The acute effects of higher versus lower load duration and intensity on morphological and mechanical properties of the healthy Achilles tendon: a randomized crossover trial

Eman Y. Merza [a*], Stephen J. Pearson [b], Glen A. Lichtwark [c], Peter Malliaras [a]

[a] *Department of physiotherapy, Faculty of Medicine, Nursing and Health Science, Monash University, Frankston Vic 3199, Melbourne, Australia,* email: iman.merza@monash.edu

[b] *Centre for Health, Sport and Rehabilitation Sciences Research, University of Salford, Greater Manchester, M5 4WT, United Kingdom,* email: s.pearson@salford.ac.uk

[c] *Centre for Sensorimotor Performance, School of Human Movement and Nutrition Sciences, The University of Queensland, St. Lucia, QLD 4072, Brisbane, Australia,* email: g.lichtwark@uq.edu.au

[a] *Department of physiotherapy, Faculty of Medicine, Nursing and Health Science, Monash University, Frankston Vic 3199, Melbourne, Australia*, email: peter.malliaras@monash.edu

[*] **Corresponding Author**: Eman Y. Merza, email: iman.merza@monash.edu

Key words: Achilles tendon, volume, stiffness, free tendon, 3D ultrasound

Summary statement: High levels of load duration and intensity had the greatest acute effect on the free Achilles tendon volume and stiffness.

Abstract

The Achilles tendon (AT) exhibits volume changes related to fluid flow under acute load which may be linked to changes in stiffness. Fluid flow provides a mechanical signal for cellular activity and may be one mechanism that facilitates tendon adaptation. This study aimed to investigate whether isometric intervention involving a high level of load duration and intensity could maximize the immediate reduction in AT volume and stiffness compared to interventions involving a lower level of load duration and intensity. Sixteen healthy participants (12 males, 4 females; age= 24.4 ± 9.4 y; body mass= 70.9 ± 16.1 kg; height= 1.7 ± 0.1 m) performed three isometric interventions of varying levels of load duration (2 s and 8 s) and intensity (35% and 75% MVICs) over a 3-week period. Freehand 3D ultrasound was used to measure free AT volume (at rest) and length (at 35%, 55%, and 75% of maximum plantarflexion force) pre and post interventions. The slope of force-elongation curve over these force levels represented individual stiffness (N/mm). Large reductions in free AT volume and stiffness resulted in response to long-duration high-intensity loading whilst less reduction resulted with a lower load intensity. In contrast, no change in free AT volume and a small increase in AT stiffness occurred with lower load duration. These findings suggest that the applied load on the AT must be heavy and sustained for a long duration to maximize immediate volume reduction, which might be an acute response that enables optimal long-term tendon adaptation via mechanotransduction pathways.

Introduction

Tendons are connective tissues comprised of densely packed collagen fibers (solid phase) and water-binding proteoglycans (fluid phase)(Kjær 2004). Tendons can adapt to a wide range of load requirements via changing their mechanical (i.e., stiffness) and morphological (i.e., thickness and cross-sectional area [CSA]) properties, and this may have implications for performance and injury (Bohm et al. 2015). For example, tendons become more extensible (Kubo et al. 2001b, 2009; Kay and Blazevich 2009; Burgess et al. 2009; Joseph et al. 2014) and smaller in thickness (Wearing et al. 2007, 2014; Grigg et al. 2009; Kristiansen et al. 2014) when subjected to acute load, and become stiffer

(Kubo et al. 2012, 2001a, 2006) and may hypertrophy (Arampatzis et al. 2007; Couppé et al. 2008; Bohm et al. 2014; Geremia et al. 2018; Kongsgaard et al. 2007) when loaded over long-term periods (> 12-week). The mechanisms responsible for tendon adaptation to load are currently unclear.

It is well accepted that mechanical load raises hydrostatic pressure within the fluid phase causing fluid flow (movement). This may include fluid exudation, that is, fluid movement out of the tendon causing reduced fluid volume which has been demonstrated in *ex vivo* tendon loading (Butler 1997; Lavagnino et al. 2003; Hannafin and Arnoczky 1994). Tendon undergoing fluid flow/exudation may become thinner or smaller in volume. From a mechanical perspective, this means higher levels of mechanical stress on the solid phase (collagen) and a greater tissue strain. Moreover, load-induced fluid flow provides a mechanical signal for cellular activity (Docking 2013; Wall et al. 2016). This is likely to occur because fluid flows away from the tendon core (it becomes thinner) so tensile tenocyte load may increase (as predicted by Poisson's ratio; Iwanuma et al. (2011)). Shear stresses from the fluid flow may also directly impact tenocyte mechanotransduction and signaling (Archambault et al. 2002; Lavagnino and Arnoczky 2005; Lavagnino et al. 2003, 2008). Therefore, it is reasonable to speculate that load-induced fluid flow/exudation is an important mechanism in tendon mechanotransduction and adaptation.

Fluctuation in fluid content (fluid loss) has been proposed as a potential mechanism responsible for the reduction in tendon thickness/diameter observed post-acute loading (Wearing et al. 2007; Grigg et al. 2012; Grigg et al. 2009). However, reduction in tendon thickness could occur with fluid redistributing within the tendon with no exudation, the latter may well change tendon morphology (volume) (Nuri et al. 2017c,a). The *in vivo* evidence on AT volume change with acute loading is unclear. There are reports of a small but significant reduction in healthy AT volume immediately after cross-country running (Syha et al. 2014; Grosse et al. 2016), or a lack of acute change after eccentric (Obst et al. 2015) and submaximal isometrics (10 reps. at 50% MVIC) (Nuri et al. 2017b) whilst others found an immediate reduction in tendinopathic Achilles volume with submaximal isometrics (at 50% MVIC) (Nuri et al. 2017c, 2018) or an immediate

increase with eccentric calf training (Shalabi et al. 2004). Understanding load parameters that may trigger maximal reduction in tendon volume (meaning substantial fluid flow and exudation) may be useful in designing future loading protocols to drive the tendon toward optimal long-term adaptation (i.e., increased stiffness and tendon hypertrophy).

Volume changes of the AT are likely to be linked to the mechanical stress applied (e.g., load duration and intensity), however there is a paucity of supporting evidence. It has been shown that tendons display greater deformation “creep” when loaded slowly or for longer duration because of their viscoelastic properties (Pearson et al. 2007) or when loaded heavily (i.e., > 70% maximal voluntary contraction)(Obst et al.). There is evidence that a combination of long-duration and high-intensity loading have the greatest acute and longer-term effects in healthy AT (Kubo et al. 2001c, 2005, 2009; Wiesinger et al. 2015; Obst et al.). Kubo et al. (2009) compared short (1 s, 5 sets × 50 reps) and long duration (15 s, 1 set × 17 reps) high-intensity isometric contractions and found an immediate increase in Achilles tendon elongation only after the long-duration contraction. Previous studies by the same group showed that long-duration and high-intensity isometric contractions over 12 weeks resulted in a greater increase in tendon stiffness compared to shorter-duration contractions (Kubo et al. 2001a, 2006). However, none of these studies has linked changes in mechanical properties (i.e., stiffness) with changes in tendon morphology (i.e., volume). Whether and to what extent long-duration and high-intensity loading could lead to reductions in tendon volume and stiffness in healthy AT, and whether change in material properties (i.e., Young’s modulus) occurs in parallel, is still unclear.

This study aimed to investigate the acute effect of long-duration and high-intensity loading on the volume and stiffness of healthy free AT (i.e., pathology free or non-injured tendon). We hypothesized that a higher level of load duration and intensity could result in greater immediate reductions in tendon volume and stiffness compared to lower load duration and intensity.

Materials and methods

Trial design

A randomized crossover trial was used to examine whether a higher level of load duration and intensity would result in a greater immediate reduction in tendon volume and stiffness compared to lower level of load duration and intensity. This crossover trial involved three different isometric interventions implemented over a period of three weeks and separated by a one-week washout period between interventions, to ensure recovery of tendon properties (volume and stiffness) to baseline. Crossover design provides the advantage of controlling individual differences (confounders) since the subjects are their own controls, thus, more statistical power and smaller sample size (Portney and Watkins 2014). This crossover trial was reported in accordance with the CONSORT statement for this trial type (Dwan et al. 2019) and was prospectively registered (website: Australian New Zealand Clinical Trials Registry; registration record: ACTRN12620000306910).

