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## RESEARCH ARTICLE

# Effects of running on human Achilles tendon length–tension properties in the free and gastrocnemius components

Glen A. Lichtwark[1,*], Andrew G. Cresswell[1] and Richard J. Newsham-West[2]

[1]Centre for Sensorimotor Neuroscience, The School of Human Movement Studies, The University of Queensland, St Lucia 4072, Australia and [2]Centre for Musculoskeletal Research, School of Rehabilitation Sciences, Griffith University, Gold Coast 4222, Australia

*Author for correspondence (g.lichtwark@uq.edu.au)

## SUMMARY

**The elastic properties of the human Achilles tendon are important for locomotion; however, *in vitro* tests suggest that repeated cyclic contractions lead to tendon fatigue – an increase in length in response to stress applied. *In vivo* experiments have not, however, demonstrated mechanical fatigue in the Achilles tendon, possibly due to the limitations of using two-dimensional ultrasound imaging to assess tendon strain. This study used freehand three-dimensional ultrasound (3DUS) to determine whether the free Achilles tendon (calcaneus to soleus) or the gastrocnemius tendon (calcaneus to gastrocnemius) demonstrated tendon fatigue after running exercise. Participants ($N$=9) underwent 3DUS scans of the Achilles tendon during isometric contractions at four ankle torque levels (passive, and 14, 42 and 70 N m) before and after a 5 km run at a self-selected pace (10–14 km h$^{-1}$). Running had a significant main effect on the length of the free Achilles tendon ($P$<0.01) with a small increase in length across the torque range. However, the mean lengthening effect was small (<1%) and was not accompanied by a change in free tendon stiffness. There was no significant change in the length of the gastrocnemius tendon or the free tendon cross-sectional area. While the free tendon was shown to lengthen, the lack of change in stiffness suggests the tendon exhibited mechanical creep rather than fatigue. These effects were much smaller than those predicted from *in vitro* experiments, possibly due to the different loading profile encountered and the ability of the tendon to repair *in vivo*.**

Key words: strain, fatigue, creep, ultrasound, biomechanics.

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## INTRODUCTION

Tendons have elastic mechanical properties: they stretch in proportion to the forces that are applied to them and also dissipate a small amount of energy when the force is removed (Abrahams, 1967; Pollock and Shadwick, 1994). The elastic properties of muscles and tendons are important for minimising energy consumption in a variety of species, with long compliant tendons being essential parts of the musculoskeletal design (Alexander and Bennet-Clark, 1977; Biewener and Roberts, 2000; Cavagna and Kaneko, 1977; Lichtwark and Barclay, 2010). The classic example of a compliant tendon in humans is the Achilles tendon, which is a long tendon attached to muscles with relatively short pennate muscle fibres. This design enables these muscles to generate large forces as a result of the increased physiological cross-sectional area (CSA), while still contracting at favourable lengths and speed for force production during locomotion (Lichtwark and Wilson, 2008; Wilson and Lichtwark, 2011) because the elastic tendon undergoes most of the length change (Fukunaga et al., 2001; Lichtwark and Wilson, 2006). Thus, the precise architecture and effective compliance of the tendon is extremely important for efficient locomotion.

*In vitro* tests have shown that when tendons undergo multiple tensile strain cycles, the tendon increases in length in response to stress (i.e. becomes more compliant) and therefore become susceptible to large strains and ultimately tendon failure (Ker et al., 2000; Wang et al., 1995). This process is known as tendon fatigue. Mechanical fatigue is typically reserved for changes in mechanical properties in response to cyclic loading, but in this case this inevitably involves some contribution from creep (deformation under constant load) because the mean strain is always greater than zero (Ker, 2008; Ker et al., 2000; Wren et al., 2003). Fatigue can be distinguished from creep in that creep does not accelerate length changes and does not induce a change in stiffness (only a change in overall length) (Ker et al., 2000).

In running, the Achilles tendon experiences up to three to five times body weight and has been shown to experience strains in excess of 6% during each stride (Komi, 1990; Lichtwark and Wilson, 2006). *In vitro* experiments on the Achilles tendon would suggest that it is susceptible to tendon fatigue and ultimately damage at such large strains (Wren et al., 2003). Therefore, it is not surprising that this tendon has a high rate of injury in athletes competing in running sports, where the tendon is repetitively loaded to high strains (Maffulli et al., 2004; Rees et al., 2006). Understanding the susceptibility of the Achilles tendon to fatigue in relation to exercise intensity may be the key to providing guidance for training load to minimise the risk of injury.

*In vivo* experiments using ultrasound imaging techniques have attempted to determine whether repetitive cyclic contractions result in a change in the stress–strain response of the Achilles tendon. Maganaris found that after as few as five maximal voluntary contractions (MVCs), the Achilles tendon increases its net strain for a maximum force effort (providing that prior activity is limited) (Maganaris, 2003). However, after the fifth contraction the net strain did not change. Similarly, low intensity (25% MVC) cyclic contractions have been shown to induce an increase in tendon strain relative to force after immobilisation; but this effect plateaued after ~5 min (Hawkins et al., 2009). In contrast, when repeated isometric or isokinetic contractions are applied through the Achilles tendon after conditioning contractions, no change in tendon elongation in response to force has been found (Mademli et al., 2006), suggesting little tendon fatigue. Recent studies have examined more rapid and forceful movements, which may be more likely to induce greater tendon strain. Farris and colleagues examined the effect of a 30 min run (12 km $h^{-1}$) (Farris et al., 2011), while Peltonen and colleagues examined single leg hopping to exhaustion (Peltonen et al., 2010) and also a marathon run (11.2 km $h^{-1}$) (Peltonen et al., 2012). Each of these studies found that there was no measurable change in tendon stiffness, while Peltonen and colleagues (Peltonen et al., 2010) also reported no change in the resting length of the tendon. In these studies, the tendon was pre-conditioned through a series of maximum voluntary isometric contractions prior to the exercise so as to remove the conditioning effect found by Maganaris (Maganaris, 2003). These results suggest that after initial conditioning, tendon fatigue effects in the Achilles tendon may not be as significant as demonstrated in the *in vitro* preparations.

