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Short communication

In vivo measurements of biceps brachii and triceps brachii fascicle lengths using extended field-of-view ultrasound

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ABSTRACT

Muscle fascicle lengths are commonly measured in vivo using static 2D ultrasound. However, static ultrasound is best suited for muscles with shorter, pennate fascicles, in which entire fascicles can be viewed in one static image. An informal review of data from cadaver dissections suggests that over 60% of muscles in the upper and lower limbs have optimal lengths longer than the field-of-view of standard ultrasound transducers. Extended field-of-view ultrasound (EFOV) has been validated for measurement of fascicle lengths, but has yet to be implemented in the upper extremity in humans. In this study, EFOV ultrasound was used to measure the lengths of fascicles sampled from the anterior portion of the biceps brachii (long head) and the distal half of the triceps brachii (lateral head). Data were collected from both limbs of eleven healthy subjects in three elbow postures under passive conditions. Image analysis was completed via Image J. Fascicle length measurements were highly reliable, with intra-class correlations ranging from .92 to .95 for biceps and .81–.92 for triceps ($p < .001$). Systematic, significant differences in measured lengths, consistent with muscle function, were observed between elbow positions. In vivo measurements for both muscles in this study were within the range of cadaver data. This work establishes the feasibility and reliability of EFOV ultrasound for measurement of the long fascicles of muscles in the upper limb.

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1. Introduction

Muscle architecture, an important determinant of an individual muscle's function, describes the number, length, and arrangement of a muscle's fibers and their orientation with respect to the axis of force generation (Lieber and Friden, 2000; Lieber and Ward, 2011). Optimal fiber length (defined as the length at which the muscle generates its maximum isometric force (Zajac, 1989)), is a critical architectural parameter that defines a muscle's excursion capacity and absolute shortening velocity (Lieber and Friden, 2000). To calculate optimal fiber length, researchers must first measure the lengths of a sample of its fascicles, the corresponding lengths of the sarcomeres within those fascicles, and then normalize the fascicle lengths (Lieber et al., 1990; Murray et al., 2000; Ward et al., 2009; 2006; Wickiewicz et al., 1983) to the optimal sarcomere length of human muscle (2.6–2.8 μm (Burkholder and Lieber,

2001; Lieber et al., 1994; Walker and Schrodt, 1974; Woledge et al., 1985)). Fascicle lengths are measured (vs. fiber lengths) due to challenges associated with isolating individual fibers from human muscle.

Traditionally, samples of fascicles for length measurements are obtained from cadaver dissections (Murray et al., 2000; Ward et al., 2009; Wickiewicz et al., 1983). However, with the rise of multiple imaging modalities, subsets of muscle architectural parameters are increasingly assessed in vivo (Bolsterlee et al., 2015; Kwah et al., 2013; Lansdown et al., 2007; Scott et al., 1993). The most common imaging modality used for the in vivo assessment of fascicle length is ultrasound, shown to be both valid and reliable for the in vivo measurement of fascicle length for a number of muscles (Kwah et al., 2013). The most problematic limitation of static ultrasound imaging for in vivo measurement of fascicle lengths is the inability to view entire fascicles that are longer than the probe field-of-view (generally 4–6 cm in standard linear transducers (Weng et al., 1997)). Thus, muscles with longer fascicles are only rarely assessed in living subjects. Importantly, recent work has established extended field-of-view ultrasound (EFOV US) as a reliable and accurate method for imaging fascicles that are longer than a standard US

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image (Ema et al., 2013; Fornage et al., 2000; Noorkoiv et al., 2010). Specifically, a gold standard study that compared fascicle lengths measured from the swine vastus lateralis using EFOV to direct measurements from dissected fascicles reported the accuracy of EFOV to be $2.4 \pm 1.2\%$, with an intra-class correlation coefficient of .99 (Noorkoiv et al., 2010).

The aim of this study is to use extended field-of-view ultrasound to quantify *in vivo* lengths of samples of fascicles from the biceps and triceps brachii and to quantify the reliability of our methodology. To our knowledge, this study represents the first application of EFOV US in the upper extremity for these purposes.

2. Materials and methods

2.1. Participants

Eleven subjects (6 female, 5 male, age range 23–71, 53.7 ± 13.4), with no history of significant shoulder or elbow injury, or any evidence of neurological disorder, volunteered to participate in the study. Northwestern University Institutional Review Board approved the human subject protocol, and all subjects provided informed consent prior to participating in the study.

