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RESEARCH ARTICLE

Three-dimensional morphology and strain of the human Achilles free tendon immediately following eccentric heel drop exercise

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ABSTRACT

Our understanding of the immediate effects of exercise on Achilles free tendon transverse morphology is limited to single site measurements acquired at rest using 2D ultrasound. The purpose of this study was to provide a detailed 3D description of changes in Achilles free tendon morphology immediately following a single clinical bout of exercise. Freehand 3D ultrasound was used to measure Achilles free tendon length, and regional cross-sectional area (CSA), medio-lateral (ML) diameter and antero-posterior (AP) diameter in healthy young adults (*N*=14) at rest and during isometric muscle contraction, immediately before and after 3×15 eccentric heel drops. Post-exercise reductions in transverse strain were limited to CSA and AP diameter in the mid-proximal region of the Achilles free tendon during muscle contraction. The change in CSA strain during muscle contraction was significantly correlated to the change in longitudinal strain (*r*=−0.72) and the change in AP diameter strain (*r*=0.64). Overall findings suggest the Achilles free tendon experiences a complex change in 3D morphology following eccentric heel drop exercise that manifests under contractile but not rest conditions, is most pronounced in the mid-proximal tendon and is primarily driven by changes in AP diameter strain and not ML diameter strain.

KEY WORDS: 3D ultrasound, Cross-sectional area, Diameter, Creep

INTRODUCTION

Repeated exposure to high tensile strains during exercise has been shown to induce immediate changes in Achilles tendon mechanical and morphological properties that could impact on tendon function and injury risk, and be important for long-term tendon adaptation (Obst et al., 2013). The mechanical and morphological properties of the distal Achilles tendon (i.e. Achilles free tendon) appear particularly susceptible to change following acute exercise (Grigg et al., 2009, 2012; Lichtwark et al., 2013; Obst et al., 2015). Increased strain of the Achilles free tendon measured at the same external load after exercise has been demonstrated following running (Lichtwark et al., 2013) and repeated eccentric heel drops (Obst et al., 2015), and is suggestive of early stage tendon fatigue due to mechanical creep (Fung et al., 2010). Because increased tendon longitudinal strain during tensile loading is closely associated with changes in tendon transverse morphology and strain (Obst et al., 2014b; Pokhai et al., 2009; Reeves and Cooper, 2014; Vergari et al., 2011), a similar relationship might be expected for the changes in tendon morphology and strain that occur in response to an exercise bout. In view of the high rate of exercise-related injuries of the Achilles free tendon and the potential role short-term changes in tendon transverse dimensions could have on the local mechanical and biological environment (Heinemeier and Kjaer, 2011; Shim et al., 2014; Smith et al., 2013), there is a need to better understand the acute effects of exercise on the tendon 3D morphology and strain.

There is evidence from 2D ultrasound (2DUS)-based measurements that resting Achilles free tendon antero-posterior (AP) diameter is reduced for up to 2.5 h after dynamic exercise and this effect is more pronounced following eccentric compared with concentric exercise (Grigg et al., 2009), and in healthy compared with injured tendons (Grigg et al., 2012). These short-term reductions in AP diameter after exercise have been suggested to reflect fluid exudation from the tendon core to the peri-tendinous space as a result of the creation of positive hydrostatic pressure within the tendon when tendon fibres stretch and pack under tensile load (Grigg et al., 2009; Hannafin and Arnoczky, 1994). While *in vitro* studies demonstrate load-dependent changes in fluid content and tendon dimensions in response to repeated loading (Hannafin and Arnoczky, 1994; Helmer et al., 2006; Wellen et al., 2005), *in vivo* studies of the human Achilles tendon report mixed findings with respect to exercise-induced changes in tendon volume (Pingel et al., 2013a, b; Shalabi et al., 2004; Syha et al., 2013) and cross-sectional area (CSA) (Burgess et al., 2009; Farris et al., 2012; Neves et al., 2014; Ooi et al., 2015); discrepancies that may reflect different exercise interventions, populations or imaging methods used to assess tendon morphology. It is also currently unclear whether changes in transverse morphology and strain following exercise are uniformly distributed across the length of the tendon, occur along the medio-lateral (ML) diameter, related to changes in longitudinal deformation, or are more pronounced under tensile load.