There are some technical limitations to the *in vivo* ultrasound studies that might limit the ability to detect Achilles tendon fatigue or creep. One major limitation is that these studies rely on measurements of tissue displacement from two-dimensional image planes that may not necessarily match that of the tendon strain and only measure strain of one region of the tendon; usually at the medial gastrocnemius muscle–tendon junction, which represents the gastrocnemius tendon elongation. Therefore, any changes in the shape of the distal region of the muscle during contraction are likely to affect the strain measurements. There are also differences in the strain along the length of both the free tendon (the thick tendon that is free of muscle attachment – distal to the soleus) and the sheath-like part of the tendon above the soleus insertion which completes the gastrocnemius tendon, with the free tendon having been shown to experience the greatest strain (Magnusson et al., 2003). Using cine-phase magnetic resonance imaging, Finni and colleagues (Finni et al., 2003) demonstrated that regions within the proximal tendon sheath experience different strains depending on where the measurement is made (e.g. distal or proximal regions). Recently, freehand three-dimensional ultrasound (3DUS) has also shown that the free tendon undergoes more strain at the same level of voluntary force than the rest of the proximal tendon sheath that connects to the gastrocnemius (Farris et al., 2013). Therefore, the measurement of the free tendon strain may differ from that of the gastrocnemius tendon or parts of the aponeurosis, thus possibly making it difficult to assess tendon fatigue.

The aim of this study was to examine whether there were changes in tendon mechanical properties that might indicate tendon fatigue or creep in response to repetitive cyclic loads induced by running. Given the large inter-individual variation in tendon strain experienced during tasks like running (Lichtwark and Wilson, 2006) and the natural variation in tendon stiffness (Lichtwark and Wilson, 2005), it was impossible to control for tendon strain and/or stress experienced during running with any accuracy. We therefore decided to use a self-paced 5 km run as this represents a typical training distance for recreational athletes (providing ecological validity) and this distance would require more than 2000 loading cycles, after which we might expect to see changes in tendon stiffness and length [see figs 2, 3 of Wren et al. (Wren et al., 2003)]. We hypothesised that when the three-dimensional structure of the tendon was taken into account, both the free and gastrocnemius tendon would exhibit structural and mechanical changes consistent with tendon fatigue (change in length and stiffness at a given force) or creep (change in length at a given force) from repetitive load cycles. Specifically, we expected that the tendon would exhibit greater strain relative to the same stress (estimated from torque) and that the average free Achilles tendon CSA would diminish to accommodate such a change. To test this hypothesis, we applied a 3DUS technique to assess tendon length in response to different loading levels and tendon CSA both before and after a 5 km run at a self-selected pace.

## MATERIALS AND METHODS

### Participants

Nine male participants (mean ± s.d. age 22±4 years, height 173±11 cm, mass 74±9 kg) who participate in regular recreational running activities (and hence were competent in running 5 km) but were not trained distance runners, volunteered to participate in this study. All participants were informed about the study *via* a participant information sheet and gave written informed consent to participate, in accordance with institutional ethics guidelines (The University of Queensland Medical Research Ethics Committee). Potential participants were excluded from the study if they had any history of Achilles tendon injury or any other major injuries to the lower limb that impeded normal gait.

### Protocol

Prior to visiting the laboratory, participants were asked to refrain from any running or vigorous activity (e.g. jumping, resistance training) for the 24 h leading up to the testing session. A test–retest protocol was used to examine the influence of running on tendon mechanical properties. Participants initially underwent a test to examine Achilles tendon strain during isometric contractions at various force levels. They were then instructed to run continuously on a treadmill for 5 km at a comfortable self-selected pace (10–14 km $h^{-1}$). All participants were able to achieve this running distance within 31 min (range 23–31 min). Immediately after the run, participants were re-tested to examine Achilles tendon strain in response to isometric contractions at the same force level. Care was taken to ensure that the participant was positioned in the same position in both pre- and post-run trials.

### Achilles tendon strain measurements

During mechanical tendon testing, participants lay prone on an examination bench with their right ankle constrained in a neutral position (90 deg) by a custom-built foot pedal with a transducer for measuring plantar flexion torque about the ankle. The axis of rotation of the ankle was aligned with that of the torque transducer. Once aligned, the foot was securely strapped to the footplate by consecutive lengths of athletic strapping tape, primarily taped over the topside of the foot close to the joint centre and looped around the rear of the footplate. Lengths of tape were also placed down the long axis of the shank from the medial to lateral sides *via* the base of the footplate to create a stirrup effect. This arrangement was necessary because ultrasound imaging of the Achilles tendon required the rear of the foot to be clear of any tape or obstruction. As a result of this configuration, it was not possible to perform MVCs without significant heel lift, which may impact on the torque measurements. Hence, contractions up to ~50% of MVC were used, which resulted in minimal heel lift. Heel lift is only likely to influence measures of torque and not Achilles tendon length, which is measured directly using the 3DUS technique (see below).

