

Mersmann, F., Seynnes, O. R., Legerlotz, K., Arampatzis, A. (2018). Effects of tracking landmarks and tibial point of resistive force application on the assessment of patellar tendon mechanical properties in vivo. *Journal of Biomechanics*, 71, 176-182.

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<http://dx.doi.org/10.1016/j.jbiomech.2018.02.005>

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<http://dx.doi.org/10.1016/j.jbiomech.2018.02.005>

1 **Effects of tracking landmarks and tibial point of resistive force application on the assessment of patellar**
2 **tendon mechanical properties in vivo**

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16 **Article type:** Original article

17 **Key words:** Tendon elongation; Ultrasound; Dynamometry; Pad position; Tracking features

18 **Word count:** 3986

19 **Abstract**

20 The different methods used to assess patellar tendon elongation *in vivo* may partly explain the large variation of
21 mechanical properties reported in the literature. The present study investigated the effects of tracking landmark
22 position and tibial point of resistive force application during leg extension in a dynamometer.

23 Nineteen adults performed isometric contractions using a proximal and distal shank pad position. Knee joint
24 moments were calculated using an inverse dynamics approach. Tendon elongation was measured using the patellar
25 apex and either the tibial tuberosity (T) or plateau (P) as tracking landmark.

26 Using P for tracking introduced a bias towards greater values of tendon elongation at all force levels from 100 N
27 to maximum tendon force TFmax ($p < 0.05$). The differences between landmarks considering maximum tendon
28 strain were greater at the proximal shank pad position ($p < 0.05$). Tendon stiffness was lower for P compared with
29 T, but only in intervals up to 50% of TFmax ($p < 0.05$). The agreement between T and P for stiffness calculated
30 between 50% and TFmax was acceptable with the distal, but poor with the proximal pad position.

31 We demonstrated that using the tibia plateau and not the insertion as tracking landmark clearly affects the
32 assessment of the force-elongation curve of the patellar tendon. However, using a distal point of resistive force
33 application and calculating tendon stiffness between 50% and TFmax seems to yield an acceptable agreement
34 between landmarks. These findings have important implications for the assessment of tendon properties *in vivo*
35 and cross-study comparisons.

36 **Introduction**

37 It is well established that the mechanical properties of tendons play a crucial role for movement performance of
38 musculoskeletal systems, since they determine the transmission of muscle force to the skeleton and allow for the
39 storage and release of mechanical strain energy (Biewener et al., 1998; Roberts, 1997). In view of the epidemiology
40 of tendon injuries (Järvinen, 1992), the association of tendon mechanical properties and pathophysiological
41 mechanisms is also of interest for the scientific community and precisely estimating maximal tendon strain and
42 stiffness could be of clinical relevance (LaCroix et al., 2013; Wren et al., 2003). The mechanical properties of
43 tendons are determined based on their force-elongation behaviour (Butler et al., 1978), which has traditionally
44 been studied using *in vitro* tensile testing (Benedict et al., 1968; Johnson et al., 1994; Woo et al., 1980). The
45 introduction of an ultrasound-based approach to determine the elongation of tendon-aponeurosis complexes
46 (Fukashiro et al., 1995; Maganaris and Paul, 1999) lay the ground for an increasing scientific interest in human *in*
47 *vivo* tendon adaptation (Arampatzis et al., 2007; Kongsgaard et al., 2007; Reeves et al., 2003; Seynnes et al., 2009),
48 muscle-tendon interaction (Fukunaga et al., 2002; Lichtwark et al., 2007; Nikolaidou et al., 2017), influence of
49 tendon mechanical properties on human movement performance (Arampatzis et al., 2006; Bojsen-Møller et al.,
50 2005; Karamanidis and Arampatzis, 2005; Stafilidis and Arampatzis, 2007) and their relation to injury (Arya and
51 Kulig, 2010; Child et al., 2010; Helland et al., 2013).