The purpose of this study was to examine the immediate effect of a single bout of eccentric heel drop exercise on the 3D morphology of the Achilles free tendon at rest, and during a submaximal isometric plantarflexion contraction, using a 3D ultrasound (3DUS)-based method (Obst et al., 2014b). We selected the eccentric heel drop as it remains an integral part of Achilles tendon rehabilitation (Beyer et al., 2015; Habets and van Cingel, 2015; Kjaer and Heinemeier, 2014), induces high tendon strains (~8.5%; Jeong et al., 2014) and has been shown to affect immediate changes in mechanical (Obst et al., 2015) and 2D morphological (Grigg et al., 2009) properties of the Achilles free tendon in healthy adults. We expected that transverse morphology and strain (CSA, AP diameter and ML diameter) of the Achilles free tendon would be altered immediately following exercise and correlated to the corresponding change in longitudinal strain.

MATERIALS AND METHODS

Participants

Fourteen recreationally active participants (8 males, 6 females; age 28.1±4.1 years, height 172.8±9.0 cm and body mass 70.0±15.4 kg) with no history of lower limb or Achilles tendon injury/surgery participated in the

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study. All participants provided written informed consent prior to participation. The study was conducted according to the Declaration of Helsinki and was approved by the institutional human research ethics committee.

Experimental design and eccentric exercise protocol

Participants were asked to avoid strenuous physical activity 24 h prior to testing. Testing commenced with maximal and submaximal isometric plantarflexion contractions (pre-exercise) during which freehand 3DUS scans were performed for measurement of Achilles free tendon parameters. Following this, participants completed 3×15 (2 min rest between sets) eccentric heel drop exercises at a constant speed of 3 s guided by a metronome (60 beats min^{-1}) that equates to an average ankle angular velocity of ~20–25 deg s^{-1}(Henriksen et al., 2009). The opposite leg was used to return to the start position, ensuring that eccentric-only contractions of the plantar flexors were performed. Verbal instruction was provided to encourage full ankle range of motion during each exercise and to ensure that the knee remained in full extension. All post-exercise tendon assessments were performed within 2 min of the exercise bout (post-exercise).

Data collection and analysis procedures

Torque and muscle activation

Ankle joint torque, muscle activation and tendon measurements were made with participants positioned prone with their left knee fully extended (0 deg flexion) and left ankle plantar flexed to 15 deg. The foot and ankle were firmly secured to a foot plate that housed a torque transducer (TFF600, Futek, Irvine, CA, USA). The position of the foot and ankle was checked to ensure that the talocrural joint axis of rotation (i.e. line bisecting the medial and lateral malleoli) aligned with the torque transducer axis of rotation. In this position the resting net ankle joint torque was negligible (mean ankle torque <1 N m) and thus measurements of resting tendon morphology were made at approximately zero stress (De Monte et al., 2006). Participants initially completed five maximal voluntary isometric contractions (MVICs) of ankle plantarflexion to pre-condition the Achilles tendon (Maganaris, 2003) and establish target plantarflexion torques for subsequent testing trials. Two 3DUS scans were then performed at rest and during an 8–12 s isometric plantarflexion contraction at 70% MVIC. Real-time visual feedback of torque was provided to assist maintenance of the target ankle torque for the duration of the trial. A double differential surface electromyography (EMG) system (band pass 30–500 Hz, gain 300 dB, common mode rejection ratio 160 dB) and commercial amplifier (Bagnoli-8™, Delsys Inc., Boston, MA, USA) were used to measure muscle activity of medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL) and tibialis anterior (TA) for all tendon measurement trials. Following skin preparation, three 24 mm surface electrodes (inter-electrode distance 20 mm; H124SG, Covidien, Kendall, USA) were placed over each muscle according to SENIAM guidelines (Hermens et al., 2000). Torque and EMG signals were analog–digital converted at a sampling rate of 1000 Hz and recorded using a custom-written LabView program (LabView 9.0, National Instruments, Austin, TX, USA) and USB data acquisition device (NI:USB-6259 BNC, National Instruments). Pre- and post-exercise target plantarflexion torques were normalised against the pre-exercise plantarflexion MVIC. EMG signals were amplified (×3000), band-pass filtered (30–500 Hz), full-wave rectified and root mean squared using a 20 ms non-overlapping window. All EMG amplitudes were normalised to pre-exercise MVIC and averaged over a 3 s window during the middle of each trial. EMG amplitudes for LG, MG and SOL were also expressed as a ratio of total triceps surae activation to account for the potential effect a change in synergistic muscle activation may have on tendon transverse strain (Arndt et al., 1998).