2.2. Experimental setup

Subjects were seated, trunk secured, with the arm in approximately the horizontal plane (shoulder abduction 85° , shoulder rotation 0° , wrist in neutral). Subjects were casted at the forearm and wrist and secured to a metal manipulator (System 3, Biodex Medical Systems, Inc., Shirley, NY), positioned in different trials to enable measurements at different elbow postures. Surface EMG electrodes (Delsys Bagnoli™ 16-channel desktop EMG system, Delsys, Inc., Natick, MA) were placed on the biceps and triceps brachii to monitor muscle activity throughout the experiment.

2.3. Ultrasound image acquisition

In order to quantify muscle fascicle lengths in the upper extremity, B-mode extended field-of-view ultrasound images (Siemens Antares™ Siescape v.5 software, Siemens Medical Solutions USA, Inc., Mountain View, CA (Weng et al., 1997)) were taken of the biceps brachii (long head) and triceps brachii (lateral head) in both arms. The EFOV algorithm used in the Siescape software has been described in detail previously (Weng et al., 1997), and validated for the measurement of muscle fascicle lengths (Noorkoiv et al., 2010). For both muscles, images were acquired using a 11.43-MHz linear array probe (45 mm width) in three elbow positions: extended (25°), neutral (55°), and flexed (80°) with each muscle relaxed, as verified with EMG.

In order to optimize image acquisition and repeatability of the imaging process, prior to image acquisition the line of action of the biceps brachii (long head) was established through palpation and the use of static ultrasound to establish fascicle orientation for each subject. After verifying fascicle orientation, a line was drawn on the skin tracking the probe path that visualized muscle fascicles using static ultrasound. A similar method was followed for establishing the orientation of the

fascicles for the distal portion of the triceps brachii (lateral head). During dynamic image acquisition, the probe was oriented parallel to the fascicle orientation and perpendicular to the skin.

2.4. Data analysis

For each position, the three best images (defined subjectively by the experimenter as images with the most continuously visible fascicles) were chosen by visual inspection for data analysis (Fig. 1). Fascicles were measured by manually tracing a fascicle along its path using an open-source digitizing software (Image J 2.0.0, Wayne Rasband, National Institutes of Health, Bethesda, MD) to measure its length. For each image, four fascicles were measured, for a total of 12 measurements per position. For the biceps brachii, all fascicle measurements were made in the anterior portion of the muscle due to optimal image quality in that region (Fig. 2). For the triceps brachii (lateral head), all measurements were made in the section of the image of the distal muscle for which fascicles were consistently viewable for their entire length, from origin to insertion (Fig. 2).

2.5. Statistical analysis

For each muscle, two-factor (arm, position) repeated measures ANOVA tested whether average fascicle length (mean of the 12 repeated measurements) differed between the three elbow postures or across arms. Intra-rater error associated with the manual digitization process was quantified via the typical error of measurement (TEM, (Bland and Altman, 1996)). Intra-rater measurement reliability was assessed using the coefficient of variation (Atkinson and Nevill, 1998; Noorkoiv et al., 2010) for repeated measurements of the same fascicle. To establish these metrics, the same rater measured one fascicle in one image per position for all 11 subjects (33 measurements), and repeated the same measurement 2 days later. TEM was calculated as the standard deviation (SD) of the difference between the fascicle measurements divided by $\sqrt{2}$. Coefficient of variation (CV%) was calculated using the following equation:

$$CV\% = \frac{\text{Standard deviation of repeated measurement}}{\text{Mean of repeated measurement}}$$

To evaluate the reliability of fascicle measurements, the intra-class correlation coefficient (ICC, two-way random effects model, absolute agreement) was calculated within each image (among the 4 measurements), and across images in a trial (12 total measurements), with an ICC of $\geq .75$ indicating excellent reliability (Andresen, 2000). Finally, we quantified differences in fascicle lengths between arms by calculating the percent difference in average fascicle length:

$$\frac{(\text{Dominant avg. fascicle length} - \text{Nondominant avg. fascicle length})}{\text{Dominant avg. fascicle length}} \times 100$$

where positive indicates longer fascicles in the dominant limb. Significance for all tests was set at $p < .05$, and Bonferroni correction was used where appropriate to adjust for multiple comparisons.

