

RESEARCH ARTICLE

Differential strain patterns of the human Achilles tendon determined *in vivo* with freehand three-dimensional ultrasound imaging

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SUMMARY

The human Achilles tendon (AT) has often been considered to act as a single elastic structure in series with the muscles of the triceps surae. As such it has been commonly modelled as a Hookean spring of uniform stiffness. However, the free AT and the proximal AT have distinctly different structures that lend themselves to different elastic properties. This study aimed to use three-dimensional freehand ultrasound imaging to determine whether the proximal AT and the free AT exhibit different elastic behaviour during sub-maximal, fixed-end contractions of the triceps surae. Six male and five female participants (mean \pm s.d. age=27 \pm 5 years) performed fixed position contractions of the plantar-flexors on an isokinetic dynamometer at 50% of their maximum voluntary contraction in this position. Freehand three-dimensional ultrasound imaging was used to reconstruct the free-tendon and proximal AT at rest and during contraction. The free-tendon exhibited significantly ($P=0.03$) greater longitudinal strain (5.2 \pm 1.7%) than the proximal AT (2.6 \pm 2.0%). The lesser longitudinal strain of the proximal AT was linked to the fact that it exhibited considerable transverse (orthogonal to the longitudinal direction) strains (5.0 \pm 4%). The transverse strain of the proximal AT is likely due to the triceps surae muscles bulging upon contraction, and thus the level of bulging may influence the elastic behaviour of the proximal AT. This might have implications for the understanding of triceps surae muscle–tendon interaction during locomotion, tendon injury mechanics and previous measurements of AT elastic properties.

Key words: transverse strain, triceps surae, gastrocnemius, elastic.

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INTRODUCTION

The human Achilles tendon (AT) exhibits elastic properties and acts in series with the triceps surae muscle group that acts to plantar-flex the ankle. The AT's elasticity allows it to store and return elastic energy during locomotion (Ishikawa et al., 2005; Lichtwark et al., 2007), which serves as an important energy-saving mechanism. The AT consists of two structurally distinct parts: (1) the proximal AT, which originates at the muscle–tendon junction (MTJ) with the gastrocnemius muscle and runs contiguous with the soleus muscle; and (2) the free AT, which continues from the distal end of the soleus to its insertion on the calcaneus (Fig. 1). In studies of AT elastic properties (e.g. Kubo et al., 1999; Maganaris and Paul, 2002) and AT behaviour during locomotion (e.g. Neptune et al., 2001; Ishikawa et al., 2007), the proximal AT and tendon are often considered as a single series elastic structure with a single value for longitudinal stiffness.

The proximal AT arises from the gastrocnemius aponeurosis to which muscle fascicles attach, and is a broad 'sheet-like' structure similar to the aponeurosis. The free AT begins at the most distal insertion of the soleus fascicles and is coiled into an elliptical 'rope-like' structure. These differing shapes give them very different aspect ratios, making the free AT better suited for stretch under longitudinal tension. Ultrasound and magnetic resonance imaging (MRI) have demonstrated that the free AT stretches more than the aponeurosis in the longitudinal direction during plantar-flexor contractions (Finni et al., 2003; Magnusson et al., 2003). This may be due to different material properties of different parts of the tendon, or

alternatively it may be because the aponeurosis is tensioned transversely as well as longitudinally, owing to muscle bulging. The latter has been demonstrated in the human tibialis anterior muscle (Maganaris et al., 2001) as well as turkey muscle (Azizi and Roberts, 2009). In the turkey muscle, this provided a mechanism for increasing aponeurosis stiffness with increasing contraction force up to 30% of maximum isometric force (Azizi and Roberts, 2009). The combined human free AT and proximal AT complex also becomes stiffer with increasing levels of plantar-flexor contraction (Sugisaki et al., 2011). If muscle bulging causes transverse strain of the aponeurosis, it seems probable that it could also transversely strain the proximal AT. This might allow the proximal AT to exhibit elastic behaviour similar to that of the aponeurosis and different from the free AT. Unfortunately, the two-dimensional (2-D) imaging used by Magnusson et al. (Magnusson et al., 2003) did not allow them to measure transverse strain of the aponeurosis or proximal AT, and Finni et al. (Finni et al., 2003) only examined a central portion of the soleus aponeurosis. Therefore, neither could confirm this mechanism.

Whilst the MRI scanning employed by Finni et al. (Finni et al., 2003) appears an attractive technique for analysing three-dimensional (3-D) AT structure, it is not widely available and is relatively expensive. Recently, 3-D ultrasound image reconstruction techniques have been developed (Gee et al., 2004) and validated for measurements of medial gastrocnemius (MG) muscle dimensions (Barber et al., 2009). This technique provides a non-invasive, low-cost approach that could be used to measure AT shape change in 3-D.

