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1 **HETEROGENEITY OF PASSIVE ELASTIC PROPERTIES WITHIN THE**
2 **QUADRICEPS FEMORIS MUSCLE-TENDON UNIT**

3
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24 **Key words:** elasticity; elastography; stress-shielding; tendinopathy; patellar; knee **Abbreviations:**
25 electromyography (EMG), rectus femoris (RF), shear wave velocity (SWV), vastus lateralis (VL), vastus
26 medialis (VM)

27 **Running Title:** QUADRICEPS MUSCLE-TENDON ELASTIC PROPERTIES

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34

35 **ABSTRACT**

36

37 **Purpose** The purpose of this study was to compare regional elastic properties between anterior and posterior

38 regions of the patellar tendon, and individual quadriceps muscles, over a range of knee flexion angles.

39 **Methods** An isokinetic dynamometer passively positioned the non-dominant knee of 19 young, healthy

40 participants, at 25°, 40°, 55°, 70° and 85° flexion. Shear wave velocity (SWV, an index of tissue elasticity) was

41 measured using ultrasound shear wave elastography in a relaxed (passive) state, confirmed by

42 electromyography.

43 **Results** SWV of the patellar tendon and quadriceps muscles increased with knee flexion (longer muscle-tendon

44 unit; $P < 0.001$). Within the proximal third of the patellar tendon, SWV was lower in the posterior than anterior

45 region at 70° ($P = 0.002$) and 85° ($P < 0.001$), but not at 25°, 40° or 55° (region-by-angle interaction, $P = 0.007$).

46 No differences were found between anterior and posterior regions within the middle third of the patellar tendon

47 ($P = 0.332$). For the quadriceps muscles, a significant muscle-by-angle ($P < 0.001$) interaction was also observed.

48 SWV of VL was greater than VM at 55° ($P = 0.005$), 70° (0.17 [$P = 0.001$]) and 85° ($P < 0.001$), but not at 25° or

49 40°. RF SWV was lower than VL at all angles (all $P < 0.002$) and lower than VM at 55°, 70° and 85° (all

50 $P < 0.002$).

51 **Conclusions** Passive knee flexion at and beyond 70° was associated with non-uniform elastic properties within

52 the proximal patellar tendon and between individual quadriceps muscles. To what extent this heterogeneity of

53 passive elastic properties contributes to injury remains unknown.

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57 **INTRODUCTION**

58

59 *In vivo* biomechanical properties of muscle and tendon have been classically inferred from inverse dynamics or
60 measurements of joint torque using dynamometers and ultrasound measurement of tissue displacement (Hug et
61 al. 2015). However, these measures provide information about the combined behaviour of several structures
62 acting around a given joint and cannot isolate the behaviour of an individual muscle or isolated region within a
63 tendon. There is growing evidence that differential passive strain may occur within tendons, such as the patellar
64 (Almekinders et al. 2002), Achilles (Lyman et al. 2004) and supraspinatus tendons (Bey et al. 2002), which has
65 been proposed to contribute to the development localised tendinopathy (Pearson et al. 2014). Likewise,
66 heterogenous passive behaviour of synergist muscles, such as within the hamstrings muscles (Le Sant et al.
67 2017) might reflect the prevalence of localised muscle strain.

68

69 With the advent of innovative shear wave elastography techniques, it is now possible to estimate the mechanical
70 properties of regional areas of soft tissues, including muscle (Koo et al. 2013) and tendon (Haen et al. 2015).

71 The shear wave elastography technique uses acoustic radiation force to generate shear waves within tissue.

72 Ultra-fast ultrasound imaging is used to measure the shear wave velocity (SWV) within tissues, which is

73 positively related to tissue shear modulus, i.e. an index of stiffness (Bercoff et al. 2004). Measurement of

74 muscle shear modulus (using elastography) has been shown to be strongly linearly related to both passive and

75 active muscle force (Hug et al. 2015). Recently, shear wave elastography was used to examine shear modulus of

76 the gastrocnemius muscle and Achilles tendon (Hug et al. 2013) and of individual quadriceps muscles (Xu et al.

