



HAL
open science

Shear-wave velocity of the patellar tendon and quadriceps muscle is increased immediately after maximal eccentric exercise

Luke Heales, Rohitha Badya, Brandon Ziegenfuss, François Hug, Jeff Coombes, Wolbert van den Hoorn, Kylie Tucker, Brooke Coombes

► To cite this version:

Luke Heales, Rohitha Badya, Brandon Ziegenfuss, François Hug, Jeff Coombes, et al.. Shear-wave velocity of the patellar tendon and quadriceps muscle is increased immediately after maximal eccentric exercise. *European Journal of Applied Physiology*, 2018, 118 (8), pp.1715-1724. 10.1007/s00421-018-3903-2 . hal-03335182

HAL Id: hal-03335182

<https://hal.science/hal-03335182>

Submitted on 25 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 SHEAR WAVE VELOCITY OF THE PATELLAR TENDON AND QUADRICEPS MUSCLE ARE INCREASED
2 IMMEDIATELY AFTER MAXIMAL ECCENTRIC EXERCISE
3

4 Luke J. Heales^{a,d}, Rohitha Badya^a, Brandon Ziegenfuss^a, François Hug^{c,e,f}, Jeff S. Coombes^b, Wolbert
5 van den Hoorn^c, Kylie Tucker^a, Brooke K. Coombes^a
6

7 a) The University of Queensland, School of Biomedical Sciences, Brisbane 4072, Australia

8 b) The University of Queensland, School of Human Movement and Nutrition Sciences, Brisbane
9 4072, Australia

10 c) The University of Queensland, NHMRC Centre of Clinical Research Excellence in Spinal Pain,
11 Injury and Health, School of Health and Rehabilitation Sciences, Brisbane 4072, Australia

12 d) Central Queensland University, School of Health, Medical and Applied Sciences, Department of
13 Exercise and Health Sciences, Rockhampton 4701, Australia

14 e) Laboratory "Movement, Interactions, Performance" (EA 4334), University of Nantes, Faculty of
15 Sport Science, Nantes 44000, France

16 f) Institut Universitaire de France (IUF), Paris, France.
17
18

19 **Corresponding Author:**

20 Dr. Brooke Coombes

21 School of Biomedical Sciences

22 University of Queensland

23 St Lucia, Brisbane, QLD 4072

24 Email: b.coombes@uq.edu.au
25

26 **ACKNOWLEDGEMENTS:**

27 This study was funded by a University of Queensland Postdoctoral Fellowship for Women (BKC) and
28 a Sports Medicine Australia Research Foundation Grant (LJH, BKC, FH, KT).
29

30 **ABSTRACT**

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

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

42 *Results:* Compared to pre-exercise, MVIC decreased and self-reported pain and stiffness increased
43 at post₀ and post₄₈ and CK levels increased at post₄₈ (all p<0.01).
44 Compared to pre-exercise, shear wave velocity was greater at post₀ for the patellar tendon (15.9
45 (24.6)%, p=0.01) and RF (23.6 (16.7)%, p<0.001), each returning to baseline by post₄₈. No significant
46 differences were observed for VL or VM post₀ or post₄₈ exercise.

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

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

51 **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

52 VL
VM

Vastus lateralis
Vastus medialis

53

54 1 INTRODUCTION

55 The response of skeletal muscle to unaccustomed eccentric exercise has been studied widely,
56 yet it remains incompletely understood. Unaccustomed eccentric contractions typically result in
57 exercise induced muscle damage (EIMD), characterised by muscle soreness, that peaks after 48
58 hours (Guilhem et al. 2016; Lacourpaille et al. 2014), reduced strength and range of motion (Noska
59 and Clarkson 1996) and increased passive joint stiffness (Porter et al. 2002; Whitehead et al.
60 2001). Traditionally, passive joint stiffness is measured by determining the overall passive torque in
61 relation to joint range of motion using an isokinetic dynamometer (Porter et al. 2002; Whitehead et
62 al. 2001). When using overall passive joint stiffness it has not been possible to isolate the mechanical
63 behaviour of structures either in series (i.e. muscle versus tendon) or structures in parallel (i.e.
64 synergistic muscles). Investigation of how eccentric exercise affects the mechanical behaviour of
65 individual synergistic muscles and tendon tissue crossing the knee joint could help improve the
66 understanding of musculotendinous adaptation to exercise.