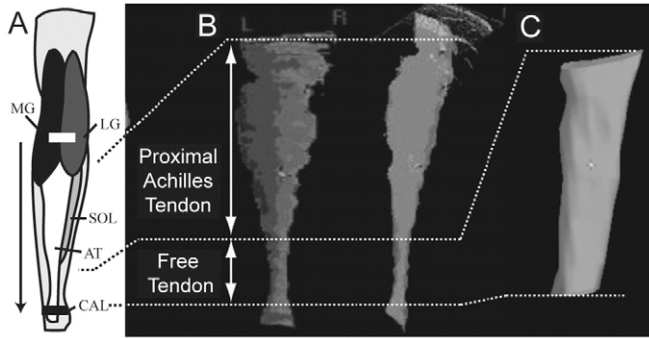


Fig. 1. (A) Positioning of the ultrasound transducer and the direction of the sweep (arrow) for the transverse scans. (B) Two views of an example of a reconstruction from a transverse scan showing the proximal Achilles tendon (AT) and the free AT. (C) An example of a reconstruction of the free AT.

Therefore, this study aimed to employ 3-D ultrasound imaging to non-invasively determine whether longitudinal strain of the free AT and proximal AT are different during plantar-flexion contraction *in vivo*. Furthermore, we aimed to measure transverse strain of the proximal AT during contraction of the plantar-flexors. It was hypothesised that the free AT would exhibit greater longitudinal strain than the proximal AT and this would be due to transverse strain of the proximal AT.

MATERIALS AND METHODS

Participants

Six male and five female participants (mean \pm s.d. age=27 \pm 5 years; height=1.69 \pm 0.15 m) gave written informed consent to take part in the study. Ethical approval for this study was granted by an institutional ethics panel and complied with the regulations set out in the 2008 Declaration of Helsinki.

Experimental protocol

Participants were required to perform plantar-flexion contractions using their dominant leg on a dynamometer (HUMAC NORM, CSMi, Stoughton, MA, USA). The ankle angle was fixed at 90 deg and participants lay in the prone position (Fig. 2A). During plantar-flexion contractions on a dynamometer where the ankle is fixed there is still potential for ankle rotation, which can passively alter muscle and tendon geometry (Maganaris, 2005). To avoid this, strapping was applied over the dorsal aspect of the foot and participants held the hand grips with the instruction to keep their heel in contact with the dynamometer foot plate (Fig. 2A). The condition that the heel did not leave the plate was visually confirmed by the experimenter.

Each participant had a familiarisation session in which they practised holding contractions at a consistent pre-defined torque level for 10 s. The torque level was the maximum torque that the participant could hold consistently for 10 s as determined from a series of contractions where the target torque was gradually increased. Feedback was given in the form of a visual display where the participant was required to keep the real-time torque plot between two lines (\pm 5 Nm) above and below the target torque. The torque that participants could hold for 10 s resulted in a group mean \pm s.d. target torque that was 52 \pm 7% of their maximum voluntary contraction (MVC) torque (MVC was determined as the mean of three attempted MVCs performed during the familiarisation session and group mean \pm s.d. MVC torque was 138 \pm 32 Nm).

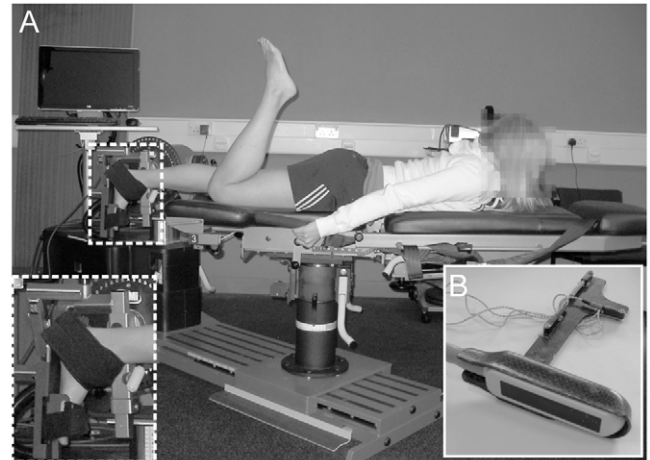


Fig. 2. (A) The dynamometer setup. Participants lay in the prone position with the foot-plate at 90 deg to the shank. Strapping was applied over the dorsal aspect of the foot (inset). (B) The ultrasound transducer with a rigid carbon fibre attachment fitted. Three motion analysis markers were attached to track the position and orientation of the probe.