3DUS measurement of Achilles free tendon mechanical and morphological properties

Our freehand 3DUS system consisted of a conventional ultrasound machine (SonixTouch, Ultrasonix, Richmond, BC, Canada) and a five-camera optical tracking system (V100:R2, Tracking Tools v 2.5.2, NaturalPoint, Corvallis, OR, USA) that enabled synchronous recording of 2D brightness-mode (B-mode) ultrasound images with 3D position and orientation of the transducer determined from four reflective markers rigidly affixed to the probe (see Obst et al., 2014a). Prior to data acquisition, the relationship between the image coordinate system and the marker coordinate system was determined using a single wall phantom calibration procedure (Prager et al., 1998). Following calibration, the 3D coordinates of a pixel within a 2DUS image were known with an approximate error of ±0.5 mm. All 3DUS calibration, acquisition and image segmentation procedures were performed using the Stradwin software package (Stradwin 4.7^1, Cambridge University, UK; http://mi.eng.cam.ac.uk/~rwp/stradwin/). Previous work has established the accuracy and repeatability of freehand 3DUS for *in vivo* measurement of human muscle morphology (length and volume) at rest (Barber et al., 2009) and Achilles free tendon morphology (length, volume, CSA, AP diameter and ML diameter) under passive and active loading conditions (Lichtwark et al., 2013; Obst et al., 2014a,b, 2015).

All ultrasound acquisition and analysis were performed by one investigator (S.J.O.) using a 58 mm linear transducer with 10 MHz central frequency (L14-5W/60 linear, Ultrasonix) and standard image settings (frame rate 60 Hz, depth 40 mm, gain 50%, dynamic range 65 dB, map 4 and power 0). For each ankle condition (i.e. rest and 70% MVIC), two transverse scans were performed over the posterior leg extending proximally from the distal calcaneus to beyond the SOL muscle–tendon junction. To enhance visualisation of the Achilles tendon cross-section, a 1.5 cm thick acoustic standoff pad (Ultra/Phonic Focus, Pharmaceutical Innovations Inc., Newark, NJ, USA) was housed within a thermoplastic case affixed to the end of the transducer. Total scan time for each trial was 8–12 s (average scan speed of 10 mm s^{-1}) with an approximate between-frame interval of less than 0.2 mm. Achilles free tendon length determined using a combination of sagittal, transverse and frontal image re-slices and was defined as the shortest distance between two 3D points corresponding to the most distal edge of the calcaneal notch and the SOL muscle–tendon junction (MTJ) (Obst et al., 2015, 2014b). Tendon cross-sections were manually segmented between these two landmarks at ~5–10 mm intervals and interpolated using a shape-based interpolation method to create a 3D surface representation of the Achilles free tendon (Treece et al., 2000) (Fig. 1A–C). The corresponding 3D point cloud was then exported into Matlab (version R2012a, The MathWorks, Natick, MA, USA) for determination of tendon transverse morphology using methods described by Obst et al. (2014b). Pre- and post-exercise longitudinal and transverse strains at 70% MVIC were normalised to the corresponding pre-exercise resting lengths. All transverse morphological parameters are presented as the average of each 10% interval and expressed relative to the normalised tendon length. Because of difficulties visualising the Achilles free tendon cross-section as it traverses the calcaneus, subsequent analysis of mean and regional transverse morphology and strain only includes data between 30% and 100% of the normalised tendon length; this approximated the tendon region between the proximal edge of the calcaneus and distal edge of the SOL MTJ (Fig. 1B).

Statistical analysis

Differences in regional transverse morphology (CSA, AP diameter and ML diameter) measured before and after exercise were assessed separately at each torque level using a two-way (measurement time×tendon region) full factorial repeated measures general linear model. Where a significant time-by-region interaction or main effect of time was found, pairwise comparisons were performed between measurement time points at each tendon region using Bonferroni *post hoc* comparisons. The same statistical approach was used to assess the effect of exercise on regional transverse strains at 70% MVIC. Differences in pre- and post-exercise tendon length and volume, ankle torque and EMG, were assessed at each torque level using a two-way (measurement time×torque level) full factorial repeated measures general linear model. Where a significant time-by-torque interaction or main effect of time was found, pairwise comparisons were performed between measurement time points at each torque level using Bonferroni *post hoc* corrections. Differences in pre- and post-exercise tendon longitudinal strain at 70% MVIC were assessed using a two-way paired Student's *t*-test. Relationships between pre- and post-exercise change in mean CSA strain and tendon longitudinal strain, mean CSA strain and mean AP diameter strain, and mean CSA strain and mean ML diameter