Participants

Sixteen healthy participants (12 males, 4 females; age= 24.4 ± 9.4 y; body mass= 70.9 ± 16.1 kg; height= 1.7 ± 0.1 m) volunteered to participate in this study. Participants were recruited from the local university student and staff population and via personal contacts of the researchers. All participants were recreationally active (see Table S1). Participants were excluded if they had current painful musculoskeletal disorders in the lower limbs, history of lower limb surgery/trauma in the past 12 months (in the dominant side) or neurological conditions (i.e., multiple sclerosis, Parkinson's, stroke) or a history of Achilles pain or injury (in the dominant side). Ultrasound examination revealed no signs of Achilles tendon disorders (i.e., thickening of the midportion and/or enthesis or hypoechoic regions) (Maffulli et al. 2003). The study was approved by Monash University Human Research Ethics Committee (Project ID: 21356). All participants provided informed written consent prior to participation.

Interventions

Each participant attended the laboratory on three occasions, at the same time of the day, with a 7-day interval between sessions. The participants were asked to refrain from strenuous physical activities 24 h prior to each testing session. One isometric intervention was performed per testing session in a random sequence (according to the allocated sequence for each participant). The isometric interventions varied in load duration (contraction duration) and load intensity but consisted of a similar volume (10 reps × 4 sets) and a similar rest time between the repetitions (10 s) and sets (3 min.) (Table 1). This allowed direct comparison amongst the interventions for the effects of different load duration and load intensity on the AT morphological and mechanical properties. To ensure that target load duration and load intensity for each intervention were consistently attained, a VBScript (Visual Basic Scripting) was created for each intervention using LabChart 8 software scripting (ADInstruments, Spechbach, Germany) and displayed on real-time visual feedback.

All interventions were undertaken with the participants seated in a custom-built dynamometer (Fig. 1A). The hip was in 120^{o} of flexion, the knee was in full extension (knee angle= 0^{o}), and the tested ankle (dominant side) was in 0^{o} plantar flexion (PF) with the lateral malleolus aligned to the axis of rotation of the dynamometer. The tested ankle was firmly strapped to the footplate of the dynamometer using a ladder strap to prevent downward foot slide during the intervention and was repositioned or tightened if movement was detected (this was done at the end of each set). To prevent knee flexion during PF contractions, a non-elastic strap was positioned above the knees. Participants were instructed to position both arms on the chest and to rest the non-tested leg down to the floor during the intervention to prevent compensation patterns.

In the first session and before commencing the isometric intervention, participants performed 3-5 trials of maximal PF isometric contractions (2-3 s) while seated in the dynamometer to establish target load intensity for the allocated intervention and subsequent interventions (i.e., 35% and 75%). Standardised instructions were provided during testing to motivate participants and ensure achieving maximal PF torque. To

prevent fatigue, participants were given 1-2 minutes rest between maximal PF isometric contractions test and commencing the first intervention.

Preconditioning and MVIC test

Tendon preconditioning was performed at each test session, followed by AT volume and stiffness assessment, the intervention, and then a repeat of the AT volume and stiffness assessment (Fig. 2). To precondition the tendon, participants performed five 80% maximal voluntary isometric contractions (MVICs) of PF (3-5 s) whilst positioned prone on the same dynamometer (Fig. 1B). Both hips were secured to the dynamometer using a nonelastic strap to prevent forward body shift during PF trials and the ankle was kept in 0^{o} plantar flexion and strapped to the footplate using a ladder strap. In the first session and with employing an identical positioning, tendon preconditioning was modified to include PF MVIC testing to establish torque levels for AT stiffness assessment (i.e., staged protocol; see below). The participants performed between 3 to 5 trials of ramped isometric PF contraction to a maximum over 3-5 s. duration. Maximal PF torque was confirmed to be attained when participants performed the two highest trials within 10%. Standardised instructions were provided prior to testing (push toward the footplate and build up your force to maximum within 2-3 s. and hold at maximum for 2-3 s) and during testing (Push as hard as possible and hold for 2-3 s) to motivate participants and ensure achieving maximal PF torque. All torque data were displayed on real-time visual feedback, recorded at 1000 Hz with a PowerLab 26T, and analyzed using LabChart 8 software (all ADInstruments, Spechbach, Germany).

Freehand-3D ultrasound measures of Achilles tendon volume and stiffness

Using freehand three-dimensional ultrasound (3DUS), the free AT volume and length were measured pre and post each intervention (Fig. 2) whilst participants were positioned prone on the same dynamometer (Fig. 1B). The hips and knees were extended, and the tested ankle was in 0^{o} plantar flexion with the lateral malleolus aligned to the axis of rotation of the force transducer. The freehand 3DUS system that

was utilized in this study has been described in previous studies investigating the mechanical and morphological properties of musculoskeletal tissues including AT (Barber et al. 2009; Farris et al. 2013; Merza et al. 2021; Obst et al. 2014a) and the set-up in the current study was identical to those we used in a previous study (Merza et al. 2021). The freehand 3DUS includes a B-mode ultrasound device (ArtUS EXT-1H, TELEMED, Vilnius, Lithuania, EU) and a four-camera optical tracking system (OptiTrack PRIME 13, Tracking Tools v 1.7.1; NaturalPoint, Corvallis, Oregon, USA) that recorded the position and orientation of the transducer during scanning by tracking four reflective markers attached rigidly to the transducer (Treece et al. 2003). The acquired B-mode images were transformed into the global coordinate system using the Stradwin software package (Version 5.4, Mechanical Engineering, Cambridge University, Cambridge, UK; http://mi.eng.cam.ac.uk/_rwp/stradwin) to construct a 3D image of the AT. Following the guidelines provided with the Stradwin software, temporal and spatial calibration of the ultrasound transducer was performed in a water bath (21^{o}C) using a single-wall phantom calibration (Treece et al. 2003). After calibration, the coordinates of any pixel within a 2-D ultrasound image were transformed into 3-D space with an error of less than ±0.4 mm (Prager et al. 1998).

The freehand 3DUS scanning was performed using a 40-mm linear transducer (L15-7 H40-A5, TELEMED, Vilnius, Lithuania) with a central frequency of 7.5 MHz, sampling frequency of 40 Hz, axial and lateral resolution of 0.5 mm, and standardized image generation parameters (depth = 40 mm, gain = 50%, dynamic range = 66 dB, power = 0). To enhance visualization of the tendon cross-section and ensure the best contact between the transducer and skin, a disposable ultrasound gel pad 2×9 cm (Parker Laboratories, Fairfield, NJ, USA) was used during all scans. A thin layer of hypoallergenic ultrasound transmission gel (Other-Sonic, Pharmaceutical Innovations, Newark, NJ, USA) was applied to the participant's skin to reduce the friction between the skin and the standoff pad.

To obtain measurements of free AT morphology (i.e., resting length and cross-sectional area), two single sweep 3DUS scans were performed at rest (pre and post intervention). Scans were performed at a steady speed from calcaneal notch to soleus-musculotendinous junction (MTJ). The duration of single scanning lasted between 15 to 20 s with approximately 0.1 mm. distance between acquired US frames.

To estimate the free AT stiffness, two 3DUS scans from calcaneal notch to soleus-MTJ were performed during sustained PF contractions at 35%, 55%, and 75% MVICs in a random order (staged protocol). The PF torque levels for the staged protocol were standardized across the three testing sessions based on the values achieved in the first testing session. The participants were requested to hold each contraction until completion of scanning while maintaining the target force level and were given 30-60 s rest between each trial to minimize fatigue. To minimize heel lift during the sustained submaximal PF contractions (also during PF MVIC test), the ankle was firmly strapped to the footplate of the dynamometer using a ladder strap (Arya and Kulig 2010). Infrequently, re-scanning was done in case the participant was unable to achieve or steadily maintain the target torque level or in case of excessive heel lift (the strap was tightened prior to re-scanning). To reduce the influence of contraction duration (time under tension) on tendon viscoelastic behaviour and prevent tendon creep, an effort was made to standardise scanning duration at 8 s for all MVIC trials.

Electromyography and muscle co-contraction

To account for the co-contraction of tibialis anterior (TA) muscle during submaximal PF contractions and to obtain a true PF torque, maximal dorsiflexion (DF) contractions were performed (Arya and Kulig 2010) (the position of DF MVIC test was identical to PF MVIC test - see above). The participants were asked to perform up to 3 trials of ramped maximal isometric DF contraction until the two highest were within 10%. The participants were instructed to flex their foot against the ladder strap (i.e., isometric DF of the ankle) as hard as possible and hold for 2-3 s.