At the start of the experiment itself, participants completed five warm-up contractions to pre-condition the AT (Maganaris et al., 2002). Ultrasound scanning was performed while the participant was at rest and while they contracted at the target torque level. Five resting measurements and five contracted measurements were taken. Because two different scanning techniques were used, this was repeated for each, giving 10 total resting scans and 10 contracted scans.

Ultrasound scanning and reconstruction procedures

The 3-D ultrasound reconstruction was performed in a manner similar to the methods described in detail by Barber et al. (Barber et al., 2009), who also demonstrated the validity of these methods for accurate reconstruction of the gastrocnemius muscle volume and length. For each reconstruction of the AT, a 'stack' of ultrasound images was obtained by sweeping the transducer over the volume of interest. The position and orientation of the transducer were tracked in 3-D space by a motion capture system tracking three markers that were attached rigidly to the transducer (Fig. 2B). A prior single-wall phantom calibration procedure (Prager et al., 1998) was used to calibrate this system for accurate reconstruction. This calibration determined the position of the ultrasound image relative to the coordinate system defined by the three markers, allowing successive images to be projected into 3-D space and effectively produce a 3-D volume. To provide good contact between the transducer and the skin and to minimise tissue deformation, an acoustic standoff and gel were used.

Ultrasound images were recorded at 50 Hz with a 128 element linear array transducer (LV7.5/60/96Z, TELEMED, Vilnius, Lithuania) operating in B-mode at 8.0 MHz. A 3 V square wave pulse produced by the beam-former was used to synchronise ultrasound imaging with the motion capture system (CODA, Charnwood Dynamics, Rothley, UK). A slight delay between ultrasound and motion capture of 0.02 s was measured and accounted for in subsequent data processing.

Two types of ultrasound scan were collected: (1) a transverse scan (Figs 1,3,4) to determine differential length changes between free AT and proximal AT and (2) a longitudinal scan (Fig. 5) to determine proximal AT width at the MTJ with the gastrocnemius. For the transverse scans, the transducer was orientated to obtain a transverse

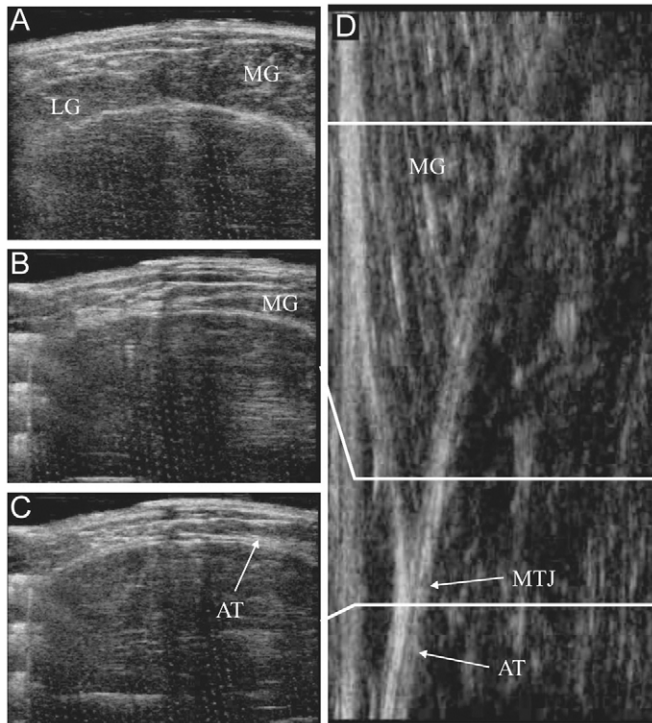


Fig. 3. Example of the first part of a transverse scan sequence at the muscle–tendon junction (MTJ) between the medial gastrocnemius (MG) and the Achilles tendon (AT) showing the MG merging into the tendon (A–C). (D) Longitudinal scan of the MTJ to help the reader orientate the transverse scans.

section of the AT and swept down the longitudinal axis of the leg from the distal end of the MG through to the calcaneus (Fig. 1A). The MTJ was identified and digitised in the first frame during the sweep in which the MG was no longer visible and the AT was a solid white line within the image (Fig. 3B–D). To obtain an anatomically consistent point on the MTJ across subjects, the most distal point on the MTJ was always digitised. This was considered to be the point where the MG had last been visible. The start of the free tendon was defined as the most distal insertion of the soleus into the tendon. Fig. 4A–F shows the soleus muscle gradually decreasing in cross-section as the probe is swept down the shank until it is no longer present in the image. Images were sampled at 50 Hz and it took ~5–7 s to complete a sweep down the long axis of the leg. This meant that ~250–300 images were recorded per scan. Given that combined free AT and proximal AT length never exceeded 250 mm in this study, this gave an approximate spatial resolution between images never greater than 1 mm. This was considered sufficient as no lengths were reported to sub-millimetre dimensions. For longitudinal scans, the transducer was orientated to obtain a longitudinal section and swept from a position marked on the medial aspect of the lower leg around the surface of the leg to the lateral side and then across the calcaneus (Fig. 5). The start and end points of the initial sweep across the leg were far enough medial and lateral on the leg that the full width of the MTJ had been included.