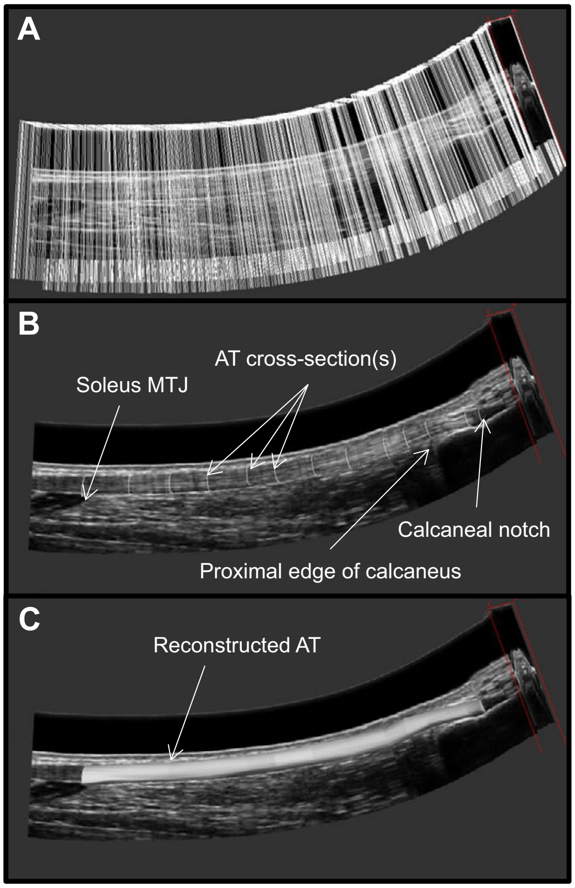

Fig. 1. 3D ultrasound analysis. Example of a reconstructed 3D stack of ultrasound images (A) with corresponding manual segmentation of the Achilles free tendon (AT) cross-sections (B) and interpolated 3D surface imbedded back into the original image stack (C). The AT was manually segmented from the transverse images at ~0.5–1 cm intervals from the most distal edge of the calcaneal notch to the soleus muscle–tendon junction (MTJ).

strain were each assessed using the Pearson's product-moment correlation coefficient (r). All statistical analyses were performed using SPSS Statistics software (version 20.0, SPSS Inc., Chicago, IL, USA) and the significance level for all tests was set at $P \leq 0.05$.

RESULTS

Effect of exercise on Achilles free tendon length, longitudinal strain and volume

There was a significant time-by-torque interaction and main effect of time for tendon length. *Post hoc* comparisons revealed a significant increase in post-exercise tendon length at rest (pre-exercise 61.4±17.7 mm, post-exercise 61.9±17.4 mm, $P=0.03$) and at 70% MVIC (pre-exercise 65.3±18.0 mm, post-exercise 66.8±17.9 mm, $P<0.001$). When normalised to pre-exercise resting length, these changes equated to a significant increase in tendon longitudinal strain at 70% MVIC (pre-exercise 6.5±2.6%, post-exercise 9.2±3.1%, $P<0.001$). There was no significant time-by-torque interaction or main effect of time for tendon volume measurements at rest (grand mean 4.1±1.3 ml) or at 70% MVIC (grand mean 4.2±1.2 ml).

Effect of exercise on Achilles free tendon regional transverse morphology and strain