Bipolar surface electrodes (Kendall 300 Foam Electrodes, Covidien, Mansfield, USA) were applied to the skin after shaving and cleansing with alcohol pads (see Hermens et al. (2000) for details). The raw EMG signals were recorded at 1000Hz. Custom software (MATLAB R2019b, MathWorks, Natick, MA, USA) was used to process EMG data. A bandpass filter [20 450] was applied to eliminate electrical noise in EMG data. The filtered signal was enveloped using the root-mean-square method over a sliding window of 200 samples. The antagonist DF torque was estimated from the relationship between TA EMG activity and recorded torque during DF contractions (Eqn. 1), assuming a linear relationship between the recorded EMG amplitude and muscle torque (Kongsgaard et al. 2011). The estimated antagonist DF torque was added to the net PF torque to obtain a true estimate of the PF torque (Geremia et al. 2018; Kongsgaard et al. 2011; Arya and Kulig 2010).

Equation 1:

$$\text{Antagonist DF torque} = P(1) \times Up1 + P(2)$$

Where P(1) is the slope of the gradient of the relationship between TA EMG activity and recorded torque during the DF contractions, Up1 is the envelope of the TA EMG signal, and P(2) is the regression constant.

Tendon moment arm

To obtain PF force, the resultant true PF torque was divided by the internal moment arm (Kongsgaard et al. 2011; Arya and Kulig 2010). Moment arm was obtained at rest using B-mode ultrasound while the ankle joint was kept in 0^{o} plantar flexion (Geremia et al. 2018). The inferior tip of the lateral malleolus of the tested ankle was marked with a permanent marker (i.e., center of rotation, COR). A 40 mm linear probe was placed longitudinally over the AT to acquire a static ultrasound image, with the center of the probe aligned with the COR. The distance from the inferior tip of the US probe to the COR was measured using a tape measure (d1). From the B-mode static US image, the distance between the skin and midline of AT (d2), also known as the line of action (LOA) (Maganaris et al. 1998) was measured using the in-built length tool provided in the Echo

wave II version 4.0.1 software. The difference between these two lines (d1-d2) represented the AT moment arm (i.e., the perpendicular distance from COR to the LOA) (Geremia et al. 2018; Kongsgaard et al. 2011). The total mean and standard deviation (SD) of the moment arm for the 16 participants was 43.3 (3.93) mm which was comparable to values previously reported in the literature (Kongsgaard et al. 2011; Maganaris et al. 1998).

Freehand 3D-ultrasound image analysis and reconstruction of free AT volume

Stradwin software was used to perform image analysis. To measure free AT length (at rest and MVICs) two anatomical sites: calcaneal notch and soleus-MTJ were manually identified on sagittal and transverse images, then marked using a landmark tool (two-point method) (Fig. 3A, B, C). The soleus-MTJ was the first visible cross-section of muscle tissue and was determined using sagittal, frontal, and transverse, image planes. The distance between the calcaneal notch and soleus-MTJ was defined as the free AT and the length was measured using the length tool within the Stradwin software. Tendon cross-sections were manually contoured from the transverse B-mode images from the calcaneal notch to soleus-MTJ at 5-10 mm interval (Fig. 3D). The 3D tendon image reconstruction was performed on the segmented cross sections using the in-built interpolation algorithm in Stradwin software (Fig. 3E).

Determination of free AT stiffness and Young's modulus

To calculate the free AT stiffness (N/mm), all torque and EMG data obtained at maximal DF and submaximal PF trials, as well as data for the free AT length (i.e., at rest and during MVICs), were exported to MATLAB software R2019b (MathWorks, Natick, MA, USA). To measure the amount of tendon elongation at 35%, 55%, and 75% MVIC, the mean resting length was subtracted from the mean length obtained at each corresponding MVIC level. The slope of the line fitted to the force-elongation curve (i.e., PF force at 35%, 55%, and 75% MVIC and corresponding tendon elongation) across all force levels represented the individual stiffness. Tendon stiffness was normalised to

CSA and length to determine Young's modulus which was calculated by dividing tendon force with the average resting CSA (i.e., stress MPa) and tendon elongation with the resting length (i.e., strain %). The average CSA was determined from the contoured cross sections using a custom MATLAB script. Young's modulus was the slope of the line fitted to the stress-strain curve across the entire stress range.

Sample size calculation

The sample size was calculated using our pilot data (unpublished). We found a Cohen's standardised effect of ≥ 0.5 between the study interventions. Therefore, to achieve an effect size of ≥ 0.5 with 5% significance level and power of 80%, the sample size required for this crossover trial was n=11. This assumed a correlation between repeated measures of 0.2.

Randomization

Each participant performed three different isometric interventions in a random sequence using a computer-based random sequence generation. An off-site researcher (P.M) not involved in data collection informed the researcher who performed data collection (E.M) of the allocation sequence. This occurred immediately prior to the first session. Due to the nature of the interventions, the participants were aware of the exercise intervention they performed each week. However, we were very careful to ensure that the participants were not aware of the hypothesis of the study, and at no time was this disclosed in any written materials or verbal interactions.

Reliability of tendon volume and stiffness measurements

Freehand 3DUS has been shown to provide accurate measures of phantom volumes (± 0.5 ml) and reliable measures for *in vivo* free AT volume (intra-class correlation coefficient (ICC) ≥ 0.98) (Obst et al. 2014a) and stiffness (ICC= 0.994) (Merza et al. 2021). A recent study by Devaprakash et al. (2019) demonstrates high agreement

between magnetic resonance imaging (MRI) and freehand 3DUS estimates of free AT volume.

Reliability of plantar flexor force

Between-sessions (using pre-interventions data) and within-session reliability of plantar flexor force at 35%, 55%, and 75% MVICs were assessed using the intra-class correlation coefficient [ICCs (3,1), two-way mixed-effects model with absolute agreement] and 95% confidence intervals (CIs), coefficient of variation (CV), and the minimal detectable change (MDC). The between-sessions and within-session ICCs for PF force at all MVIC levels were greater than 0.97 (lower band of 95% confidence interval was ≥ 0.845). The CV and MDC ranged between 0% to 9% and between 68.5 to 179 N, respectively.

Statistical methods

A (2 × 3) repeated measures ANOVA with time (pre-post intervention) and type of isometric intervention (1, 2, and 3) as within-subjects factors, was used to investigate the interaction and simple effects of the time and type of intervention on the following dependent variables i) free AT volume, and ii) free AT stiffness. Data normality was evaluated with the Kolmogorov-Smirnov test. Effect size (ES) was reported for between-group differences (ES= difference between change scores/pooled change score standard deviation) (Middel and van Sonderen 2002). All statistical analyses were performed using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 26.0, IBM Corp., Armonk, NY, USA). The level of significance for all the tests was set at $P < 0.05$. Data are expressed as mean ± standard deviation (s.d.).

Results

The acute effects of different isometric interventions on:

Free AT volume

There was a significant time-by-intervention interaction effect (F=25.098, df= (2,30), $P \leq$ 0.001) on the free AT volume. Interaction (within-subjects) contrasts analysis shows that the acute effect of the 8 s, 75% MVIC intervention on the free AT volume was significantly greater than the 2 s, 75% MVIC (ES= 2) and 8 s, 35% MVIC (ES= 1.4) interventions but there was no significant difference between the 2 s, 75% MVIC and 8 s, 35% MVIC interventions (ES= 0.5) on the free AT volume (Fig. 4C). The simple effects analysis shows that the 2 s, 75% MVIC intervention had no significant acute effects on the free AT volume (P= 0.106) which changed only by an average of < - 0.1 ml (2.3%), whilst the 8 s, 75% MVIC and 8 s, 35% MVIC interventions significantly reduced the free AT volume by an average of 0.3 ml (13.6%) ($P \leq$ 0.001) and 0.1 ml (5.3%) (P= 0.004), respectively (Fig. 4A). The same test indicates no significant differences in the free AT volume at baseline (pre-intervention) across interventions ($P \geq$ 0.05). The 8 s, 75% MVIC intervention had the greatest acute effect on free AT volume. The pre-and post-intervention mean (s.d.) for the free AT volume are shown in Table 2.