Reconstructions of the AT from ultrasound images (Figs 2, 6) were all performed in Stradwin software (v3.5, Mechanical Engineering, Cambridge University, Cambridge, UK). In each image within a stack, the perimeter of the AT was outlined manually and each of these segments was used for surface interpolation to

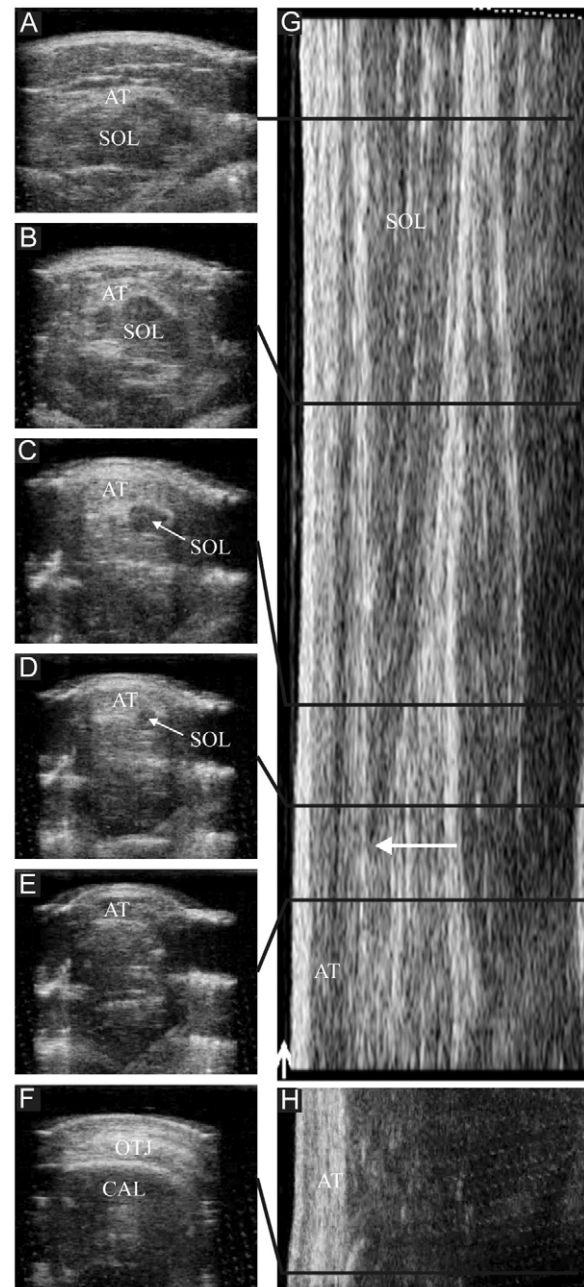


Fig. 4. Sequence of transverse scans (A–D) showing the soleus (SOL) gradually decreasing in cross-section and inserting on the Achilles tendon (AT). (E) A transverse scan of the free AT. (F) The osteotendinous junction (OTJ) of the AT with the calcaneus (CAL). (G,H) Longitudinal scans of the soleus inserting on the AT and the AT inserting on the CAL, respectively, to help the reader orientate. The white arrow in G indicates the distal attachment of the SOL on the AT.

render 3-D images. Specific 3-D coordinates were obtained from reconstructions of transverse sweeps for the most distal point on the MTJ with MG, the most distal insertion of soleus on the AT and the most proximal insertion of the free AT on the calcaneus. The insertion on the calcaneus was defined as the most proximal point at which the AT clearly attached to the bone. Three-dimensional coordinates for the position of the MTJ in each of the images within the stack of each longitudinal scan were taken to map

