



Original Article

Accuracy of long head of the biceps tendon palpation by physical therapists; an ultrasonographic study

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Abstract. [Purpose] Examination and treatment of the long head of the biceps tendon (LHBT) requires accurate palpation. The purpose of this study was to determine physical therapists' reliability and ability to accurately palpate the LHBT in two arm positions with ultrasound as the gold standard. [Participants and Methods] Examiners palpated the LHBT within the intertubercular groove (ITG) of the humerus on the bilateral shoulders of 32 asymptomatic (21 female; 24.3 ± 1.9 years) participants in 2 arm positions. The magnitude of distance between a marker and the border of the ITG was compared between 2 positions using an independent t-test. Percent accuracy was calculated. [Results] Inter-rater reliability was poor (position 1, $k=1.04$; position 2, $k=0.016$). Overall accuracy rate was 45.7% (117/256). Accuracy was 49.2% (63/128) and 42.2% (54/128) for testing position 1 and position 2 respectively. Mean distance palpated from the groove was $M=2.58$ mm (± 6.2 mm) for position 1 and $M=3.77$ mm (± 6.6 mm) for position 2. Inaccurate palpation occurred medially 72.3% (47/65) and 93.2% (69/74) in position 1 and position 2 respectively. [Conclusion] Results of this study did not support one arm position being more accurate over another for LHBT palpation.

Key words: Palpation, Accuracy, Long head of biceps tendon

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INTRODUCTION

Shoulder pain is common with a reported incidence ranging from 7–26% in the general population¹⁾, up to 53% in certain working populations²⁾ and a reported lifetime prevalence of up to 67%¹⁾. Additionally, studies have reported low rates of perceived recovery for individuals with a primary complaint of shoulder pain^{3,4)}. The prognosis is generally poor, and Rekola and colleagues⁵⁾ reported that over 50% of individuals with neck or shoulder pain are likely to experience a recurrence of their symptoms and pursue additional episodes of care within 12 months. Several authors have reported a high economic burden of shoulder pain on the medical system⁶⁻⁹⁾. The financial burden associated with the evaluation and management of shoulder pain has been estimated at 3 billion dollars annually in the United States^{10, 11)}.

Pathology of the long head of the biceps tendon (LHBT) has long been recognized as a source of shoulder pain^{12, 13)}. The condition can be debilitating and often impacts an individual's quality of life due to persistent pain with activity¹⁴⁻¹⁶⁾. The overall incidence of bicipital tendinopathy remains unclear^{14, 17)} as it is commonly associated with other pathologies of the

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shoulder including anterior glenohumeral instability, rotator cuff disease and subacromial impingement¹⁵⁻¹⁷).

LHBT pathology is difficult to identify and is therefore diagnosed through a combination of patient identified pain location, clinical palpation, and other clinical findings including clinical tests involving specific movements of the shoulder designed to reproduce the patient's pain¹⁸). Many of the clinical tests (Speed's, Yergason's) for diagnosing LHBT lesions have been shown to have high sensitivity, poor to moderate specificity, poor predictive value and low likelihood ratios¹⁹⁻²¹). Accurate diagnosis of LHBT pathology can be difficult without the use of imaging due to the relatively poor psychometric properties associated with clinical tests used to diagnose the condition¹⁸). Moreover, tenderness over the bicipital groove is still considered one of the most common clinical tests for diagnosing biceps tendinopathy^{12, 15, 22}). Therefore, accurate palpation of the LHBT is critical for accurate diagnosis and subsequent management for LHBT pathology.