Free AT Stiffness

There was a significant time-by-intervention interaction effect (F= 60.503, df= (2,30), $P \leq$ 0.001) on the free AT stiffness. Interaction (within-subjects) contrasts analysis shows that the acute effect of the 8 s, 75% MVIC intervention on the free AT stiffness was significantly greater than the 2 s, 75% MVIC (ES= 0.9) and 8 s, 35% MVIC (ES= 0.8) interventions but there was no significant difference between the 2 s, 75% MVIC and 8 s, 35% MVIC interventions (ES= 0.0) on the free AT stiffness (Fig. 4D). The simple effects analysis shows that the 2 s, 75% MVIC intervention significantly increased the free AT stiffness by an average of 37.78 N/mm (11.5%) ($P \leq$ 0.001) and the 8 s, 75% MVIC and 8 s, 35% MVIC interventions significantly reduced the free AT stiffness by an average of 67.35 N/mm (20.4%) ($P \leq$ 0.001) and 36.45 N/mm (11.2%)(P=0.001), respectively (Fig. 4B). The same test indicates no significant differences in the free AT stiffness at

baseline (pre-intervention) across interventions ($P \geq 0.05$). The 8 s, 75% MVIC intervention had the greatest acute effect on free AT stiffness. The pre-and post-intervention mean (s.d.) for the free AT stiffness, elongation, and tendon force are shown in Table 2.

Discussion

The present study investigated the acute effect of long-duration and high-intensity loading on the morphological and mechanical properties of the free AT, in healthy humans. Consistent with our hypothesis, the main finding was that a long-duration and high-intensity loading resulted in greater immediate reductions in the volume and stiffness of the free AT compared to lower levels of load duration and intensity.

The acute effect of different isometric interventions on the free AT volume

The present study found that long-duration contraction at 75% MVIC reduced free AT volume by 60% more than long-duration contraction at 35% MVIC and 82.3% more than short-duration contraction at 75% MVIC. The reduction in the free AT volume found in the current study is likely a manifestation of transient fluid flow and exudation. This is in light of the *ex vivo* investigations that demonstrated immediate fluid flow and reduced fluid volume as a response to cyclic and static load (Butler 1997; Lavagnino et al. 2003; Hannafin and Arnoczky 1994), and *in vivo* investigations using MRI (i.e., off-resonance saturation combined with ultrashort echo time sequence) that show reductions in AT volume and concomitant hydration state after cross-country running (Grosse et al. 2016; Syha et al. 2014). Fluid flow driven by the mechanical load might be attributed to increased hydrostatic pressure associated with the reorganisation of collagen fibers (Connizzo et al. 2013; Atkinson et al. 1997). On the other hand, the relatively constant tendon volume observed with short-duration contraction likely reflects a preserved fluid content.

The 13.5% reduction in AT volume following the long-duration contraction at 75% MVIC is higher than reductions reported following cross-country running (4% and 5.8%, respectively) (Syha et al. 2014; Grosse et al. 2016). This may be because running exposes the tendon to high mechanical stress but lasting only for a short duration (0.2 – 0.25 s)(Obst et al.). Our findings suggest that the change in tendon volume is occurring in a time-dependent manner (i.e., a greater volume reduction is occurring with 8 s than 2 s duration contraction) which may likely be attributed to the viscoelasticity of tendinous structure (Pearson et al. 2007). We also investigated the effect of long-duration contraction performed at a low load intensity (35% MVIC). Interestingly, we found a significant reduction in tendon volume by 5.4% (> 0.1 ml, P= 0.004) with long-duration contraction at 35% MVIC. The significant reduction in tendon volume following long-duration contractions was greater than our minimal detectable change [(MDC)= standard error of measurement × 1.96 ×√2] which was ± 0.1ml, therefore, was likely a real change, on average.

In contrast to our findings of reduced AT volume by 5.4% with 35% MVIC contractions, Nuri et al. (2017b) found that the AT volume of healthy humans, measured by freehand 3DUS, remained constant after repeated isometric contractions at 50% MVIC (10 reps x 25 s). Despite the relatively comparable PF torque values between our study and Nuri et al. (2017b) (35 Nm. *vs* 40.5 Nm., respectively), we had a higher number of loading cycles (i.e., 10 reps x 4 sets) compared to Nuri et al. (2017b) meaning longer total loading duration (i.e., 320 s *vs* 250 s). In this regard, several studies have shown that AT stiffness significantly reduced following maximal isometric contractions (Kay and Blazevich 2009, 2010; Kubo et al. 2002), and the study employing the longest total loading duration had reported the greatest response (Kubo et al. 2002).

Our free AT volume (2.5 ml) is comparable to MRI data reported by Syha et al. (2014) and Grosse et al. (2016) (2.4 cm^3 and 2.3 ml, respectively), and freehand 3DUS data reported by Devaprakash et al. (2020) (< 3 ml for trained runners and 3 ml for healthy controls). However, our free AT volume measurement is smaller than 3DUS data reported by Obst et al. (2014b) and Nuri et al. (2017b) for healthy young males (ranged between 3.9 ml to 4.9 ml). The average CSA value reported in the present study was

relatively comparable to 3DUS data reported by Nuri et al. (2017b) (52 mm^2) for the midportion of the free AT but smaller than MRI data reported by Devaprakash et al. (2020) (60 mm^2).

Comparison of volume and diameter changes

Previous *in vivo* studies had investigated the acute effect of different loading interventions on tendon structural properties (i.e., thickness and diameter) and demonstrated immediate thickness/diametral reductions post-acute loading (Wearing et al. 2007; Grigg et al. 2012; Grigg et al. 2009). For example, Wearing et al. (2007) reported a reduction of 15% in Achilles anterior-posterior diameter following resistive PF exercise and had attributed such reduction to transient fluid loss. Importantly, the diametral reduction could likely occur with fluid redistributing within the tendon without the fluid leaving the tendon core (i.e., exudation). Therefore, it is unknown to what extent the reduction in tendon diameter reflects a change in tendon volume or fluid flow/exudation.

The acute effects of different isometric interventions on the free AT stiffness

The present study found that long-duration contraction at 75% MVIC changed the free AT stiffness by 46% more than long-duration contraction at 35% MVIC and 44% more than short-duration contraction at 75% MVIC. While there were no changes in force across intervention conditions, there was a significant increase in corresponding tendon elongation following the interventions involving the long-duration contractions (8 s, 75% MVIC and 8 s, 35% MVIC) (Table 2). Therefore, the observed reduction in the free AT stiffness was also associated with an increased tendon elongation for a given force. Our stiffness value for the free AT (between 325 and 331 N/mm) is comparable to the value reported by Devaprakash et al. (2020) (410 ± 164 N/mm) for the free AT, using 3DUS.

Similar to our findings for volume, mechanical loading seems to influence change in stiffness. We found that long-duration contraction at 35% and 75% MVICs reduced the free AT stiffness but the reduction at 35% was significantly smaller than at 75% MVIC (11.3% vs 20.4%, respectively). In agreement with our finding, there is evidence that stiffness of vastus lateralis tendon significantly reduced after long-duration contractions but not after repetitive drop jumps (Kubo et al. 2005) or shorter duration (1 s) contractions (Kubo et al. 2001c). Here, we found reductions in tendon stiffness and concomitant volume immediately after long-duration loading with no changes in Young's modulus. This suggests that reductions in tendon volume through reductions in CSA (fluid flow/exudation) contribute to reductions in stiffness with acute loading. Theoretically, reduced fluid volume (i.e., reduction in the viscous element) would make the tendon thinner and may deprive the elastic matrix components of protection against tensile loading causing greater stress and strain (i.e., reduced stiffness). An unexpected finding was that the short-duration contraction at 75% MVIC induced a significant but small increase in the free AT stiffness by 11.5%. It is noteworthy that the changes in free AT stiffness following the 2 s, 75%, and 8 s, 35% interventions were smaller than our MDC value (≤ 45 N/mm), therefore, within measurement error, on average. Notably, the between-group differences support our hypothesis of greater changes in the intervention with the highest duration and intensity.

Implications for tendon adaptation

Our findings may provide evidence that the applied load on the tendon must be heavy and sustained for a long duration to maximize immediate volume reduction. This acute tendon response may be important to shift the tendon toward optimal long-term adaptation, however, this remains speculative and requires further investigation.

Load-induced fluid flow/exudation may be a remodeling signal for tendon cells. This is likely to occur because radial fluid flow to the periphery (exudation) may temporarily reduce the fluid volume within the tendon core, enabling tenocytes to perceive loading. At the same time, the flow of tendon fluid generates shear stresses on the tenocytes membrane, all of which stimulate cellular activity (Wall et al. 2016; Docking 2013; Ingber 2006). Therefore, the immediate reduction in free AT volume observed after the long-

duration high-intensity loading might be a physiological response that stimulates tenocytes mechanotransduction and signaling. We speculate that chronic exposure to long-duration load at high load intensity should lead to greater changes in tendon matrix and ultimately greater tendon adaptation (i.e., increased stiffness and tendon hypertrophy) compared to lower levels of load duration and intensity. In this context, Kubo et al. (2001a) found that tendon stiffness remarkably increased after a 12-week of high-intensity isometric training with a longer-duration contraction compared to a shorter duration.

