequence and placing the grid between the boundaries (Fig. 2). The grid covered a 15 mm region along the line of action of the tendon, comprising six rows of nodes that were placed along the assumed direction of the tendon fascicles, thereby resolving six regions (16 nodes in each region) of tendon tissue. The rows were uniformly distributed over the entire depth (sagittal plane) or width (coronal plane) of the tendon. A rectangular kernel (2x1 mm) was centered to each node. Two-dimensional normalized cross-correlation function was used to estimate nodal displacement between subsequent frames by finding the peak of the cross-correlation within a 3.6x1.6 mm search window. Subpixel displacements were found using peaks of a 2D quartic spline surface fit of the 2D cross-correlations<sup>21</sup>. If high cross-correlation (>0.7) was not found, the nodal displacement was found by linear interpolation between the neighboring frames where high cross-correlations (>0.7) was obtained. The tracking was done in forward and backward directions of the image sequence. Assuming that the motion was cyclic, the final nodal displacements were calculated as the weighted average of the forward and backward tracking.

All ultrasound image sequences (B-mode image generated from RF data) and tracking results were visually inspected. Trials with poor image quality, e.g. tendon was not visible during the whole contraction, or apparently poor tracking of tissue motion that could have been resulted from excessive out-of-plane motion or non-cyclic motion, were excluded from the analysis. Complete data set was acquired from 10 subjects in sagittal plane and from 9 subjects in coronal plane and used for further statistical analysis. The average number of trials per subject included in the analysis was  $4.7 \pm 1.4$ ,  $4.8 \pm 1.1$ ,  $3.7 \pm 1.5$  and  $3.6 \pm 2.1$  for extended and flexed knee in sagittal plane and extended and flexed knee in coronal plane, respectively and ranged from 1-7 trials.

The nodal displacements within the six regions were averaged to represent tissue displacement in each region. Peak displacement of each region was extracted and averaged across trials within a subject and condition and was used for statistical analysis of regional tendon displacements. The nodal displacement during the contractions is caused by elongation of the tendon distal to the measurement location and small but unavoidable ankle joint rotation. In order to rule out the effect of possible differences in ankle joint rotation between extended and flexed knee postures on comparison of tendon displacements between the postures, the regional peak displacements were normalized by dividing peak displacement of each region by the average peak displacement across all regions. Both absolute and normalized displacements are reported. Range of peak displacements across different regions (maximum-minimum) was calculated to represent tendon displacement non-uniformity.

### *Statistics*

Normality of the data were tested using Shapiro-Wilk test. Differences in peak force of ramped contractions, and range of peak displacements in extended and flexed knee postures were tested using paired samples t-tests. Main effects and interaction of knee joint posture (2 levels) and tissue region (6 levels) on Achilles tendon tissue displacements were tested using two-way repeated measures analysis of variance (ANOVA). Interaction between knee joint posture and tissue region in

the normalized displacements was interpreted to suggest knee joint posture effects on tissue displacements since average displacement across tissue regions (marginal mean) was set to one by the normalization. Linear contrast of the group means was used to assess increasing or decreasing trends in tissue displacements as a function of tissue depth. Due to violation of sphericity, Greenhouse-Geisser corrections were applied to the tests of within-subject effects. Pairwise post hoc comparisons were Bonferroni corrected. Critical level for statistical significance was set at  $p < 0.05$ . Statistical analyses were performed using IBM SPSS Statistics (version 21, IBM Corporation, Armonk, NY, USA).

## Results

There were no significant interaction effects between knee joint posture and tissue region for either absolute or normalized peak tendon displacements in the sagittal (absolute  $p = 0.466$ , normalized  $p = 0.841$ ) or coronal plane (absolute  $p = 0.084$ , normalized  $p = 0.534$ ).

The main effect of tissue region on peak tendon displacements was significant for both the sagittal (absolute  $p < 0.001$ , normalized  $p = 0.001$ ) and coronal (absolute  $p < 0.001$ , normalized  $p < 0.001$ ) imaging planes. In the sagittal plane, both absolute and normalized values revealed progressively larger peak displacements from the posterior to anterior tendon regions ( $p$ -value for a linear trend between regions: absolute  $p < 0.001$ , normalized  $p = 0.001$ ). Pairwise comparisons of both absolute and normalized peak displacements showed that the three most anterior tissue regions of the Achilles tendon displaced significantly more than all other regions ( $p$ 's  $< 0.05$ , Fig. 3) in the sagittal plane. In addition, the peak displacements of the four most posterior regions were all significantly different from each other for both absolute and normalized values ( $p$ 's  $< 0.05$ ). In the coronal plane, both absolute and normalized values revealed progressively larger peak displacements from lateral to medial tendon regions ( $p$ -value for a linear trend between regions: absolute  $p < 0.001$ , normalized  $p < 0.001$ ). Pairwise comparisons of both absolute and normalized peak displacements of different regions showed that the three most medial tissue regions of the Achilles tendon displaced

significantly more than all other regions ( $p's < 0.05$ ) in the coronal plane. Furthermore, the peak displacements of the four most lateral regions were all significantly different from each other in both absolute and normalized values ( $p's < 0.05$ ).