52 Soon attempts were made to determine the mechanical properties of the patellar tendon *per se* as well (Hansen et
53 al., 2006; Reeves et al., 2003), which involves the measurement of joint moments around the knee or a resultant
54 exerted force and tendon elongation during muscle contractions. The latter is achieved by tracking the
55 displacement of the tendon origin and insertion at the patellar apex and tibial tuberosity in the ultrasound images
56 (O'Brien et al., 2010; Schulze et al., 2012). This, however, is only possible with ultrasound transducers visualizing
57 both landmarks in a single field of view during the contraction (i.e. linear arrays with an approximate minimum
58 length of 8 cm). When only short transducers are available, it is common that a prominent bony landmark close to
59 the tibia plateau is used for tracking (Carroll et al., 2008; Kongsgaard et al., 2007; Kösters et al., 2014; Seynnes et
60 al., 2011), based on the assumption that its displacement in the longitudinal axis of the tendon is representative of
61 tendon elongation. Yet during knee extension contractions in the open kinetic chain, a tilt of the tibia is commonly
62 observed in the ultrasound recordings (Seynnes et al., 2015). This means that the displacement of the insertion site
63 is influenced by both a translational and rotational component and, therefore, the displacement of any other point
64 distant to the insertion site that is caused by the tibia sagittal plane rotation and measured along the longitudinal
65 axis of the tendon is different compared to the insertion site itself (see also figure 1, panel B). Therefore, the
66 assessment of patellar tendon elongation based on the displacement of a landmark at the tibia plateau is likely to

67 be associated with a measurement bias depending on the magnitude of tibia tilt during the isometric contractions.
68 The movement of the tibia relative to the femur can be influenced by the point of resistive force application at the
69 shank. Moving the point of resistive force application proximally increases the load on the posterior cruciate
70 ligament due to a progressing posterior shift of the tibia plateau (Zavatsky et al., 1994). However, it is to date
71 unknown how this affects the measurement of patellar tendon elongation based on ultrasound recordings.
72 Therefore, the objective of the present study was to investigate how the choice of the tracking landmark and point
73 of resistive force application influences the assessment of patellar tendon mechanical properties *in vivo*. We
74 hypothesised that tracking the displacement of a landmark at the tibia plateau would lead to an overestimation of
75 tendon elongation and, thus, underestimation of tendon stiffness, due to the additional displacement of the
76 landmark induced by the tilt of the tibia during isometric contractions. We further expect both the tibia tilt and the
77 associated bias on the elongation measurement be more pronounced with a proximal compared to a distal point of
78 resistive force application.

79 **Methods**

80 *Participants*

81 Twenty-five healthy adults from the university population volunteered to participate in the present study, which
82 was conducted in accordance with the recommendations of the local university ethics committee and with written
83 informed consent from all subjects. Six subjects needed to be excluded due to ultrasound artefacts during image
84 acquisition ($n = 3$), inability to follow through the whole experimental protocol ($n = 1$) or ultrasound probe
85 movement artefacts that were detected in post-processing ($n = 2$). Average age, body height and body mass of the
86 remaining 19 participants were 25 ± 4 years, 176 ± 10 cm and 69 ± 12 kg, respectively.

87 *Experimental protocol and data acquisition*

88 Following a standardised warm-up of five minutes of ergometer cycling, the participants were seated and fixated
89 on a dynamometer (Biodex Medical System 3, Shirley, NY, USA) with the knee joint angle at 90° and the trunk
90 angle at 85° ($0^\circ =$ full knee or hip extension; values refer to the angle determined by the dynamometer). The
91 dynamometer shank pad was positioned in random order at 60% or 80% tibia length (from proximal to distal;
92 measured between the medial tibia plateau and malleolus). For the use of inverse dynamics in the calculation of
93 knee joint moments (see Arampatzis et al., 2004 for details), six reflective markers were fixed to the following
94 anatomical landmarks: greater trochanter, lateral and medial femoral epicondyles and malleoli, and second
95 metatarsal head. For estimating the contribution of antagonist activity to the resultant joint moments, two bipolar

96 surface electrodes (Blue Sensor N, Ambu GmbH, Bad Nauheim, Germany) were fixed over the mid-portion of the
97 muscle belly of the lateral head of the biceps femoris with an inter-electrode distance of 2 cm after shaving and
98 cleaning the skin. The kinematic data were recorded using a Vicon motion capture system (version 1.7.1; Vicon
99 Motion Systems, Oxford, UK) integrating eight cameras operating at 250 Hz. The electromyographic (EMG) data
100 were captured at 1,000 Hz (Myon m320RX; Myon, Baar, Switzerland) and transmitted to the Vicon system via a
101 16-channel A-D converter. A 10-cm linear ultrasound probe (7.5 MHz; My Lab60; Esaote, Genova, Italy; probe:
102 linear array (LA923), depth: 7.4 cm, focal point: 0.9 and 1.9) was fixed in a modified knee brace overlying the
103 patellar tendon in the sagittal plane, capturing the elongation of the tendon during contractions at 25 Hz. A
104 schematic of the experimental setup, including a representative ultrasound image of the patellar tendon and an
105 illustration of both the elongation analysis as well as a model of how tibia tilt could affect the elongation
106 measurement is shown in figure 1.

