ORIGINAL ARTICLES

Repeatability of ultrasound in assessment of distal biceps brachii tendon

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ABSTRACT

Objective: Examine the repeatability of ultrasound imaging for capturing cross sectional area (CSA), tendon length and elongation of the distal biceps brachii (dBB) tendon at rest and during submaximal elbow flexion contractions. The secondary purpose was to assess the influence of these architectural measures on tendon mechanics of stress and strain.

Methods: Within a testing session and between two days CSA, tendon length and elongation of the dBB were captured with ultrasound. Measures were compared within a session and between days. Pearson's correlations were performed to determine the intra-class correlation coefficients. Bland and Altman plots were used to identify the agreement between measures as well as the bias in measurements. Paired *T*-test were performed to ensure the calculated variables did not differ between days.

Results: Resting tendon CSA was repeatable and strongly correlated (r = 0.98) within a session and between days; however, modest differences were observed in resting tendon length between days ($\sim 1.8 \text{ mm}$) although values were correlated (r = 0.98). During submaximal contractions of 10%-80% maximal tendon elongation (r = 0.83) and CSA (r = 0.98) were also repeatable. From the measures of elongation and CSA, the calculated values of tendon strain (r = 0.97) and stress (r = 0.96) were also repeatable.

Conclusions: Elongation and CSA of the dBB tendon captured with ultrasound are repeatable between testing sessions. From these measures tendon mechanics can be calculated to define the tendon's role in upper limb tasks, long-term adaptation and diagnostics.

Key Words: Static, Dynamic, Submaximal contractions, Tendon mechanics, Stress, Strain, Architecture, Cross-sectional area

1. INTRODUCTION

Ultrasonography is increasingly used to visualize and quantify in vivo structures of muscle and tendon architecture in research and clinical diagnostic settings.^[1,2] As with any imaging technique used to visualize and quantify in vivo structures, it is important to know the tendon reliability and repeatability of measures obtained with the technology. While magnetic resonance imaging (MRI) remains the goldstandard of imaging, ultrasonography allows for real-time assessment of structures under dynamic conditions.^[3,4] Albeit somewhat equivocal due to limited definition of the tendon border,^[5,6] measures of tendon cross-sectional area (CSA) using ultrasound are generally reliable and repeatable for the patellar^[7–9] and Achilles^[7,10–13] tendons. Additional to measures of tendon CSA, panoramic ultrasound scans are a repeatable and reliable technique for measuring Achilles tendon length.^[14–16] Given the number of studies that have reported reliability and repeatability of the patel-

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lar and Achilles tendons it is surprising that few reports are available for tendons of the upper limb. Although reliability of tendon thickness for the proximal long head tendon of the biceps brachii was reported,^[17] the distal biceps brachii (dBB) tendon was not studied. This tendon is particularly important as most upper limb movements are regulated through its contribution to forearm flexion and supination.

Repeatability and reliability of architectural measures of the tendon are especially important in order to define the involvement of the tendon in transferring muscular force to the bone. This calculated quantification of tendon mechanics relies heavily on architectural measures of CSA, length and tendon elongation under load that can be acquired with ultrasonography. Whereby the quantification of tendon stress requires acquisition of the CSA, while resting tendon length and elongation must be defined in the assessment of strain. Thus, from a methodological point of view it is important to investigate the repeatability of the dBB tendon architecture using ultrasonography. Similar to architecture there are a number of reports that identify mechanical properties of the Achilles and patellar tendons across static and dynamic situations.^[3,4,18] but no study to-date has examined the mechanics of the dBB tendon. In order to understand the mechanics of the upper limb, the repeatability of architectural measures, notably those of the dBB should be established.

For studies on tendon mechanics to progress in the upper limbs ultrasound measures of tendon CSA, length and elongation of the dBB tendon are clearly required. Thus, the present study examines the repeatability of ultrasound imaging to record CSA, length and elongation of the dBB tendon at rest and during submaximal elbow flexion contractions. The secondary purpose of this study was to assess the influence of these architectural measures on tendon mechanics of stress and strain. It was hypothesized that resting tendon length, tendon elongation and tendon CSA measures, along with the respective mechanics of the tendon would be repeatable within the same individual between two testing sessions.

2. METHODS

The first testing session for this repeatability study consisted of measurements gained from one of two separate studies. Participants from these studies were invited back for the repeatability testing. Five males $(24 \pm 4 \text{ yrs}, 180.0 \text{ cm} \pm 10.6 \text{ cm}, 75.6 \text{ kg} \pm 6.5 \text{ kg})$ volunteered for re-examination on a second day that was matched to the first. All procedures were approved by the University Research Ethics Board (BREB) (H14-00165; H16-00948). All participants were free of neuromuscular disorders, injuries to the right arm and active tendinopathy. Those who participated in the second day of testing had not changed activity patterns since the first. Body position and upper limb joint angles were matched between sessions in order for the resting measurements of the dBB on day-2 to correspond to day-1. In order to acquire the dynamic measures of tendon elongation and CSA relative submaximal forces were matched on the second day to those performed on the first.

