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Non-Uniform *In Vivo* Deformations of the Human Achilles Tendon During Walking

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Abstract

The free Achilles tendon (AT) consists of distinct fascicles arising from each of the triceps surae muscles that may give rise to non-uniform behavior during functional tasks such as walking. Here, we estimated *in vivo* deformations of the human AT during walking using simultaneous ultrasound and motion capture measurements. Ten subjects walked at three speeds (0.75, 1.00, and 1.25 m/s) on a force-measuring treadmill. A custom orthotic secured a linear array transducer in two locations: 1) the distal lateral gastrocnemius muscle-tendon junction and 2) the free AT, on average centered 6 cm superior to calcaneal insertion. We used motion capture to record lower extremity kinematics and the position and orientation of the ultrasound transducer. A 2D ultrasound elastography algorithm tracked superficial and deep tissue displacements within the free AT. We estimated AT elongation (i.e., change in length) relative to the calcaneal insertion by transforming the orthotic, transducer, and calcaneus kinematics into a common reference frame. Superficial and deep regions of the free AT underwent significantly different longitudinal displacements and elongations during walking. For example, we found that the superficial AT exhibited 16-29% greater peak elongation than the deep AT during the stance phase of walking ($p < 0.01$). Moreover, superficial-deep AT tissue deformations became less uniform with faster walking speed ($p < 0.01$). Non-uniform deformations of the free AT, which could reflect inter-fascicle sliding, may enable the gastrocnemius and soleus muscles to transmit their forces independently while allowing unique kinematic behavior at the muscle fiber level.

Keywords

Ultrasound; Elastography; Plantarflexor; Gait; Triceps Surae

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

INTRODUCTION

The Achilles tendon (AT) transmits substantial forces from the gastrocnemius and soleus muscles during movement. Considerable research has characterized the gross biomechanical behavior of the AT during walking [1-6]. However, these landmark studies generally do not consider the complex architecture of the AT and may thereby simplify *in vivo* musculotendon behavior. The free AT (i.e., calcaneus to soleus muscle-tendon junction) consists of distinct fascicle bundles arising from the triceps surae muscles; superficial fascicles arise from the medial gastrocnemius and deeper fascicles arise from the soleus and lateral gastrocnemius [7]. This unique anatomy gives rise to relative motion between fascicle bundles of the free AT [8, 9]. However, the relevance of such non-uniform AT deformations to biomechanical function remains unclear, particularly for tasks such as walking which involve complex coordination between these plantarflexor muscles [10-13].

Comparative studies in horse tendons suggest that sliding between adjacent fascicles may be a key biomechanical feature in energy-storing tendons [14]. Direct and indirect evidence for non-uniform deformations within the human free AT comes from cadaver experiments or from measurements made during isolated ankle exercises [8, 9, 15-19]. For example, varying knee flexion can elicit differential displacements of the soleus and gastrocnemius aponeuroses [19]. Similarly, our research group showed that knee flexion modulates the uniformity of AT tissue displacements [9]. In addition, Haraldsson et al. [20] found negligible inter-fascicle force transmission within the AT and suggested that sliding may allow AT fascicles to act as functionally independent structures. These results imply that inter-fascicle sliding within the AT may be important to normal function. Biomechanically, sliding would enable the gastrocnemius and soleus muscles to stretch their associated tendinous structures more independently. Such behavior could thus allow muscles to operate at independent fiber lengths, which may be more tuned for effective force generation. Indeed, there is evidence that the gastrocnemius and soleus muscles exhibit markedly different behavior. For example, Ishikawa et al. [3] found that during the late stance phase of walking, medial gastrocnemius muscle fibers remain isometric or shorten while soleus muscle fibers lengthen. Further, it has been shown that the gastrocnemius and soleus muscles perform unique biomechanical functions during walking (i.e., propulsion vs. body weight support) [10-13, 21], with greater distinction observed at faster walking speeds [13, 21]. Consequently, non-uniform deformations of the free AT may be critical to coordinate the triceps surae muscles across a range of walking speeds. Moreover, if functionally relevant, deformations within the free AT may become less uniform with faster walking speed.