There was a significant time-by-region interaction and main effect of time for tendon CSA at rest ($P<0.001$) and 70% MVIC ($P<0.001$). The data show tendon CSA was decreased across the measurement region (30–100% normalised length) following exercise at rest (pre-exercise 59.5±7.4 cm^2, post-exercise 52.6 ±6.8 cm^2, $P=0.001$) and at 70% MVIC (pre-exercise 62.1±8.5 cm^2, post-exercise 50.9±7.2 cm^2, $P<0.001$). *Post hoc* comparisons between time points at each tendon region revealed significant differences in tendon CSA at 70% MVIC between 60% and 80% of normalised tendon length (Fig. 2B, top), but no change in resting CSA (Fig. 2A, top). There was a significant time-by-region interaction and main effect of time for tendon ML diameter at rest ($P<0.001$) and 70% MVIC ($P<0.001$). Tendon ML diameter was decreased across the measurement region following exercise at rest (pre-exercise 13.7±0.33 mm, post-exercise 12.4±0.27 mm, $P<0.001$) and at 70% MVIC (pre-exercise 13.0±0.39 mm, post-exercise 11.3±0.31 mm, $P<0.001$). *Post hoc* comparisons between time points at each tendon region failed to reveal any significant differences in ML diameter at either torque level (Fig. 2A,B, middle). There was no significant time-by-region interaction for tendon AP diameter at rest or at 70% MVIC; however, there was a significant main effect of time at 70% MVIC (pre-exercise 5.4 ±0.43 mm, post-exercise 5.2±0.47 mm, $P<0.001$). *Post hoc* comparisons between time points for each region revealed a significant decrease in AP diameter at 70% MVIC between 50% and 100% of the normalised tendon length (Fig. 2B, bottom). There were no significant time-by-region interactions for any transverse strain measure; however, there was a main effect of time for CSA strain (pre-exercise 0.9±2.3%, post-exercise −2.5±2.3%, $P=0.035$) and AP diameter strain (pre-exercise 10.4±1.75%, post-exercise 5.7±1.9%, $P<0.001$), but not ML diameter strain. *Post hoc* comparisons between time points at each region revealed significant reductions in CSA strain and AP diameter strain between 60% and 90%, and 40% and 100% of the normalised tendon length, respectively (Fig. 3). Changes in mean CSA strain following exercise were strongly correlated to changes in longitudinal strain ($r=-0.72$, $P=0.002$) and mean AP diameter strain ($r=0.65$, $P=0.006$) (Fig. 4).

Effect of exercise plantarflexion torque and muscle activation

There was no time-by-torque interaction or main effect of time on ankle plantarflexion torque at rest (grand mean 0.97±0.53 N m) or at 70% MVIC (grand mean 57.1±13.7 N m). There was a significant time-by-torque interaction for normalised EMG amplitudes for all muscles; however, main effects of time were only found for MG ($P<0.001$), LG ($P<0.001$) and SOL ($P<0.001$). *Post hoc* comparisons revealed no change in resting normalised EMG amplitude; however, significant increases were found at 70% MVIC for MG (pre-exercise 0.54±0.13, post-exercise 0.72±0.24, $P=0.002$), LG (pre-exercise 0.59±0.17, post-exercise 0.89±0.31, $P=0.004$) and SOL (pre-exercise 0.5±0.17, post-exercise 0.73± 0.18, $P<0.001$). No significant time-by torque interaction or main effect of time was found for any of the EMG ratios.

DISCUSSION

This study used freehand 3DUS to determine the immediate effect of isolated eccentric exercise on the 3D morphology and strain of the Achilles free tendon. Post-exercise reductions in regional transverse morphology were detected for CSA and AP diameter during the 70% MVIC and were most pronounced in the mid-proximal region of the tendon. In contrast, no differences in ML diameter in response to the eccentric exercise were detected at

