Short Communication

Loading rate and contraction duration effects on *in vivo* human Achilles tendon mechanical properties

Christopher McCrum[1,2], Kai D. Oberländer[3], Gaspar Epro[4], Peter Krauss[4], Darren C. James[4], Neil D. Reeves[5], Kiros Karamanidis[4]

[1]NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Department of Human Movement Science, Maastricht, The Netherlands

[2]Institute of Movement and Sport Gerontology, German Sport University Cologne, Germany

[3]Media School, Fresenius University of Applied Science, Cologne, Germany

[4]Sport and Exercise Science Research Centre, School of Applied Sciences, London South Bank University, London, UK

[5]School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, U.K.

Corresponding author:

Christopher McCrum

Dept. of Human Movement Science, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands

Email: chris.mccrum@maastrichtuniversity.nl

Short title: Achilles tendon mechanical properties *in vivo*

Summary

Tendons are viscoelastic, which implies loading rate dependency, but loading rates of contractions are often not controlled during assessment of human tendon mechanical properties *in vivo*. We investigated the effects of sustained submaximal isometric plantarflexion contractions, which potentially negate loading rate dependency, on the stiffness of the human Achilles tendon *in vivo* using dynamometry and ultrasonography. Maximum voluntary contractions (high loading rate), ramp maximum force contractions with 3s loading (lower loading rate), and sustained contractions (held for 3s) at 25%, 50% and 80% of maximal tendon force were conducted. No loading rate effect on stiffness (25-80% max. tendon force) was found. However, loading rate effects were seen up to 25% of maximum tendon force, which were reduced by the sustained method. Sustained plantarflexion contractions may negate loading rate effects on tendon mechanical properties and appear suitable for assessing human Achilles tendon stiffness *in vivo*.

Keywords: Gastrocnemius muscle, M. triceps surae, tendon stiffness, tendon strain, muscle strength, ultrasonography

Introduction

Tendons transfer force generated by the muscles to the bones, leading to joint rotations and movement, and therefore, tendon mechanical properties can have a large impact on movement effectiveness. The mechanical properties of the *triceps surae* muscle-tendon unit play an important role in locomotion, with the muscles providing significant propulsive force during the push-off phase of gait and the tendinous structures storing and returning elastic energy to the joint (Biewener and Roberts, 2000; Roberts, 2002), thereby affecting the efficiency of movement (Hof *et al*., 2002; Huang *et al*., 2015; Lichtwark and Wilson, 2007; Pandy and Andriacchi; 2010). Specifically, the mechanical properties of the Achilles tendon (AT) are of interest, as the stiffness or slackness of the AT greatly influences the ability of the *triceps surae* muscle-tendon unit to contribute to forward propulsion during gait. The most common method currently for assessing human tendon mechanical properties *in vivo* is synchronous ultrasonography and dynamometry, originally proposed by Fukashiro *et al* (1995) and later further developed by Kubo *et al.* (1999), Maganaris and Paul (1999) and Maganaris (2002). However, one factor that may affect the accuracy of tendon mechanical properties assessment *in vivo* is tendon viscoelasticity.

Tendon viscoelasticity, which implies loading rate dependency (a viscous time-dependent property) of tendon tensile strain (Abrahams, 1967; Hooley *et al.* 1980; Fung, 1993), is generally accepted and has been shown in human lower limb tendons *in vivo* (Gerus *et al*., 2011; Kosters *et al*., 2014; Pearson *et al*., 2007; Theis *et al*., 2012). However, other studies have not found loading rate dependency in human tendons *in vivo* (Kubo *et al*., 2002; Peltonen *et al*., 2013). These differences in findings may be related to the tendon (AT or patellar) or tendon structure (tendon or aponeurosis)

<u>analysed, due to differences in deformation characteristics between structures or tendon elongation tracking procedures, as well as other methodological differences, such as the duration and rate of loading used or the method used to account for joint movement on the measured tendon elongation during contraction (for a detailed overview of such methodological issues, see Seynnes *et al.* (2015)).</u>