Ultrasound imaging of the distal muscle-tendon junctions has contributed much to the research on AT function during walking (though see [5, 6]). However, it is challenging to use traditional anatomical feature tracking to resolve spatial variations in tendon tissue deformation. Thus, our research group introduced a 2D ultrasound elastography algorithm to resolve regional variations in tendon tissue deformations [22]. This approach uses phase information inherent in ultrasound radiofrequency signals and, in cyclically loaded tendons, provides deformations that agree well with prescribed displacements ($R^2 > 0.97$) [22] and with digital image correlation ($R^2 > 0.92$) [23].

Our primary purpose was to use ultrasound elastography to investigate *in vivo* deformations of the human free AT during treadmill walking. We resolved superficial-deep variations in tendon tissue elongation (i.e., change in length) by coupling our ultrasound measurements with motion capture. We used these data to test the hypotheses that: 1) superficial and deep regions of the free AT would undergo different longitudinal displacements and elongations, and 2) superficial versus deep deformations would become less uniform with faster walking speed.

MATERIALS AND METHODS

Subjects and Experimental Design

We present data for 10 healthy subjects (age: 23.9 ± 4.0 years, mass: 70.3 ± 12.0 kg, height: 1.76 ± 0.14 m, 4 females and 6 males) who provided written informed consent as per the University of Wisconsin-Madison Internal Review Board. We conducted walking trials on a dual-belt, force-measuring treadmill (Bertec Corporation, Columbus, OH). Subjects walked for 2 min at each of three speeds (0.75, 1.00, 1.25 m/s) to become familiar with the treadmill and to precondition the AT. Subjects then completed a series of 2 min walking trials at each speed as outlined below. An 8-camera motion capture system (Motion Analysis, Corp., Santa Rosa, CA) recorded the 3D positions of retroreflective markers placed as detailed previously [24] on subjects' pelvis and right and left legs at 200 Hz. We recorded electromyographic (EMG) activity at 2000 Hz from single-differential electrodes (Trigno, Delsys, Inc., Boston, MA) placed over the following right leg muscles using recommendations by Cram and Kasman [25]: lateral gastrocnemius (LG), soleus (SOL), and tibialis anterior (TA). Subjects completed the experiment barefoot to facilitate placement of the ultrasound transducer near the calcaneus.

Ultrasound Measurements

Also on the right leg, a single 10 MHz, 38 mm linear array transducer (L14-5W/38, Ultrasonix Corporation, Richmond, BC) was positioned via a custom orthotic (**Figure 1**) either over: 1) the free AT, on average centered 6 cm superior to the posterior calcaneus marker (20 mm depth at 155 Hz), or 2) the distal LG muscle-tendon junction (MTJ) (30 mm depth at 128 Hz). These maximum sampling rates depended upon the depth of the tissues of interest. We first randomized the order of transducer locations. Subjects then completed 2 min walking trials at each speed in random order. We selected the LG MTJ so that the transducer would not interfere with the swing leg. We aligned the transducer to capture ultrasound radiofrequency (RF) data from a longitudinal cross-section with an image resolution of 128×1560 (0.297×0.019 mm pixels). Due to a delay introduced as the machine saved RF data, we recorded 5 strides per condition distributed over each 2 min trial. These strides were later identified in our motion analysis data to within 5 ms using a burst waveform from a signal generator triggered by the Ultrasonix system when sampling lines of RF data. We tracked transducer position and orientation using three retroreflective markers placed on the orthotic [4]. The orthotic limited axial rotation of the probe relative to the leg to values (range, $6.3 \pm 1.8^\circ$ at 1.25 m/s) less than those previously published [4].

Data Analysis

We defined strides in the ultrasound and kinematic data using synchronized ground reaction force (GRF) measurements. Low-pass filtered (10 Hz) marker trajectories from the right leg defined sagittal plane knee and ankle joint angles using standard procedures [24] and we band-pass filtered (20-450 Hz) and rectified all EMG signals. We computed average kinematic and EMG profiles from 15 consecutive strides extracted during the final 30 s of each 2 min collection. We calculated muscles' mean stance phase activity relative to the average activity at the slowest walking speed.

