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SHEAR WAVE VELOCITY OF THE PATELLAR TENDON AND QUADRICEPS MUSCLE ARE INCREASED
IMMEDIATELY AFTER MAXIMAL ECCENTRIC EXERCISE

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ABSTRACT

Purpose: To determine whether stiffness of the patellar tendon and quadriceps muscles is altered immediately after and 48hrs after a single-bout of maximal eccentric exercise of the knee extensor muscles.

Methods: Thirteen healthy individuals (group mean (SD) age 22.4 (3.5) years; 7 female) performed a single-bout of maximal eccentric exercise of the non-dominant knee extensors, using an isokinetic dynamometer. Shear wave velocity (an index of tissue stiffness) was recorded from the patellar tendon, vastus medialis (VM), rectus femoris (RF) and vastus lateralis (VL), before, following (post₀), and 48-hours after (post₄₈) exercise. To investigate features of exercise induced muscle damage, maximal voluntary isometric contraction (MVIC) and self-reported pain and stiffness (numerical rating scales 0=no pain/stiffness to 100=worst imaginable pain/stiffness) were measured before, post₀, and post₄₈ exercise. Serum creatine kinase (CK) was measured before and post₄₈ exercise.

Results: Compared to pre-exercise, MVIC decreased and self-reported pain and stiffness increased at post₀ and post₄₈ and CK levels increased at post₄₈ (all $p < 0.01$). Compared to pre-exercise, shear wave velocity was greater at post₀ for the patellar tendon (15.9 (24.6)%, $p = 0.01$) and RF (23.6 (16.7)%, $p < 0.001$), each returning to baseline by post₄₈. No significant differences were observed for VL or VM post₀ or post₄₈ exercise.

Conclusion: Maximal eccentric exercise produced an immediate increase in the stiffness of the patellar tendon and RF, resolving by 48-hours. As this change was not observed in VL and VM, future studies may explore heterogeneity within synergist muscles following eccentric exercise.

Keywords: delayed onset muscle soreness; DOMS; elasticity; stiffness; supersonic imaging

Abbreviations:

CK	Creatine kinase
EIMD	Exercise induced muscle damage
EMG	Electromyography
ICC	Intraclass correlation coefficients
IQR	Interquartile range
MVIC	Maximal voluntary isometric contraction
NRS	Numerical rating scale
RF	Rectus femoris
SWV	Shear-wave velocity

VL
VM
52

Vastus lateralis
Vastus medialis

53

1 INTRODUCTION

The response of skeletal muscle to unaccustomed eccentric exercise has been studied widely, yet it remains incompletely understood. Unaccustomed eccentric contractions typically result in exercise induced muscle damage (EIMD), characterised by muscle soreness, that peaks after 48 hours (Guilhem et al. 2016; Lacourpaille et al. 2014), reduced strength and range of motion (Noska and Clarkson 1996) and increased passive joint stiffness (Porter et al. 2002; Whitehead et al. 2001). Traditionally, passive joint stiffness is measured by determining the overall passive torque in relation to joint range of motion using an isokinetic dynamometer (Porter et al. 2002; Whitehead et al. 2001). When using overall passive joint stiffness it has not been possible to isolate the mechanical behaviour of structures either in series (i.e. muscle versus tendon) or structures in parallel (i.e. synergistic muscles). Investigation of how eccentric exercise affects the mechanical behaviour of individual synergistic muscles and tendon tissue crossing the knee joint could help improve the understanding of musculotendinous adaption to exercise.