Many studies of *in vivo* tendon mechanical properties have employed ramped isometric contractions, with a gradual increase to maximum voluntary force over a number of seconds (*e.g.*: Arampatzis *et al*., 2007b; Kubo *et al*., 1999; Kubo *et al*., 2000b; Kubo *et al*., 2000a; Maganaris and Paul, 2002; Maganaris *et al*., 2004; Reeves *et al*., 2005; Seynnes *et al*., 2009). However, if a set time (e.g. three seconds) is given to reach maximum force, the absolute loading rate may differ between participants of different strengths (Kosters *et al*., 2014). One method that may negate such loading rate effects is to instead use isometric contractions held at multiple given submaximal force levels. This contraction method has recently been used in different forms to assess AT mechanical properties (Ackermans *et al*., 2016; Farris *et al*., 2013; Lichtwark *et al*., 2013; Obst *et al*., 2016), but the method's effects on loading rate dependency have not been investigated.

The sustained method may negate loading rate dependency <u>as it addresses the phase shift (due to the time-dependent viscous properties)</u> of the reactive response of viscoelastic material (Meyers and Chawla, 1999). This can be illustrated using a simple Kelvin-Voigt model, comprised of a purely viscous damper and purely elastic spring connected in parallel (see examples of Kelvin-Voigt model application in biological tissue assessment in: Alkalay *et al*., 2015; Kiss *et al*., 2004; Tzschatzsch *et al*., 2014). When an external stress is applied to the model, the spring deforms while the damper acts against the deformation, causing a time delay in the deformation. After a certain time, the model reaches its final deformation, determined by the spring constant and the applied stress. As well as potentially negating loading rate dependency, a constant force held for a given time period potentially negates measurement error due to ultrasound sampling frequency or synchronization delays between ultrasound and force data, previously suggested by Finni *et al*. (2013) as limitations for measuring AT hysteresis *in vivo*.

Given the potential benefits of sustained isometric contractions on *in vivo* tendon mechanical property assessment, this study aimed to determine if sustained submaximal isometric plantarflexion contractions would negate potential effects of loading rate on AT stiffness measurements in comparison to traditionally used contractions (MVC and ramp contraction).

Methods

Study Participants

Ten male adults (mean[SD] age: 26.5[5.5] years) participated in this study. Volunteers with previous AT ruptures, AT injury within the last 12 months, or musculoskeletal impairments were excluded. The study was approved by the German Sport University Cologne ethical board and informed consent was obtained according to the Declaration of Helsinki.

Experimental Setup and Procedure

The experimental setup used in this study has been described previously in detail (Karamanidis *et al*., 2016). Briefly, the participants were seated on a custom made dynamometer with the knee of the dominant leg fully extended and the foot of the dominant leg positioned on the dynamometer foot plate perpendicular to the femur and tibia (see Fig. 1Ai). A custom made brace constructed using ski bindings was attached around the foot and the dynamometer foot plate to reduce any joint motion during contractions.

Insert Fig. 1

The measurements began with a standardized warm-up of five minutes hopping and stretching, 2-3 minutes of submaximal contractions guided by TEMULAB software (Protendon GmbH & Co. KG, Aachen, Germany), and three maximal isometric contractions to precondition the tendon (Maganaris, 2003). Following this, participants completed three MVCs with a high loading rate, three ramp maximum force contractions with a three second loading time (guided by visual feedback provided by the software) resulting in a lower loading rate, and nine sustained contractions at the same lower loading rate (also with visual feedback), held three times for three seconds at 25%, 50% and 80% of the maximal tendon force ascertained during the MVC protocol. The order of the ramp and sustained contractions was randomized (MVC always first). <u>The fact that MVC was always performed first was assumed not to affect the results as tendon preconditioning was conducted and no acute change in the properties would be expected as a result of further contractions within our protocol (Maganaris, 2003).</u> Sufficient rest was given between contractions (approximately two to three minutes). <u>For the MVCs, participants were instructed to produce as much force as possible, as fast as possible.</u> Representative ankle joint moment-time curves from one subject across the three tasks can be seen in Fig. 1(C). All three contraction tasks were repeated on a second day with all participants <u>and the data were</u> pooled for the analysis.