A 2D elastography speckle tracking algorithm tracked free AT longitudinal tissue displacements according to previously published and validated methods [22, 23]. Briefly, we manually positioned a $\sim 15 \text{ mm} \times 3 \text{ mm}$ region of interest (ROI) (i.e., grid of nodes with $1 \text{ mm} \times 0.5 \text{ mm}$ spacing) on a B-mode ultrasound image of the AT at toe-off. Consistent with earlier studies [3, 4], we used toe-off, an instant of negligible tendon force [6], as a reference for AT tissue deformations. The ROI width did not extend the full tendon width to ensure that tissue tracking used only data from tendinous tissue. We calculated a 2D normalized cross-correlation function between up-sampled (4x) RF data within a kernel ($2 \text{ mm} \times 1 \text{ mm}$) surrounding each nodal position and a search window ($2.8 \text{ mm} \times 1.4 \text{ mm}$) within the subsequent frame. We then calculated gross nodal displacements as the position that maximized the resulting cross-correlation function. We refined those estimates by calculating sub-pixel displacements using a 2D quartic spline centered at the gross nodal displacements. We used a correlation threshold of 0.7 to retain nodal displacement information [22, 26]. On average, we observed very strong normalized cross-correlations between successive frames ($r > 0.95$). However, for some subjects walking at the fastest speed, the frame(s) corresponding to heel-strike exhibited cross-correlations that fell below threshold. To estimate displacements in those cases, we computed displacements for the relevant frames based on a cubic interpolation of the neighboring, highly-correlated frames. Nodal positions at the subsequent frame were calculated by adding the median-filtered (3×3 nodes) nodal displacements. We implemented this tracking algorithm in reverse order and established final nodal displacements as the weighted average of forward and backward nodal displacements over a gait cycle. We evaluated tendon non-uniformity by segmenting the nodal displacements into those originating from two equally sized tendon depths (superficial, deep) at toe-off (**Figure 1. Supplementary material**). We manually tracked the positions of the distal LG MTJ using B-mode images created from the RF data and custom software implemented in Matlab (Mathworks, Inc., Natick, MA) [4].

We estimated AT elongation relative to the calcaneus marker (a proxy for calcaneal insertion) by transforming the transducer and downsampled marker kinematics into a common reference frame [4]. In an earlier session, we established the coordinate transformation between ultrasound images and the orthotic marker positions using an active motion capture system and an instrumented probe to digitize corners of the transducer face (VisualEyez, PTI, Burnaby, BC). We defined distal free (superficial and deep) AT elongations as the change in length between the calcaneus marker and the average nodal positions computed via speckle tracking. To contextualize free AT deformations, we quantified elongations of the aggregate AT defined more conventionally as the change in

length between the calcaneus marker and the distal LG MTJ. The aggregate AT included the soleus aponeurosis and the free tendon, including that superior to the nodal positions.

Statistical analysis

For each subject, we averaged for each condition the 3 strides with the highest average nodal cross-correlations (at least 2 strides used for walking at 1.25 m/s to minimize the effect of frame-to-frame correlations that fell below threshold). Based on having clear and reliable images over a complete cycle, we averaged at least 3 strides for LG MTJ measurements. Outcome measures included peak superficial and deep free AT longitudinal displacements and elongations and peak aggregate AT elongation. A Shapiro-Wilk's test first confirmed that all outcome measures were normally distributed. A repeated measures ANOVA then tested for significant main effects of and interactions between two, within-subject factors: walking speed and measurement depth/location (superficial free vs. deep free vs. aggregate). When a significant main effect or interaction was found, we performed post-hoc pairwise comparisons using a $p < 0.05$ criterion. A secondary ANOVA tested for main effects of walking speed on ankle joint range of motion and stance phase muscle activities. Unless otherwise stated, p -values in the results narrative refer to statistical main effects.