Participants initially performed five MVCs, which were held for ~1 s duration to condition the tendon (Maganaris, 2003). During these contractions the heel was allowed lift off the footplate, as the tendon length was not measured. 3DUS was then used to scan the Achilles tendon at rest (no voluntary plantar flexion torque) using two different scanning techniques: either a distal–proximal or a proximal–distal direction along the Achilles tendon (see below for details). Participants then performed two 8–12 s isometric contractions at three different sub-maximal target torque levels: 14, 42 and 70 N m. Visual feedback of the torque with respect to time was provided *via* a second computer monitor to enable participants to match the target torque levels. During the two contractions at each target torque level, a 3DUS scan was performed in each of the two scan directions (distal–proximal or proximal–distal).

### Freehand 3DUS scans and measurements

A 3DUS scan of the Achilles tendon was performed by combining conventional B-mode ultrasound imaging (SonixTouch, Ultrasonix, BC, Canada) with synchronous position and orientation data from a three-camera optical tracking system (Optitrack, NaturalPoint, OR, USA). All data were collected and analysed within the Stradwin software package (Stradwin v4.2, Mechanical Engineering, Cambridge University, Cambridge, UK) (Fig. 1). Four markers were rigidly attached to the ultrasound transducer to provide position and orientation data for the transducer. Prior to scanning, the relationship between the image coordinate system and the marker coordinate system was determined using the single wall phantom calibration protocol in the Stradwin software (Barber et al., 2009; Prager et al., 1998).

A 38 mm linear probe (L14-5/38 Linear, Ultrasonix) with a central frequency of 10 MHz was used to provide transverse images (depth 40 mm) of the soft tissues within the lower leg at ~40 frames $s^{-1}$. A custom-made casing built from plastic casting material (Rolyan Aquaplast Original splinting material, Patterson Medical, Warrenville, IL, USA) was attached to the probe to a hold a piece of 2 cm thick stand-off material (Aquaflex Ultrasound Gel Pad, Parker Labs Inc., Fairfield, NJ, USA) in contact with the transducer surface while it was swept along the leg. The stand-off conformed to the shape of the tendon when pressure was applied and ensured that images contained the entire tendon cross-section, particularly at the distal end where the surface is very narrow and convex.

At each target torque level a scan was first performed in the distal–proximal direction. Scans of the free tendon began at the base of the heel with the probe approximately perpendicular to the leg in the transverse plane. Scans progressed along the midline of the leg until either the medial or the lateral gastrocnemius muscle was visible in the image. The total scan time was ~8–12 s, which equated to an approximate average scan speed of 20 mm $s^{-1}$ and hence an average distance between frames of 0.5 mm. The distance between the tendon insertion on the calcaneous and the soleus muscle–tendon junction was determined as a straight line between the most distal aspect of the notch in the calcaneous at the osteo-tendinous junction (Hawkins et al., 2009) and most distal point of the soleus muscle–tendon junction (MTJ). This length is defined as the free tendon henceforth. A second scan began more proximally above the medial gastrocnemius and progressed distally over the most distal point of the gastrocnemius MTJ towards the midline of the leg and then continued on beyond the calcaneous. This scan was used to measure the tendon distance from the gastrocnemius MTJ to the calcaneal notch and was defined as the gastrocnemius tendon (Fig. 1). We also used this scan to re-measure the length of the free tendon (using the inverse scanning direction) during the passive trials and this was used to examine the intra-session reliability of the measurement technique.

Tendon CSA was segmented from the transverse images of the distal–proximal scan at ~5 mm intervals along the length of the free tendon. The free tendon volume was reconstructed using these slices and their three-dimensional location using the 3D rendering algorithms of the Stradwin software (Fig. 2). In some cases the tendon at the distal end was not visible for the first 5–10 mm and hence the CSA could not be segmented. To overcome this limitation, the average CSA across the volume of tendon that was segmented was determined by dividing the volume by the length of the tendon that was segmented. This mean tendon CSA of the free tendon was used for comparison between pre- and post-running measurements.

### Data analysis

Intra-session reliability of the measurement technique was assessed using the intraclass correlation (ICC) and the limits of agreement method (Bland and Altman, 1986) to examine the length of the free tendon when scanned in the distal–proximal direction in comparison to a scan in the proximal–distal direction (within the same session). The length of the free and gastrocnemius Achilles tendon at each torque level was divided by the length at rest measured before the run to quantify tendon strain relative to the relaxed position (note that the relaxed position may have induced some stress on the tendon and hence this is not a measure of tendon strain relative to zero stress). The relative stiffness of the free and gastrocnemius tendons was calculated at each torque level as the slope of the length–tension

