Title

In vivo fascicle length measurements via B-mode ultrasound imaging with single vs dual transducer arrangements

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Abstract

Ultrasonography is a useful technique to study muscle contractions in vivo, however larger muscles like vastus lateralis may be difficult to visualise with smaller, commonly used transducers. Fascicle length is often estimated using linear trigonometry to extrapolate fascicle length to regions where the fascicle is not visible. However, this approach has not been compared to measurements made with a larger field of view for dynamic muscle contractions. Here we compared two different single-transducer extrapolation methods to measure VL muscle fascicle length to a direct measurement made using two synchronised, in-series transducers. The first method used pennation angle and muscle thickness to extrapolate fascicle length outside the image (extrapolate method). The second method determined fascicle length based on the extrapolated intercept between a fascicle and the aponeurosis (intercept method). Nine participants performed maximal effort, isometric, knee extension contractions on a dynamometer at 10^0 increments from 50-100^0 of knee flexion. Fascicle length and torque were simultaneously recorded for offline analysis. The dual transducer method showed similar patterns of fascicle length change (overall mean coefficient of multiple correlation was 0.76 and 0.71 compared to extrapolate and intercept methods respectively), but reached different absolute lengths during the contractions. This had the effect of producing force-length curves of the same shape, but each curve was shifted in terms of absolute length. We concluded that dual transducers are beneficial for studies that examine absolute fascicle lengths, whereas either of the single transducer methods may produce similar results for normalised length changes, and repeated measures experimental designs.

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, estimations of fascicle length from a single transducer have not yet been compared to length measurements from a greater FOV using two transducers.

The aim of the study was to determine if dynamic measurements of VL fascicle length using extrapolation methods with one transducer during isometric knee extension contractions match those made with two synchronised, in-series transducers. We hypothesised that the absolute lengths of the fascicles would differ between the single and dual ultrasound techniques, due to the ability to visualise the fascicle endpoint. However, we also predicted that any differences would be negligible for normalised length changes, and hence, would not affect observations made using a repeated measures design.

Methods

Protocol

Nine participants (age 26 ± 2.5 years, mass 72.8 ± 7.0 kg, height 178 ± 6.3 cm) provided informed consent to participate in the study. The study was approved by an institutional ethics committee. Each participant completed maximal effort, isometric, knee extension contractions on an isokinetic dynamometer (HUMAC NORM, CSMi Inc., Stoughton, MA, USA). A familiarisation session was completed to make sure that they could perform consistent maximal efforts. A second experimental session followed within 10 days, which included the ultrasound measurements. The two sessions used the same protocol and dynamometer position.

Participants were seated in the dynamometer with a hip angle of 80^0 and the dynamometer attachment adjusted to align with the flexion/extension axis of the left knee. A 60-s isotonic warm up protocol was performed using the interactive path program on the dynamometer. The isometric protocol consisted of randomised blocks of three maximal effort, isometric contractions at 10^0 increments from 50^0-100^0 of knee flexion. A straight leg was defined as 0^0 of knee flexion. For each contraction participants were instructed to perform a ramp contraction to maximal effort over a 3-s period, and hold the maximum effort for 1-s before relaxing. Two minutes rest was given between trials to avoid any potential fatigue effects.

Dynamometer measurements

Knee extensor torque and joint angle were sampled from the analogue output of the dynamometer using a CED Micro 1401 A/D converter at a 2kHz sample rate and recorded in Spike 2 software (Cambridge Electronic Design Ltd., Cambridge, England). The torque signal was filtered using a 10 Hz, first-order, low-pass, bi-directional Butterworth filter in Matlab (MathWorks Inc., Natick, MA, USA). The maximum gravity effective torque (maxGET) was taken as the resting torque with the knee at full extension (0^0). Torque was then gravity corrected using maxGET and joint angle (Pincivero et al., 2004; Westing and Seger, 1989). Passive torque was calculated as the difference between the resting torque and gravity corrected torque prior to the contraction. The best two-out-of-three trials based on maximal torque were analysed for each joint angle.

Ultrasound measurements

Muscle fascicle measurements of VL were made using two flat ultrasound transducers (LV7.5/60/96Z, TELEMED, Vilnius, Lithuania) that were held end-to-end by a custom made frame (Figure 1). Due to the shape of the transducer, there was a 22 mm gap between the visual fields of the transducers. A custom Matlab script was written to 'stitch' the images together (Figure 1c). The transducers were placed at approximately 50% thigh length, following a line between the greater trochanter and superior patella insertion. A self-adhesive compression bandage was used to secure the transducers to the thigh. The central frequency of the transducer was set at 5 MHz, image depth at 50 mm, and sampling rate of 80 Hz. A logic pulse from the first ultrasound system triggered data capture by the other system, which produced its own logic pulse. The two pulses were recorded by the A/D board to determine any delay between the onsets of image collection. A semi-automated tracking algorithm (Cronin et al., 2011; Farris and Lichtwark, 2016; Gillett et al., 2013) tracked the positions of the visible fascicle, and the deep and superficial aponeuroses, which was subsequently used to estimate fascicle length using three different methods.

Method 1 - Extrapolation

Fascicle length for the "extrapolation" method (Figure 1a) was calculated from the proximal image using the equation:

$$FL = \text{visible fascicle length} + h/\sin(PA)$$

where 'h' equals the vertical distance between the intersection of the visible fascicle with the image border and the deep aponeurosis; and PA equals the pennation angle of the tracked fascicle (Austin et al., 2010; Finni et al., 2003; Fontana et al., 2014).