Tendon tissue and the extracellular matrix of *connective tissue which lies within a skeletal muscle* are collagen rich, and are crucial for muscle function, especially with regard to the transmission of force (Kjaer 2004). The acute physiological response to unaccustomed eccentric exercise has been studied widely in relation to changes to the extracellular matrix of connective tissue that lies within a muscle (Hylldahl et al. 2017; Hylldahl and Hubal 2014; Hylldahl et al. 2015; Mackey et al. 2004) and the Achilles tendon (Joseph et al. 2014; Obst et al. 2013). Using imaging techniques, previous work has investigated the mechanical properties of the elbow flexors (Lacourpaille et al. 2014), ankle plantarflexors (Green et al. 2012), and knee extensors (Lacourpaille et al. 2017), following eccentric exercise. However, there has been considerably less research investigating the acute effect of eccentric exercise on the mechanical properties of the tendon. Previous work has focused on the Achilles (Joseph et al. 2014; Obst et al. 2013; Obst et al. 2016) and patellar tendons (Yin et al. 2014), without concurrent investigation of the mechanical properties of the in series muscles. Morphologically, the Achilles tendon is compliant and better suited to the storage and release of elastic energy (Lichtwark and Wilson 2007; Obst et al. 2016), whereas the

patellar tendon is shorter and stiffer, making it better suited to force transmission (Malliaras et al. 2015). Previous work suggests that the Achilles tendon acts as a shock-absorber that dissipates energy and reduces muscle fibre elongation during eccentric contractions (Guilhem et al. 2010) and hence potentially the amount of EIMD. Given the morphological differences between the Achilles and patellar tendons, an improved understanding of the effect of maximal eccentric exercise on the shear wave velocity of the patellar tendon and the muscles in series is warranted.

Ultrasound shear-wave elastography provides a valid and reliable quantitative measurement of tissue mechanical properties in real-time (Dubois et al. 2015; Eby et al. 2013; Miyamoto et al. 2015; Tas et al. 2017). For this, an acoustic perturbation is delivered via an ultrasound probe, positioned above the tissue being assessed (Hug 2016). The perturbation generates a shear wave within the tissue, and the shear-wave velocity (SWV) (Hug 2016) is related to the shear modulus of the tissue, meaning the faster the SWV, the stiffer the tissue (Hug et al. 2015). Previous studies have reported shear wave elastography is a reliable technique to investigate the shear elastic modulus for skeletal muscles (Dubois et al. 2015; Lacourpaille et al. 2012; Tas et al. 2017) and the patellar tendon (Tas et al. 2017). Recent work has used shear wave elastography to investigate changes in muscle stiffness of the knee extensors (Lacourpaille et al. 2017) following a single bout of maximal eccentric exercise. Although Lacourpaille et al. (2017) identified an immediate increase in knee extensor muscle stiffness 30 minutes post exercise, which returned to baseline at 48 hours (Lacourpaille et al. 2017), there were statistical differences between the three measured quadriceps muscles, vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF), suggesting synergistic muscles are not affected equally. Given the importance of understanding the interaction between synergistic muscle and tendon tissue following eccentric exercise further investigation is warranted into the superficial quadriceps muscles and the patellar tendon in series.

Therefore, the aims of this study were to determine; (1) whether changes in SWV (a measure of stiffness) of the patellar tendon occur directly after (post₀) and/or 48 hours after (post₄₈) maximal eccentric exercise of the knee extensor muscles, and (2) whether changes in SWV differ for the three

superficial quadriceps muscles (vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF))
post₀ and post₄₈ after maximal eccentric exercise of the knee extensor muscles.

2 METHODS

2.1 Participants

Healthy, young participants who were not engaged in resistance training in the preceding 12 months were recruited by advertising on social media and university newsletters. Participants were excluded if they had: current or previous back and/or lower limb pain/injuries, previous surgery to the back or lower limb, history of corticosteroid injections in the patellar tendon and systemic neurological or metabolic conditions (e.g. epilepsy, diabetes). This study was approved by the Bellberry Human Research Ethics Committee (ID-2016-02-139) and participants signed an informed consent form prior to participation.

2.2 Protocol

Participants attended two laboratory sessions separated by 48 hours, during which period participants were advised to avoid any strenuous exercise. All measures were collected before (*PRE*), immediately (*POST₀*) and 48 hours (*POST₄₈*) after an acute eccentric exercise protocol, with the exception of blood samples taken at *PRE* and *POST₄₈* only. A standardised order of testing was performed in each session (Figure 1).

For each session, the participant sat with their back supported in an isokinetic dynamometer (Kin-Com Model 125AP, Chattanooga Group, Tennessee, USA) with their hip flexed at an angle of 60° (supine=0°) and non-dominant limb positioned at 70° knee flexion (limb dominance was determined by which leg the participant used to kick a ball).