3. Results

Measured biceps and triceps fascicle lengths were consistently longer than the field-of-view of the ultrasound probe (4.5 cm field-of-view). Across all 11 subjects and each of the 3 elbow

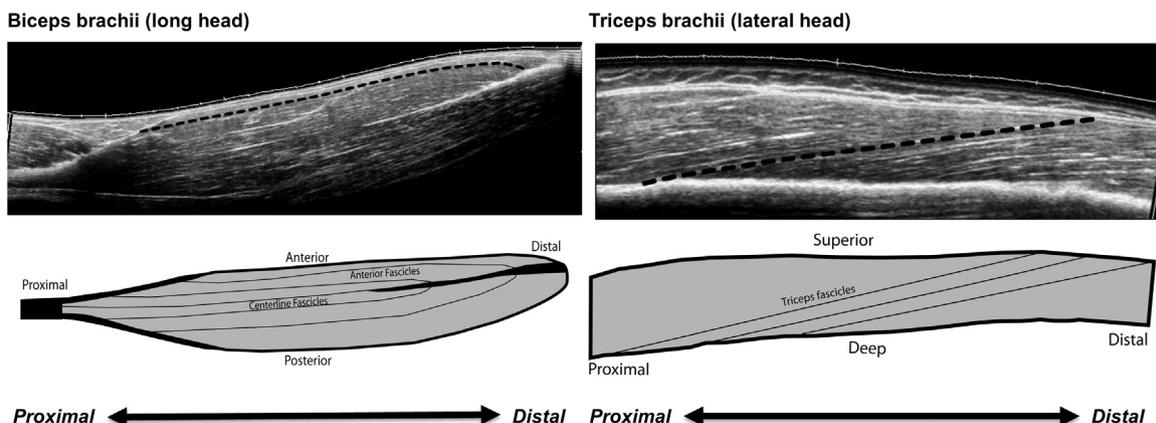


Fig. 1. (Top) Extended field-of-view ultrasound images of biceps brachii long head (left) and triceps brachii lateral head (right) in the neutral position. The dashed line represents a single fascicle as outlined during the data analysis protocol. (Bottom) Schematic diagrams of biceps brachii (left) and triceps brachii (right) illustrating each muscle's architecture.

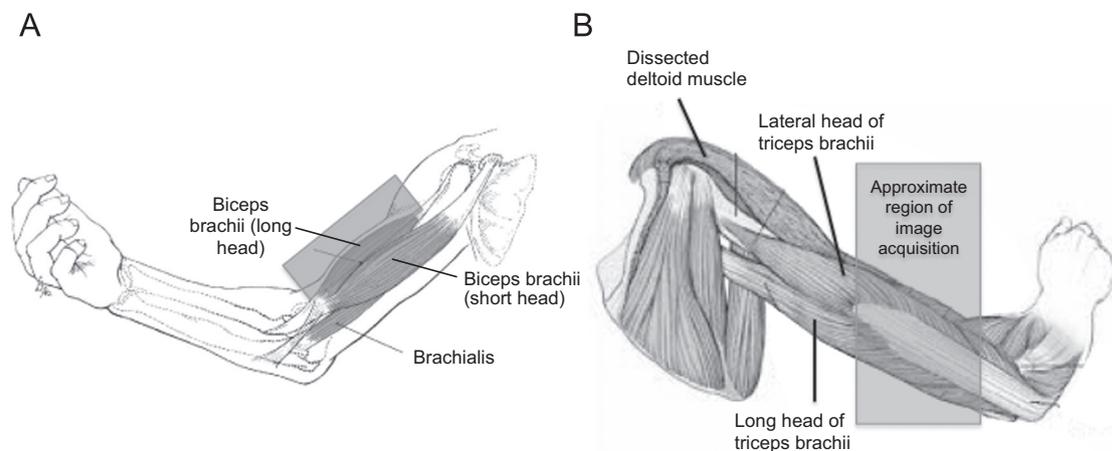


Fig. 2. Anatomy of the biceps and triceps brachii. (A) The shaded region indicates the approximate plane of image acquisition for the long head of the biceps brachii. (B) The shaded region represents the approximate region of image acquisition in the distal portion of the triceps lateral head. Adapted from *Surgical Exposures in Orthopaedics: The Anatomic Approach* (Hoppenfeld et al., 2009).