Method 2 - Intercept

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

FL = visible fascicle length + predicted length

where the predicted length is equal to the distance between the visible fascicle's intersection with the image border and the intersection of the linearly extrapolated paths of the visible fascicle and deep aponeurosis (Blazevich et al., 2009).

Method 3 – Dual

The proximal and distal images of VL were used to separately track the positions of the proximal and distal endpoints of a line assumed to be representative of a single fascicle (Figure 1c). The proximal insertion and visible fascicle length was defined first, then the distal 'fascicle' was defined as the continuation of that line within the distal image. Fascicle lengths were calculated as the distance between the origin of the fascicle in the proximal image and the distal intersection with the deep aponeuroses in the distal image.

Due to the large proportion of fascicle length that is estimated, Methods 1 and 2 (extrapolate and intercept) are highly sensitive to changes in the orientation of the deep aponeurosis. As such, the coordinates of the tracking points were filtered using a 5 Hz, second-order, low-pass, bi-directional, Butterworth filter to reduce the chances of non-physiological, high frequency length changes as a result of the calculations. Fascicle lengths were then calculated from the filtered X-Y coordinates and interpolated to the analogue sampling rate.

Analysis

Quadriceps force was calculated as active torque divided by the angle specific VL moment arm, calculated individually using a modified *gait 2392* musculoskeletal model in OpenSim software and standard scaling procedures (Delp et al., 1990). The scale factors were determined from markers placed on anatomical landmarks of the pelvis and left lower limb. Fascicle length was recorded at rest and at the time of maximal quadriceps force for each contraction at each joint position. The change in fascicle length from the resting state to maximum quadriceps force was also calculated.

For each individual a force-length curve was fitted, based on physiologically appropriate models (Azizi and Roberts, 2010)

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

where F is force, L is fascicle length, a is roundness, b is skewness, and s is width. The curve fit was optimised using a nonlinear least squares method.

A coefficient of multiple correlation (CMC) analysis was performed for each joint angle, comparing the waveform fascicle lengths of Method 3 with each of the other estimation methods, averaged across two trials. A two-way repeated measures ANOVA (method x joint angle) was performed on fascicle length and fascicle length change data, with Dunnett's multiple comparisons where interactions were found. A one-way repeated measures ANOVA was used to compare L_o across methods. The coefficient of variation (R^2) of the force-length fits was calculated to measure how well the curve fit explained the variance in the data. An alpha level of 0.05 was used for all statistical tests. Values in text are shown as mean ± standard deviation (SD).

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

The main findings of the study suggest that fascicle length measurements made by the different methods result in absolute differences in fascicle length. However, these differences appear to be systematic and the pattern of length change between the different methods is consistent. Furthermore, the effect on normalised lengths is minimal.

We observed that a second ultrasound transducer is beneficial for visualising the distal changes in muscle orientation. The greater fascicle shortening and shorter fascicle lengths at maximal force in both of the single transducer methods may be due to underestimation of fascicle length by tracking only the proximal region of the muscle. The greater shortening resulted in lower predicted absolute L_o values, however that shift was not evident when utilising normalised fascicle lengths (Figure 4). Therefore, if understanding absolute fascicle lengths is important, using a second ultrasound transducer to visualise the distal fascicle endpoint is recommended. The use of either single transducer method would provide similar results for experimental data measuring differences in muscle contraction dynamics within-participants. Thus, for a repeated measures design, the choice of estimation method may shift the overall data set but not alter the effects of experimental factors.

Limitations

We assumed that a second transducer is beneficial because it is possible to visualise the distal muscle region. However, the dual transducer method used in this study was not validated against any other fascicle measurement technique such as diffusion tensor imaging (Bolsterlee et al., 2015) or extended FOV techniques (Noorkoiv et al.,

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

Conflict of Interest Statement

The authors have no conflict of interest to disclose.

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Figure Captions

Figure 1. Schematic of the different methods of estimating fascicle length in the vastus lateralis muscle. The top of the image shows the frame used to hold the two ultrasound transducers. The extrapolate method (a) and intercept method (b) use only the information from the proximal transducer, whereas the dual transducer method (c) uses two separate fields of view. The extrapolate method calculates the remaining portion of the muscle fascicle by dividing the remaining muscle thickness (h) by the sine of the pennation angle (α). The intercept method calculates the remaining portion of the muscle fascicle length by finding the intersection of the extrapolated paths of the visible fascicle and deep aponeuroses, each defined by a respective linear equation $y=mx+c$. The dual transducer method uses information from both regions of interest (red dashed lines) to track the movement of two parts of a visible fascicle (L_1 & L_2).

Figure 2. Example data from a representative subject, showing the patterns of fascicle length change (a) and force-length curves (b) for each method. (a) Torque is plotted against the right axis (dotted). The vertical line indicates the occurrence of peak torque development and the point at which fascicle length measurements were taken during the trial. (b) The absolute force-length curves show that the curves are the same shape but fascicle length ranges vary across methods. The line types in (b) match the legend from (a).

Table 1. Coefficient of multiple correlation (CMC) values for extrapolate and intercept methods compared to the dual transducer method. Data are shown as group mean ± SD.

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.

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.