Wireless electromyography (EMG) electrodes (Trigno, Delsys, Natick, Massachusetts, USA) were used to measure myoelectric muscle activity of VM, VL and RF during elastography acquisition. After standard skin preparation, electrodes were attached using adhesive stickers. Data were sampled at 2000 Hz, and observed using Spike2 software (Cambridge Electronic Design, Cambridge,

UK). EMG recordings were used to provide real-time feedback to confirm that the participants' quadriceps muscles were relaxed during elastography data acquisition. Elastography images were discarded and the trial repeated, if EMG activity from any muscle was observed, and as such EMG is not discussed further in this manuscript.

2.3 Outcome measures

2.3.1 Ultrasound and elastography measurement

Shear-wave velocity was recorded using an Aixplorer® Ultrasound Scanner (Version 8.2; Supersonic Imagine, Aix-en-Provence, France) with a linear array transducer (4-15 MHz; SuperLinear 15-4, Vermon, Tours, France) by a musculoskeletal physiotherapist with 18 months training using sonoelastography. Methodology for elastography data acquisition and extraction are reported in detail elsewhere (Coombes et al. 2018) and summarised below. Acceptable within-day reliability for measurement of SWV of the patellar tendon and quadriceps muscles were previously determined (Intraclass correlation coefficients 0.88 to 0.92; coefficient of variation 3.2 to 6.4%) (Coombes et al. 2018). The hip remained in 60° flexion (0°= supine), with the back supported and knee passively positioned at 70° flexion (0°=full extension) for all measurements. This angle was selected as it was considered above the slack length for the VL, VM and RF (i.e. range 45° - 51° (Xu et al. 2016)) and below the maximum SWV measures when investigating tendon tissue. B-Mode ultrasound was used to identify and align the probe longitudinally to the fascicles of the patellar tendon, VM, VL and one compartment of the bi-pennate RF. Approximate positions for imaging were: 33.3% of thigh length for VM, 50-75% thigh length for RF and 33-50% of thigh length for VL (Coombes et al. 2018).. To facilitate consistency between measurements, these locations were marked on the skin using permanent marker. B-mode ultrasound was used to align the probe as carefully as possible with muscle fascicles, however it is more difficult to accurately align the ultrasound probe with fascicle direction in complex muscles such as the rectus femoris. It is important to note that minor changes in the angle of the transducer relative to the fascicles shows negligible difference in shear wave elastography measures (Miyamoto et al. 2015).

As the properties of muscle and tendon can be affected by the loading history (Haraldsson et al. 2005), passive preconditioning (ten cycles from 0-90° knee flexion/extension, 30°s⁻¹) was performed by the isokinetic dynamometer prior to elastography measurement. Participants were advised to remain as relaxed as possible throughout the preconditioning sequence, and EMG was monitored to verify this. Two, ten second elastography videos were recorded for each structure in a standardised order: VM, RF, VL and the patellar tendon. Images were captured at 1.8 iHz for muscle and 1.6 Hz for tendon. Two static B-mode images of the patellar tendon were also recorded to measure patellar tendon thickness, as changes in tissue thickness can alter the relationship between shear wave velocity and stiffness. This protocol was repeated twice more, with identical preconditioning performed between sets, resulting in six ten second videos for each muscle and patellar tendon and six static B-mode images for the patellar tendon. A standardised order was selected to minimise potential error during data collection.

Elastography videos were converted into images and a customised Matlab (R2016a, The Mathworks, Inc. Natick, MA, USA) script was used to analyse the deidentified SWV images. Images were analysed in a random order. Each image was visually inspected, using Matlab, *and the ROI were traced on overlaid B-mode images* (for example, see Fig 1). The middle third of the patellar tendon was selected using the B-mode image as a visual guide (Fig 1). The mean SWV determined from the images (6 images per measure * 6 repeated measures = total 36 images) for each muscle and tendon was used for further analysis, respectively. Tendon thickness was measured using the in-built Aixplorer measurement tool as the maximal anterior-posterior thickness at the middle of the patellar tendon.