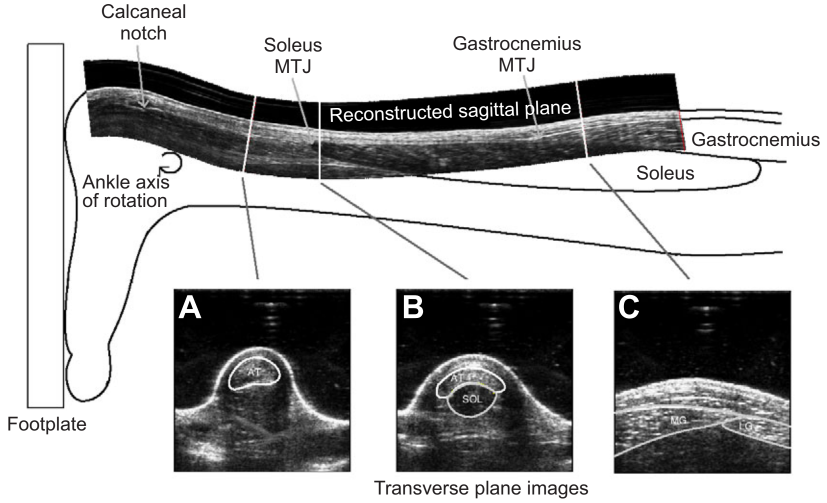

Fig. 1. Experimental setup showing a re-constructed sagittal plane view of the triceps surae muscle–tendon unit with identifying landmarks used in calculating Achilles tendon length. The reconstructed view is created using a stack of transverse plane images of which three slices are shown from the areas indicated by the white lines. Within the transverse plane images, the Achilles tendon (AT) area is outlined in A and B, while the soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) are outlined in B and C. MTJ, Muscle–tendon junction.

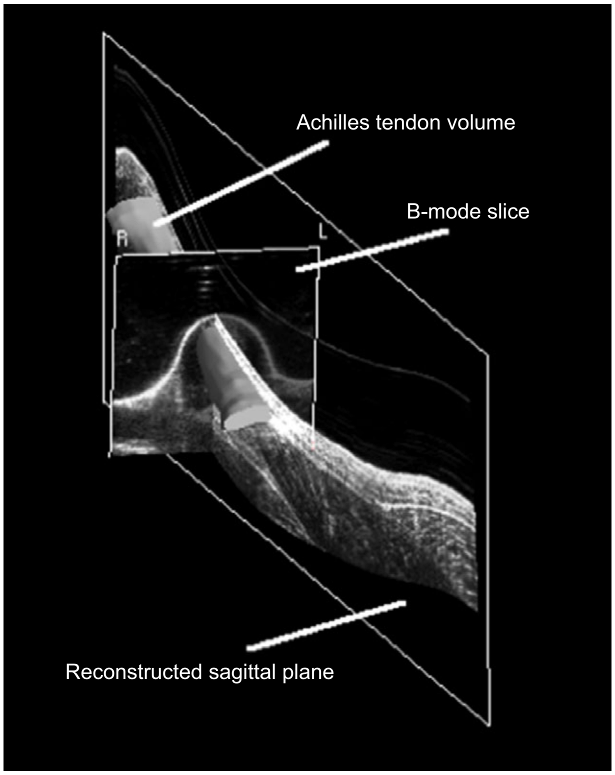

Fig. 2. Three-dimensional volume rendering of the free Achilles tendon relative to a sagittal plane reconstructed cross-section and a single B-mode image slice from which cross-sections were constructed. R, right; L, left.

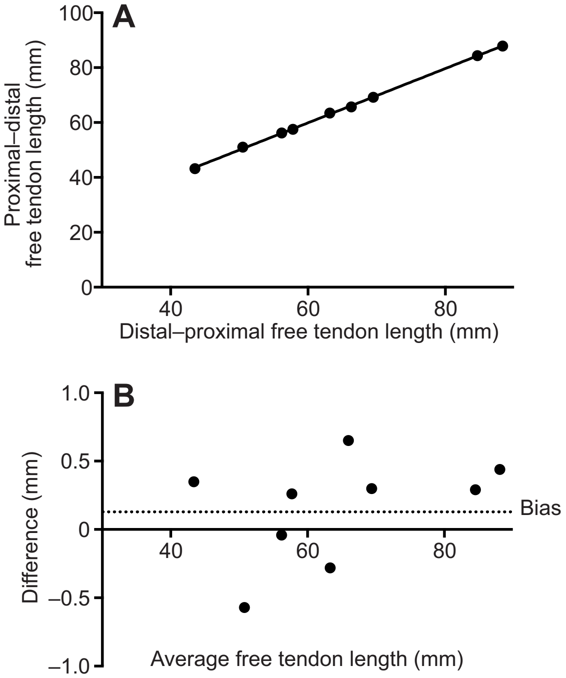

Fig. 3. (A) Correlation between free tendon length measurements obtained from a distal–proximal scan *versus* a proximal–distal scan. (B) Bland–Altman plot of average tendon length from the distal–proximal scan and proximal–distal scan *versus* the difference between each scanning direction. Bias line represents the mean difference in the two scanning directions.

relationship by dividing the change in length of the tendon (from each torque level to the one immediately below) by the change in torque. While this does not represent the instantaneous stiffness at each torque level, it does provide a means of comparing changes in the slope of the length–tension relationship at each torque level. Differences in absolute length, strain and stiffness between the two measurement sites (free or gastrocnemius tendon) were assessed at each torque level using a two-way repeated measures ANOVA (measurement site × torque level). The same statistical test was used to examine differences in either muscle length or strain levels between the pre- and post-run measurements (measurement time × torque level). Where a significant interaction existed, multiple comparisons were performed at each torque level using Bonferroni post-test corrections. A two-tailed Student's *t*-test was conducted to compare the average CSA of the free tendon. All statistical analyses were performed in Graphpad Prism (Prism 5; La Jolla, CA, USA) and the significance level was set to $P\leq 0.05$.

## RESULTS

### Reliability of measurements

A comparison of the length of the free tendon measured in opposite sweep directions within the same session resulted in an ICC of 0.998 (see correlation in Fig. 3A) and the 95% limit of agreement (difference *versus* the average) was from −1.13% (−0.59 mm) to 1.49% (0.90 mm), with a bias of less than 0.16 mm. The Bland–Altman plot is shown in Fig. 3B.