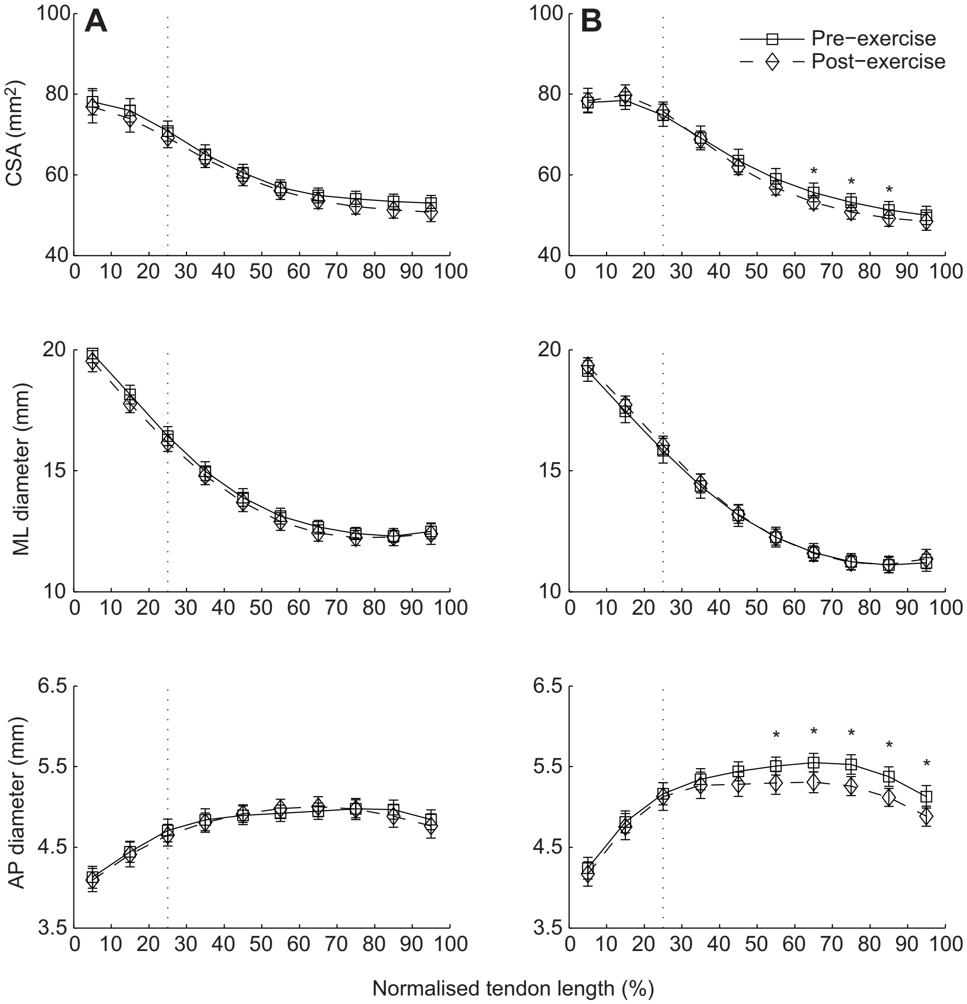

Fig. 2. Group data for Achilles free tendon cross-sectional area (CSA), medio-lateral (ML) diameter and antero-posterior (AP) diameter. Data were obtained at rest (A) and during a 70% maximal voluntary isometric contraction (70% MVIC; B) before and after a single bout of eccentric heel drop exercise. All data were averaged over 10% intervals and expressed relative to normalised tendon length (0%=calcaneal notch and 100% =soleus MTJ). Data between 0% and 30% of normalised tendon length (see vertical dotted line) were excluded from the statistical analysis. Error bars represent ±1 s.e.m. (N=14). *Significant *post hoc* pairwise difference between pre- and post-exercise value (P<0.05).

70% MVIC or for any morphological measures evaluated at rest. Although post-exercise reductions in regional AP diameter under load were small (~0.2 mm), when expressed relative to pre-exercise resting values, they equated to a 50% reduction in mean AP diameter strain under load. Consistent with our hypothesis, we also observed a strong inverse relationship between post-exercise change in longitudinal strain and mean CSA strain (r=−0.70), the latter of which was primarily derived from reduced AP diameter strain. Together, these results highlight the interaction between exercise-related changes in tendon length and transverse morphology, and the importance of characterising tendon morphology in 3D.

Effect of exercise on Achilles free tendon transverse morphology at rest

Consistent with previous *in vivo* studies, we found no change in regional CSA measured at rest immediately after exercise (Burgess et al., 2009; Farris et al., 2012; Lichtwark et al., 2013; Obst et al., 2015; Ooi et al., 2015). While our results did show a small but consistent reduction in CSA across all measurement regions (~7%), *post hoc* comparisons failed to reveal any significant differences between time points at any of the tendon regions. We also found no significant differences in resting ML diameter or AP diameter at any of the tendon regions. The latter result was unexpected, as previous studies have demonstrated large reductions in AP diameter (~0.9 mm or ~20%) measured at rest between 2 and 4 cm proximal to the superior calcaneal edge (equating to ~60–80% normalised tendon length in Fig. 2A) immediately after a similar bout of eccentric exercise in healthy individuals (Grigg et al., 2009, 2012). It is possible that the inclusion of tendon pre-conditioning contractions prior to baseline measurements in the present study may have reduced the overall effect of our exercise intervention. Consistent with this observation, previous studies that have not included tendon pre-conditioning contractions tend to report significant changes in transverse morphology immediately after exercise (e.g. AP diameter; Fahlstrom and Alfredson, 2010; Grigg et al., 2009, 2012; Wearing et al., 2013), whereas studies that have included pre-conditioning report little or no effect (e.g. CSA; Burgess et al., 2009; Farris et al., 2012; Lichtwark et al., 2013; Obst et al., 2015). In addition, the lack of change in resting AP diameter could reflect differences in the measurement method between studies. We computed tendon diameter along the tendon length by analysing the shape and dimensions of each consecutive 2D tendon cross-section determined from manual segmentation of the original transverse B-mode images. As a consequence, while our method minimises measurement errors due to tendon obliquity (Fornage, 1986; Sunding et al., 2014), the measures are inclusive of the peri-tendinous space (as opposed to Grigg et al., 2009, 2012) and therefore are unlikely to detect small changes in transverse dimensions due to possible movement of fluid from the tendon core to the peri-tendinous space (Grigg et al., 2009, 2012; Wellen et al., 2005).