107 After ten submaximal isometric knee extension contractions as additional warm-up and familiarisation, the
108 subjects performed one maximum voluntary contraction (MVC) and two blocks of five isometric ramp
109 contractions, where the participants gradually increased contraction intensity up to 90% of their MVC in about
110 five seconds. A screen set-up in front of the dynamometer provided online feedback about the moment measured
111 at the dynamometer. Every ramp contraction in each block was preceded by five preconditioning contractions at
112 40% MVC (Maganaris, 2003). An additional passive trial (i.e. passive knee extension driven by the dynamometer
113 at 5°/s with the shank of the participants fixed to the dynamometer lever pad) was recorded to account for moments
114 due to gravity (Arampatzis et al., 2004) in the inverse dynamics approach. Further, two trials of knee flexions were
115 captured to establish an EMG-activity knee flexion moment relationship for estimating the contribution of
116 antagonistic muscles to the resultant joint moments (see Mademli et al., 2004 for details). Subsequently, the
117 dynamometer shank pad position was changed to the other target position (i.e. 60% or 80% of tibia length) for the
118 second block of five ramp contractions as well as the respective passive trial and the two knee flexion contractions.

119 *Knee joint moments and tendon mechanical properties*

120 The stiffness of the patellar tendon was derived from the force-elongation relationship during the isometric ramp
121 contractions. Tendon force was calculated by dividing the knee extension moments – determined with the inverse
122 dynamics approach suggested by Arampatzis and colleagues (2004) and considering antagonistic activity (Kellis
123 and Baltzopoulos, 1997; Mademli et al., 2004) – by the tendon lever arm, which in turn was predicted using
124 anthropometric data (Mersmann et al., 2016) and adjusted to the respective knee joint angle position based on the
125 data by Herzog and Read (1993).

126 Tendon elongation during the ramp contraction was assessed by measuring the displacement of the deep origin at
127 the patellar apex and both the deep insertion at the tibial tuberosity and a prominent landmark close to the edge of
128 the tibia plateau using a semi-automatic tracking software (Tracker Video Analysis and Modeling Tool V. 4.92,
129 Open Source Physics, Aptos, California, USA). When using the tibial tuberosity, elongation was measured as
130 vector between the two landmarks, which also was defined as the longitudinal axis of the tendon. When tracking
131 the tibia plateau, the displacement along this longitudinal axis was considered for the elongation measurement
132 (figure 1, panel A) to account for the misalignment between the vector connecting the landmarks (plateau and the
133 patella apex) and the patellar tendon. To account for tracking errors, for example due to relative probe movement
134 with respect to the bony structures, the digitalisations were considered valid if the R^2 of a second order polynomial
135 fit of the resultant force-elongation curve was at least 0.9 (Seynnes et al., 2015). If less than three trials fulfilled
136 the criteria, the participant was excluded ($n = 2$). For all participants included in the final analysis, all 5 trials per
137 condition were available for further analysis, which ensures an excellent reliability of the patellar tendon
138 elongation measurement (Schulze et al., 2012). The force-elongation curves of each pad-position condition (i.e. 5
139 trials) were averaged up to a common maximum tendon force of the ramp contractions in both conditions (TF_{max}).
140 Tendon stiffness was calculated in 10%-intervals of relative tendon force (relative to the individual maximum
141 tendon force exerted during the MVC assessment: TF_{mvc}) as well as between 50% TF_{mvc} and TF_{max} as slope of a linear
142 regression. TF_{mvc} was used to determine relative tendon force thresholds due to the marked differences in the
143 capability of the individual participants to exert force during the ramp contractions (TF_{max} ranged between 73% and
144 95% TF_{mvc}). As an indication for tibia tilt, we calculated the angle between the lower border of the patellar tendon
145 and the anterior intercondylar area (see figure 1). All outcome parameters related to the ramp contractions with
146 the two pad position conditions (e.g. maximum elongation and strain, knee angular change, antagonistic moment,
147 tibia tilt) were evaluated at TF_{max} and the average of the five trials was considered for analysis.