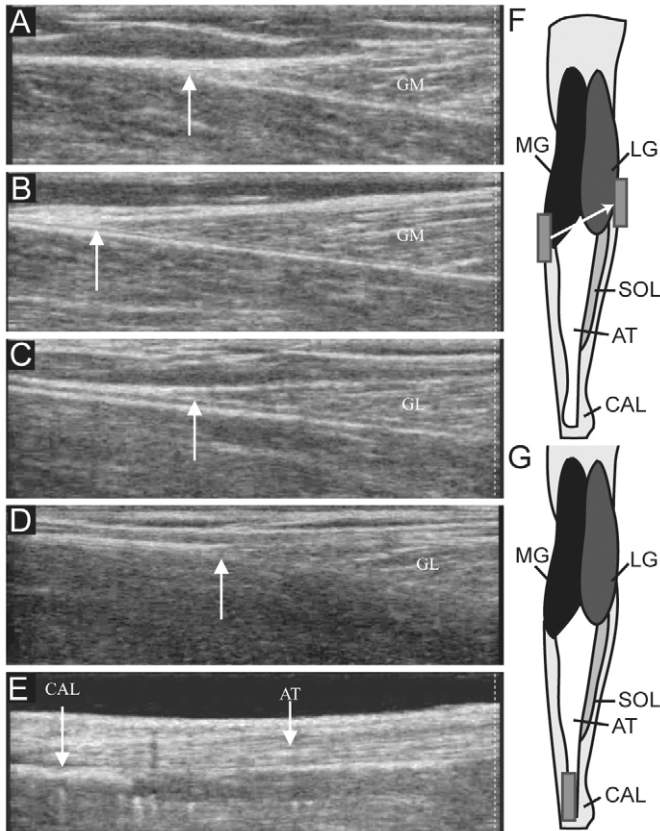


Fig. 5. (A–D) Longitudinal scans of the muscle–tendon junction (MTJ) between the medial (MG) and lateral (LG) heads of the gastrocnemius and the Achilles tendon (AT). (E) Longitudinal scan of the insertion of the AT on the calcaneus (CAL). (F) Orientation and direction of the sweep of the probe across the MTJ. (G) Positioning of the probe when scanning the insertion of the AT on the calcaneus.

out the MTJ in three dimensions. The position of the most proximal insertion on the calcaneus was also extracted from the longitudinal scans.

Data analysis

The transverse scans were used to calculate the length of the whole AT and the length of the free tendon at rest and during contractions. This was done using the coordinates of the landmarks identified in the previous section. Vectors between the MTJ and the start of the free AT and between the start of the free AT and the AT insertion were computed. The magnitude of these vectors was taken as the length of the proximal AT and the free AT, respectively. The length of the whole AT was equal to the length of the proximal AT plus the length of the free AT. Strain of the three parts of the tendon was calculated using Eqn 1:

$$\varepsilon = \frac{(L_c - L_r)}{L_r} \times 100\% , \quad (1)$$

where ε is strain, L_c is the length during contraction and L_r is the length at rest.

Proximal AT width was always measured using digitised points on the MTJ. It was taken as the medio-lateral distance between the most lateral point on the MTJ and the most medial point on the MTJ (Fig. 6). These two points were identified in the longitudinal

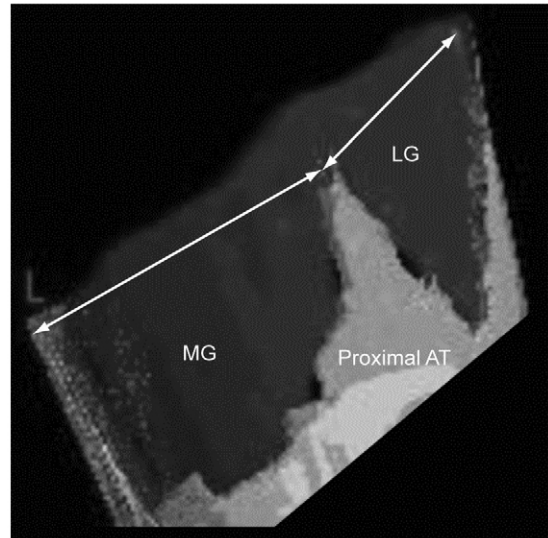


Fig. 6. An example of a 3-D reconstruction of the medial gastrocnemius (MG), lateral gastrocnemius (LG) and the proximal Achilles tendon (AT) at the level of the muscle–tendon junction (MTJ). This type of reconstruction was achieved with a longitudinal scan across the MTJ (Fig. 5).

scans. The lateral and medial end points of the MTJ were defined as the first/last frame in which the muscle was visible (i.e. a tapered darker area representing the distal end of the muscle was present in the image). These points were chosen because they were anatomical landmarks that were identifiable in the rested and contracted states. However, they probably do not represent the widest part of each muscle, which is likely nearer their mid-belly.