Recommended shoulder positions to palpate the LHBT appear to be based on anatomical theory or personal preference as no evidence exists to suggest whether one position is more effective for palpation than another. One study found that 65% of patients with chronic anterior shoulder pain with clinical findings consistent with biceps tendinopathy, also had concomitant anatomic findings of variability in the anatomy of the bicipital groove (acute angle, flat groove, small medial groove)²³) thus making accurate palpation challenging. A study by Gazzillo et al.¹⁸) investigated the overall accuracy of physicians palpating the LHBT of asymptomatic individuals in a position of 20–30° of shoulder abduction, 90° elbow flexion and full forearm supination. The examiners could rotate the humerus to fine-tune their palpation. They reported that physicians had, on average, only 5.4% agreement based on their definition of successful palpations¹⁸). Other positions that have been investigated in cadavers include the shoulder in adduction and 20° medial rotation and a position of shoulder extension with the “forearm behind the back”, which is more typically used to palpate the supraspinatus tendon²⁴). From these studies, it appears the positions with the most potential for accuracy might be with the shoulder in adduction and 20° medial rotation or the shoulder in 20–30° degrees abduction, 90° elbow flexion, full supination with the examiner's choice of rotation. However, few studies have used physical therapists as the palpating clinicians, and thus it is difficult to generalize the results of other palpation studies involving other healthcare providers to physical therapists due to differences in education related to training in the area of palpation. Physical therapists may be a patient's first point of contact to evaluate an individual's shoulder pain, therefore, it is important to determine physical therapists' ability to reliably and accurately locate and palpate the LHBT in any position. The purpose of this study was 1) to determine the inter-rater reliability and accuracy of physical therapists in palpating the LHBT and 2) to examine the accuracy of physical therapists palpation of the LHBT in two different shoulder positions.

PARTICIPANTS AND METHODS

A prospective single-blind validity study was performed to investigate the reliability and accuracy of physical therapists palpating the LHBT as compared to the location of the tendon as observed on ultrasound (US) images. A total of 32 asymptomatic male and female (21) volunteers were recruited. Participants were included if they were between the age of 18 and 65 years and were able to attend the data collection site for two hours on a specified day. Exclusion criteria included: any previous history of biceps tenotomy or tenodesis, history of shoulder surgery, shoulder pain, known bicipital tendon pathology or anatomic deformity of the shoulder. The study was approved through the Colorado Multiple Institutional Review Board (No. 17-1161) and all participants provided informed written consent before their participation. Two practicing physical therapists working full time in an outpatient orthopedic practice with 19 and 22 years of experience participated as palpating therapists. The palpating therapists did not receive any education or advanced training on how to palpate the LHBT, as we were interested in the clinical reliability and accuracy of physical therapists palpating the LHBT as they normally would in the clinical setting.

All US scans were performed on a Phillips iU22 US machine using a 12 MHz linear transducer (Philips Ultrasound Systems, Bothell, WA, USA). Short axis (transverse orientation to biceps tendon) grey scale images were taken for each palpated position and were saved for later analysis and measurement. An electronic digital inclinometer (Floureon DXL360S) was zeroed to be parallel to the surface of the examination table and was secured to the US transducer using elastic bands. The inclinometer calibration of 0° facilitated a standardized transducer position that would parallel the table for all measurements to control for consistency with how the images were taken. A standard goniometer was used to measure the two palpating positions.

All palpations occurred on the bilateral shoulders of each participant, in two positions. Therapists attempted to palpate the LHBT within the intertubercular groove (ITG) of the humerus in two test positions which were measured and stabilized before and after palpation by study investigators: position 1 was supine, with 90° elbow flexion, 0° shoulder abduction, 20° medial rotation²⁴); position 2 was supine with 90° elbow flexion, 30° shoulder abduction and neutral (0°) rotation to allow examiner preference for the desired rotation¹⁸). The two positions were randomized for each participant to eliminate the potential for within-session practice effect. Additionally, the radiologist and palpating therapist were blinded to the exact degree of shoulder rotation, flexion and abduction for the above two positions.

Study investigator 1 prepared the participants in each of the two test positions, depending on randomization, using a goniometer to measure joint angles before the palpating therapist entered the room. Study investigator 2 stabilized the humerus before the palpating therapist entered the room. Once the palpating therapists entered the room, they were given instructions to: “palpate the LHBT in the ITG and attempt to position the LHBT parallel to the surface of the examination table”. They

then attempted to palpate the LHBT without moving the extremity (position 1) or palpated the LHBT after medially and laterally rotating the shoulder to their preference (position 2). Once the palpating therapist determined they had their palpating finger on the LHBT, they marked the position by using transpore clear surgical tape to secure a disposable, blunt stainless steel needle on top of the skin with the assumption that the needle was superficial to the biceps tendon over the ITG (Fig. 1a). Using a black marker they also drew a horizontal line on the tape bisecting the needle to verify the exact location of their palpation (in the caudad-cephalad direction) of the LHBT in the ITG (Fig. 1b). Study investigator 2 measured the therapist's preferred medial/lateral rotation position with a standard long-arm goniometer (Fig. 2). The palpating therapist would then leave the room. The radiologist would use real-time US to sonographically assess the magnitude and direction of the marker from the underlying LHBT and ITG (Fig. 3). The same procedure was repeated with two palpating therapists for both test positions (position 1 and position 2) on the right and left shoulder of each participant for a total of 128 total palpations per therapist.