2.3.2 Maximal Voluntary Isometric Contraction

Maximal voluntary isometric contraction (MVIC) of the knee extensor muscles was recorded using a 100kg strain gauge (Output 3.0041 mV/V, Gedge Systems, Melbourne, Australia), attached to the non-dominant limb at 22.5cm from the medial and lateral epicondyles of the knee using a custom made strap. Chest and waist restrains were used to secure the participant and minimise

trunk involvement. Force (N) was digitized using a Power1401 data acquisition system with Spike2 software (Cambridge Electronic Design, Cambridge, UK), sampled at 2000 Hz then smoothed with a time constant of 0.1 s to provide feedback to the investigators. Participants *warmed-up* by performing 1 submaximal isometric contraction at an estimated 25%, followed by 1 submaximal isometric contraction at an estimated 50%. Participants then performed three maximal contractions, with two minutes rest between each contraction. Participants were provided strong verbal encouragement during each maximal contraction and asked to maintain the maximal contraction for approximately 5 s. If the recorded force increased by >10% compared to the previous MVIC, participants were asked to perform an additional contraction until variation of <10% was observed for the largest 2 attempts. MVIC data are reported as torque (Nm) throughout the manuscript.

2.3.3 *Self-reported pain and stiffness*

Participants were asked to rate their worst pain experienced in their non-dominant (test) leg in the previous 24 hours, using a numerical rating scale (NRS), where 0 = no pain and 100 = the worst pain imaginable (Hjermstad et al. 2011). In addition, participants were asked to rate their perceived muscle stiffness in their non-dominant (test) leg at rest, using a similar NRS, where 0 = no muscle stiffness and 100 = the worst imaginable muscle stiffness.

2.3.4 *Creatine Kinase*

Serum creatine kinase (CK) levels were measured from blood samples obtained 30 minutes prior to the eccentric exercise protocol (pre) and 48 hours after eccentric exercise. A qualified venipuncturist sampled 10ml of blood from the antecubital vein of each participant using standard procedures and collected in a serum separator tube. Serum CK activity was measured with an automated clinical analyser (Cobas e411, Roche, Rotkreuz, Switzerland) using commercial reagents (#TR14010, ThermoFisher, Waltham, MA, USA). The coefficient of variation for samples measured in duplicate in this study was 1.52%.

2.4 Eccentric exercise protocol

Participants performed maximal eccentric contractions of the knee extensor muscles, of their non-dominant lower limb, between 20° and 90° knee flexion at a speed of 60°s⁻¹, using the isokinetic dynamometer. To enable familiarisation with the eccentric action, one set of 20 contractions at approximately 25% of their estimated maximum was first performed. Following familiarisation, participants performed 5 sets of 20 maximal eccentric contractions with 2 minutes rest between each set. Comparable protocols have been previously used successfully to induce muscle damage in a similar population (Paschalis et al. 2007; Paschalis et al. 2010). Strong verbal encouragement was used to encourage participants to maintain maximal contraction throughout the entire range of motion and to relax during passive extension. Participants were able to visualise their performance on the Kin-Com screen.

2.5 Statistical analysis

Statistical analyses were performed using Stata 13.1 (StataCorp, USA) and significance level was set at $p < 0.05$. Linear mixed models, fitted via Maximum Likelihood, were used to examine the effects of eccentric exercise (Time: pre, post₀ and post₄₈ exercise) on all outcomes, with time as a fixed effect, and the intercepts of the participants as a random effect. For SWV of the quadriceps muscles, both Time, Muscle (VM, RF and VL) and Time-by-Muscle Interaction were entered as fixed effects. Model residuals were tested for normality using Shapiro-Wilk test, consequently Muscle SWV, CK, and self-reported stiffness were transformed using square root⁻¹ transformation and self-reported pain using log transformation. Post-hoc pairwise comparison was performed to assess differences between pre and post₀ and post₄₈ using Bonferroni correction for multiple comparisons. Adjusted P-values are reported. Where applicable, data is presented as median [IQR] or mean \pm SD. The percentage change from baseline to post₀ for SWV of each muscle and tendon, and MVIC were computed to provide relative changes following eccentric exercise.