77 2016) during passive ankle or knee movements respectively. Concurrent investigation of the quadriceps

78 muscles and regions within the patellar tendon has not been undertaken.

79

80 Anatomical studies (Basso et al. 2001, Andrikoula et al. 2006, Hansen et al. 2006) describe the patellar tendon

81 as comprising superficial (anterior) fascicles, which primarily extend from the rectus femoris muscle and pass

82 over the anterior surface of the patellar; and deep (posterior) fascicles, some of which attach to the posterior

83 surface of the patellar's inferior pole. Anterior fibres are longer than their posterior counterparts and both

84 regions merge as they approach the tibial tuberosity (Basso et al. 2001). The ability to accurately quantify the

85 mechanical properties of isolated regions of the musculotendinous unit will provide a more integrated

86 understanding of passive muscle-tendon biomechanics. This information is important for both basic and clinical

87 sciences and may advance our understanding of localised musculotendinous injury.

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The objectives of this study were to 1) determine the reliability of localised shear wave velocity (SWV) measurement (within day and within rater); 2) compare SWV between anterior and posterior regions of the patellar tendon, and between the superficial quadriceps muscles (vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF)); and 3) correlate SWV values between muscle and tendon regions; over a range of knee flexion angles (25°, 40°, 55°, 70° and 85°). We hypothesised that knee angle would affect the regional SWV within the proximal patellar tendon and individual quadriceps muscles.

MATERIALS AND METHODS

Participants

Twenty one healthy participants who were free of musculoskeletal injury in the back or lower limb in the preceding six months were recruited from The University of Queensland student community by flyers and word of mouth. Participants were asked to refrain from exercising on the day of testing. This study was approved by The University of Queensland's Human Research Ethics Committee and all participants provided written informed consent. Two participants were unable to fully relax their quadriceps muscles, as confirmed by surface electromyography (EMG) recordings, and were excluded shortly after commencing testing. Nineteen participants, aged 19 to 24 years (10 males, 9 females, age: 21.8±1.4 years; height: 171.5±9.4cm and weight: 66.1±10.3kg), completed the testing.

Experimental equipment

For the duration of testing, participants were seated in an isokinetic dynamometer (Kin Com, model 125AP, Chattanooga, USA), with the backrest declined by 30° from upright. All testing was undertaken on the non-dominant leg (dominant leg defined as the leg they would use to kick a ball). The dynamometer axis was aligned with the lateral epicondyle of the test leg (Maffiuletti et al. 2007) and the tibial strap was fixed at a distance of 20cm from this location. Chest straps were applied to limit trunk movement and foam wedges placed under the lateral thigh used where necessary to maintain neutral hip rotation. Using a manual goniometer, a reference point of 0° knee extension was calibrated on the isokinetic dynamometer, and the test-angles of 25°, 40°, 55°, 70° and 85° of knee flexion were determined.

117 Ultrasound and elastography examination was conducted using an Aixplorer Ultrasound Scanner (version 9.3;
118 Supersonic Imagine, Aix-en-Provence, France) with a linear transducer (4-15MHz, Vermon, Tours, France) by a
119 single examiner with 18 months of elastography imaging experience. Standard musculoskeletal muscle and
120 tendon presets were used. The upper limit (16.3m/s) of the system was adopted for measurement of tendon
121 SWV. Images were captured at 1.8 images/s for muscle and 1.6 images/s for tendon.