148 *Statistics*

149 All statistical procedures were carried out in SPSS (version 20.0; IBM, Armonk, NY, USA). We performed an
150 analysis of variances for repeated measures (RM ANOVA) with the within-subject factors *pad position* (i.e.
151 proximal, distal) *tracking landmark* (i.e. tuberosity, plateau) and *force level* (i.e. from 100 N to 1800 N every
152 100 N and TF_{max}) on the tendon force-elongation data. Normality of the data was tested using the Shapiro-Wilk test
153 and Mauchly's test was applied to test sphericity. Due to a violation of both assumptions at some of the force
154 levels, we applied the Huynh-Feldt correction, as it has demonstrated robust error I control upon violation of
155 normality and sphericity at similar sample sizes and factor levels (Oberfeld and Franke, 2012). In case of

156 significant landmark- or pad position-by-force level interactions, we used the Wilcoxon signed-rank test and
157 Bonferroni adjustment to test differences between landmarks or pad positions at each force level, respectively. A
158 similar approach was taken to analyse tendon stiffness calculated in 10%-intervals of TF_{mvc} . With regard to
159 maximum strain and stiffness calculated between 50% TF_{mvc} and TF_{max} , no force level factor was applied in the RM
160 ANOVA. Considering all other parameters (resting knee joint angle and angular rotation, antagonistic moment,
161 tibia tilt), the two pad position conditions were compared with students t-tests after testing for normality. The alpha
162 level for all tests was 0.05 and effect sizes f were calculated for significant effects, interactions and differences
163 using G*Power (Version 3.1.6; HHU, Düsseldorf, Germany; (Faul et al., 2007)). Effect sizes of $0.1 \leq f < 0.25$ can
164 be considered as small, $0.25 \leq f < 0.5$ as medium and $f \geq 0.5$ as large (Cohen, 2013). To examine the agreement
165 between the results obtained from using either the tibia plateau or tuberosity as tracking landmark we used Bland-
166 Altman plots (Bland and Altman, 1986). The agreements were considered *good*, if their limits (mean ± 1.96
167 standard deviation of the differences between methods) were smaller than the differences that can be expected
168 following interventions or between cohorts, *acceptable* in case of similar limits and *poor* in case of greater limits.

169 **Results**

170 There was no difference in the resting knee joint angle ($p = 0.86$) or in the moment due to the activation of the
171 antagonistic knee flexors during the maximum ramp contractions ($p = 0.86$), yet a significantly greater angular
172 change from rest to maximum in the proximal compared to the distal pad position condition ($p < 0.001, f = 1.43$;
173 table 1). Similarly, the tibia tilt (i.e., angular change between the tendon and anterior intercondylar area; see figure
174 1) was significantly greater during contractions with the proximal pad position ($p < 0.001, f = 0.84$; table 1). Figure
175 2 illustrates the tibia tilt as a function of relative tendon force for both pad positions, respectively.

176 TF_{mvc} and TF_{max} were on average 4119 ± 1114 N and 3401 ± 842 N (i.e. $\sim 83\%$ TF_{mvc}), respectively. Figure 3 illustrates
177 the force-elongation behaviour yielded by the analysis using the two landmarks and pad position conditions for
178 every 100 N of tendon force and TF_{max} . There was a main effect of landmark ($p < 0.001, f = 1.51$), force level
179 ($p < 0.001, f = 2.92$) and a significant landmark-by-force level interaction ($p < 0.001, f = 0.99$), yet no significant
180 main effect of pad position ($p = 0.468$) or interaction with force level ($p = 0.798$). *Post hoc* testing revealed
181 significant differences between landmarks for all force levels ($p < 0.001, 0.48 \leq f \leq 0.67$), indicating an
182 overestimation of elongation when using the plateau as tracking landmark with increasing effect sizes up to
183 1200 N. Similarly, there was a significant main effect of tracking landmark ($p < 0.001, f = 1.16$), force level
184 ($p < 0.001, f = 1.19$), and landmark-by-force level interaction ($p < 0.001, f = 1.03$), on stiffness calculated in 10%-
185 intervals of TF_{mvc} , while no significant main effect of pad position ($p = 0.901$) or interaction with force level

186 ($p = 0.054$) was present (figure 4). *Post hoc* comparisons showed significant differences between landmarks in the
187 intervals up to 50% TF_{mvc} ($p \leq 0.002$, $0.35 \leq f \leq 0.62$), while the differences in the intervals 50-60% and 60-70%
188 TF_{mvc} were not statistically significant ($p \geq 0.06$).