The peak force during the ramped contraction did not significantly differ between the extended and flexed knee postures for either imaging plane (sagittal  $p=0.307$ , coronal  $p=0.605$ , Fig. 4). Hence, overall loading of the Achilles tendon could be considered similar between extended and flexed knee postures. As stated earlier, there was no significant interaction effect between knee joint posture and tissue region for normalized peak tendon displacements. There was also no significant main effect of knee posture on absolute peak displacements in the sagittal ( $p=0.067$ ) or coronal plane ( $p=0.471$ ). Effect of knee joint posture on Achilles tendon displacement non-uniformity was additionally assessed as the range of peak displacements across tissue regions. There were no significant differences in this measure between the extended and flexed knee postures in the sagittal (absolute range: extended  $2.3 \pm 1.5$  mm, flexed  $1.8 \pm 0.7$  mm,  $p=0.200$ ; normalized range extended  $0.4 \pm 0.3$ , flexed  $0.4 \pm 0.2$ ,  $p=0.943$ ) or in the coronal plane (absolute range: extended  $4.5 \pm 2.1$  mm, flexed  $3.6 \pm 1.2$  mm,  $p=0.087$ ; normalized range: extended  $0.8 \pm 0.3$ , flexed  $0.7 \pm 0.3$ ,  $p=0.439$ ).

## Discussion

This study used ultrasound imaging performed in the sagittal and coronal planes to investigate Achilles tendon displacement non-uniformity during isometric plantarflexion contractions. Our major finding was that non-uniform Achilles tendon displacements were observed in both imaging planes during the contractions but in contrast to our hypothesis, knee joint posture had little to no effect on tendon tissue displacement non-uniformity. The sagittal plane results largely confirmed previous findings of non-uniform Achilles tendon tissue displacements observed in antero-posterior axis *in vivo*<sup>12-15</sup>. Here, we add that Achilles tendon tissue displacement non-uniformity also exists in lateral-medial axis. Below, we elaborate how these findings may arise from the complex anatomical architecture of the human Achilles tendon.

The biplanar investigation approach used in the current study revealed a non-uniform displacement pattern in the Achilles tendon in which the anterior part displaced more than posterior in sagittal plane imaging and the medial part more than lateral in coronal plane imaging consistent with our hypothesis. Additionally, it was observed that displacement non-uniformity was approximately two times greater in the coronal plane compared to that in sagittal plane. We propose that most of the non-uniform motion within the tendon is caused by motion between sub-tendons and hence these finding could be linked with sub-tendon anatomy. This is supported by the correlation observed between the relative shortening of gastrocnemius and soleus muscle bellies and the relative motion between anterior and posterior parts of the Achilles tendon<sup>23</sup>. However, it should be noted that previous research has shown potential for relative motion also between collagen fascicles<sup>24</sup> and fibres<sup>26</sup> and hence contribution of the potential non-uniform motion within these hierarchical levels cannot be excluded. In our sagittal plane images, the posterior regions of the tendon most likely arise from the medial gastrocnemius and anterior regions most likely arise from the soleus sub-tendon. In our coronal plane images, lateral regions likely arise from the lateral gastrocnemius and medial regions likely arise from the soleus<sup>7,16</sup>. Therefore, we interpret the results to suggest that soleus sub-tendon displaced the most followed by medial gastrocnemius and with smallest displacement in the lateral gastrocnemius sub-tendon. Tissue displacement in the current study set-up is due to tendon elongation distal to the measurement location and due to unavoidable small amount of ankle joint rotation. We further suppose that the ankle joint rotation displaces all sub-tendons by approximately similar amounts. Therefore, we interpret the observed differences in local tendon tissue displacements to represent differences in sub-tendon elongation and further differences in sub-tendon strain due to similar length of each sub-tendon from insertion to the measurement site. Hence, the results suggest that soleus sub-tendon experienced the largest strains and lateral gastrocnemius sub-tendon the smallest.