122

123 With the knee positioned at 70° knee flexion, B-mode ultrasound was used to identify and align the probe
124 longitudinally to the fascicles of the patellar tendon, VM, VL and one compartment of the bipennate RF. Note
125 that, of the muscles tested, the alignment with muscle fascicles is most difficult for the RF where a more
126 complex muscle architecture can be observed within the B-mode image (Ema et al. 2013). For RF, the angle of
127 the probe was tilted relative to the skin, to align with the three dimensional fascicle arrangement (Ema et al.
128 2013). Approximate positions of ultrasound and elastography imaging measurements were: 33.3% of thigh
129 length for VM, 50-75% thigh length for RF and 33-50% thigh length for VL. The probe locations were drawn
130 on the participant's skin with a waterproof marker to facilitate consistency in the repeated measurements. EMG
131 surface electrodes (Trigno, Delsys, UK) were placed over each of the tested muscles, within 2cm proximal of
132 the probe locations. Before electrode placement, skin was prepared with abrasive gel (NuPrep, Weaver &
133 Company) and cleaned with alcohol. EMG recordings were used to provide real-time feedback to confirm that
134 participants quadriceps muscles were relaxed throughout the experiment. Elastography images were discarded
135 and the trial repeated if there was any evidence of concurrent EMG activity for any of the muscles, and as such
136 EMG data is not discussed further here-in.

137

138 **Procedure**

139 As loading history affects tendon and muscle properties (Haraldsson et al. 2005), standardised preconditioning
140 consisting of 10 passive cycles (0-85° knee flexion, 30°/s) was performed prior to each (of two) sets of
141 measurements. Then the knee was passively moved to the following angles 25°, 40°, 55°, 70° and 85° (in this
142 order), where two ten second elastography videos were recorded over VM, followed by RF, VL and then the
143 patellar tendon. The order of testing was not randomised. Care was taken to minimise probe pressure during
144 recording periods. Each set of measures took 12-15 minutes to complete. The average SWV measure, calculated
145 from sets one and two, was used for further analysis.

146

147 In order to examine the within-day / within rater reliability of each measure, six participants were asked to
148 return to the laboratory 60-90 minutes after completion of the first trial, where the entire protocol was repeated.
149 Participants were free to move around the University grounds, but were asked to avoid strenuous activity for the
150 period between testing sessions. The location for recording ultrasound images (i.e. the probe locations) was
151 marked on the skin, and the dynamometer position was recorded to facilitate set-up consistency between trials.

152

153 **Data Extraction**

154 Elastography videos were converted into images and customised MATLAB (R2016a, Mathworks Inc., Natick,
155 MA, USA) scripts were used to extract the mean SWV in m/s for each series of images. The examiner was
156 blinded for knee angle and participant by randomisation of the order of image processing. Regions of interest
157 were manually determined using the B-mode image as visual guide (Fig. 1). The proximal one-third of the
158 patellar tendon, originating at the distal edge of the patella's inferior pole, was evenly divided into anterior and
159 posterior regions. Similarly, the middle third of the patellar tendon was evenly divided into anterior and
160 posterior regions. For the quadriceps muscles, a representative area within the elastogram, that avoided
161 overlying fascia or areas void of colour was selected (Fig. 2). Mean±SD ROI areas (cm²) were: 0.22±0.06 and
162 0.20±0.05 for the anterior and posterior proximal patellar tendon; 0.24±0.05 and 0.24±0.05 for anterior and
163 posterior middle patellar tendon; and 1.18±0.22; 1.04±0.29 and 1.33±0.27 for VM, RF and VL respectively.

164

165 **Statistical analysis**

166 Intraclass coefficients (ICC) and 95% CI were calculated for anterior and posterior regions within the proximal
167 patellar tendon and each muscle using linear mixed effects regression models, with angle as a fixed factor.
168 Standard error of measurement (SEM) and coefficient of variation (CV) (typical error expressed as a percent of
169 the participant's mean score) were also calculated as measures of within-day reliability (Hopkins 2000).

170

171 Linear mixed-effects regression models were used to examine the effect of region (anterior and posterior
172 tendon) and angle (25°, 40°, 55°, 70° and 85°) on SWV measured within the proximal and middle thirds of the
173 patellar tendon. Region and angle were entered as fixed factors and individuals and angle as random intercept
174 and random slope, respectively. Differences in SWV between individual quadriceps muscles was also examined,
175 substituting muscle (VM, RF and VL) for region in the model. Different within subject covariance models were
176 considered, and Akaike information criteria and Bayesian information criteria were used to select the best fitting

177 model. An unstructured covariance pattern was selected for tendon and an independent covariance pattern was
178 selected for muscle. Model residual plots were checked to ensure the assumptions of the model were satisfied.
179 Post hoc pairwise comparisons were performed using Bonferroni-adjusted *P*-values.