189 Maximum strain demonstrated a significant effect of landmark ($p < 0.001$, $f = 1.40$) with greater maximum strain
190 values when using the plateau as tracking landmark, while no significant main effect of the pad position was
191 present ($p = 0.41$; figure 5A). A significant landmark-by-pad position interaction ($p = 0.003$, $f = 0.81$) and *post*
192 *hoc* analysis indicated greater differences between landmarks at the proximal pad position ($p = 0.013$) as well as
193 greater differences between pad positions for the analysis using the plateau as landmark ($p = 0.013$). The Bland-
194 Altman plots in figure 6 illustrate the poor agreement between maximum strain obtained from the analysis using
195 the landmark at the tibia plateau and tuberosity at the distal (limits of agreement of 2.02 to -4.12%; A) and proximal
196 pad position (limits of agreement of 0.54 to -5.24%; B), respectively. Though there was neither a significant main
197 effect of landmark or pad position nor a landmark-by-pad position interaction on stiffness calculated between 50%
198 TF_{mvc} and TF_{max} (figure 5B), the agreement between methods was only acceptable at the distal pad position (limits
199 of agreement of 249 N/mm to -308 N/mm; figure 5C) and poor at the proximal pad position (limits of agreement
200 of 704 to -558 N/mm; figure 6D).

201 **Discussion**

202 The present study investigated the effect of the choice of tracking landmark and point of resistive force application
203 at the shank on the assessment of patellar tendon elongation during isometric contractions *in vivo*. In line with our
204 hypotheses, we found that tracking a bony landmark close to the tibia plateau overestimates the elongation of the
205 tendon throughout the whole force-elongation curve and results in lower values of the first derivative of the force
206 elongation curve up to 50% of maximum tendon force. A proximal position of the point of resistive force
207 application appears to amplify this bias and compromises the agreement between tracking landmarks.

208 Our hypothesis that using a landmark at the tibia plateau instead of the tendon insertion at the tibial tuberosity
209 would overestimate tendon elongation was based on the common observation of a tibia tilt during isometric
210 contractions in the open kinetic chain (Seynnes et al., 2015). In the present study, the tilt of the tibia was determined
211 as change in the angle between the tendon and the anterior intercondylar area (α), as a change in this angle leads
212 to a displacement of the tibia plateau along the longitudinal axis of the tendon that is not representative of the
213 actual tendon elongation (see figure, panel B). The bias associated with this angular rotation (ΔL) can be calculated
214 as $\Delta L = r \cdot \cos(\alpha) - r \cdot \cos(\alpha + \Delta\alpha)$, under the simplified assumption that the vector r from the landmark at the

215 tuberosity to the one at the plateau is constant, and it explained 44% of the variance of the between-method
216 differences in maximum elongation ($r = 0.66$; $p < 0.001$).

217 The major contraction-induced tilt of the tibia can be observed up to about 50% TF_{MVC} , which explains both that a)
218 the elongation measured at a given force differs substantially between the two landmarks already at low force
219 levels and b) that stiffness calculated at force levels below 50% TF_{MVC} is significantly lower when the plateau was
220 used for tracking instead of the tuberosity. This is an important finding with respect to the interpretation of
221 experimental results as well as for the conduction of future *in vivo* studies investigating the mechanical properties
222 of the patellar tendon. For example, tendon strain during maximum isometric contractions can be used as a marker
223 for the mechanical demand placed upon the tendon by the working muscle, as there is convincing evidence that
224 ultimate strain can be assumed to be more or less constant (Abrahams, 1967; LaCroix et al., 2013; Loitz et al.,
225 1989; Shepherd and Screen, 2013). Recent *in vivo* studies on humans support the notion that tendon overuse injury
226 might be related to increased tendon strain during high-effort muscle contractions (Arya and Kulig, 2010; Child
227 et al., 2010; Mersmann et al., 2016; 2017). Though the outcomes of tracking of the patellar plateau have shown to
228 be reproducible (Hansen et al., 2006; Kösters et al., 2014), maximum tendon strain values were significantly
229 greater compared to those measured using the actual insertion at the tuberosity. When the precision of measuring
230 maximum tendon strain *in vivo* is compromised due to tibia sagittal plane rotation, it might not be possible to
231 identify associations between maximum *in vivo* tendon strain other outcome parameters. This limitation also needs
232 to be considered when investigating tendon stiffness at lower levels or tendon force, which might be of scientific
233 interest considering the different micromechanical mechanisms underlying the force-elongation behaviour of
234 tendons at different force levels (Screen et al., 2004). Surprisingly, maximum strain values (of young recreationally
235 active adults) reported by authors that used the displacement of the plateau in the assessment of patellar tendon
236 elongation (Carroll et al., 2008; e.g. 5.8-6.9%; Hansen et al., 2006; Kongsgaard et al., 2007; Seynnes et al., 2013)
237 are actually lower compared to those obtained in the present study when tracking the tuberosity (i.e. 7.3% at the
238 distal pad position). However, this might be related to the respective tracking procedures, since studies that used
239 the same automatic tracking algorithms as applied in the present study reported similar strain values as we found
240 as a result of plateau-tracking (8.3% vs. 8.2-8.9%; Kösters et al., 2014; Wiesinger et al., 2016).