Assessment of Achilles Tendon Mechanical Properties

The *triceps surae* mechanical properties of the dominant leg were assessed during isometric plantarflexion contractions by integrating dynamometry (using three strain gauge load cells [100Hz] placed at pre-defined positions on the foot plate; Fig 1Aii) and ultrasonography (Aloka α7, Tokyo, Japan). Eight light emitting diodes (four on the lower limb and four on the force plate; Fig. 1Ai & ii) were used as active markers, whose 2D trajectories were recorded by two digital high-speed cameras (15Hz; Basler, Germany) and tracked automatically by the TEMULAB software (Karamanidis *et al*., 2016). The resultant ankle joint moments were calculated using inverse dynamics following compensation for moments resulting from gravitational and compression forces (Arampatzis *et al*., 2005; Karamanidis and Arampatzis, 2005). Reaction forces under the foot and their respective lever arms to the ankle joint centre were assessed as described previously (Karamanidis *et al*., 2016). AT force (N) was calculated by dividing the ankle joint moment (Nm) by the AT moment arm (m). The AT moment arm was estimated as the perpendicular distance from the ankle joint centre of rotation to the AT (Scholz *et al*., 2008). The *m. gastrocnemius medialis* (GM) tendon was examined using a 7.5MHz linear array ultrasound probe. The probe was placed longitudinally over the GM myotendinous junction with a black rubber band placed between the skin and the probe to determine any probe motion relative to the skin (as in previous work: Arampatzis *et al*., 2007a; Arampatzis *et al*., 2005). All recordings were saved at 73Hz. Tendon elongation was determined by manually tracking the GM myotendinous junction during loading (Fig. 1B). The effect of potential ankle joint angular rotation on the measured tendon elongation during contractions (Magnusson *et* *al*., 2001) was taken into account by multiplying the estimated AT moment arm by the ankle joint angular changes during contraction. In this way, the actual tendon elongation caused by the exerted tendon force could be estimated. Tendon elongation was analysed at 25%, 50% and 80 % of MVC for all three contraction types. Tendon stiffness was determined as the ratio of the increase in the calculated tendon force and the increase in the elongation from 25 to 80% of maximum tendon force (AT $\text{Stiffness}_{25\text{-}80\%}$). Additionally, a post hoc analysis of the slope of the force-elongation relationship from 0% to 25% of maximal tendon force was conducted (AT $\text{Stiffness}_{0\text{-}25\%}$; see Results and discussion section).

Statistics

The data from the two different measurement days were pooled together. The data of three participants on day two were excluded due to measurement errors, leaving 17 samples for the analysis. Normality was checked using the Shapiro Wilk test. Wilcoxon Signed Rank tests were used to assess loading rate differences between MVC and ramp. A two-way ANOVA with method (MVC, ramp and sustained) and normalized tendon force (25%, 50% and 80%) as factors was used to determine method and tendon force-related differences in AT elongation. One-way ANOVAs with contraction method as a factor were used to determine method-related differences in AT $\text{Stiffness}_{25\text{-}80\%}$ and AT $\text{Stiffness}_{0\text{-}25\%}$. Homogeneity of variance was checked with Levene's test. Significance was set at α=0.05. Analyses were performed using IBM SPSS Statistics (Armonk, NY: IMB Corp.).