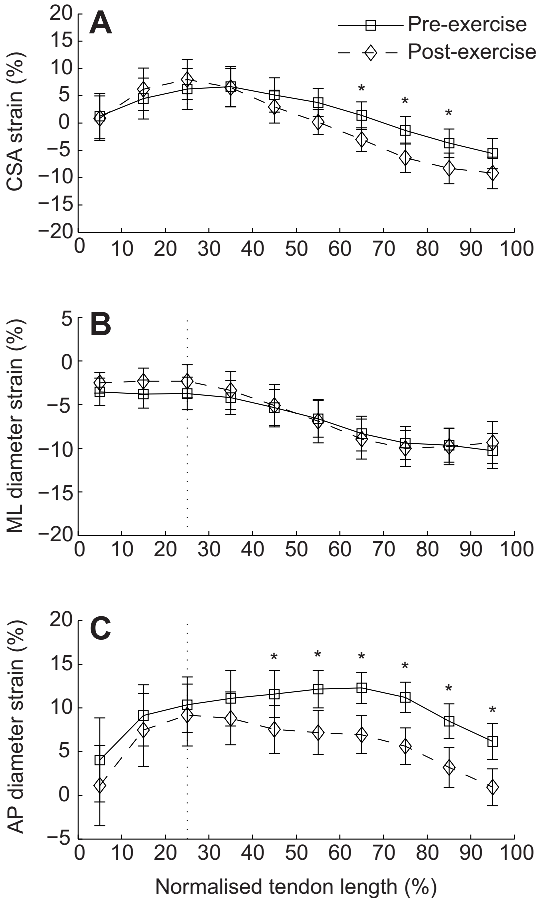

Fig. 3. Group data for Achilles free tendon strain at 70% MVIC measured before and after a single bout of eccentric heel drop exercise. (A) CSA, (B) ML diameter and (C) AP diameter strain. All data were averaged over 10% intervals and expressed relative to normalised tendon length (0%=calcaneal notch and 100%=soleus MTJ). Data between 0% and 30% of normalised tendon length (see vertical dotted line) were excluded from the statistical analysis. Error bars represent ±1 s.e.m. (*N*=14). *Significant *post hoc* pairwise difference between pre- and post-exercise value (*P*<0.05).

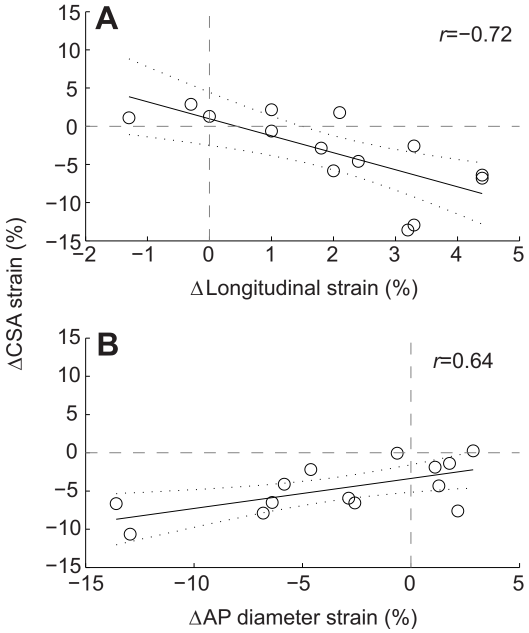

Fig. 4. Correlations (±95% confidence intervals) between changes in Achilles free tendon parameters post-exercise for each participant (*N*=14). (A) CSA strain (ΔCSA strain) and longitudinal strain (Δlongitudinal strain; *P*=0.002) and (B) CSA strain and AP diameter strain (ΔAP diameter strain; *P*=0.006). *r*, Pearson's correlation coefficient.