241 In accordance with models for cruciate ligament loading (Zavatsky et al., 1994), shifting the point of resistive
242 force application proximally led to significantly greater angular rotation of the knee joint and tibia tilt at a similar
243 knee extension moment. The magnitude of the reactive force at the shank pad increases at a given resultant knee
244 joint moment when the pad is located more proximally, as the product of the reactive force and its lever arm (with
245 respect to the knee joint) needs to be in equilibrium with the moment exerted at the knee during isometric muscle

246 contractions. This increase of reactive force is likely to contribute to the posterior shift of the plateau and increasing
247 sagittal plane rotation as well. In consequence of the increase of tibia tilt, the overestimation of maximum tendon
248 strain upon the use of the plateau for tracking was significantly greater (indicated by the tracking landmark-by-
249 pad position interaction) and we found a marked impairment of the agreement with tracking the tuberosity in the
250 measurement of tendon stiffness at the proximal shank pad position. It is interesting to note that shifting the point
251 of resistive force application proximally did not introduce a systematic bias but increased the spread of the
252 differences in stiffness between the two tracking landmarks. It could well be that an increase in angular rotation
253 and tibia tilt during isometric contractions (even above 50% of TF_{mvc}) is associated with out-of-plane movements
254 of the ultrasound probe, which introduces different tracking errors at the two sites (Seynnes et al., 2015). However,
255 even at the more favourable pad position condition, the limits of agreement of 22% and -27% indicate considerable
256 differences in stiffness calculated from the elongation measured using the two tracking methods. Assuming that
257 random tracking errors occur using both landmarks, the tibia tilt generates an additional uncertainty affecting only
258 the elongation measurement at the plateau. It is thus likely that a given effect that is present during an *in vivo*
259 investigation remains concealed or can only be detected at greater sample sizes due to a reduced precision of that
260 approach compared to tuberosity-tracking. Nevertheless, group mean values of stiffness at high force levels seem
261 to be similar and allow comparison of values obtained using both tracking-approaches, as the major contraction-
262 induced tibia tilt occurs up to 50% TF_{mvc} and reaches a plateau afterwards.

263 In conclusion, the choice of tracking landmark affects the measurement of patellar tendon elongation during
264 isometric contractions *in vivo*. Tracking a bony landmark close to the tibia plateau introduces a measurement bias
265 towards greater elongation of the tendon throughout the whole force-elongation curve due to its sensitivity to the
266 tibia tilt that occurs during isometric knee extension contractions. Consequently, the approach yields lower values
267 of the first derivative of the force elongation curve up to 50% of maximum tendon force, which corresponds well
268 with the tibia tilt as a function of exerted force. A proximal position of the point of resistive force application
269 appears to amplify the measurement-bias of the plateau-tracking and compromises the agreement to the results
270 from tracking the tuberosity. However, the use of a distal point of resistive force application together with a
271 calculation of tendon stiffness at higher tendon force levels yields results that allow for cross-study comparisons.
272 These results have important implications for the design of future *in vivo* studies on the mechanical properties of
273 the patellar tendon as well as the interpretation of the results published in the literature.

274 **Conflict of interest statement**

275 All authors disclose any financial and personal relationships with other people or organisations that could

276 inappropriately influence (bias) the study.