### Free tendon *versus* gastrocnemius tendon strain

Prior to the run, tendon measurement site (free or gastrocnemius tendon) had a significant main effect on tendon strain measurements across the torque levels ($P<0.01$). A significant interaction was not found ($P=0.53$). The data reveal that the free tendon experiences more strain than the gastrocnemius tendon relative to torque (Fig. 4). A peak strain of 3.81% was measured in the free tendon compared with 2.74% in the gastrocnemius tendon. The peak torque level of 70 N m corresponded to 48±7% of MVC across all participants (neglecting error in MVC torque measurements due to heel lift in these pre-conditioning contractions).

### Effects of 5 km run

A comparison of pre- and post-running measurements from the free tendon showed that the 5 km run had significant main effects on both the absolute tendon length ($P<0.01$) and tendon strain ($P<0.01$). Torque also had a main effect ($P<0.01$), but there was no interaction. The data show that the free tendon was significantly longer across the torque range for the post-run measurements compared with the pre-run values (Fig. 5A). The multiple comparison tests found no significant differences in comparing pre- with post-run length or strain at any torque level (including the passive condition). Therefore, while the increase in length was consistent, the overall effect was small and only equated to a mean difference in passive length of 0.57 mm (0.96%) (Table 1). There was no detectable change in free tendon stiffness in response to the run ($P=0.5$). There was no difference between the passive tension at rest pre- and post-run ($P=0.56$).

In contrast to the free tendon results, no main effect of the 5 km run was found on the gastrocnemius tendon length or strain ($P=0.12$ and $P=0.1$, respectively), with no interaction between torque and measurement time (pre- or post-run). Therefore, the gastrocnemius tendon length did not seem to change significantly in response to

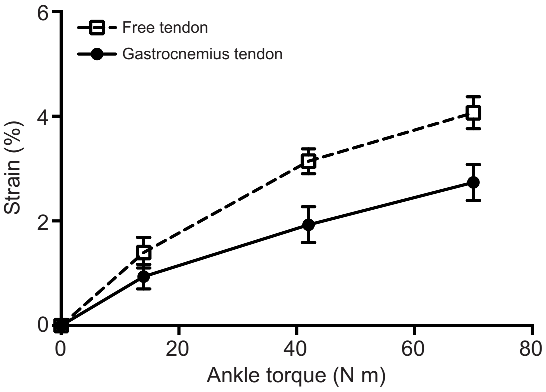

Fig. 4. The mean relationship between ankle torque and Achilles tendon strain for the gastrocnemius tendon and free tendon from the pre-run measurement. Error bars represent ±1 s.e.m. (*N*=9). There was a significant main effect of the tendon measurement site (free *versus* gastrocnemius) on tendon strain ($P<0.001$), with the free tendon straining further than the gastrocnemius tendon.

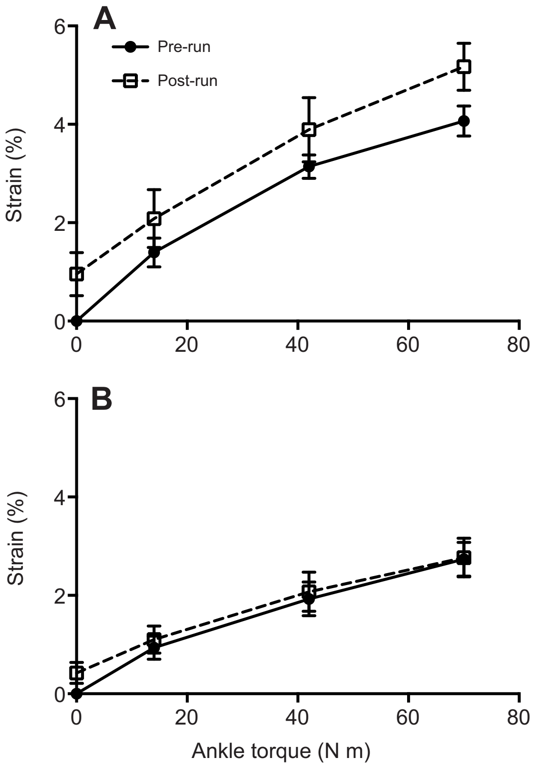

Fig. 5. Comparison of the mean relationship between ankle torque and Achilles tendon strain for pre- and post-run measurements for the free tendon (A) and gastrocnemius tendon (B). Error bars represents ±1 s.e.m. There was a significant main effect of the run on tendon strain of the free tendon ($P<0.001$) and no main effect on the gastrocnemius tendon ($P=0.098$).

torque generation, although there was a trend towards lengthening at lower torques (Fig. 5B). Like the free tendon, there was a small overall increase in passive length post-run of 0.87 mm (0.43%) (Table 1), but this was not significant. There was no detectable change in gastrocnemius tendon stiffness in response to the run ($P=0.46$).

The 5 km run resulted in no change in the mean tendon CSA (−0.1%, $P=0.79$) (Table 1).