RESULTS

Non-uniform deformations of the distal free AT

Relative to toe-off, the deep AT underwent significantly larger distal displacements than the superficial AT at all walking speeds ($p < 0.01$) (**Figure 2**). Across walking speeds, longitudinal stance phase displacements of the deep AT were on average 36-43% larger than those of the superficial AT ($p < 0.01$) (**Table 1**). Consequently, we observed significantly larger peak elongation in the superficial vs. deep AT (e.g., 7.6 mm vs. 5.9 mm at 1.25 m/s, $p < 0.01$) (**Figure 3**). These deformations became significantly less uniform with faster walking speed (depth $\times$ speed, longitudinal displacement: $p < 0.01$, elongation: $p < 0.01$) (**Table 1**). Moreover, peak elongation in the superficial, but not deep, AT increased significantly with faster walking speed (**Table 1**). Thus, compared to the slowest walking speed, walking at 1.25 m/s elicited a threefold greater increase in superficial vs. deep AT elongation (1.2 mm vs. 0.4 mm, depth $\times$ speed, $p < 0.01$). However, walking faster elicited similar increases in gastrocnemius (+47%, $p < 0.01$) and soleus (+47%, $p < 0.01$) muscle activities (muscle $\times$ speed, $p = 0.92$).

Distal free vs. aggregate AT deformations

Across the range of walking speeds, peak elongation of the distal free AT averaged 42-53% of peak aggregate AT elongation measured from calcaneal insertion to distal LG MTJ (**Table 1, Figure 3**). Moreover, walking faster elicited the same increase in relative elongations of the aggregate and free AT (e.g., 2.0 ± 1.2 % increase (aggregate) and 2.0 ± 2.6 % increase (superficial free) for 1.25 m/s vs. 0.75 m/s, $p < 0.01$).

DISCUSSION

The free AT consists of distinct fascicles arising from each of the triceps surae muscles that may give rise to non-uniform behavior during functional tasks such as walking. Our findings reveal that, as hypothesized, superficial and deep regions of the free AT undergo different longitudinal displacements and elongations during walking. We observed that the deep AT underwent significantly larger distal displacements with ankle dorsiflexion, and consequently smaller peak elongation than the superficial AT. These non-uniform deformations suggest that inter-fascicle sliding within the human free AT may arise during normal walking.

Biomechanically, non-uniform deformations of the free AT may arise from differential loading of the triceps surae muscles during walking. There is certainly evidence for unique gastrocnemius and soleus musculotendon behavior [3, 10-13, 21]. For example, Ishikawa et al. [3] found that medial gastrocnemius muscle fibers remain isometric or shorten during the late stance phase of walking while soleus muscle fibers lengthen. This observation is consistent with our observations within the AT. At the location we imaged, the superficial tendon would most likely arise from the medial gastrocnemius and the deep tendon would arise from the soleus. We found that the deep AT underwent 16-29% smaller elongation than the superficial AT, which may require soleus muscle fibers to lengthen more during stance than medial gastrocnemius muscle fibers as previously observed [3]. Such independent musculotendon behavior may be important for the gastrocnemius and soleus muscles to operate at lengths effective for force production and power generation.

The gastrocnemius and soleus muscles are also known to perform unique biomechanical functions during walking (i.e., propulsion vs. body weight support), with greater differences in function emerging at faster walking speed [10-13, 21]. Similarly, and as hypothesized, differences between superficial and deep AT deformations became larger with faster walking speed. Specifically, we found that walking faster elicited a disproportionate increase in superficial vs. deep AT elongation. Consistent with these tendon elongations, Miyoshi et al. [21] previously found that medial gastrocnemius muscle activity increased more with faster walking speed than soleus muscle activity. Although we did not find concomitant differences between gastrocnemius and soleus muscle activities, we collected EMG only from the lateral gastrocnemius and studied a much smaller range of walking speeds than that previously reported [21]. Further studies are needed to determine the extent to which AT non-uniformity is associated with differences between medial and lateral gastrocnemius and soleus muscle fiber behavior.