Whole AT lengths were calculated at 20 evenly spaced points (every 5% of the muscle width in that condition) across the MTJ for each head of gastrocnemius. To achieve this, lengths were calculated as the distance between each digitised point on the MTJ and the insertion of the AT on the calcaneus (Fig. 7). An interpolating cubic spline was then used to find the length at intervals of 5% across the width of each muscle (i.e. to normalise where measurements were taken across each muscle head). The corresponding length measurements at rest and during contraction were used to calculate strain at these 20 points across the MTJ. From these 20 strain values the mean of each consecutive window of five points was taken to represent four equally sized regions across the width of each head of the gastrocnemius (i.e. four regions from the lateral to medial border of each muscle). A mean value of all 20 points was also taken for each head of gastrocnemius in order to compare strain between the two heads of the muscle. Proximal AT width was calculated as the distance between the medial end of the MTJ of MG and the lateral end of the MTJ of the lateral gastrocnemius (LG) as defined above. It was also separated into changes in width of the MG portion and the LG portion.

Statistical analyses

The main outcome measures to be compared were: longitudinal strain of the free AT *versus* the proximal AT; proximal AT width at rest and during contraction; mean strain of the AT measured on the MG against mean strain of the AT measured on the LG; and strain of the AT in each of the four different regions of the MTJ for MG and LG. To test for a statistically significant ($P < 0.05$) difference in strain between the free tendon and proximal AT and

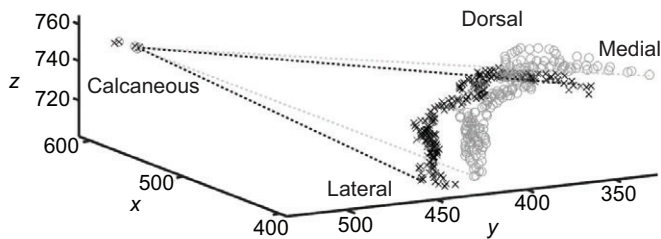


Fig. 7. Example data from five resting (black crosses) and five contracted (grey circles) trials from one participant showing raw digitised 3-D coordinates of points across the muscle–tendon junction (MTJ) of the medial gastrocnemius and the lateral gastrocnemius. The points nearest the z-axis are the digitised positions of the calcaneus. The dotted lines show how a vector was drawn between individual points on the MTJ and the calcaneus to compute tendon lengths at different points on the MTJ.

between the two heads of the gastrocnemius, a paired Student's *t*-test was used. For determining whether there were statistically significant ($P < 0.05$) differences in longitudinal strain between the four different regions of the MTJ on each head of the gastrocnemius, a repeated-measures ANOVA with a Bonferroni adjustment and a Huynh–Feldt epsilon correction was used. In the event of the ANOVA returning a significant *F*-ratio, a Tukey's honestly significant difference *post hoc* test was used to determine between which regions of the tendon any difference existed. Data are presented as means \pm s.d. unless otherwise indicated.

RESULTS

The group mean resting free AT and proximal AT lengths were 55 ± 15 and 136 ± 33 mm, respectively. Length change from rest to contraction was 3 ± 1 and 3 ± 2 mm for the free AT and proximal AT, respectively (i.e. length change of the whole tendon, from the MTJ to the calcaneus, was 6 mm). This equated to a mean strain of $5.2 \pm 1.7\%$ of the free AT and $2.6 \pm 2.0\%$ of proximal AT (Fig. 8). This difference was statistically significant ($P = 0.03$). Proximal AT width increased on average by 4 ± 2 mm, which gave a mean transverse strain of $5.0 \pm 4\%$ (Fig. 8). Most of this transverse strain occurred at the MTJ with the MG (medial \pm s.d. transverse strain = $6 \pm 4\%$) whereas the MTJ with the LG exhibited less or sometimes negative transverse strain ($2 \pm 4\%$). Group mean length changes and strains at different regions of the MTJ with the MG and LG did not differ significantly and there was no difference in the average strain across the whole MTJ with the MG *versus* the whole MTJ with the LG (Fig. 9).

DISCUSSION

This study used freehand 3-D ultrasound reconstructions to compare strain in the free AT and its associated proximal AT during static contractions of the plantar-flexors. It was hypothesised that the free AT would strain more than the proximal AT longitudinally and that this would be related to transverse strain occurring in the proximal AT. Our results supported this hypothesis, showing significantly greater longitudinal strain in the free AT (5.2%) than in the proximal AT (2.6%) and that the proximal AT strained transversely by 6% on average at the distal end of the MG muscle (5% for MG and LG combined).