Results

The ankle joint moment loading rates during the MVC and ramp contractions were (mean and SD) 3181(2032) and 688(151)Nm/s, respectively (Fig. 2A; approximately 79% MVC per second and 18% MVC per second, respectively). The Wilcoxon Signed Rank tests revealed significantly ($P<0.001$) lower loading rates during ramp, compared to MVC. A two way repeated measures ANOVA with method and tendon force level as factors found significant method ($F_{[1.5,\ 24]}=15.5$, $P<0.001$) and tendon force ($F_{[1.4,\ 23]}=277.5$, $P<0.0001$) effects on tendon elongation (Fig. 2B). *Post hoc* tests with Bonferroni corrections revealed significant differences for tendon elongation between SUS and both RAMP and MVC, as well as between RAMP and MVC, for all tendon force levels ($P<0.01$; see Table 1). The one way ANOVA with method (MVC, ramp and sustained) as a factor found no significant effect on AT $\text{Stiffness}_{25\text{-}80\%}$ (Fig. 2C; MVC: 654[221]N/mm; ramp: 695[190]N/mm; sustained: 564[148]N/mm; $F_{[2,\ 32]}=2.5$, $P=0.079$).

The fact that elongation, but not stiffness was significantly different between methods in the current study suggests that the change in elongation observed between methods occurred prior to the force levels used in this study (i.e. up to 25% of AT force) and that the difference in elongation remained constant between the methods thereafter, which agrees with our theory that the sustained contraction method accommodates the phase shift of the reactive response to applied force due to tendon viscoelasticity. Therefore, a *post hoc* analysis of the slope of the force-elongation relationship from 0% to 25% of maximal tendon force (AT $\text{Stiffness}_{0\text{-}25\%}$) was conducted in a similar manner to Lichtwark *et al*. (2013). A one way ANOVA with method (MVC, ramp and sustained) as a factor revealed a significant method effect on AT $\text{Stiffness}_{0\text{-}25\%}$ (Fig. 2D; MVC: 190[46]N/mm; ramp: 165[43]N/mm; sustained: 150[37]N/mm; $F_{[1.5,\ 24]}=14$, $P<0.001$).

Post hoc tests with Bonferroni corrections (see Fig. 2D) revealed significant differences in AT Stiffness$_{0\text{-}25\%}$ between MVC and RAMP (P=0.0455), MVC and SUS (P=0.0002) and RAMP and SUS (P=0.0353).

Insert Fig. 2

Discussion

In the current study, we aimed to determine if sustained submaximal isometric plantarflexion contractions would negate potential effects of loading rate on AT stiffness measurements in comparison to traditionally used contractions (MVC and ramp contraction). Loading rate dependency was seen for AT elongation, as fast (MVC: mean of 3181Nm/s) and slower (ramp: 688Nm/s) loading rate contractions led to differences in elongation (Fig. 2B and Table 1). However, an effect of loading rate was not observed in AT stiffness, as no significant differences were found between MVC and ramp contractions. In order to further investigate the change in tendon elongation across methods, we conducted a *post hoc* analysis of AT Stiffness$_{0\text{-}25\%}$ (Fig. 2D) in a similar manner to Lichtwark *et al*. (2013). We were able to confirm that, at this region of the force-elongation relationship, significant differences in the slope could be seen, confirming loading rate dependency (Fig. 2D; MVC vs. RAMP) and at least a partial negation of loading rate dependency using the sustained contraction method (Fig. 2D; SUS vs. MVC and SUS vs. RAMP). This finding seems to support our suggestion that the sustained contraction method accommodates the phase shift of the reactive response to applied force due to tendon viscoelasticity.