There are several mechanisms that may underlie the non-uniform deformations exhibited by the free AT during walking. Noting that free AT deformations became less uniform with faster walking speed implies that differences in triceps surae muscle forces may at least modulate the displacements and elongations experienced by the superficial vs. deep AT. However, others [8, 9] have observed these non-uniform deformations during passive ankle flexion in the absence of complex muscle activation patterns. In addition, Slane and Thelen [9] recently found that increasing knee flexion, and presumably relative soleus vs. gastrocnemius tissue tension, altered the uniformity of AT deformations during an isolated

ankle flexion task. Together, these findings allude to the possible contribution of at least three additional, and perhaps interdependent, mechanisms. First, tendon fascicles arising from the MG may exhibit different material properties than those arising from the SOL or LG. Indeed, elastic modulus is known to vary as much as two-fold between leg tendons extracted from the same animal [27]. Even with the same elastic modulus, absolute tendon stiffness may vary considerably between bundles due to the relatively longer and smaller cross-section of tendon fascicles arising from the gastrocnemius vs. soleus muscles [7]. Second, musculotendon slack lengths of the triceps surae may occur at different ankle angles, leading to differential elongation of tendon fascicles arising from each muscle. Finally, depth differences between the superficial and deep regions of the AT could lead to differences in muscle-tendon gearing [28] for a given external load.

Consistent with studies on gross AT function during walking [1-4], our results demonstrate that aggregate AT elongation increases progressively during stance, paralleling the rise in plantarflexor muscle activity and, presumably, muscle force. Moreover, our elongation estimates are nearly identical to those previously reported [4]. The mechanical energy stored in the aggregate AT is subsequently returned rapidly during push-off. We found here that the free AT undergoes progressive elongation and rapid recoil during the stance phase that temporally mirrors that of the aggregate AT. However, across the range of walking speeds, average elongation of the distal ~6 cm of free AT averaged approximately half the elongation of the ~22 cm aggregate AT. Thus, relative to their respective lengths at toe-off, peak longitudinal strains of the superficial and deep free AT (e.g., 13% and 10% at 1.25 m/s, respectively) were notably larger than that of the aggregate (7%) and hence proximal AT (5%). Consistent with this finding, Farris et al. [29] found that during isometric plantarflexor contractions, the free AT exhibited twice the longitudinal strain of the proximal AT (from distal SOL MTJ to distal gastrocnemius MTJ). Noting that this differential behavior does not occur during passive tendon stretch [30], those authors reported that plantarflexor muscle contractions may stiffen the aponeuroses leading to smaller longitudinal strains of the proximal AT. Our results extend these earlier observations and suggest that a similar mechanism may contribute to series elasticity that varies longitudinally along the human AT during walking.

There are several limitations of this study. We used a 2D elastography algorithm based on ultrasound RF data. We have previously shown that the use of RF signals provides greater frame-to-frame tracking accuracy than traditional B-Mode images [23], but bias errors can accumulate when incremental displacements are integrated over time. By assuming cyclic motion trajectories, we used forward-backward integration to address this problem [31]. Also, due to the delay in writing RF data and using a minimum criteria for frame-to-frame correlations, we analyzed a relatively small number of strides. A third limitation is that 2D image data does not allow for tracking out-of-plane motion [22], which we know can arise due to the helical structure of tendons. However, our elastography approach has reliably estimated tissue motion within axially loaded tendons free to exhibit transverse rotation [23]. Moreover, our orthotic limited rotation of the probe relative to the leg due to probe inertia or skin movement. A ~90° twisting of the AT [7, 32], from the aponeuroses to the calcaneal insertion, precipitates a complex arrangement of fascicle bundles arising from the gastrocnemius and soleus muscles. Recognizing that inter-subject variability in AT