Limitations

The present study has some limitations that need to be considered. First, the level of PF force in the present study was not maximal (the highest force was exerted at 75% MVIC). The AT viscoelastic behavior would likely differ under higher/maximal absolute tendon forces, that is, a higher force level would have caused the free AT to display higher stiffness (Rigby et al. 1958). However, with freehand 3DUS scanning, sustaining a maximal contraction for long enough to attain high quality images is extremely challenging. Second, we examined changes in volume and stiffness at the level of the free AT, which according to the evidence exhibits distinct material properties (i.e., higher longitudinal strain) compared to the proximal gastrocnemii tendon (Magnusson et al. 2003; Iwanuma et al. 2011). Therefore, our findings may not be generalisable to the gastrocnemius portion of the AT. Third, our analysis only presents volumetric changes across the entire free AT, and without conducting regional analysis, it is not possible to determine if there are any region-specific changes in tendon volume. Fourth, although the direction of loading response for each intervention was the same in every subject, there was an inter-subject variability in the magnitude of change. Various levels of activity and plantar flexor strength amongst the participants may explain this variability. Future studies with specific inclusion criteria may be needed to examine whether there is a differential adaptive response between athletic and recreationally active populations and between men and women, separately. Finally, we had a small sample size of healthy participants (n=16) with a greater percentage of men (n = 12) than women (n =

4) (M: 75%). Therefore, we suggest caution in generalizing our results to other populations.

Conclusion

The present study demonstrates that long-duration and high-intensity loading resulted in greater immediate reductions in the volume and stiffness of the free AT compared to lower levels of load duration and intensity.

Acknowledgments: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest: The authors declare that they have no competing interests.

References

Arampatzis, A., Karamanidis, K. & Albracht, K. (2007). Adaptational responses of the human achilles tendon by modulation of the applied cyclic strain magnitude. *Journal of Experimental Biology.* 210**,** 2743-2753.

Archambault, J. M., Elfervig-Wall, M. K., Tsuzaki, M., Herzog, W. & Banes, A. J. (2002). Rabbit tendon cells produce mmp-3 in response to fluid flow without significant calcium transients. *J Biomech.* 35**,** 303–309.

Arya, S. & Kulig, K. (2010). Tendinopathy alters mechanical and material properties of the achilles tendon. *Journal of Applied Physiology.* 108**,** 670-675.

Atkinson, T. S., Haut, R. C. & Altiero, N. J. (1997). A poroelastic model that predicts some phenomenological responses of ligaments and tendons. *J Biomech Eng.* 119.

Barber, L., Barrett, R. & Lichtwark, G. (2009). Validation of a freehand 3d ultrasound system for morphological measures of the medial gastrocnemius muscle. *J Biomech.* 42**,** 1313-9.

Bohm, S., Mersmann, F. & Arampatzis, A. (2015). Human tendon adaptation in response to mechanical loading: A systematic review and meta-analysis of exercise intervention studies on healthy adults. *Sports Medicine - Open.* 1.

Bohm, S., Mersmann, F., Tettke, M., Kraft, M. & Arampatzis, A. (2014). Human achilles tendon plasticity in response to cyclic strain: Effect of rate and duration. *The Journal of experimental biology.* Part 22. 217**,** 4010-4017.

Burgess, K. E., Graham-Smith, P. & Pearson, S. J. (2009). Effect of acute tensile loading on gender-specific tendon structural and mechanical properties. *Journal of Orthopaedic Research.* 27**,** 510-515.

Butler, S. L., Kohles, S.S.,Thieike, R.J. , Chen, C. , Vanderby Jr S, R. (1997). Interstitial fluid flow in tendons or ligaments: A porous medium finite element simulation. *Med. Biol. Eng. Comput.* 35**,** 742-746.

Connizzo, B. K., Sarver, J. J., Iozzo, R. V., Birk, D. E. & Soslowsky, L. J. (2013). Effect of age and proteoglycan deficiency on collagen fiber re-alignment and mechanical properties in mouse supraspinatus tendon. *J Biomech Eng.* 135.

Couppé, C., Kongsgaard, M., Aagaard, P., Hansen, P., Bojsen-Moller, J., Kjaer, M. & Magnusson, S. P. (2008). Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *J Appl Physiol.* 105**,** 805-810.

Devaprakash, D., Lloyd, D. G., Barrett, R. S., Obst, S. J., Kennedy, B., Adams, K. L., Hunter, A., Vlahovich, N., Pease, D. L. & Pizzolato, C. (2019). Magnetic resonance imaging and freehand 3-d ultrasound provide similar estimates of free achilles tendon shape and 3-d geometry. *Ultrasound Med Biol.* 45**,** 2898-2905.

Devaprakash, D., Obst, S. J., Lloyd, D. G., Barrett, R. S., Kennedy, B., Ball, I., Adams, K. L., Collings, T. J., Davico, G., Hunter, A., Vlahovich, N., Pease, D. L. & Pizzolato, C. (2020). The free achilles tendon is shorter, stiffer, has larger cross-sectional area and longer t2(*) relaxation time in trained middle-distance runners compared to healthy controls. *Front Physiol.* 11**,** 965.

Docking, S. T., Samiric. Ebonie, Scase. Craig Purdam, Jill Cook (2013). Relationship between compressive loading and ecm changes in tendons. *Muscles Ligaments Tendons J.* 3**,** 7-11.

Dwan, K., Li, T., Altman, D. G. & Elbourne, D. (2019). Consort 2010 statement: Extension to randomised crossover trials. *BMJ.* 366**,** l4378.

Farris, D. J., Trewartha, G., Mcguigan, M. P. & Lichtwark, G. A. (2013). Differential strain patterns of the human achilles tendon determined in vivo with freehand three-dimensional ultrasound imaging. *Journal of Experimental Biology.* 216**,** 594-600.

Geremia, J. M., Baroni, B. M., Bobbert, M. F., Bini, R. R., Lanferdini, F. J. & Vaz, M. A. (2018). Effects of high loading by eccentric triceps surae training on achilles tendon properties in humans. *European journal of applied physiology.* 118**,** 1725-1736.

Grigg, N. L., Wearing, S. C. & Smeathers, J. E. (2012). Achilles tendinopathy has an aberrant strain response to eccentric exercise. *Medicine and Science in Sports and Exercise.* 44**,** 12-17.

Grigg, N. L., Wearing, S. C., Smeathers, J. E., Grigg, N. L., Wearing, S. C. & Smeathers, J. E. (2009). Eccentric calf muscle exercise produces a greater acute reduction in achilles tendon thickness than concentric exercise. *British Journal of Sports Medicine.* 43**,** 280-283.

Grosse, U., Syha, R., Gatidis, S., Grozinger, G., Martirosian, P., Partovi, S., Nikolaou, K., Robbin, M. R., Schick, F. & Springer, F. (2016). Mr-based in vivo follow-up study of achilles tendon volume and hydration state after ankle-loading activity. *Scandinavian journal of medicine & science in sports.* 26**,** 1200-1208.

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

Hermens, H. J., Freriks, B., Disselhorst-Klug, C. & Rau, G. (2000). Development of recommendations for semg sensors and sensor placement procedures. *J Electromyogr Kinesiol.* 10**,** 361–374.

Ingber, D. E. (2006). Cellular mechanotransduction: Putting all the pieces together again. *Faseb j.* 20**,** 811-27.

Iwanuma, S., Akagi, R., Kurihara, T., Ikegawa, S., Kanehisa, H., Fukunaga, T. & Kawakami, Y. (2011). Longitudinal and transverse deformation of human achilles tendon induced by isometric plantar flexion at different intensities. *J Appl Physiol (1985).* 110**,** 1615-21.

Joseph, M. F., Lillie, K. R., Bergeron, D. J., Cota, K. C., Yoon, J. S., Kraemer, W. J. & Denegar, C. R. (2014). Achilles tendon biomechanics in response to acute intense exercise. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 28**,** 1181-1186.

Kay, A. D. & Blazevich, A. J. (2009). Isometric contractions reduce plantar flexor moment, achilles tendon stiffness, and neuromuscular activity but remove the subsequent effects of stretch. *Journal of Applied Physiology.* 107**,** 1181-1189.