180

181 Analyses were performed using Stata 13.1 (StataCorp, USA) and two-tailed tests were considered significant if
182 $P < 0.05$. Data are presented as mean \pm SD or mean difference (MD) and [95% CI] as appropriate throughout the
183 results section. Correlations between muscle and tendon regions SWV were visualised graphically and Pearson
184 correlation coefficients are presented.

185

186 RESULTS

187 The within-day ICC and CV for measurement of SWV for tendon and each muscle ranged from 0.88 to 0.95 and
188 3.2% to 6.4% respectively (Table 1).

189

190 A significant effect of angle ($P < 0.001$), region ($P < 0.001$), and region-by-angle interaction ($P = 0.007$) was
191 observed for SWV within the proximal third of the patellar tendon (Table 2; Fig. 3). There were no differences
192 in SWV between anterior and posterior parts of the proximal patellar tendon for more extended angles (25°, 40°
193 and 55°). In contrast, significantly lower SWV was detected for the posterior proximal region than anterior
194 proximal region at 70° and 85° (all $P \leq 0.002$). For the middle third of the patellar tendon, a significant effect of
195 angle ($P < 0.001$), but no region or region-by-angle interaction was found. All mean differences between tendon
196 regions and their 95% CI data are reported in Table 2.

197

198 For SWV within the quadriceps femoris muscle group, a significant effect of angle, muscle, and angle-by-
199 muscle interaction (all $P < 0.001$) was observed (Table 2, Fig 4). No differences in SWV were found between 25°
200 and 40° for any muscle ($P > 0.18$), or between 40° and 55° for RF. SWV increased after 40° in the monoarticular
201 muscles (VM and VL) and after 55° for the biarticular RF ($P < 0.04$). No differences were detected between VL
202 and VM at 25° or 40° ($P > 0.784$), however VL was greater than VM at higher knee flexion angles (55° to 85°,
203 all $P < 0.005$). RF was significantly lower than VL at all knee angles and lower than VM at 55°, 70° and 85° (all
204 $P < 0.002$). All pairwise mean differences between muscles at each knee angle and their 95% CI data are reported
205 in Table 2.

206

207 Significant correlations were found between the anterior proximal patellar tendon and individual quadriceps
208 muscles, with Pearson correlation coefficients being higher for the monoarticular muscles than RF (VL $r=0.74$;
209 VM $r=0.61$; RF $r=0.45$; *all* $P<0.001$). Correlation coefficients between the posterior proximal patellar tendon
210 and the respective muscles (VL $r=0.66$; VM $r=0.54$; RF $r=0.38$; *all* $P\leq 0.001$) were lower than for the anterior
211 counterpart.

212

213 **DISCUSSION**

214 Our first observation that knee angle influenced the distribution of shear wave velocity between anterior and
215 posterior regions within the proximal third of the patellar tendon, aligns with previous cadaveric and in vivo
216 work. By inserting strain gauges within the patellar tendon of eight fresh frozen limbs, Almekinders et al (2002)
217 demonstrated similar tensile strain behaviour between regions with the knee in full extension (0°). During knee
218 flexion, strain increased on the anterior side, while a relative decrease was observed on the posterior side of the
219 proximal tendon (full extension was designated as the reference point). No regional differences were found at a
220 more distal tendon location at any angle. In another study of 16 healthy volunteers, regional patellar tendon
221 strain was examined with the knee fixed at 90° flexion using an ultrasound based automated pixel tracking
222 technique (Pearson et al. 2014). Participants performed a series of ramped isometric knee extension contractions,
223 ranging from 10% to 100% of maximal force. They demonstrated lower strain for the posterior compared to
224 anterior region of the proximal patellar tendon, a pattern which was observed across force levels (Pearson et al.
225 2014). Extension of this work in ten healthy males was recently published (Pearson et al. 2017), demonstrating
226 that during maximal ramped isometric knee extension, the anterior to posterior strain ratio was greatest at 90°
227 knee flexion (7.8%) and lowest at 30° (2.9%) of flexion. Interestingly, these ratios (generated during maximal
228 isometric contraction) are similar to those for our study which measured SWV in a relaxed state (1.1% at 25° ,
229 2.2% at 40° , 3.0% at 55° , 5.6% at 70° and 8.4% at 85°).