Effect of exercise on Achilles free tendon longitudinal and transverse strain under load

In contrast to the resting measurements, both CSA and AP diameter during the 70% MVIC were reduced immediately after exercise and represent the first *in vivo* evidence of altered transverse morphology and strain of the human Achilles free tendon measured during a muscle contraction. Consistent with our hypothesis, reduced mean CSA strain post-exercise was inversely correlated to increased longitudinal strain during the isometric muscle contraction. Our results provide the first evidence that links acute changes in transverse morphology to corresponding changes in tendon length after exercise. Short-term changes in Achilles free tendon transverse morphology after exercise (Fahlstrom and Alfredson, 2010; Grigg et al., 2010, 2009, 2012; Ooi et al., 2015; Wearing et al., 2011, 2013, 2008) may therefore be indicative of transient mechanical creep of the tendon (Lichtwark et al., 2013; Obst et al., 2015), whereby increased collagen fibril packing linked to longitudinal creep and reduction of collagen crimp could promote fluid redistribution within, and out of, the tendon (Grigg et al., 2009; Hannafin and Arnoczky, 1994; Wellen et al., 2005). It should be noted that although we did not detect any change in tendon volume at rest or during 70% MVIC, we cannot discount small changes in volume (minimal detectable change for 3DUS volume ~0.2 ml; Obst et al., 2014b) or redistribution of fluid along the tendon length (Sun et al., 2015). Regardless, our findings demonstrate that acute changes in tendon transverse morphology after exercise may be more pronounced when measured under tensile load, and that such changes may largely be dependent on changes in tendon length due to mechanical creep.

Consistent with our hypothesis, increased negative CSA strain measured during the 70% MVIC appeared to be primarily driven by reduced positive AP diameter strain, with little change in ML diameter strain. The latter result was somewhat surprising considering the inverse and proportional relationship that exists between free tendon AP diameter and ML diameter deformation and strain under tensile load during isometric plantarflexion contraction (Obst et al., 2014b). These results could therefore represent evidence of altered biaxial strain of the free tendon, during muscle contraction, in response to acute exercise. These changes were not explained by post-exercise differences in muscle activation patterns or torque production, but could reflect non-uniform fatigue or creep of Achilles tendon fascicles, due to differences in mechanical properties and/or tensile loading during eccentric heel drop (Arndt et al., 2011, 1998; Slane and Thelen, 2014). Irrespective of the cause, acute alterations in the normal biaxial strain could have implications for intra-tendinous force distribution (Haraldsson et al., 2008), fluid flow (Reese et al., 2010; Yin and Elliott, 2004) and tissue homeostasis (Smith et al., 2013) and are therefore relevant in the context of exercise-dependent regional adaptation of Achilles free tendon structure and function. Our findings also suggest that AP diameter may be more responsive to change following exercise, compared with CSA or ML diameter, and support the use of AP diameter to evaluate acute, and possibly chronic, exercise-dependent changes in Achilles free tendon transverse morphology (Grigg et al., 2012).

Limitations

There are a number of limitations that must be considered when interpreting the findings of the present study. Firstly, because we did not measure regional differences in longitudinal strain, we can only speculate upon the role non-uniform strains along and between Achilles tendon fascicles, during the eccentric heel drop, may have had on our regional transverse strain results. Secondly, in the present study, we were unable to delineate between the epi-tendon and paratendon using 3DUS and so were unable to quantify possible fluid exudation from the tendon core into the peri-tendinous space in response to exercise. Finally, caution is warranted in generalising the findings of the present study to different exercise modes, time points, age groups or tendinopathic tendons.

Conclusions

The mid-proximal Achilles free tendon experiences a reduction in CSA and AP diameter during active contractions performed immediately following a clinical dose of eccentric heel drop exercise. These findings suggest that the free tendon has different mechanical properties along its length and along the AP compared with the ML axis (anisotropy) and/or experiences non-uniform strains along its length and along the AP compared with the ML axis during heel drop exercise. The observed reductions in CSA and AP diameter strain after eccentric heel drop exercise were correlated with longitudinal strain and are likely to reflect fluid redistribution along the tendon and a corresponding increase in packing density of collagen fibrils under load.

Competing interests
The authors declare no competing or financial interests.

Author contributions
S.J.O., R.N.-W. and R.S.B. all contributed to the conception and design of the study, interpretation of the results and drafting of the manuscript. S.J.O. undertook all data collection and analysis, and creation of figures.

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