277 **Acknowledgements**

278 The authors thank Arne Schlausch for supporting the data analysis and Arno Schroll for sharing his expertise on
279 the statistical analysis.

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392

1 **Figure captions**

2 **Figure 1** Schematic representation of the experimental setup and ultrasound analysis. Dynamometry (1) and
3 kinematic recordings (2) were used to calculate knee joint moments based on an inverse dynamics approach.
4 Electromyographic activity of the biceps femoris and gastrocnemius medialis (3) was recorded because of their
5 contribution to the resultant knee joint moment and tibio-femoral movement, respectively. Ultrasound imaging
6 was integrated to assess patellar tendon elongation (4). In the ultrasound image (panel A), the crosses indicate
7 the tracking landmarks for the elongation measurement at the deep insertion at the apex of the patella, the tibial
8 tuberosity and the tibia plateau. Only the displacement in the longitudinal axis of the tendon (upper dashed line)
9 was considered in the elongation-measurement at the tibia plateau (arrow). The change of the angle (α) between
10 the lower border of the patellar tendon and the anterior intercondylar area was used as an indication of tibia tilt
11 during contractions. Panel B illustrates that the use of a tracking landmark at the tibia plateau is associated with a
12 measurement bias (see vertical arrow heads) in case of sagittal plane rotation of the tibia. In the schematic, a
13 rotation around the tendon insertion point at the tuberosity is assumed for simplification. The grey lines and the
14 black lines represent the contours of the bones and the position of the tracking landmarks before and during
15 contraction, respectively.

16
17 **Figure 2** Mean and standard error (error bars) of the angle between the patellar tendon and anterior intercondylar
18 area (see also figure 1) – used as an indication for tibia tilt – as a function of tendon force (relative to maximum
19 tendon force exerted during a maximum voluntary contraction) and using a distal (white) or proximal (black)
20 dynamometer shank pad position.

21
22 **Figure 3** Patellar tendon force-elongation relationship (mean \pm standard error of elongation) derived from
23 isometric knee extension contractions with a dynamometer shank pad position either proximal (black) or distal
24 (white) and using either the tibial tuberosity (triangles) or tibia plateau (circles) as tracking landmark in the
25 evaluation of tendon elongation. # significant main effect of tracking landmark; ‡ significant main effect of force
26 level; significant landmark-by-force level interaction; * significant post hoc difference between tracking
27 landmarks; $p < 0.05$.

28
29 **Figure 4** Tendon stiffness as first derivative of the force-elongation curve in 10%-intervals of maximum tendon
30 force using either the tibial tuberosity (triangles) or tibia plateau (circles) as tracking landmark point in the
31 evaluation of tendon elongation. Due to the absence of a significant main effect of pad position or pad position-

32 by-force level interaction, data was collated for clarity. # significant main effect of tracking landmark; ‡
33 significant main effect of force level; significant landmark-by-force level interaction; * significant post hoc
34 difference between tracking landmarks; $p < 0.05$.

35

36 **Figure 5** Maximum tendon strain (A) and tendon stiffness (B) obtained from isometric ramp contractions with a
37 distal (white) and proximal (black) dynamometer shank pad position and using either the tibial tuberosity or tibia
38 plateau as tracking landmark in the evaluation of tendon elongation. Tendon strain was evaluated at the common
39 maximum tendon force achieved during five ramp contractions and tendon stiffness was calculated between 50%
40 of maximum MVC tendon force and the common maximum during the ramp contractions. # significant main
41 effect of tracking landmark; † significant landmark- by-pad position interaction, indicating significantly greater
42 differences between landmarks at the proximal pad position and between pad positions when using tibia plateau
43 as tracking landmark; $p < 0.05$.

44

45 **Figure 6** Bland and Altman plots illustrating the agreement between the use of the tibial tuberosity or plateau as
46 tracking landmarks in the assessment of patellar tendon strain (A, B) and stiffness (C, D), and using a distal
47 (white) or proximal (black) dynamometer shank pad position for the isometric contractions. The abscissa shows
48 the absolute values for strain and stiffness averaged between reference points, the ordinate shows the differences
49 between the values measured using the tuberosity (T) and plateau (P) as tracking landmark. The horizontal solid
50 line shows the mean difference between landmarks; the dashed lines show the mean difference $\pm 1.96SD$.

51











