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1 **Title**

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3 dual transducer arrangements

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5 **Authors & Affiliations**

6 Scott F Brennan[1], Andrew G Cresswell[1], Dominic J Farris[1], Glen A Lichtwark[1]

7

8 [1]School of Human Movement & Nutrition Sciences, The University of Queensland,

9 Brisbane, QLD, Australia, 4072.

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11 **Corresponding author**

12 Scott F Brennan

13 School of Human Movement & Nutrition Sciences

14 E: s.brennan@uq.edu.au

15 P: (+61) 7 3365 6482

16 Fax: (+61) 7 3365 6877

17

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20

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23

24 **Abstract**

25 Ultrasonography is a useful technique to study muscle contractions in vivo, however

26 larger muscles like vastus lateralis may be difficult to visualise with smaller, commonly

27 used transducers. Fascicle length is often estimated using linear trigonometry to

28 extrapolate fascicle length to regions where the fascicle is not visible. However, this

29 approach has not been compared to measurements made with a larger field of view

30 for dynamic muscle contractions. Here we compared two different single-transducer

31 extrapolation methods to measure VL muscle fascicle length to a direct measurement

32 made using two synchronised, in-series transducers. The first method used pennation

33 angle and muscle thickness to extrapolate fascicle length outside the image

34 (extrapolate method). The second method determined fascicle length based on the

35 extrapolated intercept between a fascicle and the aponeurosis (intercept method).

36 Nine participants performed maximal effort, isometric, knee extension contractions on

37 a dynamometer at 10^0 increments from 50-100^0 of knee flexion. Fascicle length and

38 torque were simultaneously recorded for offline analysis. The dual transducer method

39 showed similar patterns of fascicle length change (overall mean coefficient of multiple

40 correlation was 0.76 and 0.71 compared to extrapolate and intercept methods

41 respectively), but reached different absolute lengths during the contractions. This had

42 the effect of producing force-length curves of the same shape, but each curve was

43 shifted in terms of absolute length. We concluded that dual transducers are beneficial

44 for studies that examine absolute fascicle lengths, whereas either of the single

45 transducer methods may produce similar results for normalised length changes, and

46 repeated measures experimental designs.

47

48

Introduction

Ultrasonography allows for non-invasive measurement of muscle fascicle geometry during muscle contractions. For muscles with relatively short fascicles, like *gastrocnemius* or *tibialis anterior*, dynamic imaging is relatively simple because the majority of the muscle fascicle is visible within the field of view (FOV) of the transducer (Brennan et al., 2017; Cronin et al., 2013; Day et al., 2013; Kawakami et al., 1998; Maganaris, 2003). Measurements of longer fascicles in muscles like *vastus lateralis* (VL) are more difficult due to the required FOV being larger.

Different methods are available to overcome the FOV issue. The first method is to use a longer transducer that can image a larger FOV (Sharifnezhad et al., 2014). However, longer transducers (e.g. 10 cm) often have a limited frame rate because of the greater time it takes to obtain data along the length of the transducer, and can have reduced image quality depending on the number of crystal elements per unit length. Another method is to use extended FOV techniques (Noorkoiv et al., 2010), which is a valid and reliable method for static measurements (i.e. minimal changes in muscle force and/or fascicle length). The most common method to overcome FOV issues during dynamic contractions is to use linear trigonometry to estimate the length of the portion of the fascicle that is outside the FOV of a single transducer (Austin et al., 2010; Finni et al., 2003; Fontana et al., 2014). An alternative is to utilise a second, in-series transducer to simultaneously record images of the part of the fascicle not visible by the first transducer (Bolsterlee et al., 2016; 2015; Herbert et al., 2011; 2015). Using a second transducer, both fascicle endpoints are visible, reducing much of the uncertainty in fascicle length measurements. For dynamic fascicle tracking,

73 estimations of fascicle length from a single transducer have not yet been compared to

74 length measurements from a greater FOV using two transducers.

75

76 The aim of the study was to determine if dynamic measurements of VL fascicle length

77 using extrapolation methods with one transducer during isometric knee extension

78 contractions match those made with two synchronised, in-series transducers. We

79 hypothesised that the absolute lengths of the fascicles would differ between the single

80 and dual ultrasound techniques, due to the ability to visualise the fascicle endpoint.

81 However, we also predicted that any differences would be negligible for normalised

82 length changes, and hence, would not affect observations made using a repeated

83 measures design.