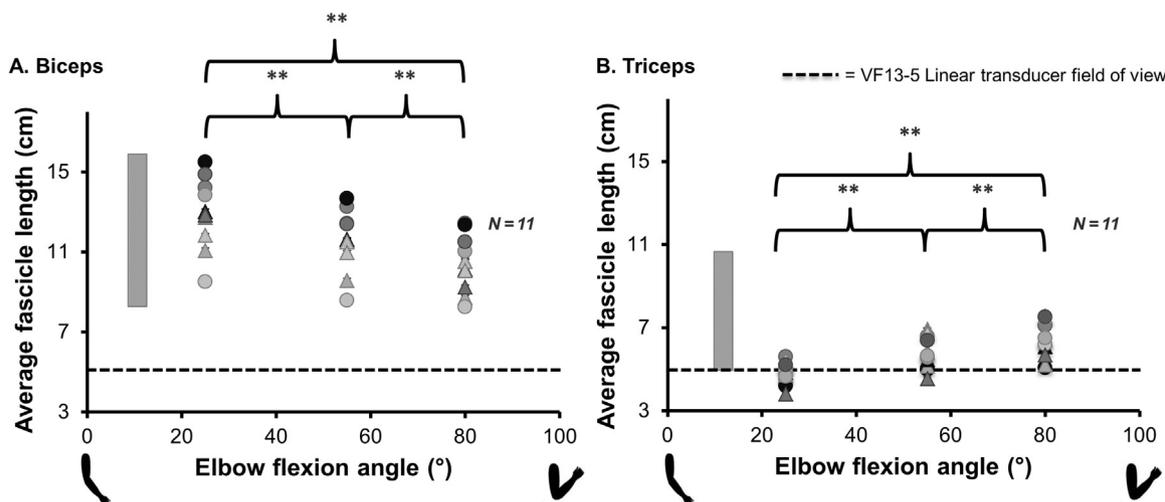


Fig. 3. Biceps brachii long head (A) and triceps brachii lateral head (B) average fascicle lengths measured in $N=11$ healthy control subjects using extended field-of-view ultrasound imaging. The shaded bar to the left in each plot represents the range of non-normalized fascicle measurements seen in 5 male cadaver specimens (Murray et al., 2000). Biceps fascicle lengths were substantially longer than the field-of-view available for the linear transducer used in our study (4.5 cm). For triceps brachii, only two subjects had average fascicle lengths that fell within the range of the transducer's field-of-view. Even in these subjects, these lengths only occurred in the most extended elbow position, where triceps is the shortest. Fascicle lengths were significantly different between positions ($p < .001$) for both muscles.

positions evaluated, biceps fascicles ranged from 8.26 cm to 15.51 cm (Fig. 3A). These lengths exceeded the field-of-view of the probe for static imaging by at least 1.8 times, with the largest value measured (15.51 cm) being almost 3.5 times longer than the probe field-of-view. Triceps fascicles sampled from the distal half of the lateral head were substantially shorter than the fascicles measured from the biceps brachii (Fig. 3B); the longest average triceps fascicle length measured among our subjects (7.53 cm) was still shorter than the shortest biceps fascicle lengths. Despite this, triceps fascicle lengths for all subjects ranged from 1 to 1.7 times the field-of-view of the ultrasound probe in the neutral and flexed positions.

Fascicle measurements for both muscles were highly reliable. The typical error of measurement (TEM) for the digitizing process was on average $.84 \pm .56$ mm for biceps and $.53 \pm .39$ mm for triceps. The CV% calculated for the intra-rater measurement process was on average $1.0 \pm .7\%$ for biceps brachii and $1.3 \pm 1.0\%$ for triceps brachii. ICC analysis revealed highly reliable fascicle measurements within single images (4 measurements per image), and across images, with ICCs for biceps across both arms ranging from

Table 1

ICC values with 95% confidence intervals for fascicle measurements within each image (4 measurements) and across all three images (12 total measurements) across all positions for both arms. ICC values ranged from .916–.949 for biceps brachii and from .812–.919 for triceps brachii, indicating excellent reliability of fascicle measurements. p -Values for all ICC values were $< .001$.

Arm	Biceps (within images)	Biceps (across 3 images)	Triceps (within images)	Triceps (across 3 images)
Dominant	.949 95% CI: (.931–.963)	.929 95% CI: (.892–.959)	.919 95% CI: (.890–.942)	.897 95% CI: (.841–.943)
Non-dominant	.933 95% CI: (.911–.952)	.916 95% CI: (.872–.951)	.889 95% CI: (.852–.920)	.812 95% CI: (.722–.892)

.916 to .949 ($p < .001$, Table 1), and triceps ICC values ranging from .812 to .919 ($p < .001$).