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

81 patellar tendon is shorter and stiffer, making it better suited to force transmission (Malliaras et al.
82 2015). Previous work suggests that the Achilles tendon acts as a shock-absorber that dissipates
83 energy and reduces muscle fibre elongation during eccentric contractions (Guilhem et al. 2010) and
84 hence potentially the amount of EIMD. Given the morphological differences between the Achilles
85 and patellar tendons, an improved understanding of the effect of maximal eccentric exercise on the
86 shear wave velocity of the patellar tendon and the muscles in series is warranted.

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

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

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

109

110 **2 METHODS**

111 **2.1 Participants**

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

119 **2.2 Protocol**

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

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

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

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

137 **2.3 Outcome measures**

138 *2.3.1 Ultrasound and elastography measurement*

139 Shear-wave velocity was recorded using an Aixplorer® Ultrasound Scanner (Version 8.2;
140 Supersonic Imagine, Aix-en-Provence, France) with a linear array transducer (4-15 MHz; SuperLinear
141 15-4, Vermon, Tours, France) by a musculoskeletal physiotherapist with 18 months training using
142 sonoelastography. Methodology for elastography data acquisition and extraction are reported in
143 detail elsewhere (Coombes et al. 2018) and summarised below. Acceptable within-day reliability for
144 measurement of SWV of the patellar tendon and quadriceps muscles were previously determined
145 (Intraclass correlation coefficients 0.88 to 0.92; coefficient of variation 3.2 to 6.4%) (Coombes et al.
146 2018). The hip remained in 60° flexion (0°= supine), with the back supported and knee passively
147 positioned at 70° flexion (0°=full extension) for all measurements. This angle was selected as it was
148 considered above the slack length for the VL, VM and RF (i.e. range 45°- 51° (Xu et al. 2016)) and
149 below the maximum SWV measures when investigating tendon tissue. B-Mode ultrasound was used
150 to identify and align the probe longitudinally to the fascicles of the patellar tendon, VM, VL and one
151 compartment of the bi-pennate RF. Approximate positions for imaging were: 33.3% of thigh length
152 for VM, 50-75% thigh length for RF and 33-50% of thigh length for VL (Coombes et al. 2018).. To
153 facilitate consistency between measurements, these locations were marked on the skin using
154 permanent marker. B-mode ultrasound was used to align the probe as carefully as possible with
155 muscle fascicles, however it is more difficult to accurately align the ultrasound probe with fascicle
156 direction in complex muscles such as the rectus femoris. It is important to note that minor changes
157 in the angle of the transducer relative to the fascicles shows negligible difference in shear wave
158 elastography measures (Miyamoto et al. 2015).

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

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

180 2.3.2 Maximal Voluntary Isometric Contraction

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

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

195 2.3.3 *Self-reported pain and stiffness*

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

201 2.3.4 *Creatine Kinase*

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

209 **2.4 Eccentric exercise protocol**

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

220 **2.5 Statistical analysis**

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

233

234 **3 RESULTS**

235 **3.1 Participants**

236 Fifteen participants, aged 19 to 30 years were screened. Two individuals were excluded due
237 to pain within the lower limb. Thirteen individuals were included (7 females; group mean (SD) age
238 22.4 (3.5) years; height 168.4 (8.1) cm; body mass 65.1 (13.0) kg). Based on the Active Australia
239 Survey, (Australian Institute of Health and Welfare 2003) participants self-reported performing a
240 median [interquartile range - IQR] total activity of 300 [150, 360] minutes of physical activity within
241 the preceding week.

242

243 **3.2 Indicators of exercise induced muscle damage**

244 A significant effect of Time was found for MVIC ($\text{Chi}^2=28.18$, $p<0.001$), self-reported pain
245 ($\text{Chi}^2=15.82$, $p<0.001$) and stiffness ($\text{Chi}^2=17.24$, $p<0.001$). Compared to pre exercise, MVIC was
246 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]
247 at post_{48} ($p<0.001$). Self-reported pain and stiffness were greater at post_0 ($p=0.028$ and $p=0.001$,
248 respectively) and at post_{48} ($p<0.001$ and $p=0.001$, respectively) compared to pre exercise. Serum CK
249 was greater at post_{48} compared to pre exercise levels ($\text{Chi}^2=13.72$, $p<0.001$). All data are presented
250 in Table 1.