84

85 **Methods**

86 **Protocol**

87 Nine participants (age 26 ± 2.5 years, mass 72.8 ± 7.0 kg, height 178 ± 6.3 cm)

88 provided informed consent to participate in the study. The study was approved by an

89 institutional ethics committee. Each participant completed maximal effort, isometric,

90 knee extension contractions on an isokinetic dynamometer (HUMAC NORM, CSMi

91 Inc., Stoughton, MA, USA). A familiarisation session was completed to make sure that

92 they could perform consistent maximal efforts. A second experimental session

93 followed within 10 days, which included the ultrasound measurements. The two

94 sessions used the same protocol and dynamometer position.

95

96 Participants were seated in the dynamometer with a hip angle of 80^0 and the

97 dynamometer attachment adjusted to align with the flexion/extension axis of the left

98   knee. A 60-s isotonic warm up protocol was performed using the interactive path

99   program on the dynamometer. The isometric protocol consisted of randomised blocks

100   of three maximal effort, isometric contractions at 10^0 increments from 50^0-100^0 of knee

101   flexion. A straight leg was defined as 0^0 of knee flexion. For each contraction

102   participants were instructed to perform a ramp contraction to maximal effort over a 3-

103   s period, and hold the maximum effort for 1-s before relaxing. Two minutes rest was

104   given between trials to avoid any potential fatigue effects.

105

106   **Dynamometer measurements**

107   Knee extensor torque and joint angle were sampled from the analogue output of the

108   dynamometer using a CED Micro 1401 A/D converter at a 2kHz sample rate and

109   recorded in Spike 2 software (Cambridge Electronic Design Ltd., Cambridge,

110   England). The torque signal was filtered using a 10 Hz, first-order, low-pass, bi-

111   directional Butterworth filter in Matlab (MathWorks Inc., Natick, MA, USA). The

112   maximum gravity effective torque (maxGET) was taken as the resting torque with the

113   knee at full extension (0^0). Torque was then gravity corrected using maxGET and joint

114   angle (Pincivero et al., 2004; Westing and Seger, 1989). Passive torque was

115   calculated as the difference between the resting torque and gravity corrected torque

116   prior to the contraction. The best two-out-of-three trials based on maximal torque were

117   analysed for each joint angle.

118

119   **Ultrasound measurements**

120   Muscle fascicle measurements of VL were made using two flat ultrasound transducers

121   (LV7.5/60/96Z, TELEMED, Vilnius, Lithuania) that were held end-to-end by a custom

122   made frame (Figure 1). Due to the shape of the transducer, there was a 22 mm gap

123 between the visual fields of the transducers. A custom Matlab script was written to

124 'stitch' the images together (Figure 1c). The transducers were placed at approximately

125 50% thigh length, following a line between the greater trochanter and superior patella

126 insertion. A self-adhesive compression bandage was used to secure the transducers

127 to the thigh. The central frequency of the transducer was set at 5 MHz, image depth

128 at 50 mm, and sampling rate of 80 Hz. A logic pulse from the first ultrasound system

129 triggered data capture by the other system, which produced its own logic pulse. The

130 two pulses were recorded by the A/D board to determine any delay between the onsets

131 of image collection. A semi-automated tracking algorithm (Cronin et al., 2011; Farris

132 and Lichtwark, 2016; Gillett et al., 2013) tracked the positions of the visible fascicle,

133 and the deep and superficial aponeuroses, which was subsequently used to estimate

134 fascicle length using three different methods.

135

136 *Method 1 - Extrapolation*

137 Fascicle length for the "extrapolation" method (Figure 1a) was calculated from the

138 proximal image using the equation:

139

140 $\quad$ FL = visible fascicle length + h/sin(PA)

141

142 where 'h' equals the vertical distance between the intersection of the visible fascicle

143 with the image border and the deep aponeurosis; and PA equals the pennation angle

144 of the tracked fascicle (Austin et al., 2010; Finni et al., 2003; Fontana et al., 2014).

145 *Method 2 - Intercept*

146 Fascicle length for the "intercept" method (Figure 1b) was calculated from the proximal

147 image using:

149   FL = visible fascicle length + predicted length

150

151 where the predicted length is equal to the distance between the visible fascicle's

152 intersection with the image border and the intersection of the linearly extrapolated

153 paths of the visible fascicle and deep aponeurosis (Blazevich et al., 2009).