All examinations were performed by a radiologist with 8 years of experience. The radiologist placed the transducer transversely over the blunt needle at the marked point superficial to the humerus with ample gel on the skin and with minimal pressure over the needle so as not to depress the underlying soft-tissue structures. This process assured standardization of the transducer position for each measurement. The transducer was placed on the black marker point and the needle was identified sonographically by its echogenic appearance, superficial location, and posterior reverberation artifact. When the needle, the LHBT, and the tuberosities were all visualized, an image was saved. Later, images were analyzed, and distances were measured between the needle and the medial or lateral borders of the ITG were recorded (Fig. 4). Measurements were taken based on the placement of a blunted stainless-steel 18-gauge needle which was used to mark and verify the palpation site for each therapists' palpation.

The primary aim was to determine both inter-rater reliability and the accuracy of palpation of the LHBT by physical therapists as compared to the actual position of the LHBT as viewed under US. The secondary aim was to determine which shoulder position was most ideal for LHBT palpation based on successful LHBT palpation accuracy rate. Successful palpation rate was defined as the therapists' palpation location being within the ITG as identified by US, or if outside of the groove, then within at least 2 mm from its medial or lateral border. The distance of 2 mm was utilized to account for the varying ITG widths along the length of the groove from superior to inferior combined with difficulty identifying clear medial ITG margins on ultrasonographic imaging due to natural variability in medial wall inclination²⁵). Thus, needle placement relative to the groove was graded as being inside (inside or within 2 mm of either medial or lateral borders) or outside of the groove. The binary outcome was therefore: successful palpation or not. The accuracy of this binary outcome for each test position (position 1 and position 2) was determined by calculating the percent accuracy. The palpation location measurements within and <2 mm outside the ITG (our threshold for success) were negative numbers, and palpations occurring greater than 2 mm outside of the ITG were recorded as positive values. This variable was used to determine if there were significant differences between the two test positions, such that one position was more accurate for palpating than the other.

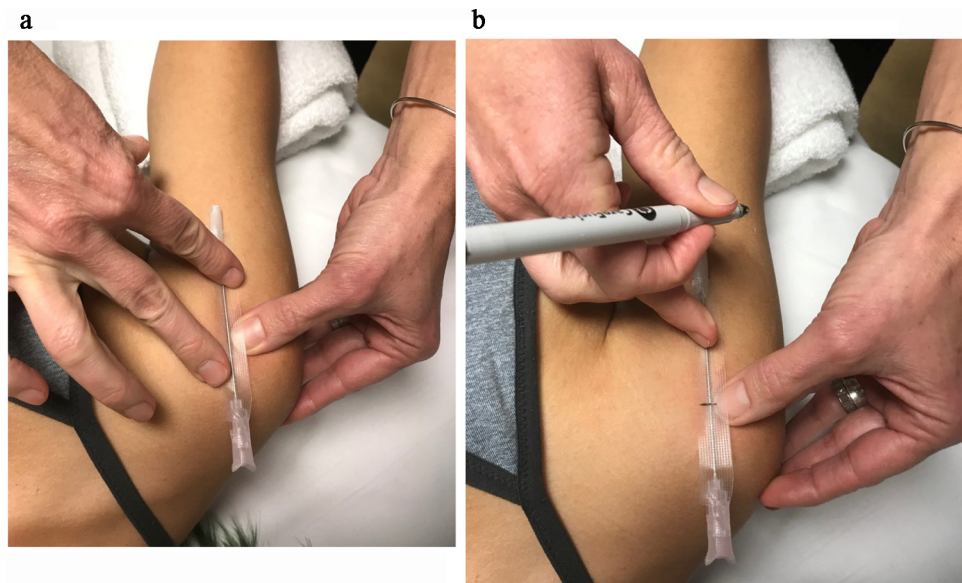


Fig. 1. a) Once the therapist determined they were on the LHBT, the position was marked by using clear surgical tape to secure a disposable, blunt stainless steel needle on top of the skin running parallel to the biceps tendon over the intertubercular groove. b) The palpating therapist drew a horizontal line on the tape with a black pen to verify the exact location of their palpation of the LHBT in the intertubercular groove.