Kay, A. D. & Blazevich, A. J. (2010). Concentric muscle contractions before static stretching minimize, but do not remove, stretch-induced force deficits. *Journal of Applied Physiology.* 108**,** 637-645.

Kjær, M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological Reviews.* 84**,** 649-698.

Kongsgaard, M., Nielsen, C. H., Hegnsvad, S., Aagaard, P. & Magnusson, S. P. (2011). Mechanical properties of the human achilles tendon, in vivo. *Clinical Biomechanics.* 26**,** 772-777.

Kongsgaard, M., Reitelseder, S., Pedersen, T. G., Holm, L., Aagaard, P., Kjaer, M. & Magnusson, S. P. (2007). Region specific patellar tendon hypertrophy in humans following resistance training. *Acta Physiologica.* 191**,** 111-121.

Kristiansen, M. S., Uhrbrand, A., Hansen, M., Shiguetomi-Medina, J. M., Vissing, K., Stødkilde-Jørgensen, H. & Langberg, H. (2014). Concomitant changes in cross-sectional area and water content in skeletal muscle after resistance exercise. *Scandinavian Journal of Medicine and Science in Sports.* 24**,** e260-e268.

Kubo, K., Ikebukuro, T., Maki, A., Yata, H. & Tsunoda, N. (2012). Time course of changes in the human achilles tendon properties and metabolism during training and detraining in vivo. *European Journal of Applied Physiology.* 112**,** 2679-2691.

Kubo, K., Ikebukuro, T., Yaeshima, K. & Kanehisa, H. (2009). Effects of different duration contractions on elasticity, blood volume, and oxygen saturation of human tendon in vivo. *European Journal of Applied Physiology.* 106**,** 445-455.

Kubo, K., Kanehisa, H. & Fukunaga, T. (2001a). Effects of different duration isometric contractions on tendon elasticity in human quadriceps muscles. *Journal of Physiology.* 536**,** 649-655.

Kubo, K., Kanehisa, H. & Fukunaga, T. (2002). Effects of transient muscle contractions and stretching on the tendon structures in vivo. *Acta Physiologica Scandinavica.* 175**,** 157-164.

Kubo, K., Kanehisa, H. & Fukunaga, T. (2005). Influences of repetitive drop jump and isometric leg press exercises on tendon properties in knee extensors. *Journal of Strength and Conditioning Research.* 19**,** 864-870.

Kubo, K., Kanehisa, H., Kawakami, Y. & Fukunaga, T. (2001b). Effects of repeated muscle contractions on the tendon structures in humans. *European Journal of Applied Physiology.* 84**,** 162-166.

Kubo, K., Kanehisa, H., Kawakami, Y. & Fukunaga, T. (2001c). Influences of repetitive muscle contractions with different modes on tendon elasticity in vivo. *Journal of Applied Physiology.* 91**,** 277-282.

Kubo, K., Ohgo, K., Takeishi, R., Yoshinaga, K., Tsunoda, N., Kanehisa, H. & Fukunaga, T. (2006). Effects of isometric training at different knee angles on the muscle–tendon complex in vivo. *Scand J Med Sci Sports.* 16**,** 159-167.

Lavagnino, M. & Arnoczky, S. P. (2005). In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. *J Orthop Res.* 23**,** 1211-8.

Lavagnino, M., Arnoczky, S. P., Kepich, E., Caballero, O. & Haut, R. C. (2008). A finite element model predicts the mechanotransduction response of tendon cells to cyclic tensile loading. *Biomech Model Mechanobiol.* 7**,** 405-416.

Lavagnino, M., Arnoczky, S. P., Tian, T. & Vaupel, Z. (2003). Effect of amplitude and frequency of cyclic tensile strain on the inhibition of mmp-1 mrna expression in tendon cells: An in vitro study. *Connect Tissue Res.* 44**,** 181-7.

Maffulli, N. V., Kenward, M. G., Testa, V., Capasso, G., Regine, R. & King, J. B. (2003). Clinical diagnosis of achilles tendinopathy with tendinosis. *Clin J Sport Med.* 13**,** 11-15.

Maganaris, C. N., Baltzopoulos, V. & Sargeant, A. J. (1998). Changes in achilles tendon moment arm from rest to maximum isometric plantarflexion: In vivo observations in man. *Journal of Physiology.* 510**,** 977-985.

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

Merza, E., Pearson, S., Lichtwark, G., Garofolini, A. & Malliaras, P. (2021). Reliability of human achilles tendon stiffness measures using freehand 3-d ultrasound. *Ultrasound Med Biol.*

Middel, B. & Van Sonderen, E. (2002). Statistical significant change versus relevant or important change in (quasi) experimental design: Some conceptual and methodological problems in estimating magnitude of intervention-related change in health services research. *Int J Integr Care.* 2**,** e15.

Nuri, L., Obst, S., Newsham-West, R. & Barrett, R. (2017a). Three-dimensional deformation of the achilles tendon during load in people with unilateral mid-portion achilles tendinopathy. *Journal of Science & Medicine in Sport.* 20**,** e71-e71.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. (2017b). Regional three-dimensional deformation of human achilles tendon during conditioning. *Scandinavian journal of medicine & science in sports.* 27**,** 1263-1272.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. (2017c). The tendinopathic achilles tendon does not remain iso-volumetric upon repeated loading: Insights from 3d ultrasound. *Journal of Experimental Biology.* 220**,** 3053-3061.

Nuri, L., Obst, S. J., Newsham-West, R. & Barrett, R. S. (2018). Three-dimensional morphology and volume of the free achilles tendon at rest and under load in people with unilateral mid-portion achilles tendinopathy. *Experimental Physiology.* 103**,** 358-369.

Obst, S. J., Barrett, R. S. & Newsham-West, R. Immediate effect of exercise on achilles tendon properties: Systematic review. *Medicine & Science in Sports & Exercise.* 45**,** 1534-44.

Obst, S. J., Newsham-West, R. & Barrett, R. S. (2014a). Invivo measurement of human achilles tendon morphology using freehand 3-d ultrasound. *Ultrasound in Medicine and Biology.* 40**,** 62-70.

Obst, S. J., Newsham-West, R. & Barrett, R. S. (2015). Three-dimensional morphology and strain of the human achilles free tendon immediately following eccentric heel drop exercise. *Journal of Experimental Biology.* 218**,** 3894-3900.

Obst, S. J., Renault, J. B., Newsham-West, R. & Barrett, R. S. (2014b). Three-dimensional deformation and transverse rotation of the human free achilles tendon in vivo during isometric plantarflexion contraction. *Journal of Applied Physiology.* 116**,** 376-384.

Pearson, S. J., Burgess, K. & Onambele, G. N. (2007). Creep and the in vivo assessment of human patellar tendon mechanical properties. *Clin Biomech (Bristol, Avon).* 22**,** 712-7.

Portney, L. G. & Watkins, M. P. 2014. *Foundations of clinical research application to practice.*, Pearson

Prager, R. W., Rohling, R. N., Gee, A. H. & L., B. (1998). Rapid calibration for 3-d freehand ultrasound. *Ultrasound in Med. & Biol.* 24**,** 855–869

Rigby, B. J., Hirai Nishio, Spikes John D. , A. & Henry, E. (1958). The mechanical properties of rat tail tendon. *J Gen Physiol.*

Shalabi, A., Kristoffersen-Wiberg, M., Aspelin, P. & Movin, T. (2004). Immediate achilles tendon response after strength training evaluated by mri. *Medicine & Science in Sports & Exercise.* 36**,** 1841-1846.

Syha, R., Springer, F., Grozinger, G., Wurslin, C., Ipach, I., Ketelsen, D., Schabel, C., Gebhard, H., Hein, T., Martirosian, P., Schick, F., Claussen, C. D. & Grosse, U. (2014). Short-term exercise-induced changes in hydration state of healthy achilles tendons can be visualized by effects of off-resonant radiofrequency saturation in a three-dimensional ultrashort echo time mri sequence applied at 3 tesla. *Journal of Magnetic Resonance Imaging.* 40**,** 1400-1407.

Treece, G. M., Gee, A. H., Prager, R. W., Cash, C. J. C. & Berman, L. H. (2003). High-definition freehand 3-d ultrasound. *Ultrasound in Medicine & Biology.* 29**,** 529-546.