251

252 **3.3 Patellar tendon stiffness and thickness**

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

258

259 3.4 Effects of exercise on muscle stiffness

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

267

268 4 DISCUSSION

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

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

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

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

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

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

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

336

337 Insert figure 3 near here.

338

339 Our finding of an increased stiffness in RF and the patellar tendon but not VM or VL suggest
340 heterogeneous changes in stiffness between synergistic quadriceps muscles following a single bout
341 of maximal eccentric exercise. Despite differences in protocols, our findings are supported by
342 previous work showing a greater stiffness of the RF compared to the VM and VL following high and
343 low volume eccentric contractions (150 and 75 repetitions, respectively) (Lacourpaille et al. 2017).
344 Conflictingly, Green et al. (2012) demonstrated, using magnetic resonance elastography, an increase
345 in soleus stiffness 1 hour following a bout of backward downhill walking and an increase in
346 gastrocnemius stiffness at 48 hours post exercise. Although there was a delay in the increased
347 stiffness of gastrocnemius compared to soleus, both muscles increase stiffness when measured
348 using magnetic resonance elastography. Studies investigating the stiffness of the elbow flexors
349 following a single bout of maximal eccentric exercises have shown homogenous changes
350 (Lacourpaille et al. 2014) and heterogeneous changes (Lacourpaille et al. 2017) between biceps
351 brachii and brachialis. This suggests EIMD may not always be homogeneously distributed between
352 synergist muscles. It is tempting to speculate that several factors may contribute to heterogeneous
353 distribution of mechanical properties within synergists muscle groups. First, despite being requested
354 to undertake a maximum voluntary contraction, the contribution of muscle activation may have
355 varied between synergist muscles during the eccentric task, with less muscle drive suggesting less
356 potential for EIMD. However, during submaximal eccentric and concentric quadriceps loading (20 -
357 90% of maximal), RF shows a lower muscle activation compared to VM and VL, which are not
358 different (Pincivero et al. 2008). Second, without direct measures of EIMD (e.g. muscle biopsy) it is
359 not possible to identify the amount of muscle damage within each muscle. Although our study
360 showed a significant increase in self-reported muscle soreness and stiffness at post₀ and post₄₈, and
361 an increase of serum CK levels at 48 hours, not all studies have undertaken these measures. Third,
362 differences in muscle fibre type (e.g. fast twitch, type II), muscle architecture (e.g. pennation angle)
363 and relative muscle lengths (e.g. bi- versus mono-articular muscle) might also explain this
364 heterogeneity, although future studies are required to investigate these possibilities.

365 4.1 Methodological considerations

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

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

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

402

403 **5 CONCLUSION**

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

412

413 **6 COMPLIANCE WITH ETHICAL STANDARDS**

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

418

419 **7 REFERENCES**

- 420 Andrikoula S, Tokis A, Vasiliadis HS, Georgoulis A (2006) The extensor mechanism of the knee joint: An
421 anatomical study. *Knee Surgery, Sports Traumatology, Arthroscopy* 14:214-220 doi:10.1007/s00167-
422 005-0680-3
- 423 Australian Institute of Health and Welfare (2003) *The Active Australia Survey: a guide and manual for*
424 *implementation, analysis and reporting.* Canberra: AIHW
- 425 Brum J, Bernal M, Gennisson JL, Tanter M (2014) In vivo evaluation of the elastic anisotropy of the human
426 Achilles tendon using shear wave dispersion analysis. *Physics in Medicine and Biology* 59:505-523
427 doi:10.1088/0031-9155/59/3/505
- 428 Coombes BK, Ziegenfuss B, David M, Badya R, Van Den Hoorn W, Hug F, Tucker K (2018) Heterogeneity of
429 passive elastic properties within the quadriceps femoris muscle-tendon unit. *European Journal of*
430 *Applied Physiology* 118:213-221
- 431 Dubois G et al. (2015) Reliable protocol for shear wave elastography of lower limb muscles at rest and during
432 passive stretching. *Ultrasound in Medicine and Biology* 41:2284-2291
433 doi:10.1016/j.ultrasmedbio.2015.04.020
- 434 Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An KN (2013) Validation of shear wave elastography in skeletal
435 muscle. *Journal of Biomechanics* 46:2381-2387 doi:https://doi.org/10.1016/j.jbiomech.2013.07.033
- 436 Green MA, Sinkus R, Gandevia SC, Herbert RD, Bilston LE (2012) Measuring changes in muscle stiffness after
437 eccentric exercise using elastography. *NMR in biomedicine* 25:852-858 doi:10.1002/nbm.1801
- 438 Guilhem G, Cornu C, Guebel A (2010) Neuromuscular and muscle-tendon system adaptations to isotonic and
439 isokinetic eccentric exercise. *Annals of Physical and Rehabilitation Medicine* 53:319-341
- 440 Guilhem G, Doguet V, Hauraix H, Lacourpaille L, Jubeau M, Nordez A, Dorel S (2016) Muscle force loss and
441 soreness subsequent to maximal eccentric contractions depend on the amount of fascicle strain in
442 vivo. *Acta Physiologica* 217:152-163 doi:10.1111/apha.12654
- 443 Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP (2005) Region-specific mechanical
444 properties of the human patella tendon. *Journal of Applied Physiology* 98:1006-1012
- 445 Helfenstein-Didier C, Andrade RJ, Brum J, Hug F, Tanter M, Nordez A, Gennisson JL (2016) In vivo quantification
446 of the shear modulus of the human Achilles tendon during passive loading using shear wave
447 dispersion analysis. *Physics in Medicine and Biology* 61:2485-2496 doi:10.1088/0031-9155/61/6/2485