## DISCUSSION

We have used 3DUS to quantify the strain of both the free and gastrocnemius Achilles tendon at different levels of plantar flexor torque generation to determine whether this relationship changes in response to cyclic loading at high forces (5 km run). Using the 3DUS technique we found that there was a small, significant increase in the free tendon strain across the prescribed torque levels in response to running. While this partly supports our initial hypothesis, the increase in length was very small (<1%) and was not shown for the gastrocnemius tendon (which underwent less net strain), and we did not find any change in tendon stiffness that might be apparent in response to mechanical fatigue. The apparent change in length was also not associated with a detectable decrease in mean tendon CSA of the free tendon. Overall, this suggests that the free and gastrocnemius Achilles tendons did not exhibit fatigue; however, the small increase in length of the free tendon may suggest that there was tendon creep in response to the net tensile load during the running task. The change in length of the free tendon was small in comparison to previous *in vitro* mechanical testing of tendon fatigue (Wang et al., 1995; Wren et al., 2003). Therefore, the *in vivo* Achilles tendon mechanical properties seem relatively robust under the running conditions tested here. Repeated small tendon fatigue events, such as those demonstrated here, without adequate tendon recovery could, however, potentially lead to overuse injury.

In support of previous *in vivo* (Farris et al., 2013; Magnusson et al., 2003) and *in vitro* (Wren et al., 2003) studies, we have shown that the free Achilles tendon undergoes significantly more strain in response to a given load under isometric conditions than the gastrocnemius tendon, which consists of both the free tendon and the aponeurotic tendon sheath. This difference in strain may be due to differences in the geometrical shape or composition of the tendon (Thomopoulos et al., 2003), torsion effects in the free tendon (van Gils et al., 2009) and forces applied by the different muscles of the triceps surae to different parts of the tendon (Szaro et al., 2009). Importantly, we did not find evidence of increased strain relative to torque level after the run for the gastrocnemius tendon, while we did for the free tendon, which supports previous ultrasound studies examining the influence of rigorous exercise on gastrocnemius tendon mechanical properties (Farris et al., 2012; Peltonen et al., 2010; Peltonen et al., 2012). This may be related to the fact that the strains were significantly smaller at the level of the gastrocnemius tendon, particularly within the aponeurotic sheath. We do not currently know what the strain attributable to the proximal gastrocnemius tendon sheath is during running, but these isometric tests suggest that the strain of the sheath is ~30% that of the free tendon.

Our results suggest that while the free tendon undergoes a small amount of lengthening relative to applied stress after a 5 km run, it does not decrease in stiffness and therefore our results are not necessarily comparable to the results from *in vitro* preparations of the Achilles tendon (Wren et al., 2003). In the 5 km run, participants would have experienced a minimum of 2000 cyclic contractions with initial peak tendon strains exceeding 6–7% (Lichtwark and Wilson, 2006). Comparable *in vitro* data presented by Wren et al. [in their figs 2, 3 (Wren et al., 2003)] suggest that such a loading profile would result in increased strain at comparable stresses of more than 2% strain and a decrease in stiffness of almost 20%. In contrast, our results show increases in the free tendon of only <1%, no difference in strain of the gastrocnemius tendon and no change in tendon stiffness. Given that we have not shown a change in

Table 1. Passive free tendon length, passive gastrocnemius tendon length and free Achilles tendon cross-sectional area in response to a 5 km run

| | Free tendon length (mm) | | Gastrocnemius tendon length (mm) | | Mean tendon CSA (mm$^2$) | |
|---|---|---|---|---|---|---|
| | Pre-run | Post-run | Pre-run | Post-run | Pre-run | Post-run |
| Mean | 64.00±15.35 | 64.57±15.29 | 208.39±25.80 | 209.26±25.76 | 67.59±11.09 | 67.29±9.34 |
| Mean change (%) | 0.96±1.31 | | 0.42±0.63 | | −0.01±5.14 | |

Data are group mean (s.d.) and mean change as a percentage of the pre-run value (±s.d.). There was no significant change in passive free tendon length, gastrocnemius tendon length or cross-sectional area (CSA) between conditions (*P*=0.40).

stiffness, the length changes measured cannot be attributed to fatigue and are more likely to be just the time-dependent loading effects, commonly classified as creep. However, classifying whether we are observing early stages of fatigue (where changes in tendon stiffness are not measurable using our technique) as opposed to creep would require an analysis of the time-dependent nature of the length changes in response to the load applied. Future experiments might assess this by determining changes in tendon length and stiffness after different periods of cyclic loading (e.g. different periods of running) and under different total stress conditions (e.g. different speeds or loads). Assessing longer periods of running or running with heavy loads to increase tendon stress may indeed increase the likelihood of finding evidence of tendon fatigue, but such studies are hard to implement practically because carrying out these tasks requires aerobic training, which may in turn induce changes in the mechanical properties of the tendon to protect it from creep or fatigue effects.

Overall, our results suggest that both the free and gastrocnemius portions of the Achilles tendon are rather robust in terms of the length–tension relationship within the distance travelled. The lack of tendon fatigue and small amount of creep may be related to differences in loading profile compared with the *in vitro* experiment of Wren and colleagues (Wren et al., 2003). For instance, loading occurred at a higher rate during our running task compared with the 1 Hz cyclic loading performed by Wren and colleagues. However, wallaby tail tendon experiments (Wang et al., 1995) suggests that higher rates of loading make a tendon more susceptible to early rupture relative to cycle number; therefore, we might have expected greater fatigue rather than less fatigue. Instead, it is more likely that the reduced fatigue is related to the fact that the tendon was under load for less than 40% of the time during running (stance only) and hence creep effects may be limited. In addition, the tendon has the capacity to be repaired in response to any minor damage; however, the time course over which this occurs is unknown.