154 *Method 3 – Dual*

155 The proximal and distal images of VL were used to separately track the positions of

156 the proximal and distal endpoints of a line assumed to be representative of a single

157 fascicle (Figure 1c). The proximal insertion and visible fascicle length was defined first,

158 then the distal 'fascicle' was defined as the continuation of that line within the distal

159 image. Fascicle lengths were calculated as the distance between the origin of the

160 fascicle in the proximal image and the distal intersection with the deep aponeuroses

161 in the distal image.

162

163 Due to the large proportion of fascicle length that is estimated, Methods 1 and 2

164 (extrapolate and intercept) are highly sensitive to changes in the orientation of the

165 deep aponeurosis. As such, the coordinates of the tracking points were filtered using

166 a 5 Hz, second-order, low-pass, bi-directional, Butterworth filter to reduce the chances

167 of non-physiological, high frequency length changes as a result of the calculations.

168 Fascicle lengths were then calculated from the filtered X-Y coordinates and

169 interpolated to the analogue sampling rate.

170

171 **Analysis**

172 Quadriceps force was calculated as active torque divided by the angle specific VL

173 moment arm, calculated individually using a modified *gait 2392* musculoskeletal model

174 in OpenSim software and standard scaling procedures (Delp et al., 1990). The scale

175 factors were determined from markers placed on anatomical landmarks of the pelvis

176 and left lower limb. Fascicle length was recorded at rest and at the time of maximal

177 quadriceps force for each contraction at each joint position. The change in fascicle

178 length from the resting state to maximum quadriceps force was also calculated.

179

180 For each individual a force-length curve was fitted, based on physiologically

181 appropriate models (Azizi and Roberts, 2010)

182
$$F_{active} = e^{-\left|(L^b-1)/s\right|^a}$$

183

184 where F is force, L is fascicle length, a is roundness, b is skewness, and s is width.

185 The curve fit was optimised using a nonlinear least squares method.

186

187 A coefficient of multiple correlation (CMC) analysis was performed for each joint angle,

188 comparing the waveform fascicle lengths of Method 3 with each of the other estimation

189 methods, averaged across two trials. A two-way repeated measures ANOVA (method

190 x joint angle) was performed on fascicle length and fascicle length change data, with

191 Dunnett's multiple comparisons where interactions were found. A one-way repeated

192 measures ANOVA was used to compare L_o across methods. The coefficient of

193 variation (R^2) of the force-length fits was calculated to measure how well the curve fit

194 explained the variance in the data. An alpha level of 0.05 was used for all statistical

195 tests. Values in text are shown as mean ± standard deviation (SD).

196

Results

CMC's between the dual transducer method and the two single transducer methods showed that the pattern of fascicle length changes was consistent across methods (Table 1, Figure 2a). The extrapolate method had higher CMC values at shorter lengths (smaller joint angle) and lower CMC values at longer lengths, whereas the intercept method was consistent across joint angles. The pattern of fascicle length change had consistent temporal phases across methods, with high values for CMCs (Table 1, Figure 2a), but the absolute fascicle length range varied between methods (Figure 2b).

There was a significant main effect of method on fascicle shortening (F = 28.71, p < 0.01), with no significant interaction (F = 1.52, p = 0.15, Figure 3b). The extrapolate and intercept methods showed greater fascicle shortening compared to the dual transducer method by a mean of 24.64 mm (95% CI = 16.75 – 32.53) and 11.38 mm (95% CI = 3.49 – 19.27) respectively across all joint angles.

The dual transducer method (106 ± 10 mm) predicted the largest L_o, where both the intercept (90 ± 17 mm) and the extrapolation (89 ± 16 mm) resulted in a significantly lower predicted L_o (F = 18.7, p < 0.01). The normalised force-length curves for each of the methods are shown in Figure 4. The R-squared values for the extrapolation, intercept and dual transducer curve fits were 0.72 ± 0.14, 0.72 ± 0.13, and 0.74 ± 0.10 respectively.

Discussion

221 The main findings of the study suggest that fascicle length measurements made by

222 the different methods result in absolute differences in fascicle length. However, these

223 differences appear to be systematic and the pattern of length change between the

224 different methods is consistent. Furthermore, the effect on normalised lengths is

225 minimal.

226

227 We observed that a second ultrasound transducer is beneficial for visualising the distal

228 changes in muscle orientation. The greater fascicle shortening and shorter fascicle

229 lengths at maximal force in both of the single transducer methods may be due to

230 underestimation of fascicle length by tracking only the proximal region of the muscle.