2.1 Experimental set-up

Participants were seated in a custom-built chair with knees and hips positioned at $\sim 90^{\circ}$ of flexion. The right arm was abducted 10-15 degrees, the elbow flexed at 110° (full elbow extension being 180°), and the forearm rested on a padded support with the wrist in neutral and grasping the handgrip bar (see Figure 1). Force was recorded using a linear calibrated force transducer (MLP150, 68 kg, 266 V sensitivity; Transducer Techniques, Temecula, CA, USA) positioned under the participant's wrist, and sampled at a rate of 2,381 Hz for analog to digital conversion (1401 Plus; Cambridge Electronic Design (CED), Cambridge, England). Force was collected and stored for offline analysis (Spike 2; CED, Cambridge, England). A real-time force tracing was displayed on a monitor located 1 meter in front of the subject for visual feedback. The ultrasound probe (ML6-15, 5-15 MHz linear array probe, LOGIQ E9, General Electric, Fairfield, CT, USA) was encased in a custom designed probe holder and secured to the arm over the dBB muscle-tendon junction (MTJ) (see Figure 2). The probe was oriented in the longitudinal or cross-sectional planes to record tendon elongation and CSA, respectively. Through monitoring of the ultrasound images, care was taken to ensure consistent positioning of the probe throughout the protocol, and that the tendon and subcutaneous tissue were not compressed due to pressure from the probe. A hyperechoic marker was placed between the probe and the skin surface to ensure the probe did not move during contraction.

2.2 Anatomical measures

The moment arm of the dBB tendon was obtained by locating the distal MTJ of the biceps brachii and the insertion of the tendon onto the radius using ultrasound. These points were marked on the skin surface and a linear edge was placed between these two points along the line of the tendon. The perpendicular distance form this linear edge to the lateral epicondyle of the humerus was measured as the biceps brachii moment arm. The lever arm length was measured as the distance from the head of the radius to the force transducer located immediately below the handgrip bar.



Figure 1. (A) The arm of the chair for positioning of the participant's arm, (B) the forearm apparatus is shown in an anterior view for positioning of the subjects to measure force and, (C) the ultrasound probe. The ultrasound probe is positioned over the tendon in a custom designed apparatus and visible on the picture are EMG electrodes to acquire muscle activation. EMG, electromyography



Figure 2. Representative ultrasound images of tendon elongation (A) and tendon CSA (B) of the dBB at rest (top panel) and 75% MVC (bottom panel). MVC, maximal voluntary contraction; dBB, distal biceps brachii tendon; LH, long head; SH, short head

2.3 Resting ultrasound measures

Resting length of the dBB tendon was recorded using a Logiqview® (GE LOGIQ E9; General Electric, Fairfield, CT, USA) scan that enables panoramic capture of the structure of interest. The oblique course of the tendon was visualized from the superficial distal MTJ overlying the brachialis to its deep insertion onto the radial tuberosity. Unlike the tendons of the wrist,^[19] the fibrillated structure of the dBB tendon is not easily visualized on ultrasound. Tendon CSA was recorded using a single image capture of the tendon in cross-section 1 cm distal to the dBB MTJ.

2.4 Experimental Protocol

Day 1

Following resting anatomical and ultrasound measures, participants performed two-three practice isometric elbow flexion contractions to familiarize themselves with the device. Participants then performed three maximal voluntary contractions (MVC) from which the highest force level achieved was used to calculate submaximal force levels. The submaximal contractions consisted of a three-second ramp increase in force, a ten-second plateau followed by a three-second relaxation to baseline. Ultrasound videos of the dBB tendon were recorded at a frame rate of 31 Hz throughout the duration of the tracking tasks. Each submaximal tracking task was repeated four times to obtain two recordings of tendon elongation (see Figure 2A) and two of CSA (see Figure 2B) at each submaximal force level. The order of ultrasound scans (elongation and CSA) were randomized in blocks for each subject and the order of submaximal force levels were randomized within each block.

Day 2

Ultrasound measures were repeated in the opposite order from which they were conducted on Day-1. The two testing days were separated by five months (range of two-eight months). Physical activity patterns and injury were discussed to ensure that it did not change between tests thereby, use or disuse would have minimal influence on the tendon measures. Identical to the first session, subjects performed MVCs and subject inclusion required the achievement of the maximal force from the first day in order to execute similar submaximal efforts on day-2. The order of the tracking tasks was randomized for each participant within each testing session. The submaximal force levels used for experiment one were (10%, 40% and 80% MVC), while (10%, 50% and 75%) were used for experiment two. These force levels were matched on day-2 to the day-1 data collection session.