Despite its widespread use, a number of methodological challenges exist that may preclude the precise assessment of tendon mechanical properties *in vivo*, as recently highlighted by Seynnes *et al*. (2015). Synchronization of ultrasound, dynamometer and computer systems is one of these challenges. Synchronization can introduce error, whereby computer processing time or the typically lower sampling frequency of ultrasound devices may introduce lag in comparison to the higher frequency force measurements (6). This has been demonstrated experimentally in the AT *in vivo* by Finni *et al*. (2013), where an artificial desynchronisation between force and ultrasound recordings (one ultrasound frame; 10ms) resulted in a 4-5% change in calculated AT stiffness, although the change was not as high when compared to AT hysteresis (9-10% change). Importantly, *in vivo* methodologies are limited to the loading rates achievable during voluntary contractions, which are much lower and less controllable than those possible in *in vitro* setups. The wide range in achieved loading rates during the MVCs in the current study (Fig. 2A) demonstrates the large variation between young, healthy participants in their ability to achieve high loading rates *in vivo*. The sustained contraction method on the other hand, as outlined in the current study, may well be a solution for negating measurement error due to ultrasound sampling frequency or synchronization delays between ultrasound and force data, and appears to negate the effects of loading rate dependency on the mechanical properties of the AT. With this in mind, it is worth noting that the variability in AT Stiffness$_{25\text{-}80\%}$ was lowest for the sustained contraction (Fig. 2C). Additionally, image processing and digitizing time is greatly reduced. Finally, while not currently conducted, electromyography signals may be more repeatable when taken during sustained contractions due to the longer observation window (Rainoldi *et al*., 1999), which would benefit the examination of the effect of tibialis anterior co-activation on the resultant ankle joint moment during plantarflexion contractions (Mademli *et al*., 2004).

When interpreting the current findings, it is important to note that the AT force is estimated *in vivo* using the resultant ankle joint moment. As a result, the influence of synergistic and antagonist muscles, which may differ between different loading rates and contraction types, have not been accounted for. This, in turn, may lead to errors in the tendon force-elongation relationship calculation, potentially reducing the ability to detect small changes in tendon stiffness between loading rates and contraction types. That being said, the effect of co-contraction of the tibialis anterior, for example, is relatively low in young healthy subjects (accounting for co-contraction of the tibialis anterior results in approximately a 4% increase in the maximal ankle joint moments generated by the *triceps surae* muscle-tendon unit during an MVC; Arampatzis *et al*., 2005) and therefore, a large effect on the force-elongation relationship would not be expected. It is also noteworthy that it was not possible to measure the loading rates during the sustained contractions in our protocol; however, these should have been similar to the ramp rates, as the same guidance software and settings were used. Finally, AT Stiffness$_{0\text{-}25\%}$ does not represent true tendon stiffness at this tendon force level due to the non-linearity of the force-elongation relationship and is only used to give an indication of changes in the slope of the force-elongation relationship at the different regions in general (Lichtwark *et al*., 2013). Regarding the difference in stiffness results, it is important to note that the time under load in the 0 to 25% period differed more between the methods than during the 25 to 80% period and that the magnitude of the change in tendon elongation was greater in the 0 to 25% in comparison to the 25 to 80% region for all contraction durations (MVC: 2.8mm vs. 1.9mm; ramp: 3.3mm vs. 1.6mm; sustained: 3.4mm vs. 2mm). Due to lower absolute elongation of the tendon in the higher region of the force-length relationship, small differences between methods are more difficult to detect due to the potential measurement error of the ultrasound method. In conclusion, the current results indicate that tendon stiffness results do not greatly differ between MVC, ramp and sustained plantarflexion contractions. Within the range of loading rates used in this study, which represent those experienced in daily life, no measureable effect of loading rate on stiffness measurements was found. However, loading rate effects were seen in the force-elongation relationship up to 25% of maximum tendon force, which appeared to be reduced by the sustained contraction method. Therefore, sustained plantarflexion contractions may negate potential loading rate effects on the force-elongation relationship of the human AT *in vivo* and represent a valid alternative to MVC and ramp contractions.

Acknowledgements

CM was funded by the Kootstra Talent Fellowship awarded by the Centre for Research Innovation, Support and Policy (CRISP) and by the NUTRIM Graduate Programme of Maastricht University Medical Center+. The Forschungsservicestelle of the German Sport University Cologne (Hochschulinterne Forschungsförderung) also provided funding for this study.