448 Hjerstad MJ et al. (2011) Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual
449 Analogue Scales for assessment of pain intensity in adults: a systematic literature review. *Journal of*
450 *Pain and Symptom Management* 41:1073-1093 doi:10.1016/j.jpainsymman.2010.08.016

451 Hug F (2016) Advancing musculoskeletal rehabilitation using elastography. *Aspetar Sports Medicine Journal*
452 5:166-171

453 Hug F, Tucker K, Gennisson JL, Tanter M, Nordez A (2015) Elastography for muscle biomechanics: Towards the
454 estimation of individual muscle force. *Exercise and Sport Sciences Reviews* 43:125-133

455 Hyldahl RD, Chen TC, Nosaka K (2017) Mechanisms and mediators of the skeletal muscle repeated bout effect.
456 *Exercise and Sport Sciences Reviews* 45:24-33 doi:10.1249/JES.0000000000000095

457 Hyldahl RD, Hubal MJ (2014) Lengthening our perspective: Morphological, cellular, and molecular responses to
458 eccentric exercise. *Muscle & Nerve* 49:155-170 doi:10.1002/mus.24077

459 Hyldahl RD et al. (2015) Extracellular matrix remodeling and its contribution to protective adaptation following
460 lengthening contractions in human muscle. *The FASEB Journal* 29:2894-2904 doi:10.1096/fj.14-
461 266668

462 Joseph MF, Lillie KR, Bergeron DJ, Cota KC, Yoon JS, Kraemer WJ, Denegar CR (2014) Achilles tendon
463 biomechanics in response to acute intense exercise. *Journal of Strength & Conditioning Research*
464 28:1181-1186

465 Kjaer M (2003) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading.
466 *Physiological Reviews* 84:649-698

467 Kjaer M (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading.
468 *Physiological Reviews* 84:649-698 doi:10.1152/physrev.00031.2003

469 Lacourpaille L, Hug F, Bouillard K, Hogrel JY, Nordez A (2012) Supersonic shear imaging provides a reliable
470 measurement of resting muscle shear elastic modulus. *Physiological measurement* 33:N19-28
471 doi:10.1088/0967-3334/33/3/n19

472 Lacourpaille L, Nordez A, Hug F, Couturier A, Dibie C, Guihem G (2014) Time-course effect of exercise-induced
473 muscle damage on localized muscle mechanical properties assessed using elastography. *Acta*
474 *Physiologica* 211:135-146

475 Lacourpaille L, Nordez A, Hug F, Doguet V, Andrade R, Guilhem G (2017) Early detection of exercise-induced
476 muscle damage using elastography. *European Journal of Applied Physiology* 117:2047-2056
477 doi:10.1007/s00421-017-3695-9

478 Lichtwark GA, Wilson AM (2007) Is Achilles tendon compliance optimised for maximum muscle efficiency
479 during locomotion? *Journal of Biomechanics* 40:1768-1765

480 Mackey AL, Donnelly AE, Turpeenniemi-Hujanen T, Roper HP (2004) Skeletal muscle collagen content in
481 humans after high-force eccentric contractions. *Journal of Applied Physiology* 97:197-203
482 doi:10.1152/jappphysiol.01174.2003

483 Malliaras P, Cook J, Purdam C, Rio E (2015) Patellar tendinopathy: Clinical diagnosis, load management, and
484 advice for challenging case presentations. *Journal of Orthopaedic and Sports Physical Therapy* 45:887-
485 898 doi:10.2519/jospt.2015.5987