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However, it should be mentioned that strains interpreted from tissue motion represent total tissue strain from insertion to the measurement location that may not represent local tissue strains. In fact, Bogaerts et al. found a similar pattern of non-uniform tissue displacements in the sagittal plane of the Achilles tendon to our findings but with regional strain estimates that may be opposite to one's intuition – tissue displacements were largest in the anterior region whereas strains were largest in posterior region<sup>11</sup>. The discrepancy between total and local tissue strain estimates suggests that soleus sub-tendon may have considerable slackness at its distal end or alternatively different mechanical properties along the length of the sub-tendon allowing large proximal displacements of the sub-tendon without large local strains observed at proximal regions. Finally, since the length of the external tendon is shortest in soleus muscle and longest in lateral gastrocnemius<sup>22</sup>, the supposed differences in total sub-tendon strains may result in total elongations of the sub-tendons that are relatively similar between the sub-tendons. We speculate that the functional relevance of this could be to minimize shear between the adjacent muscle bellies and potentially reduce risk of rupture of the connective tissue structures observed between adjacent muscles<sup>18</sup>.

*Effect of knee joint posture on displacement non-uniformity*

Interestingly, the normalized sub-tendon tissue displacements were nearly identical between the extended and flexed knee postures, independent of imaging plane. This was unexpected, as knee joint posture affects force generation capacity of gastrocnemius muscles<sup>17</sup>, has been shown to affect passive muscle tension in the gastrocnemius<sup>25</sup>, and has been also shown to affect relative displacements between the medial gastrocnemius and soleus aponeuroses when measured at the level of muscle bellies<sup>18</sup>. We anticipated that these effects would affect local tissue displacement patterns. However, we have previously shown that knee joint angle had little effect on tendon tissue displacement patterns during passive ankle joint rotations<sup>27</sup> – a finding consistent with the current study. In addition, also Bogaerts et al. recently reported that knee joint angle did not have an effect on regional Achilles tendon tissue displacement patterns during isometric contractions as measured in the sagittal plane<sup>28</sup>. In contrast, while Beyer et al. also observed that

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displacement of the anterior region of the Achilles tendon remained almost similar between plantarflexion tasks performed in knee extended and flexed (100° flexion) postures, the posterior region experienced smaller displacement in the knee flexed posture resulting in greater relative motion within the Achilles tendon in the knee flexed posture<sup>29</sup>. However, in the study by Beyer et al. the outcome may be affected by the fact that loading conditions greatly differed between the knee extended and flexed postures whereas in the current study a maximal isometric contraction was done in both joint postures.

Two different conclusions may be drawn from the findings related to the effect of knee joint posture. First, altering relative muscle force contributions from different heads of triceps surae may not appreciably affect relative displacements of different tissue regions. This could be explained by lateral force transmission within the Achilles tendon or via inter-muscular myofascial structures that would serve to limit non-uniform distribution of forces within the Achilles tendon and differential displacements of the tissue regions – a hypothesis recently presented by Maas & Finni<sup>30</sup>. The hypothesis that alterations in triceps surae muscles forces induce only limited changes in Achilles tendon non-uniform motion due to lateral force transmission is supported by a recent experiment in rat in which isolated contractions of different triceps surae muscles via electrical stimulations resulted in only small variations in displacements of the sub-tendons<sup>31</sup>. Although, rat Achilles tendon may not be functionally representative of human Achilles tendon, it nevertheless serves as a model for a tendon in which the distal tendon is a union of tendon fascicles arising from muscles with different anatomical and functional properties similar to human Achilles tendon. Such mechanisms of lateral force transmission could be important for protecting the Achilles tendon from excessive shearing that may lead to cellular damage at the interfaces between tendon fascicles<sup>32</sup>. In the current, study it is also possible that changes in muscle-tendon unit passive tension due to alterations in knee joint angle contributed to the observed results. Knee flexion may induce slackness to the gastrocnemius muscle-tendon unit allowing gastrocnemius sub-tendon to displace similarly in both knee flexed and extended postures regardless of the presumably lower force output

of the gastrocnemius in the knee flexed posture. This may allow similar displacement of the gastrocnemius sub-tendon in knee flexed compared to extended posture although force generation of the gastrocnemius was presumably lower in the knee flexed posture. Second, our underlying assumptions regarding changes in muscle force generation between knee flexed and extended postures may be incomplete. For example, if soleus muscle force dominates that transmitted from the triceps surae independent of knee flexion, the change in relative muscle forces transmitted through the Achilles with altered knee joint posture may be smaller than anticipated. In support of this, although MVIC force tended to be 10 to 15% greater in knee extended than flexed posture, we found that these values did not differ significantly in our experimental setup.