Systematic, significant differences in measured fascicle lengths were observed between elbow positions for both the biceps and triceps. Across all subjects, biceps fascicles increased an average of

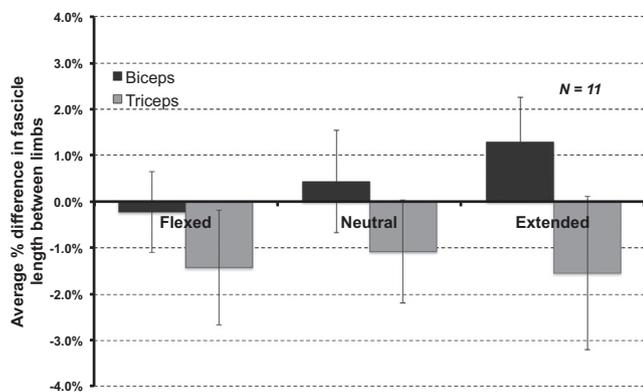


Fig. 4. Average interlimb % differences in average fascicle length for biceps brachii (long head) and triceps brachii (lateral head) for different elbow postures, shown with ± 1 standard error. Percent differences were normalized to the dominant arm. $N = 11$.

2.50 cm (SE .23) as the elbow was extended, while triceps fascicles decreased an average of 1.53 cm (SE .19) as the elbow was extended (Fig. 3). For both biceps and triceps, there was a significant main effect for position ($p < .001$), as well as significant pairwise comparisons between individual positions ($p < .001$, Bonferroni correction for multiple comparisons). There were no significant main effects for the factor of arm ($p = .455$ biceps, $p = .375$, triceps). Across all positions, the largest average biceps percent difference between limbs was 1.3% (Fig. 4). Similarly, the largest average percent difference in triceps fascicle lengths between limbs was -1.5% (Fig. 4).

4. Discussion

This study provides the first known application of extended field-of-view ultrasound technology for measurement of muscle fascicle lengths in the upper extremity. We demonstrate excellent reliability in repeated fascicle measurements, showing high reliability in measurements made both within an image and across different images within a session. We also demonstrate that this methodology characterizes differences in fascicle lengths that are expected from anatomy as a muscle is lengthened (biceps during elbow extension) or shortened (triceps during elbow extension) over a joint's range of motion.

The range of biceps and triceps fascicle lengths measured using EFOV US were within the range of non-normalized fascicle length measurements quantified in a previous cadaveric study (Murray et al., 2000). The observed variation in average fascicle lengths across all 11 subjects was large; previous cadaver research has shown similar interspecimen variability and a significant correlation between subject size and optimal fiber length (Murray et al., 2000). Although we did not quantify anthropometric data from our subjects, the similar variability observed in cadavers for the same muscles suggests different sizes among our various participants as a reasonable explanation. We have also shown that fascicle lengths are significantly different between various elbow positions, and the magnitude of change is greater than the typical error of measurement (TEM) for the digitizing process we report for both muscles.

We were able to demonstrate high reliability in our fascicle measurements as evidenced by ICC values greater than .8 for both biceps and triceps fascicle lengths. Although ultrasound has been shown to be a reliable and valid method for measurement of muscle fascicle length, previous research has also shown that some error in fascicle length can occur when the probe orientation is tilted away from the true fascicular plane (Bénard et al., 2009; Klimstra et al., 2007; Weng et al., 1997). Therefore, any variation in

ultrasound probe orientation or position between images in this study could contribute to small differences in measured fascicle measurements. In the present study, multiple practice scans were used to maximize image quality and visualization of complete fascicles. The use of a visual cue for line of action of the muscle also assisted the experimenter to achieve consistent scans within a trial. Despite slightly lower ICC values for fascicle measurements across images, ICC values were still in a range considered excellent, which would indicate that any difference in measurements across images due to probe orientation was minimal. Finally, the image reconstruction algorithm employed in this study takes into account small tissue motion artifacts (Weng et al., 1997) that could be present due to variable transducer pressure during the length of the scan. Therefore, any measurement error resulting from variation in transducer pressure during the scan is expected to be minimal.

5. Conclusions

We demonstrate application of extended field-of-view ultrasound to measurement of the long fascicles of muscles in the upper limb. We establish that these methods produced reliable length measurements for samples of fascicles from the long head biceps and the lateral head of triceps brachii, and were sensitive enough to quantify changes in fascicle lengths resulting from changes in joint position. This study initiates the evaluation of in vivo biceps and triceps brachii fascicle architecture, previously ignored due to the limitations of traditional static US methods.

Conflict of interest statement

I declare that none of the authors of this work have had any financial or personal relationships with other individuals or organizations that could inappropriately influence or bias this work.

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