Conflicts of interest

KDO, PK and KK have equity in Protendon GmbH & Co. KG, whose software was used for the data processing and analysis in this study. No other authors declare any conflicts of interest.

References

Abrahams M. Mechanical Behaviour of Tendon *In Vitro*. *Med Biol Engng* (1967); **5**: 433-443.

Ackermans TMA, Epro G, McCrum C, Oberländer KD, Suhr F, Drost MR, Meijer K & Karamanidis K. Aging and the effects of a half marathon on Achilles tendon force–elongation relationship. *Eur J Appl Physiol* (2016); **116**: 2281-92.

Alkalay RN, Vader D, Hackney D. The degenerative state of the intervertebral disk independently predicts the failure of human lumbar spine to high rate loading: an experimental study. *Clin Biomech* (2015); **30**: 211-8.

Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J Exp Biol* (2007a); **210**: 2743-53.

Arampatzis A, Karamanidis K, Morey-Klapsing G, De Monte G, Stafilidis S. Mechanical properties of the triceps surae tendon and aponeurosis in relation to intensity of sport activity. *J Biomech* (2007b); **40**: 1946-52.

Arampatzis A, Stafilidis S, De Monte G, Karamanidis K, Morey-Klapsing G, Brüggemann G-P. Strain and elongation of the human gastrocnemius tendon and aponeurosis during maximal plantarflexion effort. *J Biomech* (2005); **38**: 833-41.

Biewener AA, Roberts TJ. Muscle and Tendon Contributions to Force, Work, and Elastic Energy Savings: A Comparative Perspective. *Exerc Sport Sci Rev* (2000); **28**: 99-107.

Farris DJ, Trewartha G, Mcguigan MP, Lichtwark GA. Differential strain patterns of the human Achilles tendon determined in vivo with freehand three-dimensional ultrasound imaging. *J Exp Biol* 2013; **216**: 594-600.

Finni T, Peltonen J, Stenroth L, Cronin NJ. Viewpoint: On the hysteresis in the human Achilles tendon. *J Appl Physiol* (2013); **114**: 515-7.

Fukashiro S, Itoh M, Ichinose Y, Kawakami Y, Fukunaga T. Ultrasonography gives directly but noninvasively elastic characteristic of human tendon in vivo. *Eur J Appl Physiol* (1995); **71**: 555-557.

Fung, Y. C. *Biomechanics: Mechanical Properties of Living Tissues* (1993); Springer-Verlag, New York.

Gerus P, Rao G, Berton E. A method to characterize in vivo tendon force–strain relationship by combining ultrasonography, motion capture and loading rates. *J Biomech* (2011); **44**: 2333-2336.

Hof AL, Van Zandwijk JP, Bobbert MF. Mechanics of human triceps surae muscle in walking, running and jumping. *Acta Physiol Scand* (2002); **174**: 17-30.

Hooley CJ, McCrum NG, Cohen RE. The Viscoelastic Deformation of Tendon. *J Biomech* (1980); **13**: 521-528.

Huang T-WP, Shorter KA, Adamczyk PG, Kuo AD. Mechanical and energetic consequences of reduced ankle plantar-flexion in human walking. *J Exp Biol* (2015); **218**: 3541-3550.

Karamanidis K, Arampatzis A. Mechanical and morphological properties of different muscle-tendon units in the lower extremity and running mechanics: effect of aging and physical activity. *J Exp Biol* (2005); **208**: 3907-23.

Karamanidis K, Travlou A, Krauss P, Jaekel U. Use of a Lucas-Kanade-Based Template Tracking Algorithm to Examine In Vivo Tendon Excursion during Voluntary Contraction Using Ultrasonography. *Ultrasound Med Biol* (2016); **42**: 1689-700.

Kiss MZ, Varghese T, Hall TJ. Viscoelastic characterization of in vitro canine tissue. *Phys Med Biol* (2004); **49**: 4207-18.