Fig. 2. A study investigator used a goniometer to measure and record the medial/lateral rotation of the shoulder which was utilized in palpation position 2 while a study investigator stabilized the arm prior to the palpation.



Fig. 3. A digital inclinometer was attached to the transducer in order to standardize how the ultrasonographic images were taken. The radiologist used real time ultrasound to sonographically assess the magnitude and direction of the marker in relation to the underlying LHBT and borders of the intertubercular groove with the transducer head in a 0° position (parallel to the examination table).

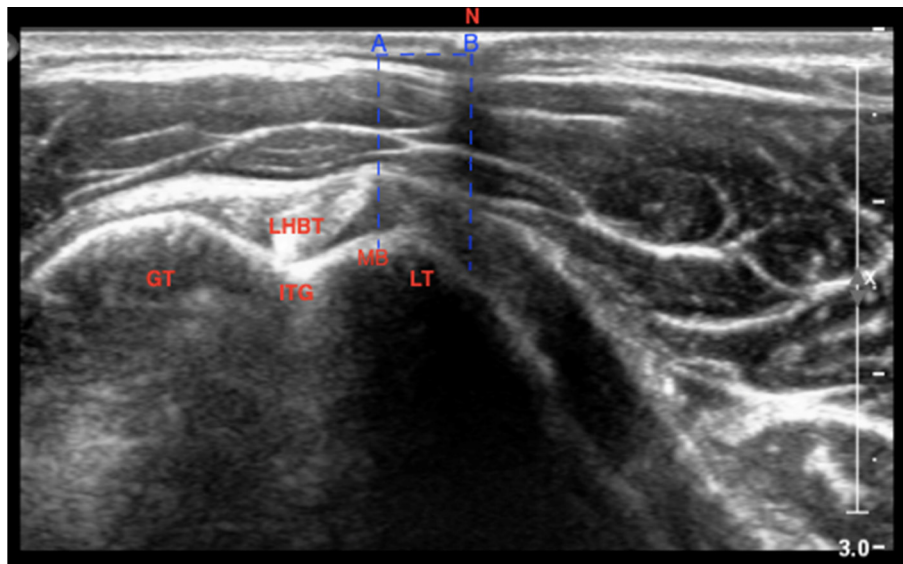


Fig. 4. When the needle, the LHBT, and the tuberosities were all visualized, an image was saved to be further analyzed at a later time. Distances from a line perpendicular to the medial (A) or lateral border of the intertubercular groove to the needle (B) were recorded. Abbreviations: GT, greater tuberosity; LT, lesser tuberosity; ITG, intertubercular groove; LHBT, long head of the biceps tendon; MB, medial border of ITG; N, needle.

To determine which shoulder position was best for achieving the highest palpation accuracy, the distance (mm) between the location palpated by the therapists (based on needle placement) and the location of the LHBT in the ITG as identified by US was measured in order to determine a magnitude of accuracy or inaccuracy. This was recorded as a continuous variable, in mm. An independent t-test was used to determine the difference between the mean distances (palpation location to location identified with US) for each test position. If there was a difference between test positions, then the position with the smallest mean distance would be considered the most accurate.

Sample size calculation was based on determining inter-rater reliability and accuracy (as a binary outcome) between two test positions however, the measure of accuracy was percent accuracy versus utilizing sensitivity and specificity as the participants were healthy individuals. Based on a prior study investigating the accuracy of LHBT palpation in physicians with a sample size of 25¹⁸⁾, a determination was made to exceed that sample size and include 32 participants to account for missing data or US image failure in order to assure adequate power. Descriptive data were reported for participants characteristics. Inter-rater reliability was calculated using Cohen's Kappa (*k*) coefficient. The overall accuracy and magnitudes of accuracy for each position were reported using percent accuracy and independent t tests respectively. A chi-square (χ^2) test was performed to determine the difference between the magnitudes of accuracy of the two palpation positions.