architecture exists [7], we segmented the free AT by half to generalize the actual anatomical arrangement. Consequently, we interpreted our data under the assumption that the superficial tendon primarily arises from the MG whereas the deep AT primarily arises from the SOL [7]. Further, estimating tendon elongation relative to the calcaneus marker neglects anatomical complexities due to coronal plane ankle motion and the natural dorsoventral curvature of the AT [33]. These factors, together with TA muscle activity and our use of toe-off as a reference position, may explain the tendon stretch we and others [4] report during the swing phase. Finally, we assumed that motion of the LG MTJ represented gross behavior of the aggregate AT. Farris et al. [29] recently substantiated this assumption and found that, while architectural differences exist, regional measurements from the lateral gastrocnemius MTJ were identical to those from the medial gastrocnemius MTJ.

In summary, our findings reveal that the human free AT exhibits non-uniform *in vivo* deformations which may reflect inter-fascicle sliding. This non-uniform behavior may allow for the coordination of gastrocnemius and soleus muscle forces during walking. Future work should consider the relevance of non-uniform tendon deformations to changes due to tendon pathology and age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research Highlights

1. Quantified *in vivo* deformations of the human free Achilles tendon during walking.
2. Coupled custom ultrasound elastography with quantitative motion capture.
3. Superficial and deep free Achilles tendon regions undergo different deformations.
4. Non-uniform deformations may reflect sliding between tendon fascicles.
5. Relevant to the coordination of triceps surae muscle forces during walking.

Figure 1.

We used a custom orthotic and 2D ultrasound elastography speckle tracking method to track superficial and deep tissue displacements within the AT on average centered 6 cm superior to the posterior calcaneus marker on the right leg. Subjects walked at three speeds (0.75, 1.00, and 1.25 m/s) on a force-measuring treadmill.

Figure 2.

Group mean (standard error) longitudinal displacements of superficial vs. deep regions of the free AT during walking plotted over an averaged gait cycle, from toe-off to toe-off. Negative values for longitudinal displacements indicate motion distal to the reference position at toe-off. Single asterisks (*) indicate superficial vs. deep significantly different in post-hoc pairwise comparisons ($p < 0.01$). Longitudinal displacements became significantly less uniform with faster walking speed.

Figure 3.

Group mean (standard error) calf muscle activities and elongation (i.e., change in length) of the free (superficial and deep) and aggregate AT plotted over an averaged gait cycle, from toe-off to toe-off. We estimated AT elongation as the change in length between the calcaneus marker and the average nodal positions or muscle-tendon junction.. Single asterisks (*) indicate superficial vs. deep free AT significantly different ($p < 0.01$) and double asterisks (**) indicate aggregate AT vs. free AT significantly different ($p < 0.01$) in post-hoc pairwise comparisons. Free AT elongation became significantly less uniform with faster walking speed.

Table 1

Mean (SD) peak Achilles tendon deformations (stance phase)

Speed (m/s)	Ankle RoM (°)	Longitudinal Displacement (mm)		Elongation (mm)			Reference AT Length (mm)
		Superficial	Deep	Superficial	Deep	Aggregate	
0.75 m/s	13.6 (3.3)	-2.1 (1.2)	-3.0 (1.4) *	6.4 (2.1)	5.5 (1.8) *	11.2 (3.8) *	223.2 (9.2)
1.00 m/s	16.0 (3.1) ^a	-3.6 (1.5) ^a	-4.9 (1.6) *, ^a	7.4 (2.6) ^a	6.2 (2.3) *	13.4 (3.4) **, ^a	221.6 (9.8)
1.25 m/s	18.3 (3.3) ^a	-5.5 (1.7) ^a	-7.5 (1.8) *, ^a	7.6 (2.6)	5.9 (2.6) *	15.7 (2.9) **, ^a	216.7 (9.7) ^a

We report all measures relative to toe-off. Negative values for longitudinal displacements indicate net motion distal to the reference position.

* Deep vs. superficial significantly different (pairwise, $p < 0.05$).

** Aggregate vs. free significantly different (pairwise, $p < 0.05$).

^a Significantly different from the next slowest walking speed (pairwise, $p < 0.05$).