The finding of greater longitudinal strain in the free AT was consistent with previous studies that have shown a similar result using 2-D ultrasound imaging (Magnusson et al., 2003) and MRI (Finni et al., 2003). The actual strain values observed in this study

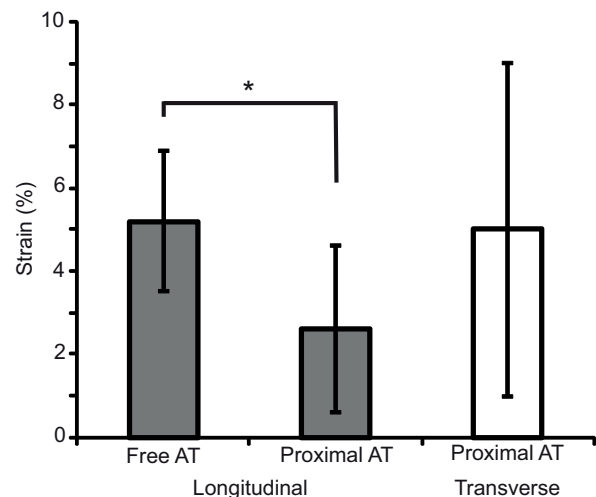


Fig. 8. Group mean longitudinal strain for the free Achilles tendon (AT) and the proximal AT (grey). Transverse strain of the proximal AT at the muscle–tendon junction is shown in white. Error bars are \pm s.d. ($*P < 0.05$).

(5.2% for free AT and 2.6% for proximal AT at $\sim 50\%$ MVC) were similar to those from Finni et al. (Finni et al., 2003) at a similar level of muscular contraction (4.7 and 2.2% at 40% MVC). In addition to the differential longitudinal strain, the present study observed transverse strain of the proximal AT at the MTJ with the MG of 6%. This is considerably less than the 21% that has been observed during MVC in the tibialis anterior muscle (Maganaris et al., 2001), but this difference may be due to contraction intensity or architectural differences between the two muscles. The differential strain patterns observed in the proximal AT and the free AT may have implications for series elastic function, AT injury and 2-D ultrasound analyses.

Implications for series elastic function

The elastic nature of the AT has frequently been cited as important for efficient function of the gastrocnemii and the soleus muscles during walking and running (Ishikawa et al., 2007; Lichtwark and Wilson, 2007; Sawicki and Ferris, 2008). The AT acts in series with these muscles and its stretch and recoil allows them to contract at low or even isometric velocities for much of stance (Lichtwark et al., 2007). This promotes efficient force production in these muscles and the storage and return of energy in the AT. In fact, it has been postulated that at preferred walking speed the elastic properties are optimised for this efficient behaviour (Lichtwark and Wilson, 2007).

However, many studies have assumed the AT to act as one linear spring of uniform stiffness, incorporating both the free AT and the proximal AT. For this assumption to be accurate, both the free AT and the proximal AT should have similar elastic properties. In the present study, the free AT and the proximal AT exhibited similar length changes (≈ 3 mm) during contraction, which, because of their different resting lengths, equated to different strains. Without measurements of force in each part of the AT, it was not possible to compare the stiffness of the free AT and proximal AT; however, the relatively low strain of the proximal AT is interesting when considering the transverse strain it exhibited. Transverse strain of the aponeurosis has been demonstrated during contractions of the human tibialis anterior muscle (Maganaris et al., 2001) and the LG of turkeys (Azizi and Roberts, 2009). This transverse strain does not occur during passive force development and may serve to stiffen

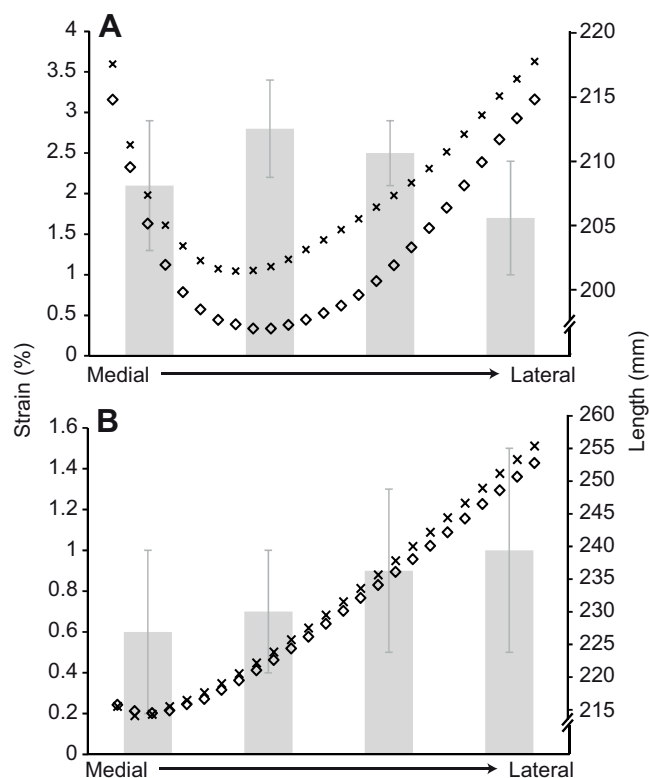


Fig. 9. Group mean resting (diamonds) and contracted (crosses) lengths of the whole proximal Achilles tendon (AT) and free AT complex plotted at intervals of 5% of the width of the medial gastrocnemius (A) and the lateral gastrocnemius (B). The normalisation masks any transverse strain of the proximal AT upon contraction. The grey bars represent the group mean strain of the whole tendon and proximal AT over four evenly sized windows across the width of the muscle–tendon junction. Error bars are \pm s.d.