486 Miyamoto N, Hirata K, Kanehisa H, Yoshitake Y (2015) Validity of measurement of shear modulus by ultrasound
487 shear wave elastography in human pennate muscle. *PloS ONE* 10:e0124311

488 Noska K, Clarkson PM (1996) Changes in indicators of inflammation after eccentric exercise of the elbow
489 flexors. *Medicine and Science in Sports and Exercise* 28:953-961

490 Obst SJ, Barrett RS, Newsham-West R (2013) Immediate effect of exercise on Achilles tendon properties:
491 Systematic review. *Medicine and Science in Sports and Exercise* 45:1534-1544
492 doi:10.1249/MSS.0b013e318289d821

493 Obst SJ, Newsham-West R, Barrett RS (2016) Changes in Achilles tendon mechanical properties following
494 eccentric heel drop exercise are specific to the free tendon. *Scandinavian Journal of Medicine &
495 Science in Sports* 26:421-431

496 Paschalis V, Nikolaidis MG, Giakas G, Jamurtas AZ, Pappas A, Koutedakis Y (2007) The effect of eccentric
497 exercise on position sense and joint reaction angle of the lower limbs. *Muscle & Nerve* 35:496-503
498 doi:10.1002/mus.20723

499 Paschalis V, Nikolaidis MG, Theodorou AA, Giakas G, Jamurtas AZ, Koutedakis Y (2010) Eccentric exercise
500 affects the upper limbs more than the lower limbs in position sense and reaction angle. *Journal of
501 Sports Sciences* 28:33-43 doi:10.1080/02640410903334764

502 Pincivero DM, Coelho AJ, Campy RM (2008) Contraction mode shift in quadriceps femoris muscle activation
503 during dynamic knee extensor exercise with increasing loads. *Journal of Biomechanics* 41:3127-3132
504 doi:10.1016/j.jbiomech.2008.09.003

505 Porter MM, Andersson M, Hellström U, Miller M (2002) Passive resistive torque of the plantar flexors following
506 eccentric loading as assessed by isokinetic dynamometry. *Canadian Journal of Applied Physiology*
507 27:612-616

508 Reider B, Marshall JL, Koslin B, Ring B, Girgis FG (1981) The anterior aspect of the knee joint. An anatomical
509 study. *Journal of Bone and Joint Surgery - Series A* 63:351-356

510 Tas S, Onur MR, Yilmaz S, Soylu AR, Korkusuz F (2017) Shear Wave elastography is a reliable and repeatable
511 method for measuring the elastic modulus of the rectus femoris muscle and patellar tendon. *Journal*
512 *of Ultrasound in Medicine* 36:565-570 doi:10.7863/ultra.16.03032

513 Whitehead NP, Weerakkody NS, Gregory JE, Morgan DL, Proske U (2001) Changes in passive tension of muscle
514 in humans and animals after eccentric exercise. *Journal of Physiology* 533:593-604
515 doi:10.1111/j.1469-7793.2001.0593a.x

516 Xu J, Hug F, Fu SN (2016) Stiffness of individual quadriceps muscle assessed using ultrasound shear wave
517 elastography during passive stretching. *Journal of Sport and Health Science Article in Press*
518 doi:<http://dx.doi.org/10.1016/j.jshs.2016.07.001>

519 Yin NH, Chen WS, Wu YT, Shih TT, Rolf C, Wang HK (2014) Increased patellar tendon microcirculation and
520 reduction of tendon stiffness following knee extension eccentric exercises. *Journal of Orthopaedic*
521 *and Sports Physical Therapy* 44:304-312 doi:10.2519/jospt.2014.4872

522

523 **Table Legend**

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

Outcome	Pre	Post₀	Post₄₈
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]

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

531

532

533 **Figure legend**

534 **Fig 1** A schematic diagram of the testing protocol.

535

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

541

542

543

544 **Fig 3** Pearson's correlation for the percentage decrease in maximum voluntary isometric contraction
545 (MVIC) and the percentage increase in shear wave velocity (SWV) for the patellar tendon and the
546 rectus femoris muscle. A significant negative correlation was observed for the patellar tendon ($r = -$
547 0.65 , $p=0.016$) and rectus femoris muscle ($r = -0.62$, $p=0.024$). For both RF and tendon, the
548 correlation was greater for participants who had more than 20% reduction