RESULTS

Participants consisted of 32 asymptomatic individuals (21 female) with a mean age of 24.3 (\pm 1.9 years) and a body mass index mean of 23.5 (\pm 1.9 kg/m²). An alpha level of 0.05 was used as an indication of significance for all statistical tests. The calculated Cohen's Kappa to determine inter-rater reliability was *k*=0.04 for position 1 and *k*=0.016 for position 2. The overall accuracy rate was 45.7% (117/256). Accuracy with position 1 was 49.2% (63/128) and position 2 was 42.2% (54/128). The overall accuracy of therapist 1 was 52.3% (67/128) and therapist 2 was 39.1% (50/128). A chi-square test of independence was performed to determine if one position was more accurate over the other. The chi-square value demonstrated no difference between the two positions, χ^2 (2, N=256)=1.275, p=0.259. Overall, palpations were localized by a mean (*M*)=2.58 mm (\pm 6.17 mm) outside the defined border of success (within 2 mm of the ITG) in position 1 and *M*=3.72 mm (\pm 6.56 mm) in position 2. Missed palpations occurred, more commonly, medial to the ITG rather than lateral: 72.3% (47/65) of misses occurring medially in position 1 and 93.3% (69/74) of misses occurring medially in position 2 (Table 1).

DISCUSSION

The present study found that therapists exhibited poor inter-rater reliability palpating the LHBT in both tested positions based on the low Cohen's Kappa value. Additionally, the present study reported accuracy to be just under 50% in asymptomatic participants in 2 positions (position 1 was supine, with 90° elbow flexion, 0° shoulder abduction, 20° medial rotation²⁴⁾; position 2 was supine with 90° elbow flexion, 30° shoulder abduction and neutral (0°) rotation to allow examiner preference for the desired rotation¹⁸⁾). The palpation accuracy rate in the current study was higher than that previously reported for physicians palpating the LHBT¹⁸⁾ (5.3%), using the presented methods and positions. In the present study, both study positions for palpation of the LHBT had similar accuracy rates (49.2% (63/128) for position 1 and 42.2% (54/128) for position 2) and magnitude of accuracy (no difference between positions), with the majority of missed palpations occurring medially in both positions. These results suggest neither of the chosen supine positions can be highly recommended for clinical practice, and due to a lack of additional evidence on the most ideal position, either may be appropriate for palpating the biceps tendon. It remains plausible that palpation in positions other than supine may be more accurate. Additionally, it is unknown if palpation accuracy would have been higher if therapists had been trained on the two study positions.

A number of measurement factors may have influenced the results of palpation accuracy including the prescriptive nature of the US transducer head and subsequent images and the difficulty in clearly identifying the ITG margins via ultrasonographic imaging due to the inter-subject variability in medial wall inclination of the ITG. Therefore, it is difficult to determine the potential magnitude of measurement error versus therapists' palpation error. Nevertheless, the reported palpation methods

Table 1. Accuracy in palpating the LHBT in the intertubercular groove

Position	Therapist 1	Therapist 2	Medial misses	Overall accuracy	Average distance*	Average difference**
Position 1 (n=128)	51.6% (33/64)	46.9% (30/64)	72.3% (47/65)	49.2% (63/128)	2.58 mm (\pm 6.2)	p=0.1514
Position 2 (n=128)	53.1% (34/64)	31.3% (20/64)	93.2% (69/74)	42.2% (54/128)	3.77 mm (\pm 6.6)	CI (-2.17 to 0.422)
Position 1 and Position 2	52.3% (67/128)	39.1% (50/128)	83.4% (116/139)	45.7% (117/256)		χ^2 (2, N=256)=1.275, p=0.259

SD: Standard deviation; mm: millimeters; CI: confidence interval (95%); χ^2 : chi-square; p: p value corresponding to the difference between average distances of positions.

*Average distance from needle to edge of the groove, mm (\pm SD).