the aponeurosis in the longitudinal direction during contraction (up to 30% of maximum isometric force), resulting in smaller longitudinal strains than during passive force development (Azizi and Roberts, 2009). Therefore, the considerable transverse strain (5%) of the proximal AT during contraction that was observed in the present study could explain why the proximal AT exhibited low longitudinal strains.

It has previously been shown that the combined free AT and proximal AT complex exhibits greater total longitudinal stiffness at higher contraction levels in humans (Sugisaki et al., 2011). Interestingly, the experiments with turkey muscle showed that transverse strain of the aponeurosis increased with contraction force up to 30% of maximum isometric force. This provided a mechanism by which some of the increase in longitudinal aponeurosis stiffness with contraction force was explained (Azizi and Roberts, 2009). Considering that transverse strain of the proximal AT was observed in the present study in a contraction at 50% the MVC, it is possible that the result of Sugisaki et al. (Sugisaki et al., 2011) could be partially explained by a similar mechanism. However, studies measuring transverse proximal AT strain at multiple contraction levels are needed to confirm this, and increases in longitudinal stiffness at high force levels may depend on other mechanisms.

Implications for AT injury

The AT is among the most frequently injured tendons in the human body and Achilles tendinopathies are common among athletic populations (Kujala et al., 2005). Of particular note is that the most

common site for these injuries is the free AT (Józsa and Kannus, 1997). As demonstrated here, the free AT is more prone to strain than other parts of the AT. The strain exerted on the AT during locomotion has been cited as a potential contributing factor in tendinopathies (Wilson and Goodship, 1994; Smith et al., 2002; Farris et al., 2011). Specifically, large strains exerted on the tendon during running may lead to increases in tendon core temperature to the point where thermal degradation of the tendon tissues could occur (Wilson and Goodship, 1994; Farris et al., 2011). Therefore, the greater longitudinal strain of the free AT might be a property that predisposes it to injury. Furthermore, most studies of AT strain during locomotion have measured strain of the whole free AT and proximal AT complex (Ishikawa et al., 2007; Lichtwark et al., 2007). These studies might be underestimating free AT strain and thus the free AT may be straining to even greater levels than previously thought during locomotion.

Implications for 2-D measurements

Ultrasound imaging has quite often been used to examine length change of the combined free AT and proximal AT by taking 2-D images in the longitudinal plane of the MG or LG MTJ during contractions or movements (Fukunaga et al., 1996; Maganaris and Paul, 2000). Typically, the transducer has been placed in the central region of that muscle's MTJ and elongation there was assumed to be representative of the whole MTJ. Results presented here support this assumption as strain was not significantly different within the different regions of the MTJ for either the MG or the LG. Furthermore, average strain across the MG MTJ was not different from average strain across the LG MTJ. This suggests relative uniformity of longitudinal elastic behaviour of the two heads. Thus, it may be acceptable to image either head in a 2-D analysis. However, it should be noted that this was for one moderate level of contraction. Also, the transverse strains of the MG and LG proximal AT were not the same. This suggests that different amounts of transverse muscle bulging may occur in these two muscles during contraction.

Study limitations

As noted previously, this study only examined one level of contraction and did not determine free AT or proximal AT stiffness because we lacked an estimate of tendon force. A comparison of transverse strain and tendon stiffness across multiple contraction levels would definitively reveal whether transverse strain is linked to stiffness and contraction level in the human triceps surae. The level of contraction in the present study (\approx 50% MVC) is modest but still relevant in terms of human movements. In a recent study of walking and slow running it was estimated that the human MG was often generating peak forces 50% or less of predicted maximum isometric force for the MG (Farris and Sawicki, 2012).

Conclusions

Here we employed a 3-D freehand ultrasound approach to measure differential strain in the human AT. It was shown that the longitudinal strain of the free AT was greater than that of the proximal AT during sub-maximal plantar-flexor contractions. Considerable transverse strain of the proximal AT was demonstrated and considered to be a factor in determining its lesser longitudinal strain. Further studies should include multiple levels of contraction from passive movement to maximum contraction and determine tendon stiffness for each contraction level.

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