230

231 Our second observation that knee angle influenced the distribution of shear wave velocity (and therefore of
232 shear modulus) of both monoarticular (VM and VL) and biarticular (RF) quadriceps muscles is only partially
233 supported by the literature. In a recent study of nine healthy males, a different pattern of muscle shear modulus
234 was observed between monoarticular and biarticular muscles, but no differences were observed between
235 monoarticular muscles throughout the range of knee flexion. In contrast, we demonstrated greater shear modulus
236 for VL than VM at 55° , 70° and 85° . Of note, this between muscle difference was not observed when tested in

237 extended knee positions, which corresponds to when the quadriceps muscles are below their slack length (Xu et
238 al. 2016). Given a strong relationship exists between muscle shear modulus and muscle force (Hug et al. 2015)
239 and because VL cross-sectional area is larger than that of VM (Hug et al. 2015), these findings provide strong
240 evidence that knee flexion induces a larger increase in VL than VM passive force. Differences in hip position
241 between our study (measured in hip flexion) and that of Xu et al, 2016 (measured in hip extension) may have
242 influenced the relative stiffness of the biarticular RF compared to mono-articular muscles. Spatial variation
243 within muscles (Le Sant et al. 2017) and orientation of the probe with muscle fascicles may have also differed
244 between studies.

245
246 To our knowledge, this is the first study to perform concurrent investigation of the elastic properties of
247 individual components of the quadriceps-patellar musculotendinous unit. When tested in the current position
248 (with the hip flexed), muscle-tendon SWV correlations were greater for the monoarticular muscles than the
249 biarticular RF. Correlations were consistently greater for the anterior proximal patellar tendon than for its
250 posterior counterpart. We postulate that this may be because fascicles from the anterior proximal tendon are
251 more continuous with the quadriceps muscles. Our results also suggest the slack angle (angle at which there is a
252 significant rise in passive SWV) may differ between muscle and tendon, as well as between monoarticular and
253 biarticular quadriceps muscles ($\sim 25^\circ$ for tendon; $\sim 40^\circ$ for VM/VL; $\sim 55^\circ$ for RF). The former result is consistent
254 with a study measuring SWV of the gastrocnemius-achilles musculotendinous unit (Hug et al. 2013), which
255 found a lower slack angle for tendon. The latter finding differs from Xu et al (2016) who observed the slack
256 angle was similar among the quadriceps muscles ($41\text{-}44^\circ$ knee flexion), when tested in supine lying with the hip
257 extended (placing greater stretch on RF).

258
259
260 The shear wave elastography technique overcomes some limitations of traditional measures by non-invasively
261 estimating the shear wave propagation velocity within individual muscles and isolated regions of tendon. A
262 distinct advantage is that it provides a real time elastogram, enabling visualisation of tissue regions of greater
263 SWV (represented by red) and lower SWV (represented by blue). However, there are some limitations to this
264 approach. Our within-day ICC was lower for RF (0.88), compared to VM and VL (0.95), which may be because
265 of difficulty in aligning the probe with the muscle fascicles within RF. Probe orientation relative to fascicle
266 direction may have also contributed to lower SWV values for RF (Aubry et al. 2013). For measurement of

267 tendon SWV, our ICCs (0.92-0.93) indicated acceptable repeatability, while regional differences within the
268 proximal patellar tendon (~8.4%) exceeded the typical error values (5.7-6.4%), highlighting the potential utility
269 of the method to study regional in vivo patellar tendon biomechanics.