2.5 Data analysis

Data analysis was performed on two separate days by one experimenter. Measurements of ultrasound recordings from the first day of testing were completed prior to the second day of testing; however, measures were not reviewed or recalled due to the time difference between sessions. All measurements were completed using the inherent measurement tool platform contained on the ultrasound software (GE LOGIQ E9). Tendon length of the dBB tendon was measured as the distance from the MTJ to its insertion onto the radial tuberosity using an open spline trace. Tendon CSA was measured by tracing the border of the tendon and quantified at rest and for the submaximal contractions. Tendon elongation was measured by marking the distance from the edge of the screen to the distal MTJ of the biceps brachii along the tendon in rested and contracted states, and the difference in these two measures was considered tendon elongation. The distance from the edge of the screen to the hyperechoic marker was measured to ensure no movement of the probe occurred during contraction.

2.6 Statistical analysis

To determine the repeatability of the experimenter measures (contraction one and two, day one) and measurement day (day-1 and day-2) tendon CSA, tendon length and elongation were compared. Pearson's correlations were performed to determine the intra-class correlation coefficients between measures on day-1 and day-2. Bland and Altman plots^[20] were used to identify the agreement between measures from day-1 and day-2, as well as determine the bias in measurements and the 95% limits of agreement. A paired *T*-test was also performed between the MVCs of day-1 and day-2 to ensure the calculated relative force levels did not differ between days. Values are expressed as means \pm standard deviation (*SD*) and probability of statistical significance was set at $p \leq .05$.

3. RESULTS

Maximal voluntary contraction force $(257.8 \pm 28.7N)$ did not differ between test days (p > .05), thus measures of elongation and CSA are presented for the same absolute and relative force levels between days.

3.1 Resting ultrasound measures

Resting tendon length for day-1 (68.52 mm \pm 10.5 mm) compared to day-2 (66.7 mm \pm 10.4 mm) differed statistically (p < .05). Yet, there was a strong correlation between the two sessions for resting tendon length (r: 0.98, p < .01) with a bias difference of 2.52 mm \pm 2.16 mm and limits of agreement between -1.71 mm and 6.7 mm (see Figure

 $(24.8 \text{ mm}^2 \pm 2.5 \text{ mm}^2)$ and day-2 $(24.5 \text{ mm}^2 \pm 2.1 \text{ mm}^2)$ (p > .05). A strong correlation was observed between test Figure 3C, D).

3A, B). Resting tendon CSA did not differ between day-1 days (r: 0.95, p < .01) with a bias of 0.3 mm² \pm 0.8 mm² and limits of agreement between -1.2 mm^2 and 1.9 mm^2 (see



Figure 3. Resting architecture measures. Bland and Altman plots (A, C) illustrating the agreement of the measures between day-1 and day-2 and correlation plots (B, D) between day-1 and day-2 for the resting tendon length (A, B) and CSA (C, D). CSA, cross-sectional area

3.2 Dynamic ultrasound measures

The within session measures of elongation and CSA did not differ (p < .05) and had strong correlations (r: 0.83-0.98). The between day comparisons of day-1 and day-2 also had a strong correlation for CSA (r: 0.89, p < .0001) with a bias of 0.64 mm² \pm 0.84 mm² and limits of agreement between -1.0 mm² to 2.28 mm² for submaximal forces of 10%-80% MVC. Similarly the average elongation between days did not differ and had a strong and significant correlation (p < .0001; r: 0.944) with a bias of -0.64 mm \pm 1.3 mm and limits of agreement between -3.22 mm and 1.95 mm (see Figure 4A, B). Strain also did not differ between sessions and had a strong significant correlation (r: 0.97, p < .0001), with a bias of -1.41% \pm 2.11% and limits of agreements between -5.50% and 2.73% (see Figure 4C, D). Tendon force did not differ (p > .05) and had a strong correlation between days (p < .0001; r: 0.96) with a bias of -48.4 N/mm \pm 135.0 N/mm and limits of agreement between -13.6 N/mm and 7.92 N/mm. Tendon stress did not differ between day-1

and day-2 (p > .05) and had a strong correlation between days (r: 0.97, p < .0001), with a bias of -2.86 N/mm² ± 5.50 N/mm² and limits of agreement between -13.6 N/mm² to 7.92 N/mm² (see Figure 4E, F).