**Difference in 'average distance' between Position 1 and Position 2.

resulted in higher palpation accuracy rates as compared to a previous study¹⁸⁾ however, study population and methodologies between studies differed. There are additional factors that may influence palpation accuracy including clinician experience, participant body mass index, participant age, and US methodology.

Examination of musculoskeletal pathology relies heavily on accurate palpation of musculoskeletal structures. The LHBT originates at the supraglenoid tubercle and superior glenoid labrum and is extra synovial despite its intra-articular origin¹⁴⁾. The LHBT becomes extra-articular when it enters the bicipital groove by way of the contours of the tuberosities¹⁵⁾. The groove has been defined as the area between the greater and lesser tuberosities extending superiorly from the margin and the greater tuberosity of the humerus inferiorly to where the depth was less than 2 mm²⁶⁾. The tendon itself is approximately 9 cm long with a diameter of 5–6 mm¹⁵⁾. The mean diameter of the biceps tendon sheath has been shown to range from 4.1 mm²⁷⁾ to 4.3 mm²³⁾, and may increase in size when inflammation is present. Based on the reported variability in the size of the tendon diameter combined with the relatively small size of the ITG and LHBT, the authors would argue that accuracy with manual palpation would be expected to be challenging. We believe that an accuracy rate of just under 50% combined with palpations localized at 2.58 mm (position 1) and 3.72 mm (position 2) may be acceptable in an asymptomatic population, however, higher accuracy rates would be necessary to provide targeted interventions. Physical therapists rely on both their knowledge of anatomical structures and digital palpation to examine and treat individuals with shoulder pain, however, the most ideal position to palpate the biceps tendon remains unknown.

Inconsistency exists regarding the most optimal position to palpate the biceps tendon. The position used in a palpation accuracy study of the LHBT was supine with 20–30° degrees of shoulder abduction, 90° elbow flexion, and full forearm supination with the examiners' preference for medial and lateral rotation in supine¹⁸⁾. Conversely, Mattingly and Mackerey found that the best position to expose and access the LHBT in cadavers was 0° of shoulder abduction/adduction with 20° degrees of medial rotation²⁴⁾. We found that neutral shoulder rotation places the LHBT under the middle anterior deltoid and lateral shoulder rotation places the LHBT under the lateral aspect of the deltoid muscle²⁴⁾. However it is difficult to generalize recommendations based on this study as it was performed on cadavers²⁴⁾. While the patient is positioned in sitting, Matsen and Kirby recommend palpating the tendon 3 to 6 cm below the anterior acromion with the shoulder in 10° of medial rotation; while Gill and colleagues also suggest 10° shoulder medial rotation with the shoulder in adduction¹²⁾. As a result of this variability, we sought to determine the palpation accuracy of two previously described positions. Position 1 was supine, with 90° elbow flexion, 0° shoulder abduction, 20° medial rotation as reported by Mattingly and Mackerey²⁴⁾; position 2 was supine with 90° elbow flexion, 30° shoulder abduction and neutral (0°) rotation to allow the examiner preference for desired rotation as studied by Gazzillo et al¹⁸⁾.

The overall accuracy of palpating the LHBT in healthy individuals by a sports medicine board-certified staff physician, a sports medicine fellow, and a physical medicine and rehabilitation resident was reported to be 5.3%¹⁸⁾. According to Gazzillo et al.¹⁸⁾, inaccurate palpations occurred medial to the ITG with a mean distance of 1.4 cm (14mm) away from the border of the ITG. Based on the results of our study, the overall accuracy of physical therapists palpating the LHBT in the same position reported by Gazzillo et al.¹⁸⁾ was higher than that of physicians¹⁸⁾ with most of the inaccurate palpations also occurring medial to the ITG with a mean distance of 2.58 mm away from the border. The study by Gazzillo et al.¹⁸⁾ did not include <2 mm outside the medial or lateral border as being accurate palpation, therefore, the accuracy results are difficult to compare due to differences in methodology.