231 The greater shortening resulted in lower predicted absolute L_o values, however that

232 shift was not evident when utilising normalised fascicle lengths (Figure 4). Therefore,

233 if understanding absolute fascicle lengths is important, using a second ultrasound

234 transducer to visualise the distal fascicle endpoint is recommended. The use of either

235 single transducer method would provide similar results for experimental data

236 measuring differences in muscle contraction dynamics within-participants. Thus, for a

237 repeated measures design, the choice of estimation method may shift the overall data

238 set but not alter the effects of experimental factors.

239

240 *Limitations*

241 We assumed that a second transducer is beneficial because it is possible to visualise

242 the distal muscle region. However, the dual transducer method used in this study was

243 not validated against any other fascicle measurement technique such as diffusion

244 tensor imaging (Bolsterlee et al., 2015) or extended FOV techniques (Noorkoiv et al.,

245   2010) because there is not currently a gold standard measurement for dynamic muscle

246   contractions.

247

248   **Conflict of Interest Statement**

249   The authors have no conflict of interest to disclose.

250

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254

255

References

Austin, N., Nilwik, R., Herzog, W., 2010. In vivo operational fascicle lengths of vastus lateralis during sub-maximal and maximal cycling. Journal of Biomechanics. 43, 2394–2399.

Azizi, E., Roberts, T.J., 2010. Muscle performance during frog jumping: influence of elasticity on muscle operating lengths. Proceedings of the Royal Society B: Biological Sciences. 277, 1523–1530.

Blazevich, A.J., Coleman, D.R., Horne, S., Cannavan, D., 2009. Anatomical predictors of maximum isometric and concentric knee extensor moment. European Journal of Applied Physiology. 105, 869–878.

Bolsterlee, B., Gandevia, S.C., Herbert, R.D., 2016. Effect of transducer orientation on errors in ultrasound image-based measurements of human medial gastrocnemius muscle fascicle length and pennation. PLoS ONE. 11, e0157273.

Bolsterlee, B., Veeger, H.E.J.D., van der Helm, F.C.T., Gandevia, S.C., Herbert, R.D., 2015. Comparison of measurements of medial gastrocnemius architectural parameters from ultrasound and diffusion tensor images. Journal of Biomechanics. 48, 1133–1140.

Brennan, S.F., Cresswell, A.G., Farris, D.J., Lichtwark, G.A., 2017. The effect of cadence on the muscle-tendon mechanics of the gastrocnemius muscle during walking. Scandinavian Journal of Medicine & Science in Sports. 27, 289–298.

Cronin, N.J., Avela, J., Finni, T., Peltonen, J., 2013. Differences in contractile behaviour between the soleus and medial gastrocnemius muscles during human walking. Journal of Experimental Biology. 216, 909–914.

Cronin, N.J., Carty, C.P., Barrett, R.S., Lichtwark, G., 2011. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. Journal of Applied

282   Physiology. 111, 1491–1496.

283   Day, J.T., Lichtwark, G.A., Cresswell, A.G., 2013. Tibialis anterior muscle fascicle

284   dynamics adequately represent postural sway during standing balance. Journal of

285   Applied Physiology. 115, 1742–1750.

286   Delp, S.L., Loan, J.P., Hoy, M.G., Zajac, F.E., Topp, E.L., Rosen, J.M., 1990. An

287   interactive graphics-based model of the lower extremity to study orthopaedic

288   surgical procedures. IEEE Transactions on Biomedical Engineering. 37, 757–767.

289   Farris, D.J., Lichtwark, G.A., 2016. UltraTrack: Software for semi-automated tracking

290   of muscle fascicles in sequences of B-mode ultrasound images. Computer

291   Methods and Programs in Biomedicine. 128, 111–118.

292   Finni, T., Ikegawa, S., Lepola, V., Komi, P.V., 2003. Comparison of force-velocity

293   relationships of vastus lateralis muscle in isokinetic and in stretch-shortening cycle

294   exercises. Acta Physiologica Scandinavica 177, 483–491.