3 RESULTS

3.1 Participants

Fifteen participants, aged 19 to 30 years were screened. Two individuals were excluded due to pain within the lower limb. Thirteen individuals were included (7 females; group mean (SD) age 22.4 (3.5) years; height 168.4 (8.1) cm; body mass 65.1 (13.0) kg). Based on the Active Australia Survey, (Australian Institute of Health and Welfare 2003) participants self-reported performing a median [interquartile range - IQR] total activity of 300 [150, 360] minutes of physical activity within the preceding week.

3.2 Indicators of exercise induced muscle damage

A significant effect of Time was found for MVIC ($\text{Chi}^2=28.18$, $p<0.001$), self-reported pain ($\text{Chi}^2=15.82$, $p<0.001$) and stiffness ($\text{Chi}^2=17.24$, $p<0.001$). Compared to pre exercise, MVIC was reduced by median 11.1% [IQR -32.7, -10.0] at post_0 ($p<0.001$) and by median 12.1% [IQR -29.4, -8.7] at post_{48} ($p<0.001$). Self-reported pain and stiffness were greater at post_0 ($p=0.028$ and $p=0.001$, respectively) and at post_{48} ($p<0.001$ and $p=0.001$, respectively) compared to pre exercise. Serum CK was greater at post_{48} compared to pre exercise levels ($\text{Chi}^2=13.72$, $p<0.001$). All data are presented in Table 1.

3.3 Patellar tendon stiffness and thickness

A significant effect of Time ($\text{Chi}^2=9.27$, $p=0.01$) on SWV of the patellar tendon was found. Compared to pre exercise measures, SWV of the patellar tendon was 15.9 (24.6) % greater at post_0 ($p=0.011$), but was not significantly different at post_{48} compared to baseline ($p=1$) (Table 1). There was no effect of Time on patellar tendon thickness ($\text{Chi}^2=0.1$, $p=0.95$). Figure 2 presents an example of the SWV measures taken pre and post_0 exercise for the patellar tendon.

3.4 Effects of exercise on muscle stiffness

A significant effect of Muscle ($\text{Chi}^2 = 110.74$, $p < 0.001$), Time ($\text{Chi}^2 = 36.12$, $p < 0.001$) and Muscle-by-Time interaction ($\text{Chi}^2 = 12.34$, $p = 0.015$) was observed for SWV for the quadriceps muscles. Compared to pre exercise, RF SWV was 23.6 (16.7) % greater at post_0 ($p < 0.001$), however this increase was not observed in VM ($p = 0.06$) and VL ($p = 1.00$) post_0 . RF SWV returned to baseline values by post_{48} ($p = 0.65$). At post_{48} VM and VL SWV were not significantly different from pre exercise values (both $p = 1.00$). All data are reported in Table 1. Figure 2 presents an example of the SWV measures taken pre and post_0 for VM, RF, and VL from a representative participant.

4 DISCUSSION

Using shear wave elastography, we demonstrated a concurrent increase in stiffness within the patellar tendon and RF immediately post exercise, returning to baseline by 48 hours. Increased muscle stiffness was not observed in VL and VM, suggesting that individual muscles within a synergist group may display different responses to an eccentric exercise bout. As a measure of EIMD we identified a significant decrease in MVIC and increase in self-reported pain post_0 exercise, both of which remained for 48 hours and an increase in serum CK at post_{48} , following eccentric exercise. These measures of EIMD provide indirect evidence of muscle damage in our sample population. Our findings contribute to the growing body of evidence supporting the utility of shear wave elastography to provide quantitative information about the adaptive responses of both muscular and tendinous tissue to acute exercise.