Kosters A, Wiesinger HP, Bojsen-Moller J, Muller E, Seynnes OR. Influence of loading rate on patellar tendon mechanical properties in vivo. *Clin Biomech* (2014); **29**: 323-9.

Kubo K, Kanehisa H, Kawakami Y, Fukunaga T. Elastic properties of muscle-tendon complex in long-distance runners. *Eur J Appl Physiol* (2000a); **81**: 181-7.

Kubo K, Kanehisa H, Kawakami Y, Fukunaga T. Elasticity of tendon structures of the lower limbs in sprinters. *Acta Physiol Scand* (2000b); **168**: 327-35.

Kubo K, Kawakami Y, Fukunaga T. Influence of elastic properties of tendon structures on jump performance in humans. *J Appl Physiol* (1999); **87**: 2090-6.

Kubo K, Kawakami Y, Kanehisa H, Fukunaga T. Measurement of viscoelastic properties of tendon structures in vivo. *Scand J Med Sci Sports* (2002); **12**: 3-8.

Lichtwark GA, Cresswell AG, Newsham-West RJ. Effects of running on human Achilles tendon length-tension properties in the free and gastrocnemius components. *J Exp Biol* (2013); **216**: 4388-94.

Lichtwark GA, Wilson AM. Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J Biomech* (2007); **40**(8): 1768-75.

Mademli L, Arampatzis A, Morey-Klapsing G, Brüggemann GP. Effect of ankle joint position and electrode placement on the estimation of the antagonistic moment during maximal plantarflexion. *J Electromyogr Kinesiol* (2004); **14**(5): 591-7.

Maganaris CN. Tensile properties of in vivo human tendinous tissue. *J Biomech* (2002); **35**: 1019-1027.

Maganaris CN. Tendon conditioning: artefact or property? *Proc Biol Sci* (2003); **270**(Suppl 1): S39–S42.

Maganaris CN, Narici MV, Reeves ND. In vivo human tendon mechanical properties: effect of resistance training in old age. *J Musculoskelet Neuronal Interact* (2004); **4**(2): 204-8.

Maganaris CN, Paul JP. In vivo human tendon mechanical properties. *J Physiol* (1999); **521**(1): 307-313.

Maganaris CN, Paul JP. Tensile properties of the in vivo human gastrocnemius tendon. *J Biomech* (2002); **35**(12): 1639-46.

Magnusson SP, Aagaard P, Dyhre-Poulsen P, Kjaer M. Load-displacement properties of the human triceps surae aponeurosis in vivo. *J Physiol* (2001); **531**: 277-88.

Meyers AM, Chawla KK. *Mechanical Behavior of Materials* (1999); Prentice Hall, Upper Saddle River, NJ.

Obst SJ, Newsham-West R, Barret RS. Changes in Achilles tendon mechanical properties following eccentric heel drop exercise are specific to the free tendon. *Scand J Med Sci Sports* (2016); **26**(4): 421-31.

Pandy MG, Andriacchi TP. Muscle and joint function in human locomotion. *Annu Rev Biomed Eng* (2010); **12**: 401-33.

Pearson SJ, Burgess K, Onambele GN. Creep and the in vivo assessment of human patellar tendon mechanical properties. *Clin Biomech* (2007); **22**(6): 712-7.

Peltonen J, Cronin NJ, Stenroth L, Finni T, Avela J. Viscoelastic properties of the Achilles tendon in vivo. *Springerplus* (2013); 2: 212.

Rainoldi A, Galardi G, Maderna L, Comi G, Lo Conte L, Merletti R. Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle. *J Electromyogr Kinesiol* (1999); **9**(2): 105-19.

Reeves ND, Maganaris CN, Ferretti G, Narici MV. Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. *J Appl Physiol* (2005); **98**(6): 2278-86.

Roberts TJ. The integrated function of muscles and tendons during locomotion. *Comp Biochem Physiol A Mol Integr Physiol* (2002); **133**: 1087-99.