295   Fontana, H. de B., Roesler, H., Herzog, W., 2014. In vivo vastus lateralis force-velocity

296   relationship at the fascicle and muscle tendon unit level. Journal of

297   Electromyography & Kinesiology. 24, 934–940.

298   Gillett, J.G., Barrett, R.S., Lichtwark, G.A., 2013. Reliability and accuracy of an

299   automated tracking algorithm to measure controlled passive and active muscle

300   fascicle length changes from ultrasound. Computer Methods in Biomechanics and

301   Biomedical Engineering. 16, 678–687.

302   Herbert, R.D., Clarke, J., Kwah, L.K., Diong, J., Martin, J., Clarke, E.C., Bilston, L.E.,

303   Gandevia, S.C., 2011. In vivo passive mechanical behaviour of muscle fascicles

304   and tendons in human gastrocnemius muscle-tendon units. Journal of Physiology.

305   589, 5257–5267.

306   Herbert, R.D., Héroux, M.E., Diong, J., Bilston, L.E., Gandevia, S.C., Lichtwark, G.A.,

307   2015. Changes in the length and three-dimensional orientation of muscle fascicles

308   and aponeuroses with passive length changes in human gastrocnemius muscles.

309   Journal of Physiology. 593, 441–455.

310   Kawakami, Y., Ichinose, Y., Fukunaga, T., 1998. Architectural and functional features

311   of human triceps surae muscles during contraction. Journal of Applied Physiology.

312   85, 398–404.

313   Maganaris, C.N., 2003. Force-length characteristics of the in vivo human

314   gastrocnemius muscle. Clinical Anatomy. 16, 215–223.

315   Noorkoiv, M., Stavnsbo, A., Aagaard, P., Blazevich, A.J., 2010. In vivo assessment of

316   muscle fascicle length by extended field-of-view ultrasonography. Journal of

317   Applied Physiology. 109, 1974–1979.

318   Pincivero, D.M., Salfetnikov, Y., Campy, R.M., Coelho, A.J., 2004. Angle- and gender-

319   specific quadriceps femoris muscle recruitment and knee extensor torque. Journal

320   of Biomechanics. 37, 1689–1697.

321   Sharifnezhad, A., Marzilger, R., Arampatzis, A., 2014. Effects of load magnitude,

322   muscle length and velocity during eccentric chronic loading on the longitudinal

323   growth of the vastus lateralis muscle. Journal of Experimental Biology. 217, 2726–

324   2733.

325   Westing, S.H., Seger, J.Y., 1989. Eccentric and concentric torque-velocity

326   characteristics, torque output comparisons, and gravity effect torque corrections

327   for the quadriceps and hamstring muscles in females. International Journal of

328   Sports Medicine. 10, 175–180.

329
330

333   **Figure 1.** Schematic of the different methods of estimating fascicle length in the vastus

334   lateralis muscle. The top of the image shows the frame used to hold the two ultrasound

335   transducers. The extrapolate method (a) and intercept method (b) use only the

336   information from the proximal transducer, whereas the dual transducer method (c)

337   uses two separate fields of view. The extrapolate method calculates the remaining

338   portion of the muscle fascicle by dividing the remaining muscle thickness (h) by the

339   sine of the pennation angle (α). The intercept method calculates the remaining portion

340   of the muscle fascicle length by finding the intersection of the extrapolated paths of

341   the visible fascicle and deep aponeuroses, each defined by a respective linear

342   equation $y=mx+c$. The dual transducer method uses information from both regions of

343   interest (red dashed lines) to track the movement of two parts of a visible fascicle (L_1

344   & L_2).

345

346   **Figure 2.** Example data from a representative subject, showing the patterns of fascicle

347   length change (a) and force-length curves (b) for each method. (a) Torque is plotted

348   against the right axis (dotted). The vertical line indicates the occurrence of peak torque

349   development and the point at which fascicle length measurements were taken during

350   the trial. (b) The absolute force-length curves show that the curves are the same shape

351   but fascicle length ranges vary across methods. The line types in (b) match the legend

352   from (a).

353

354   **Table 1.** Coefficient of multiple correlation (CMC) values for extrapolate and intercept

355   methods compared to the dual transducer method. Data are shown as group mean ±

356   SD.

357

358

Figure 3. Fascicle length at maximum force (a) and fascicle shortening (b) determined by each of the three different methods. Data are shown as group mean ± SE. Annotations show significant differences between all groups at the relevant joint angle.

362

Figure 4. Force-length curves of the normalised data for the extrapolate method (a), the intercept method (b), and dual transducer method (c). Each point represents a data point on an individual force-length curve, normalised to the respective F_{max} and L_o. The curve fits represent a new fit of the normalised data points for each method.

367