Wall, M. E., Dyment, N. A., Bodle, J., Volmer, J., Loboa, E., Cederlund, A., Fox, A. M. & Banes, A. J. (2016). Cell signaling in tenocytes: Response to load and ligands in health and disease. *Adv Exp Med Biol.* 920**,** 79-95.

Wearing, S. C., Smeathers, J. E., Hooper, S. L., Locke, S., Purdam, C. & Cook, J. L. (2014). The time course of in vivo recovery of transverse strain in high-stress tendons following exercise. *British journal of sports medicine.* 48**,** 383-387.

Wearing, S. C., Smeathers, J. E., Urry, S. R. & S.L., H. 2007. The time-course of acute changes in achilles tendon morphology following exercise. *In:* FUSS, F. K., SUBIC, A. & UJIHASHI, S. (eds.) *The impact of technology on sport ii.* London.

Wiesinger, H. P., Kosters, A., Muller, E. & Seynnes, O. R. (2015). Effects of increased loading on in vivo tendon properties: A systematic review. *Med Sci Sports Exerc.* 47**,** 1885-95.

Figures and Tables

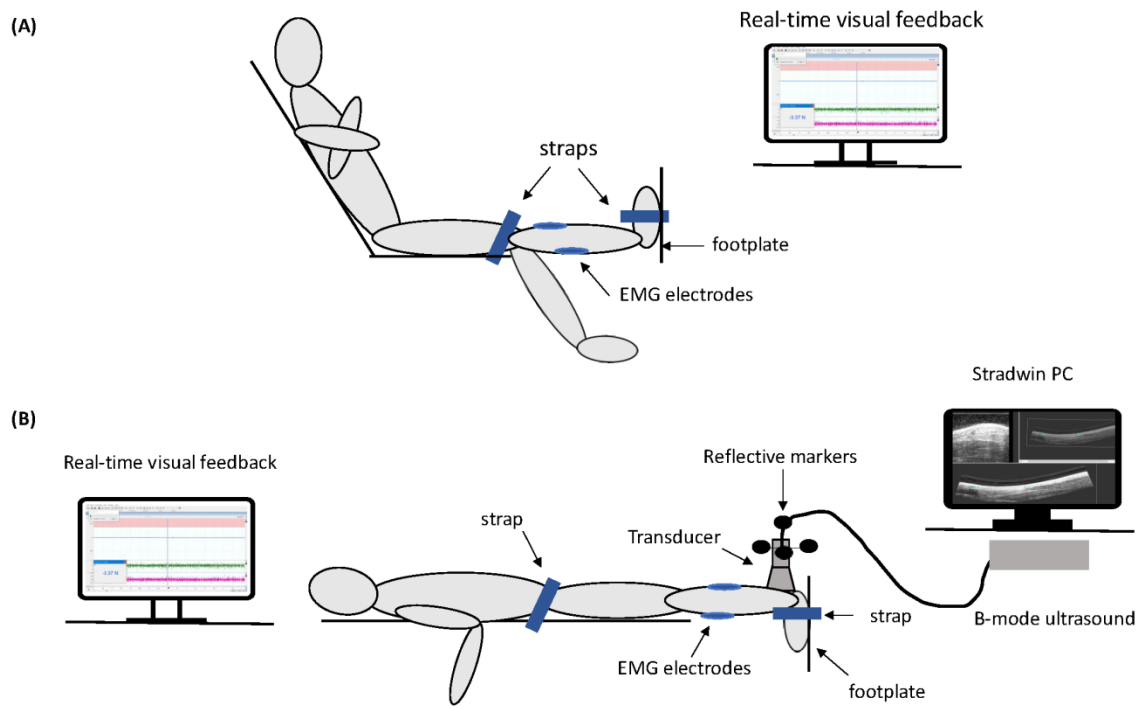

Figure 1

Fig. 1. Schematic diagram of the experimental setup. (A) during the interventions (B) during the MVIC tests and freehand 3D scanning. The interventions were performed from a sitting position to avoid any discomfort associated with a prolonged prone position.

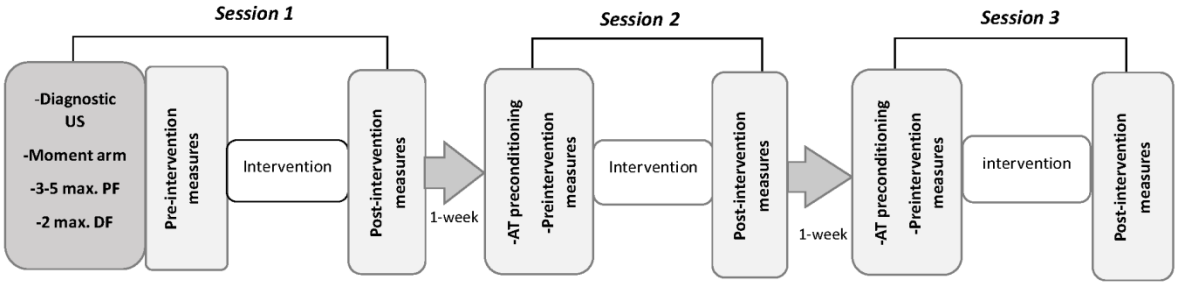

Fig. 2. Diagram of testing procedure.

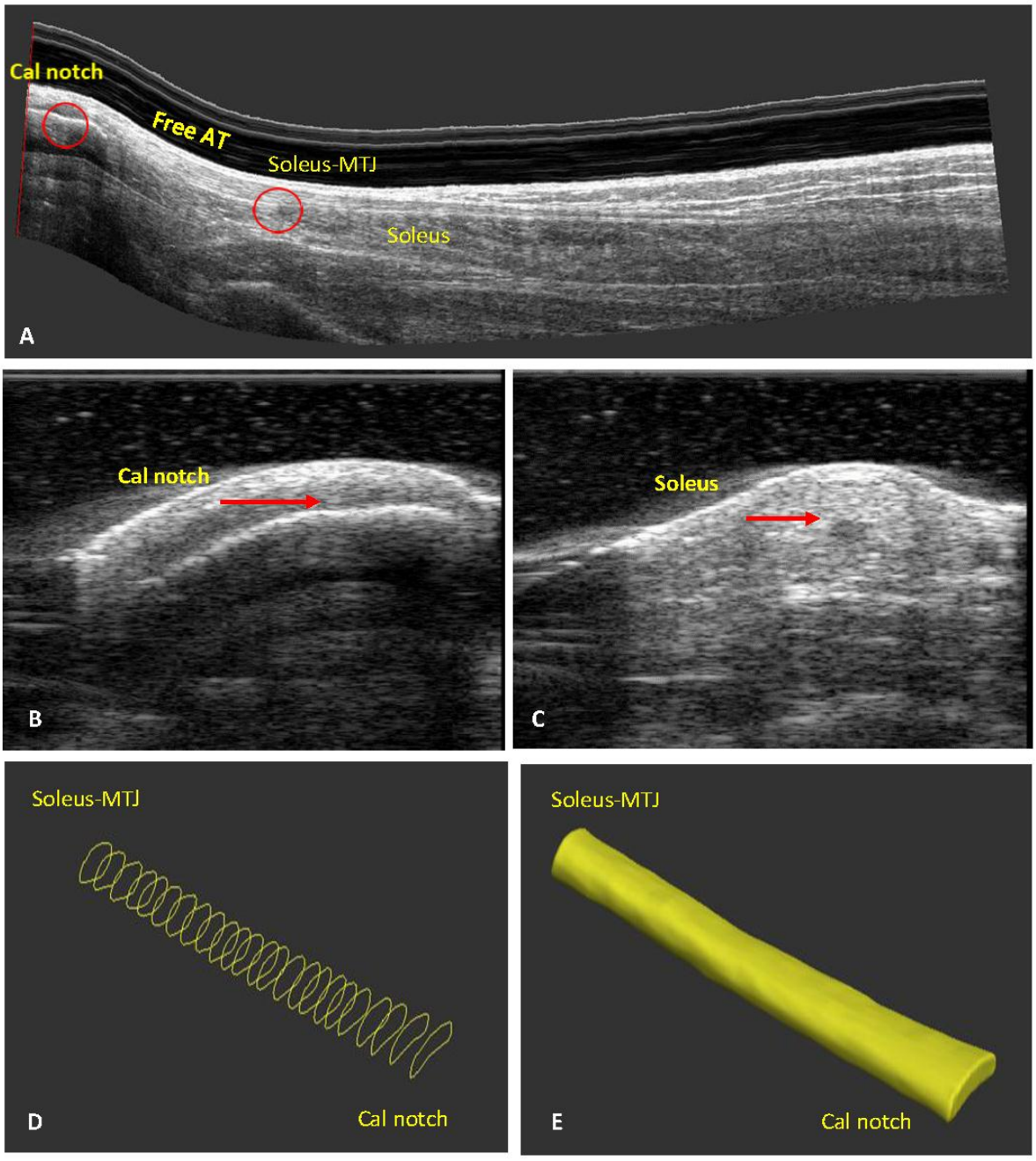

Fig. 3. Three dimensional (3D) Achilles tendon image analysis. (A) Sagittal plane of a 3D ultrasound image of the free Achilles tendon. Two anatomic landmarks (calcaneal notch and soleus-MTJ) were identified on the (A) sagittal plane and (B, C) transverse plane for calculation of Achilles tendon length. Cal notch = calcaneal notch; MTJ = muscle-tendon junction. (D) Manual contouring of free Achilles tendon cross sections from calcaneal notch to soleus-MTJ and (E) free Achilles tendon volume reconstruction.