To the best of our knowledge no previous studies have investigated the mechanical properties of the patellar tendon following maximal eccentric exercises using shear wave elastography, highlighting the importance of our work. In contrast to the results of our study, Yin et al. (2014) identified a decrease in patellar tendon stiffness following a single bout of maximal eccentric exercise of the knee extensors. The study by Yin et al. (2014) calculated stiffness from the slope of the tendon-length curve during a ramped MVIC test between 90% and 100% of maximum force. Using consistent methods to Yin et al. (2014), previous work has shown a decreased stiffness

of the Achilles tendon following submaximal eccentric exercises (Obst et al. 2016). Obst et al. (2016) suggests the decrease in stiffness may represent tendon creep, which could indicate mechanical fatigue. Although no studies have compared the relationship between stiffness measured from the slope of the tendon-length curve during a ramped MVIC and shear wave elastography in vivo, differences in methodology will likely contribute to inconsistent findings. For example, shear wave elastography is often measured in a passive state or during extremely low load isometric contractions, where as traditional methods of measuring tendon mechanical properties are taken during high load contractions. In addition, shear wave elastography measures the SWV of the tendon from a small and localised area, which may not be representative of the entire tendon. Despite inclusion of a similar population (i.e. untrained young adults), Yin et al. (2014) did not report indirect measures of EIMD (e.g. self-reported pain, MVIC, and serum CK), making it difficult to compare measures of muscle damage between studies.

In our current study, we utilized shear wave elastography, and recorded SWV of the patellar tendon at rest (hip 60° flexion and knee 70° flexion), with no active muscle force applied. Our study assumes that the measured SWV is related to the shear modulus of the tissue, meaning the stiffer the tissue, the faster the SWV (Hug et al. 2015). Shear wave elastography techniques have been validated in muscle tissue ex-vivo (Eby et al. 2013; Miyamoto et al. 2015). Given our findings of heterogenous increases in exercise induced stiffness between synergist quadriceps muscles, as well as heterogenous increases in patellar tendon stiffness during passive knee flexion of the knee (Coombes et al. 2018; Xu et al. 2016) it is plausible that eccentric exercise in different hip and knee positions might influence the distribution of changes in muscle stiffness between synergists. It is important to consider therefore that we are assessing the passive mechanical properties from a thin portion of the tendon which might represent a localised effect of the exercise. Our observed increased in stiffness of the patellar tendon may reflect increased passive tension of muscle fibres in-series with it. For instance, the tendon fibres of RF exclusively insert into the patellar and thus the patellar tendon (Andrikoula et al. 2006; Reider et al. 1981), whereas VM and VL also share their insertion into the patellar and medial and lateral retinaculum, respectively (Andrikoula et al. 2006).

Alternatively, changes within the tendon extracellular matrix, such as by altered cross-linking of collagen, may give rise to increase tendon stiffness (Kjaer 2003).

Consistent with our findings, recent work has identified an increase in muscle stiffness following eccentric exercise in the elbow flexors (Lacourpaille et al. 2014; Lacourpaille et al. 2017) and the knee extensor muscles (Lacourpaille et al. 2017), assessed using shear wave elastography. Lacourpaille et al. (2017) investigated changes in the elastic modulus of RF, VM, and VL with either a high or low volume of maximal eccentric contractions (150 and 75 repetitions, respectively), measured at three different knee angles (i.e. 30°, 90° and 110° of knee flexion). At 30 minutes post exercise there were no changes in stiffness between the high and low volume groups when measured at 30° of knee flexion (Lacourpaille et al. 2017). However at 90° the high load group showed significant increase in muscle elasticity and at 110° both groups showed a significant increase in stiffness (Lacourpaille et al. 2017). This suggests that changes in stiffness could be related to length of the muscle being examined. Based on these findings, it is possible that changes in VM and VL might occur if measured at longer muscle lengths.

The study by Lacourpaille et al. (2017) highlighted a significant correlation between the percentage decrease in MVIC and the percentage increase in elastic modulus of the knee extensor muscles post exercise. Although not a direct aim of our study, we conducted post-hoc correlation analysis of changes in SWV of the patellar tendon and RF with changes in MVIC (Figure 3) to compare with previous work (Lacourpaille et al. 2017). Our exploratory analysis revealed a significant correlation for RF ($r=-0.65$, $p=0.016$) and the patellar tendon ($r=-0.62$, $p=0.024$), which is in line with the findings from Lacourpaille et al. (2017). Although there is individual variability, this suggests that those with greater reduction in MVIC also have greater increase in patellar tendon and RF muscle stiffness.