#### *Limitations*

We cannot exclude the possibility that out-of-plane tendon tissue motion affected our speckle tracking results. Out-of-plane motion could have resulted from: 1) a change in the orientation of the ultrasound probe over the course of each contraction and/or 2) from orientation or twist of the tendon fascicles and sub-tendons relative to imaging plane<sup>7</sup>. Care was taken to visually inspect that ultrasound images showed a clear striated echo pattern within the tendon, typical for tendon tissue when imaging plane is perpendicular to collagen fiber direction. It can be assumed that any out-of-plane motions would probably affect both knee extended and flexed postures similarly and therefore would not bias our conclusion regarding the effect of knee joint posture.

Positioning the ultrasound probe for coronal plane images of the Achilles tendon was challenging. The probe's orientation was preserved during those contractions by manually supporting the probe, which may have increased the possibility of out-of-plane motion and allowed for rocking of the transducer, which would result in apparent motion within the image plane. In addition, due to individual differences in soft tissues in the area of Kager's triangle, the Achilles tendon is more prominent in some subjects making it easier to position the probe for coronal plane

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imaging. The individual anatomical differences between subjects may have caused the orientation of the probe to be slightly different for each subject.

Finally, we originally designed our experimental to collect coronal plane images from the lateral aspect of the Achilles tendon for ease of instrumentation and experimental setup. After completion, we revisited our approach by repeating our measurements on two subjects using coronal plane images taken from the lateral and medial aspects of the tendon. Ideally, this validation would have shown anatomically consistent tissue displacements irrespective of imaging direction if the imaging planes coincided with each other. However, data from these two subjects did not conform to this ideal scenario. Instead, we observed slightly larger displacement of the medial aspect of the tendon when imaged from the lateral side and slightly larger displacement of the lateral aspect when imaged from the medial side (see supplementary material). Such findings could arise from an imaging plane that was not exactly coronal and could be explained by a presumed anatomical organization of sub-tendons arising from the triceps surae that is consistent with our interpretation of sagittal plane displacement patterns (see supplementary material for details and further discussion). Finally, in our supplementary material, we also present tendon displacements from each individual subject to highlight inter-individual differences in tendon tissue motion that may arise from differences in sub-tendon arrangement<sup>7,16</sup>.

#### *Future directions*

Here we present findings that suggest that non-uniform displacement patterns arise throughout the Achilles tendon, adding to the growing body of literature suggesting that the Achilles tendon cannot be considered a single tendon, neither anatomically nor biomechanically. Results from the current study in combination with previous *in vivo* observations of Achilles tendon displacement patterns suggests that the soleus sub-tendon may experience larger total tissue strains compared to gastrocnemius sub-tendons. This has potential clinical relevance since it has been shown that although tendon material properties and ultimate tensile strength may vary

considerably, ultimate strain is consistent across tendons<sup>33,34</sup>. Therefore, higher strains in the soleus sub-tendon could place that tissue at a greater risk of both fatigue damage<sup>35</sup> and acute ruptures. However, it should be noted that since we do not know the level of pretension in the sub-tendons, it is possible that elongation and strain of the whole soleus sub-tendon includes tissue straightening (e.g. reduced waviness or curvature) in addition to tissue stretch. To verify the hypothesis of differential strains of the sub-tendons, future studies should aim to characterize mechanical properties of the sub-tendons and relate those to force generation capacity of the individual triceps surae muscles. This characterization should be made also longitudinally within a sub-tendon.

Ultrasound speckle tracking shows promise to study tendon motion *in vivo*. To further improve understanding of normal Achilles tendon function, efforts should be made toward more complete three-dimensional characterization of sub-tendon tissue displacement patterns. Although challenging, this may be possible by using 3D imaging probes or acquiring images through multiple imaging planes and co-registering the results utilizing known transducer and tendon location information. Cadaver experiments could be used as a first step toward complete three-dimensional displacement characterization and this approach could be used to clarify the effect of different muscle force distributions on tendon tissue non-uniformity. Finally, future studies should aim to clarify if the non-uniform tendon motion we and others<sup>11-15</sup> have observed arises from relative movement between sub-tendons or is this a phenomenon that also occurs within sub-tendons.