4. DISCUSSION

To our knowledge this is the first study to examine the repeatability of ultrasound in quantifying tendon architecture of the distal biceps brachii at rest and during submaximal contractions. We found resting tendon CSA was repeatable within a session and between days; however, modest differences were observed in resting tendon length between days. Additional to measures gained at rest, ultrasonography was used to capture the tendon during submaximal contractions of 10%-80% MVC. Across force levels tendon elongation and CSA were also repeatable within a session and across two separate testing sessions for the same individual. From the measures of elongation and CSA, the calculated values of tendon strain and stress were also repeatable between sessions.



Figure 4. Dynamic architecture measures. Bland and Altman plots (A, C, E) highlighting the agreement of the measures between day-1 and day-2 and correlation plots (B, D, E) between day-1 and day-2 for the submaximal forces ranging from 10%-80% MVC. Tendon elongation (A, B), strain (C, D) and stress (E, F) did not differ between sessions and showed strong correlations. MVC, maximal voluntary contraction; %, percentage; mm, millimeter; N, newton

Repeatability of resting dBB tendon CSA aligns with previous studies on the patellar^[7–9] and Achilles^[7,10–13] tendons and extends the findings of repeatability to the upper limb. In contrast to our hypothesis and previous studies on the Achilles tendon^[14–16] resting dBB tendon length differed statistically between days. The absolute difference between day-1 and day-2 was 1.82 mm but the correlation between the two measures was very high (r: 0.98). The small sample size is a limitation of the current study and likely contributes

to the difference in resting tendon length observed between sessions. Although increasing sample size would assist in reporting for this measure, all other images acquired and variables measured with ultrasound showed no differences between days. The statistical difference could be accounted for by subtle changes in subject positioning between day-1 and day-2 which might alter joint angle in which the image was acquired. For all subjects the resting tendon length on day-2 was shorter, thus positioning of the elbow is likely a key factor. Optimizing joint angle is necessary to maximize force production when evaluating mechanics. Yet, at 110° an additional imaging challenge is created at this joint angle that is not present when the forearm is extended or in other tendons that have a relatively flat surface. In Logiqview(R) (GE LOGIQ E9; General Electric, Fairfield, CT, USA) to gain these measures the operator is required to monitor and maintain consistent contact with the undulating anatomy and this challenge is minimized when scanning flat and straight surfaces. However, this case study identifies that ultrasound is a viable tool to capture the dBB tendon architecture given the repeatability of tendon elongation and CSA during submaximal contractions both within and between days. Repeatability of these resting and dynamic measures is crucial for the subsequent quantification of tendon mechanics.

From the resting and dynamic ultrasound measures, tendon mechanics of elongation, strain, tendon force and tendon stress were also repeatable across two separate test days. Tendon mechanics have been widely reported for the lower limbs;^[3,4,18] however, this is the first study to examine tendon mechanics of the upper limb and more specifically the dBB tendon. As the mechanics of the dBB are repeatable, this provides the opportunity for future studies to pursue tendon mechanics in the upper limbs. Quantification of tendon mechanics of the upper limb is necessary in understanding variability of performance of upper limb tasks and in susceptibility to injury. In this study consistency was established across a range of submaximal forces from 10%-80% as well as a lengthy interval between measurement points. A strength of establishing repeatability of tendon mechanics between measurement days that range two-eight months is the demonstrable consistency of the tendon when activity remains constant and this will assist in diagnostics and long-term monitoring of the tendon.

Despite the repeatability of ultrasound measures between separate sessions, ultrasound may not be as accurate or reliable as MRI for the quantification of static measures of tendon CSA.^[5,10] Further studies are required to compare measurements of the dBB tendon obtained with ultrasound and MRI, as Bohm et al.^[5] and Kruse et al.^[10] identified blurring of the Achilles tendon border with ultrasound which increases measurement error relative to MRI. In addition to comparisons with MRI, repeatability studies of the dBB should be performed in populations where the sonographic appearance of structures may be reduced, such as in older adults^[21] as well as muscle disease populations.^[22] The contact between the ultrasound probe and the skin also creates the possibility of added pressure displacing the internal structures, however extreme care was taken to ensure this did not occur during resting or contracted measures. But, one limitation of MRI that can be overcome with ultrasound is the ease and flexibility in acquiring the tendon during muscular contractions where the architecture does not remain static but changes in longitudinal as well as lateral planes.^[23] Despite the lack of comparative data from MRI, ultrasound remains a useful technique in quantifying tendon mechanics of the upper limb during resting and submaximal muscular contractions.

5. CONCLUSION

The primary finding of this study is that elongation and CSA of the dBB tendon captured with ultrasound are repeatable between testing sessions, and from these measures tendon mechanics can be calculated. Determination of repeatability of the dBB tendon is crucial for advancing studies of tendon mechanics to the upper limbs. This study provides the first step towards establishing the dBB tendon's role in upper limb function.

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CONFLICTS OF INTEREST DISCLOSURE

The authors have declared no conflicts of interest.

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