270

271 Although the relationship between SWV and shear modulus is well known for muscles, it remains unknown for
272 tendons. Indeed, the guided wave propagation that occurs within tendon is known to affect the estimation of
273 tissue shear modulus from SWV measurements (Hug et al. 2015, Helfenstein-Didier et al. 2016). However, the
274 good relationship between tendon shear modulus estimated through guided wave propagation and tendon shear
275 modulus measured using the shear wave dispersion technique that accounts for guided wave propagation
276 (Helfenstein-Didier et al. 2016) makes us confident that the differential changes in SWV reported herein
277 represent true differential changes in shear modulus. Scanning depth has also been shown to have an effect on
278 SWV recorded from both elasticity phantoms and from muscle, having a greater impact on stiffer tissue such as
279 the tendon (Carlsen et al. 2015, Ewertsen et al. 2016). However, we did not find a significant difference between
280 anterior and posterior regions within the middle third of the patellar tendon, suggesting that scanning depth
281 alone does not fully account for the regional differences observed within the proximal tendon. Importantly, only
282 relatively low loads can be applied to tendon before the upper limit of 16.3m/s (Aixplorer Ultrasound Scanner,
283 version 9.3) is reached, limiting current measurement of tendon stiffness to passive conditions only. While
284 tendon stiffness during contraction would be expected to be greater by an order of magnitude than during
285 passive conditions, the relative difference between anterior and posterior regions (~8%) does not appear to differ
286 between maximal isometric contraction and passive conditions. Finally, although we performed passive
287 preconditioning prior to each set of recordings, stress-relaxation may have affected the measurement of stiffness
288 at successively greater knee angles (Haraldsson et al. 2005), which were not randomised in this study.

289

290 Our sample included young, active students without knee injury. Further extensive studies are needed to address
291 the intriguing possibility that differential muscle and tendon passive elastic properties might contribute to the
292 development of injury. “Stress-shielding” of the posterior relative to anterior patellar tendon has been proposed
293 as an aetiological factor in tendon pathology (Pearson et al. 2014), which is typically located in the deep parts of
294 the proximal tendon insertion onto the patellar (Johnson et al. 1996, Khan et al. 1996). Non-uniform tendon
295 behaviour during knee flexion might result in shear type stresses and potentially underpin why volleyball

296 athletes who display greater knee flexion angle during jump landing are more likely to have symptomatic
297 patellar tendinopathy (Richards et al. 1996).

298

299 An imbalance of force generation between VM and VL has also been speculated to contribute to the develop-
300 ment and/or persistence of patellofemoral pain (Hug et al. 2015), which is typically noted by patients when the
301 knees are more flexed (Tang et al. 2001). Greater passive stiffness (and thus passive force) for VL than VM in
302 knee flexion may be necessary to accommodate heterogenous muscle activation observed between VM and VL
303 (Tang et al. 2001). Given the biomechanics of the patellofemoral joint are controlled by active and passive
304 components, future studies may wish to consider concurrent measurement of muscle stiffness using shear wave
305 elastography, muscle activation using EMG and muscle architecture to assess the balance of forces between
306 muscles in people with and without patellofemoral pain (Hug et al. 2015).

307

308 This study benefits from usage of a highly innovative ultrasound shear wave elastography technique that allows
309 for novel insights into the regional heterogeneity of passive mechanical properties within the quadriceps femoris
310 muscle-tendon unit. Such insights are critical for future biomechanical models and research to quantify changes
311 in muscle or tendon stiffness in clinical populations.

312

313

314

315 **Table 1.** Within-day reliability for shear wave velocity for each region within the proximal patellar tendon and
316 quadriceps muscle.