### **Perspective**

The results of our study show non-uniform Achilles tendon displacements in both sagittal and coronal imaging planes during high force isometric contractions. Knee joint posture during the contractions did not have significant effect on tendon displacement non-uniformity. Based on the biplanar investigation approach utilized in the current study, the largest tendon tissue displacements during isometric plantarflexor contractions occurred in the antero-medial portion of the free Achilles tendon, which we suspect reflects soleus sub-tendon behavior. Non-uniform Achilles tendon

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displacements may have an important functional role in allowing independent mechanical performance of the individual triceps surae muscles<sup>36</sup> and this independence may be lost after injury<sup>12</sup> or with aging<sup>37</sup>. Future studies are warranted to examine the functional and pathological implications of non-uniform Achilles tendon tissue displacements, which have now been shown to occur in both isolated muscle contractions and during human locomotion.

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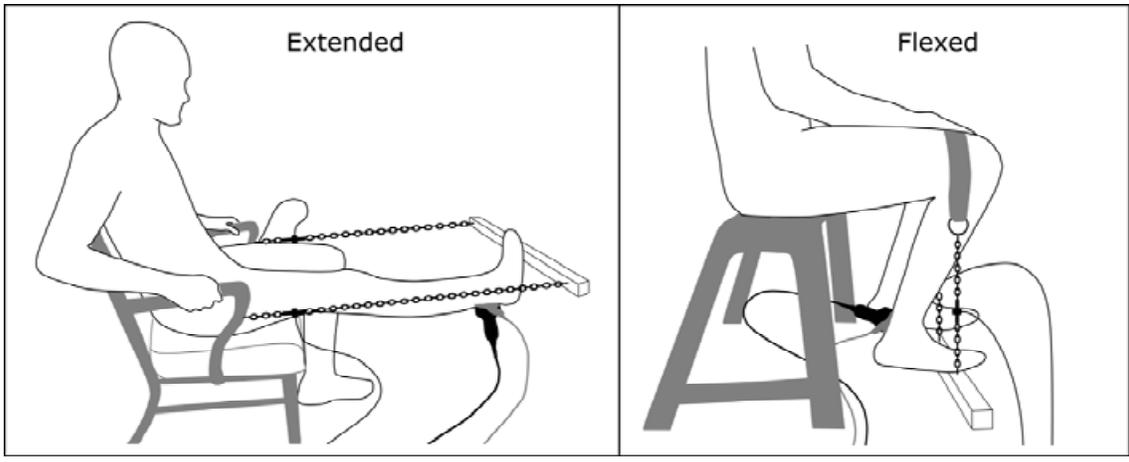
## Figure legends

Figure 1. Measurement set-up. Ultrasound probe was attached over the posterior (sagittal plane, black probe) or lateral (coronal plane, gray probe) aspect of the free Achilles tendon. Two load cells attached in the cables of the foot fixation device measured the force exerted by ankle plantarflexors.

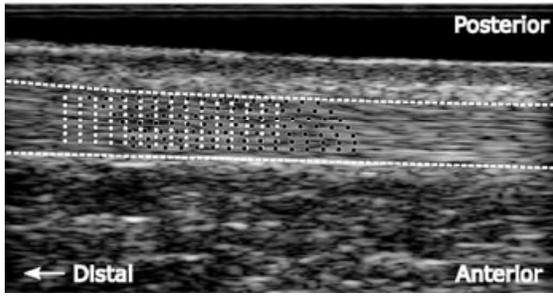
Figure 2. An example of the placement of tracking nodes from a single subject. The ultrasound image shown is from the beginning of the contractions. White dots indicate the locations of the nodes placed over this image. Black dots indicate the locations of the nodes at the instant of peak displacement. White dotted lines depict borders of the tendon. A gel pad and ultrasound gel were used in the sagittal plane between the probe and the skin (dark area in top of the image). In the coronal plane, only ultrasound gel was used.

Figure 3. The Achilles tendon peak tissue displacements in different regions for sagittal and coronal plane imaging. Data obtained in extended knee posture are shown in blue and in flexed knee in red. Greatest displacement were observed in anterior (sagittal plane imaging) and in medial (coronal plane imaging) regions of the Achilles tendon irrespective for knee joint posture. Statistical differences between regions are not shown for clarity. Refer to the text for the statistical differences.

Figure 4. Average force generation profiles of the isometric plantarflexor contractions performed in extended and flexed knee postures. Peak force generation (foot reaction force) in the ramped isometric contractions did not differ between trials performed in extended or flexed knee postures either in sagittal (left) or in coronal (right) plane imaging.



Sagittal



Coronal