One possible mechanism for increases in length in the free tendon is that the stress of the tendon increases through a change in tendon CSA. It has been shown that the tendon CSA decreases as tensile stress increases (Pokhai et al., 2009) and that repetitive cyclic loading results in changes in water content (Hannafin and Arnoczky, 1994) and tendon thickness (Grigg et al., 2009). Such changes may reduce tendon CSA and ultimately result in a higher stress for the same force. Here, we did not find any changes in the average CSA of the tendon as assessed by measurement of volume of the free tendon. Hence, the real stress applied to the tendon would have been constant in both pre- and post-run conditions, assuming the force was consistent across trials. Our measurements certainly do not discount potential changes in the tendon shape that may lead to differences in tendon thickness (Grigg et al., 2009) and perhaps shear loading. A limitation of measuring tendon CSA with ultrasound is the difficulty of gaining quality contrast between the tendon collagenous structures and other echogenic surrounding tissue. Hence, the accuracy of the CSA measurements in quantifying potential fluid shift with fatigue is one area of the 3DUS that could be improved.

There are some further limitations to our 3DUS approach that prevent solid conclusions being made about the susceptibility of the tendon to fatigue in response to running events. Firstly, we only tested up to 50% of the maximum isometric force. Testing the strain at higher loads may possibly have led to an increase in the differences in tendon strain between pre- and post-running measurements, which is expected if the tendon has also become more compliant. However, given that no interaction between torque level and testing condition was found across the torque range tested, and that there was no detectable difference in stiffness in response to the run, it is unlikely that changes in stiffness would be detected at higher force levels given that the tendon–length–tension relationship is likely to be more linear at higher force levels. However, the ability to examine a greater range in forces would improve the certainty of the results. We also restricted our measurements of tendon displacement under isometric stress to the most distal point of the MTJs, which provided a three-dimensionally consistent point to measure. This neglects any regional differences in tensile strain that might occur (e.g. medial to lateral or superficial to deep). Using 3DUS reconstructions, it is possible to see that points along the MTJ have a longitudinal strain that differs in the medial to lateral direction as the muscle changes shape during contraction (Farris et al., 2013). Hence, the measurements of tendon strain used here may not have provided the complete story regarding aponeurotic strain within the gastrocnemius tendon. Finally, the restriction of our 3DUS measurements to isometric contractions limits the findings to static strain measurements, where the static loading may itself contribute to tendon creep. This also prevents the possible measurement of the modulus or energy dissipation as further indicators of tendon fatigue or creep.

The 3DUS technique was shown to have sub-millimetre reliability for measuring tendon length when scans were compared from the same session but performed with different scan directions. Therefore, this technique is able to reliably distinguish a typical force–length relationship within both the free and gastrocnemius tendons where length changes range from 0.5 to 10 mm at the force levels used here. While the differences in length that were detected in response to the running exercise were within the 95% limits of agreement, the statistical design ensures that measurement variability is accounted for in the analysis, given that there was no systematic bias and the measurements were normally distributed. We found that there was a 0.17% chance of randomly observing an effect this big (or bigger) in an experiment of this size. However, further examination with a greater number of tendons is still required to establish the full validity and reliability of the technique, including the influence of tendon curvature on the outcome measure.

In conclusion, our results have provided the first *in vivo* evidence of Achilles tendon fatigue after running exercise. This was, however, only evident in the free tendon and the effect was small and restricted to changes in the length of the tendon relative to the stress applied, not tendon stiffness *per se*. These results may have implications for understanding Achilles tendon injury, because they suggest that some changes in material properties occur in the free tendon, which is commonly associated with tendinopathy. The 3DUS technique has significant potential for further research that might examine specific questions such as whether specific populations that are more susceptible to tendinopathies (e.g. middle-age runners) are more prone to tendon fatigue, whether the material properities of the tendons might be changed through interventions such as steroid injections or whether specific training loads induce either safe or dangerous levels of tendon fatigue.

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## AUTHOR CONTRIBUTIONS

G.A.L., A.G.C. and R.J.N.-W. all contributed to the conception, design, interpretation and drafting of the manuscript. G.A.L. and R.J.N.-W. were involved in executing the experiments. G.A.L. undertook all data analysis and creation of figures.

## COMPETING INTERESTS

No competing interests declared.

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## REFERENCES

**Abrahams, M.** (1967). Mechanical behaviour of tendon *in vitro*. A preliminary report. *Med. Biol. Eng.* **5**, 433-443.

**Alexander, R. M. and Bennet-Clark, H. C.** (1977). Storage of elastic strain energy in muscle and other tissues. *Nature* **265**, 114-117.

**Barber, L., Barrett, R. and Lichtwark, G.** (2009). Validation of a freehand 3D ultrasound system for morphological measures of the medial gastrocnemius muscle. *J. Biomech.* **42**, 1313-1319.

**Biewener, A. A. and Roberts, T. J.** (2000). Muscle and tendon contributions to force, work, and elastic energy savings: a comparative perspective. *Exerc. Sport Sci. Rev.* **28**, 99-107.

**Bland, J. M. and Altman, D. G.** (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307-310.

**Cavagna, G. A. and Kaneko, M.** (1977). Mechanical work and efficiency in level walking and running. *J. Physiol.* **268**, 467-481.

**Farris, D. J., Trewartha, G. and McGuigan, M. P.** (2012). The effects of a 30-min run on the mechanics of the human Achilles tendon. *Eur. J. Appl. Physiol.* **112**, 653-660.

**Farris, D. J., Trewartha, G., McGuigan, M. P. and Lichtwark, G. A.** (2013). Differential strain patterns of the human Achilles tendon determined *in vivo* with freehand three-dimensional ultrasound imaging. *J. Exp. Biol.* **216**, 594-600.

**Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. and Sinha, S.** (2003). Nonuniform strain of human soleus aponeurosis–tendon complex during submaximal voluntary contractions *in vivo*. *J. Appl. Physiol.* **95**, 829-837.

**Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. and Maganaris, C. N.** (2001). *In vivo* behaviour of human muscle tendon during walking. *Proc. Biol. Sci.* **268**, 229-233.

**Grigg, N. L., Wearing, S. C. and Smeathers, J. E.** (2009). Eccentric calf muscle exercise produces a greater acute reduction in Achilles tendon thickness than concentric exercise. *Br. J. Sports Med.* **43**, 280-283.

**Hannafin, J. A. and Arnoczky, S. P.** (1994). Effect of cyclic and static tensile loading on water content and solute diffusion in canine flexor tendons: an *in vitro* study. *J. Orthop. Res.* **12**, 350-356.

**Hawkins, D., Lum, C., Gaydos, D. and Dunning, R.** (2009). Dynamic creep and pre-conditioning of the Achilles tendon in-vivo. *J. Biomech.* **42**, 2813-2817.

**Ker, R. F.** (2008). Damage and fatigue. In *Collagen: Structure and Mechanics*, pp. 111-131. New York, NY: Springer.

**Ker, R. F., Wang, X. T. and Pike, A. V.** (2000). Fatigue quality of mammalian tendons. *J. Exp. Biol.* **203**, 1317-1327.

**Komi, P. V.** (1990). Relevance of *in vivo* force measurements to human biomechanics. *J. Biomech.* **23 Suppl. 1**, 23-34.

**Lichtwark, G. A. and Barclay, C. J.** (2010). The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. *J. Exp. Biol.* **213**, 707-714.

**Lichtwark, G. A. and Wilson, A. M.** (2005). *In vivo* mechanical properties of the human Achilles tendon during one-legged hopping. *J. Exp. Biol.* **208**, 4715-4725.

**Lichtwark, G. A. and Wilson, A. M.** (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J. Exp. Biol.* **209**, 4379-4388.

**Lichtwark, G. A. and Wilson, A. M.** (2008). Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J. Theor. Biol.* **252**, 662-673.

**Mademli, L., Arampatzis, A. and Walsh, M.** (2006). Effect of muscle fatigue on the compliance of the gastrocnemius medialis tendon and aponeurosis. *J. Biomech.* **39**, 426-434.

**Maffulli, N., Sharma, P. and Luscombe, K. L.** (2004). Achilles tendinopathy: aetiology and management. *J. R. Soc. Med.* **97**, 472-476.

**Maganaris, C. N.** (2003). Tendon conditioning: artefact or property? *Proc. Biol. Sci.* **270 Suppl. 1**, S39-S42.

**Magnusson, S. P., Hansen, P., Aagaard, P., Brønd, J., Dyhre-Poulsen, P., Bojsen-Moller, J. and Kjaer, M.** (2003). Differential strain patterns of the human gastrocnemius aponeurosis and free tendon, *in vivo*. *Acta Physiol. Scand.* **177**, 185-195.

**Peltonen, J., Cronin, N. J., Avela, J. and Finni, T.** (2010). *In vivo* mechanical response of human Achilles tendon to a single bout of hopping exercise. *J. Exp. Biol.* **213**, 1259-1265.

**Peltonen, J., Cronin, N. J., Stenroth, L., Finni, T. and Avela, J.** (2012). Achilles tendon stiffness is unchanged one hour after a marathon. *J. Exp. Biol.* **215**, 3665-3671.

**Pokhai, G. G., Oliver, M. L. and Gordon, K. D.** (2009). A new laser reflectance system capable of measuring changing cross-sectional area of soft tissues during tensile testing. *J. Biomech. Eng.* **131**, 094504.

**Pollock, C. M. and Shadwick, R. E.** (1994). Relationship between body mass and biomechanical properties of limb tendons in adult mammals. *Am. J. Physiol.* **266**, R1016-R1021.

**Prager, R. W., Rohling, R. N., Gee, A. H. and Berman, L.** (1998). Rapid calibration for 3-D freehand ultrasound. *Ultrasound Med. Biol.* **24**, 855-869.

**Rees, J. D., Wilson, A. M. and Wolman, R. L.** (2006). Current concepts in the management of tendon disorders. *Rheumatology* **45**, 508-521.

**Szaro, P., Witkowski, G., Śmigielski, R., Krajewski, P. and Ciszek, B.** (2009). Fascicles of the adult human Achilles tendon – an anatomical study. *Ann. Anat.* **191**, 586-593.

**Thomopoulos, S., Williams, G. R., Gimbel, J. A., Favata, M. and Soslowsky, L. J.** (2003). Variation of biomechanical, structural, and compositional properties along the tendon to bone insertion site. *J. Orthop. Res.* **21**, 413-419.

**van Gils, C. C., Steed, R. H. and Page, J. C.** (1996). Torsion of the human Achilles tendon. *J. Foot Ankle Surg.* **35**, 41-48.

**Wang, X. T., Ker, R. F. and Alexander, R. M.** (1995). Fatigue rupture of wallaby tail tendons. *J. Exp. Biol.* **198**, 847-852.

**Wilson, A. and Lichtwark, G.** (2011). The anatomical arrangement of muscle and tendon enhances limb versatility and locomotor performance. *Philos. Trans. R. Soc. B* **366**, 1540-1553.

**Wren, T. A. L., Lindsey, D. P., Beaupré, G. S. and Carter, D. R.** (2003). Effects of creep and cyclic loading on the mechanical properties and failure of human Achilles tendons. *Ann. Biomed. Eng.* **31**, 710-717.