Insert figure 3 near here.

Our finding of an increased stiffness in RF and the patellar tendon but not VM or VL suggest heterogeneous changes in stiffness between synergistic quadriceps muscles following a single bout of maximal eccentric exercise. Despite differences in protocols, our findings are supported by previous work showing a greater stiffness of the RF compared to the VM and VL following high and low volume eccentric contractions (150 and 75 repetitions, respectively) (Lacourpaille et al. 2017). Conflictingly, Green et al. (2012) demonstrated, using magnetic resonance elastography, an increase in soleus stiffness 1 hour following a bout of backward downhill walking and an increase in gastrocnemius stiffness at 48 hours post exercise. Although there was a delay in the increased stiffness of gastrocnemius compared to soleus, both muscles increase stiffness when measured using magnetic resonance elastography. Studies investigating the stiffness of the elbow flexors following a single bout of maximal eccentric exercises have shown homogenous changes (Lacourpaille et al. 2014) and heterogeneous changes (Lacourpaille et al. 2017) between biceps brachii and brachialis. This suggests EIMD may not always be homogeneously distributed between synergist muscles. It is tempting to speculate that several factors may contribute to heterogeneous distribution of mechanical properties within synergists muscle groups. First, despite being requested to undertake a maximum voluntary contraction, the contribution of muscle activation may have varied between synergist muscles during the eccentric task, with less muscle drive suggesting less potential for EIMD. However, during submaximal eccentric and concentric quadriceps loading (20 - 90% of maximal), RF shows a lower muscle activation compared to VM and VL, which are not different (Pincivero et al. 2008). Second, without direct measures of EIMD (e.g. muscle biopsy) it is not possible to identify the amount of muscle damage within each muscle. Although our study showed a significant increase in self-reported muscle soreness and stiffness at post₀ and post₄₈, and an increase of serum CK levels at 48 hours, not all studies have undertaken these measures. Third, differences in muscle fibre type (e.g. fast twitch, type II), muscle architecture (e.g. pennation angle) and relative muscle lengths (e.g. bi- versus mono-articular muscle) might also explain this heterogeneity, although future studies are required to investigate these possibilities.

4.1 Methodological considerations

There are several considerations regarding the shear wave elastography technique that must be taken into account when interpreting the results of this study. First, changes in tendon thickness can affect the interpretation of stiffness, due to the shear wave lengths being greater than the thickness of the tendon, resulting in guided wave propagation (Brum et al. 2014; Helfenstein-Didier et al. 2016). However, our study did not identify any change in the mean patellar tendon thickness, post₀ or post₄₈ following eccentric exercise, suggesting our findings represent a true change in tendon stiffness. Second, the lack of EMG recordings during the eccentric contractions is a limitation of the study. Recording of muscle activity could add further insight into the joint angle and muscle activation relationship. Third, muscle and tendon stiffness was measured at a single knee angle. Given that other studies have shown greater changes in muscle stiffness at greater muscle lengths this might provide a possible explanation for a lack of differences in VM and VL. However, it is important to note that the Aixplorer Ultrasound scanner has an upper limit when measuring the SWV of tendon under high tensioned. In addition, the restriction to one knee angle limits inferences that can be drawn in regards to changes in torque-angle relationships following eccentric exercise. To shed further insight into this, we recommend future studies perform measurement at several angles near to the optimal angle for force production and record torque values at each angle.

Methodological constraints may also have impacted upon the results of the study. First, our study did not include a gold standard approach to measurement of tendon stiffness or modulus derived from force elongation curves, which would allow direct comparison to SWV measures. Second, although shear wave elastography has been shown to be reliable for quadriceps muscle and patellar tendon in previous work (Coombes et al. 2018; Tas et al. 2017), our study did not assess reliability data. Third, the inclusion of passive preconditioning prior to each SWV measurement may have reduced the size of the effect in our study. Fourth, our study included a small sample size and future research with a greater number of individuals is required to confirm the differences between superficial quadriceps muscles. Fifth, although elastography examination was not blinded, off-line image processing was performed by an assessor blinded to the participant and to the test condition