Seynnes OR, Bojsen-Moller J, Albracht K, Arndt A, Cronin NJ, Finni T, Magnusson SP. Ultrasound-based testing of tendon mechanical properties: a critical evaluation. *J Appl Physiol* (2015); **118**(2): 133-41.

Seynnes OR, Erskine RM, Maganaris CN, Longo S, Simoneau EM, Grosset JF, Narici MV. Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *J Appl Physiol* (2009); **107**(2): 523-30.

Scholz MN, Bobbert MF, van Soest AJ, Clark JR, van Heerden J. Running biomechanics: shorter heels, better economy. *J Exp Biol* (2008); **211**: 3266-71.

Theis N, Mohagheghi AA, Korff T. Method and strain rate dependence of Achilles tendon stiffness. *J Electromyogr Kinesiol* (2012); **22**: 947-53.

Tzschätzsch H, Ipek-Ugay S, Guo J, Streitberger KJ, Gentz E, Fischer T, Klaua R, Schultz M, Braun J, Sack I. In vivo time-harmonic multifrequency elastography of the human liver. *Phys Med Biol* (2014); **59**(7): 1641-54.

Tables

Table 1. Bonferroni multiple comparisons tests for Achilles tendon elongation during MVC, ramp and sustained contraction methods

Tendon Force Level [% max.]	Contraction Methods	Mean Difference [mm]	95% Confidence Intervals of Differences	Adjusted P Value
25	MVC vs. RAMP	-0.9663	-1.421 to -0.5115	<0.0001
	MVC vs. SUS	-1.532	-1.987 to -1.077	<0.0001
	RAMP vs. SUS	-0.5658	-1.021 to -0.1109	0.0097
50	MVC vs. RAMP	-0.8324	-1.287 to -0.3776	<0.0001
	MVC vs. SUS	-1.623	-2.078 to -1.169	<0.0001
	RAMP vs. SUS	-0.791	-1.246 to -0.3362	0.0002
80	MVC vs. RAMP	-0.7165	-1.171 to -0.2617	0.0008
	MVC vs. SUS	-1.847	-2.302 to -1.392	<0.0001
	RAMP vs. SUS	-1.13	-1.585 to -0.6755	<0.0001

Figure Legends

Fig. 1. Experimental setup and methodology. **(A) i**: Lateral camera view of the participant and dynamometer setup; **ii**: position of the foot and strain gauge load cells (black circles) on the dynamometer foot plate; **(B)**: examples of myotendinous junction tracking to examine tendon elongation using the ultrasound images at rest and at 25%, 50% and 80% of MVC force. (A) and (B) adapted from Karamanidis *et al*. (2016). **(C)**: Representative plantarflexion ankle joint moment data of one subject for an isometric maximum voluntary contraction with a high loading rate (MVC), an isometric ramp contraction (RAMP) and isometric sustained contractions (SUS). The black circles represent the time points when the 25%, 50% and 80% MVC measures were taken for each method.

Fig. 2. *Triceps surae* muscle-tendon unit mechanical properties during maximum voluntary contractions with a high loading rate (MVC), isometric ramp contractions with a three second loading time (RAMP) and isometric sustained contractions (SUS) at force levels of 25%, 50% and 80% of maximal tendon force. Results are medians with error bars of the 95% confidence intervals. *, ** and *** represent significant contraction method differences ($P<0.05$, $P<0.01$ and $P<0.001$, respectively). **(A):** Ankle joint moment loading rates during the MVC and RAMP contractions. **(B):** Achilles tendon elongation at 25%, 50% and 80% of maximal tendon force during MVC, RAMP and SUS contractions at each force level. Significant method ($P<0.001$) and tendon force level ($P<0.0001$) effects were found. **(C):** Achilles tendon stiffness determined from 25% to 80% of maximal tendon force during MVC, RAMP and SUS contractions.

(D): *Post hoc* analysed Achilles tendon stiffness determined from 0% to 25% of maximal tendon force during MVC, RAMP and SUS contractions.