In a study by Woods et al.²⁸⁾, the accuracy of LHBT palpation using the same position labeled position 2 in the current study, increased from 20% to 51.7% after medical residents went through real-time US training with palpation²⁸⁾. Overall accuracy rates in the current study were 46–49% without specific training, however our therapists were experienced clinicians rather than clinicians in training which may have contributed to their increased accuracy. The use of US or other mechanisms of training may improve the accuracy of correctly palpating the LHBT or other musculoskeletal structures. The current study did not include a training component and examiners were not informed of the two LHBT palpation positions, before the study. The goal of the current study was to emulate the palpation abilities of physical therapists in clinical practice, consequently a training period or the use of US guidance was not utilized in the methodology. Therefore, it is hypothesized that accuracy rates may have increased if we had included intentional training. The literature supports increased accuracy with US-guided palpation over surface palpation alone with guided interventions of lateral joint line palpation of the knee²⁹⁾ acromioclavicular joint palpation³⁰⁾ and palpation of the sinus tarsi³¹⁾. Less experienced clinicians may have decreased accuracy with palpation guided injections according to Curtiss et al.³²⁾, however our accuracy rates (therapist 1: 52.3% (67/128); therapist 2: 39.1% (50/128) were not significantly different between 2 practicing physical therapists with similar years of experience (22 year and 19 years respectively).

Due to the high prevalence of LHBT injuries, it is important to have a better understanding of the accuracy of a health care provider's ability to palpate the potentially pathological structure. Palpation over the bicipital groove, which elicits tenderness, is a common provocation maneuver used to differentially diagnose LHBT pathology^{12, 15)} over other sources of anterior shoulder pain. Additionally, inaccurate palpation may result in incorrect placement of potentially therapeutic bicipital tendon sheath injections or dry needles¹⁸⁾.

There were limitations to our study. First, all of the participants were healthy, young individuals with a relatively low BMI. Results may have been different in older individuals with a higher BMI, or in individuals with painful LHBTs. Accuracy of LHBT palpation may be decreased or increased in individuals with suspected LHBT pathology. Palpation as an examination

finding in individuals with LHBT pathology typically includes the presence of point tenderness of the tendon within the bicipital groove¹⁴⁾ which may potentially enhance the palpation accuracy, however conversely, broad referral patterns associated with shoulder pain may make accurate palpation more difficult.

A second limitation is that the therapist positioned the participants' shoulder so the LHBT was pointing directly towards the ceiling to standardize the ultrasonographic transducer position. The ultrasonographic scan was saved when the needle was identified based on its hyperechoic appearance and the transducer was positioned at 0° and parallel to the table for every palpation. Attempting to standardize a transducer position can present a number of challenges, and radiologists usually prefer to manually position a transducer for visualization rather than be restricted to a particular position. In a study investigating methods to increase the reliability of lumbar multifidus measures by US, a transducer position template did not enhance or increase the reliability³³⁾ and the authors recommended that transducer position templates are used. A third limitation of our study is that we recorded all measurements based on the use of a single ultrasonographic image per palpation. An ultrasonographic reliability study concluded that optimal US measurement reliability requires the use of a single rater using an average score based on three images³³⁾.

Additional limitations include difficulty identifying clear medial ITG margins on ultrasonographic imaging due to natural variability in medial ITG wall inclination. This may have led to an error in the overall measurement of the images. Further limitations may include: the possibility of participant movement after palpation and before imaging, errors in the therapist placing the needle on the skin after palpation, or errors in goniometric measurement. We attempted to carefully control these using procedures designed to minimize error, such as having a separate therapist stabilize the shoulder and arm of the participant throughout palpation and imaging.

In conclusion, the results of this study suggest that the inter-rater reliability of LHBT palpation by physical therapists are poor. Additionally, we did not find therapists to be significantly more accurate palpating the LHBT in either of the two tested positions. The vision of this study was to determine if physical therapists could accurately palpate the LHBT prior to performing other manual physical therapy interventions including soft tissue techniques, deep friction massage and dry needling. Due to the high prevalence of LHBT injuries, accurate palpation of this tendon is important when considering invasive interventions such as injections and dry needling and as such, the authors believe that reliability and accuracy studies of this nature are important to serve as a foundation for future research. Further research may be necessary to determine the best position to optimally palpate and examine the LHBT.

Presentation at a Conference

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Conflict of interest

We affirm that we have no financial affiliation (including research funding) or involvement with any commercial organization that has a direct financial interest in any matter included in this manuscript. We do not declare conflicts of interest.

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