(pre/post₀/post₄₈) in a randomised order. Finally, a standardised exercise protocol consisting of 5 sets of 20 repetitions was performed by all participants. This protocol induced significant changes in self-reported pain, CK, and MVIC, but with large inter-individual variability. This individual variability is highlighted in both SWV measures and the traditional indirect measures of muscle damage (e.g. change in MVIC, Figure 3). Our exploratory analysis of correlations in Figure 3 suggests that those with greater reduction in MVIC also have greater increase in patellar tendon and RF muscle stiffness. Based on this finding, it is recommended that future studies, either consider varying the amount of eccentric exercise performed in attempt to cause a given percentage change in MVIC (e.g., 20% impairment in MVIC), or employ a greater sample size in order to consider the effect of individual variability on the outcomes measures assessed here.

5 CONCLUSION

Utilising shear wave elastography, this study revealed an immediate and similar increase in the stiffness of the patellar tendon and RF, but not of VM and VL, following a single-bout of maximal eccentric exercise of the knee extensor muscles. Following maximal eccentric contractions performed between 20 and 70° of knee flexion, our findings highlight potential heterogeneity in the mechanical properties between synergistic knee extensor muscles, suggesting eccentric exercises do not affect synergistic muscles equally. Future research to validate these findings against other gold standard approaches and to evaluate the effects of eccentric exercise in different ranges and doses is warranted.

6 COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflict of interests, financial or otherwise. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

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522

Table Legend

Table 1. Shear wave velocity, patellar tendon thickness measures, and indicators of exercise induced muscle damage obtained before (Pre), immediately post (Post₀) and 48 hours (Post₄₈) following a single bout of eccentric exercise of the knee extensor muscles.

Outcome	Pre	Post ₀	Post ₄₈
SWV Vastus medialis (m/s)	2.22 [2.15, 2.42]	2.48 [2.27, 2.80]	2.24 [2.22, 2.35]
SWV Rectus femoris (m/s)	2.02 [1.94, 2.32]	2.58 [2.20, 2.99]	2.14 [1.96, 2.46]
SWV Vastus lateralis (m/s)	2.74 [2.54, 2.90]	2.68 [2.62, 2.93]	2.75 [2.61, 2.85]
SWV Patellar tendon (m/s)	9.61 (2.10)	10.84 (2.03)	9.75 (1.36)
Tendon Thickness (cm)	0.33 (0.06)	0.33 (0.05)	0.33 (0.06)
MVIC Torque (Nm)	158 (31)	126 (32)	134 (32)
CK (units/L)	93 [75, 136]	NA	178 [106, 424]
Self-reported Pain (0-100 NRS)	0 [0, 0]	3.5 [1.0, 10]	10 [7.5, 20]
Self-reported Stiffness (0-100 NRS)	0 [0, 10]	15 [5, 25]	10 [10, 20]

Data are presented as mean (SD) or median [IQR]. SWV – shear wave velocity, MVIC – maximum voluntary isometric contraction, Pre – pre exercise measures, Post₀ – measures taken immediately after eccentric exercise, Post₄₈ – measures taken 48 hours after eccentric exercise. CK – Creatine Kinase, NRS – numerical rating scale.

Figure legend

Fig 1 A schematic diagram of the testing protocol.

Fig 2 Shear wave velocity measures for one participant shown pre and immediately post exercise for the vastus medialis (yellow), rectus femoris (red), vastus lateralis (green), and the patellar tendon (blue). The scales on the left hand side represent the SWV in m/s. Different scales are used for muscle and tendon mechanical properties due to different settings within the machine. The position of ultrasound head is indicated in the image of the lower limb on the right of the figure

Fig 3 Pearson's correlation for the percentage decrease in maximum voluntary isometric contraction (MVIC) and the percentage increase in shear wave velocity (SWV) for the patellar tendon and the rectus femoris muscle. A significant negative correlation was observed for the patellar tendon ($r = -0.65$, $p=0.016$) and rectus femoris muscle ($r = -0.62$, $p=0.024$). For both RF and tendon, the correlation was greater for participants who had more than 20% reduction