Location	ICC (95%)	SEM (m/s)	CV (%)
Anterior patellar tendon	0.92 (0.84, 0.96)	0.58	6.4
Posterior patellar tendon	0.93 (0.86, 0.97)	0.47	5.7
Vastus medialis	0.95 (0.90, 0.98)	0.07	3.2
Rectus femoris	0.88 (0.77, 0.94)	0.10	4.8
Vastus lateralis	0.95 (0.90, 0.98)	0.09	3.8

317 Intraclass coefficients (ICC), calculated using a linear mixed-effects regression model. Standard error of
318 measurement (SEM) and Coefficient of variation (CV) calculated per Hopkin (Hopkins 2000).

319

320

321 **Table 2.** Results of linear mixed-effects regression models for patellar tendon and quadriceps muscle shear
 322 wave velocity.

Model effects	Comparison	Angle	Mean difference in m/s [95% CI]	<i>P</i> values
Proximal patellar tendon				
Region P<0.001 Angle P<0.001 Interaction P=0.007	Posterior vs anterior tendon	25°	-0.07 [-0.59, 0.37]	1.00
		40°	-0.16 [-0.59, 0.28]	1.00
		55°	-0.27 [-0.71, 0.16]	0.52
		70°	-0.60 [-1.03, -0.16]	<0.01
		85°	-0.97 [-1.40, -0.53]	<0.01
Middle third of patellar tendon				
Region P=0.332 Angle P<0.001 Interaction P=0.174	Posterior vs anterior tendon	25°	0.12 [-0.29, 0.54]	1.00
		40°	0.11 [-0.31, 0.53]	1.00
		55°	-0.06 [-0.48, 0.36]	1.00
		70°	-0.17 [-0.59, 0.25]	1.00
		85°	-0.36 [-0.78, 0.05]	0.13
Quadriceps muscle				
Muscle P<0.001 Angle P<0.001 Interaction P<0.001	Vastus lateralis vs Vastus medialis	25°	0.05 [-0.07, 0.17]	1.00
		40°	0.08 [-0.05, 0.20]	0.78
		55°	0.15 [0.03, 0.27]	<0.01
		70°	0.17 [0.05, 0.29]	<0.01
		85°	0.23 [0.11, 0.35]	<0.01
	Vastus lateralis vs Rectus femoris	25°	0.16 [0.04, 0.29]	<0.01
		40°	0.18 [0.05, 0.30]	<0.01
		55°	0.31 [0.19, 0.43]	<0.01
		70°	0.45 [0.33, 0.57]	<0.01
		85°	0.55 [0.43, 0.67]	<0.01
	Rectus femoris vs Vastus medialis	25°	-0.11 [-0.23, 0.01]	1.00
		40°	-0.10 [-0.22, -0.02]	0.21
		55°	-0.16 [-0.28, -0.04]	<0.01
		70°	-0.28 [-0.40, -0.16]	<0.01
		85°	-0.32 [-0.44, -0.20]	<0.01

323

324

325 Pairwise comparisons with bonferroni-adjusted *P*-values are listed. Mean differences [95%] for first (compared
 326 to second) listed comparison.

327 **Fig. 1** Elastogram of the patellar tendon with patellar (P) and tibial tuberosity (T) labelled. Regions of interest
328 for anterior (red) and posterior (white) regions of the proximal third of the patellar tendon. Probe position, see
329 bottom right.

330

331 **Fig. 2** Elastograms for each of the quadriceps muscle regions of interest and probe positions.

332

333 **Fig. 3** Shear wave velocity (mean, standard deviation) for anterior (shaded) and posterior (unshaded) regions of
334 the proximal patellar tendon for each knee angle. Significant differences were found between all angles (not
335 shown) and between regions as indicated by astericks.

336

337 **Fig. 4** Shear wave velocity (mean, standard deviation) for the quadriceps muscles for each knee angle.

338 Significant differences between muscles are indicated by astericks. No significant differences were found

339 between 25° and 40° for VM and VL and between 25°, 40° and 55° for RF. All other angles were significantly

340 different (not shown).

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