Figure 4

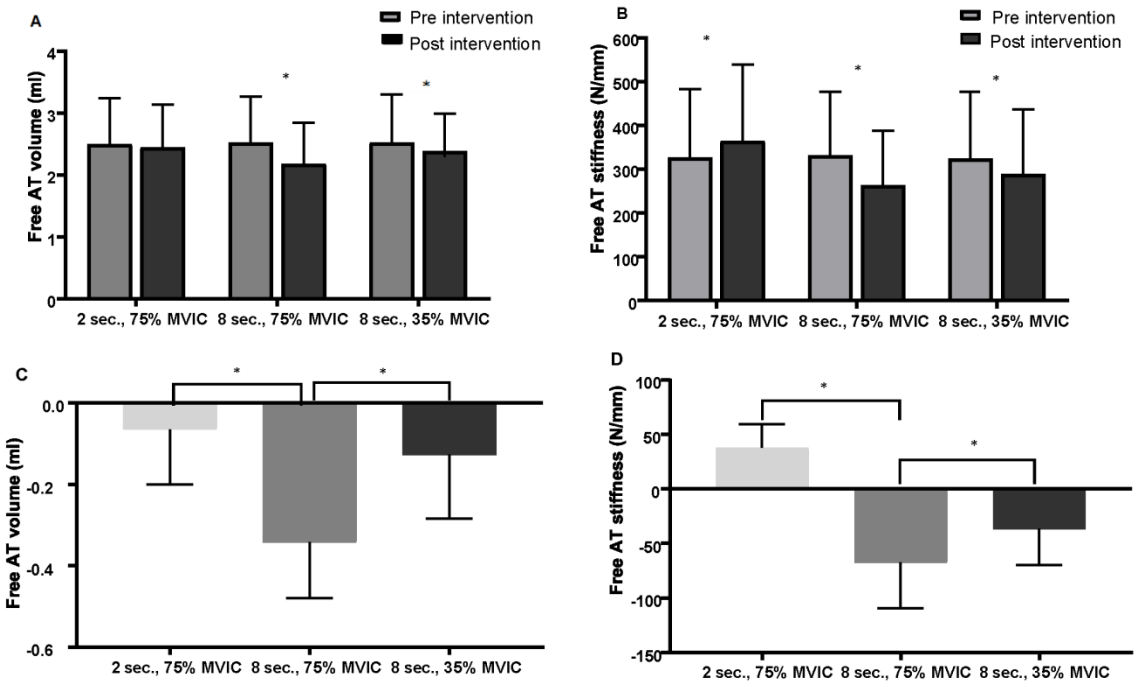

Fig. 4. Free Achilles tendon volume and stiffness data. The pre and post interventions values of free AT volume (A) and stiffness (B). * Significant *post hoc* pairwise difference from baseline ($P < 0.05$, two-tailed). The change score (post- to pre-intervention values) of the free AT (C) volume and (D) stiffness for all interventions. * Statistically significant difference between interventions ($P < 0.05$, two-tailed) according to interaction contrasts analysis. Error bars represent s.d. (n=16).

Table 1 Describes load parameters for the interventions

	Intervention 1	Intervention 2	Intervention 3
Time to peak (s)	1	1	1
Hold at peak (s)	1	7	7
Contraction duration (s)	2	8	8
Load intensity (%MVIC)	75%	75%	35%

MVIC: maximal voluntary isometric contraction.

Table 2. Comparison of the free Achilles tendon mechanical, material, and morphological properties before (pre) and after (post) interventions.

	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Interventions	2 s, 75% MVIC		8 s, 75% MVIC		8 s, 35% MVIC	
Force (N)						
at 35%	898.79 ± 211.01	908.04 ± 210.44	886.89 ± 212.84	918.32 ± 222.81*	888.06 ± 214.69	899.29 ± 212.29
at 55%	1374.11 ± 337.43	1395.56± 359.72	1354.26 ± 348.04	1379.47 ± 338.58	1376.01 ± 345.27	1381.32 ± 349.07
at 75%	1843.26 ± 461.46	1873.98 ± 459.13	1839.23 ± 466.60	1827.66 ± 456.07	1868.79 ± 493.55	1871.55 ± 478.09
Elongation (mm)						
at 35%	1.81 ± .59	2.01 ± .89	1.79 ± .66	2.97 ± 1.12*	1.79 ± .64	2.69 ± 1.13*
at 55%	3.28 ± 1.01	3.35 ± .93	3.22 ± .96	4.61 ± 1.55*	3.35 ± 1.05	4.45 ± 1.44*
at 75%	4.96 ± 1.31	4.91 ± 1.52	4.88 ± 1.32	6.64 ± 1.85*	5.05 ± 1.41	6.39 ± 1.84*
Stiffness (N/mm)	326.40 ± 156.22	364.18 ± 170.37*	330.91 ± 146.36	263.56 ± 124.23 *	324.72 ± 152.28	288.26 ± 148.72*
CSA (mm^2)	42.53 ± 7.92	41.81 ± 6.78	44.09 ± 7.60	37.85 ± 6.96*	44.09 ± 7.58	41.4 ± 7.01*
Resting length (mm)	57.43 ± 13.04	57.44 ± 13.02	57.43 ± 13.04	57.43 ± 13.00	57.43 ± 13.04	57.45 ± 13.05
Volume (ml)	2.49 ± .74	2.44 ± .69	2.51 ± .75	2.17 ± .67*	2.51 ± .78	2.38 ± .69*
Stress (MPa)						
at 35%	21.39 ± 4.22	21.85 ± 4.14	20.36 ± 4.39	25.02 ± 5.45*	20.34 ± 4.14	21.9 ± 4.44*
at 55%	32.64 ± 6.51	33.49 ± 7.09	31.02 ± 6.92	37.57 ± 8.32*	31.47 ± 6.57	33.5 ± 6.88*
at 75%	43.79 ± 8.91	45.1 ± 9.37	42.12 ± 9.25	49.8 ± 11.27*	42.75 ± 9.54	45.44 ± 9.55*
Strain (%)						
at 35%	3.36 ± 1.41	3.67 ± 1.63	3.31 ± 1.49	5.51 ± 2.55*	3.33 ± 1.5	4.92 ± 2.49*
at 55%	6.1 ± 2.6	6.11 ± 2.16	5.98 ± 2.43	8.54 ± 3.68*	6.22 ± 2.6	8.18 ± 3.28*
at 75%	9.24 ± 3.94	9.09 ± 3.84	9.07 ± 3.81	12.31 ± 4.88*	9.38 ± 3.96	11.83 ± 4.77*
Young's modulus (GPa)	0.44 ± 0.23	0.49 ± 0.27	0.43 ± 0.22	0.42 ± 0.23	0.43 ± 0.23	0.41 ± 0.22

CSA: cross-sectional area. *Statistically significant difference from baseline ($P < 0.05$, two-tailed). Data are expressed as group mean (s.d.).

Table 1. Type of physical activity with corresponding hours per week among the participants.

Participant	Type of physical activity or exercise	Hours per week (number)
1	Football and basketball	5
2	Pilates and tap dancing	3
3	Basketball	2
4	Rock climbing	8
5	Gym classes and netball	4
6	Walking	3
7	Football, basketball, and gym	10
8	Basketball	2
9	Soccer, weightlifting, and basketball	6
10	Soccer and volleyball	10
11	Power lifting	8-10
12	Skating	30
13	Weightlifting	5
14	None	-
15	None	-
16	Swimming	2