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## Research Article

# Speckle Track Analysis of Ultrasound Images Produce Reliable Measures of Supraspinatus Tendon Strain During a Maximal Isometric Contraction

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#### ABSTRACT

**Introduction:** Ultrasound speckle tracking is an emergent method in studying musculoskeletal physiology and disease. The precision and reliability of supraspinatus tendon strain measurements have not been explored. The purpose of this study was to examine the reliability of speckle tracking to measure supraspinatus tendon strain.

**Methods:** Forty-two (42) participants participated in this study. Five (5) ultrasound images of the participant's right shoulder supraspinatus tendon were collected during a maximal voluntary isometric abduction contraction. Cine loop video files of the 5 imaging trials were imported into Ncorr software for speckle track analysis. Axial and longitudinal strain measurements were made for the bursal side, midsubstance, and joint side of the thickest portion of the supraspinatus tendon. Reliability of the strain measures was determined using interclass correlation coefficients (ICC). Bland-Altman plots were created in order to explore systematic error.

**Results:** Mean strain of the supraspinatus tendon ranged from 1.791 to -2.120 %. ICC values for the longitudinal and axial strains of both within and between images were high (>0.9) for all locations of the tendon, demonstrating very good reliability. The 95% for the MDC was large for all measurements of strain, except the axial strain at the mid-substance demonstrating poor precision.

Conclusion: The results of the investigation show evidence of very good reliability, poor precision, and some evidence of systematic error. The very good ICC values support the hypothesis that speckle tracking does produce reliable strain measurements. The large MDC values do not support the hypothesis that speckle tracking produces precise strain measurements. Improvements in ultrasound image quality and the shoulder stabilization process need to be made before ultrasound speckle tracking will be a viable research method for the supraspinatus tendon.

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#### Introduction

The complexity of the shoulder anatomy poses challenges in the assessment and treatment of shoulder injury. The supraspinatus tendon curves and twists over the head of the humerus, traversing through the subacromial space, under the acromion process and above the humeral head before inserting into the greater tubercle of the humerus [1-3]. Contraction of the supraspinatus results in an increase in the stress within the tendon while decreases in the width of the subacromial spaces leads

to increased compression of the supraspinatus tendon within the subacromial space [1-3]. The stress within the tendon causes special deformation, leading to mechanical strain within the tendon.

Ultrasound imaging is used to make measurements of the shoulder anatomy in vivo in order to assist in the assessment of shoulder injury [4-6]. Quantitative and qualitative assessments of the anatomy are made using ultrasound imaging [5, 6]. However, the anatomy of the supraspinatus tendon produces difficulty when making ultrasound images of the tendon because the tendon cannot be imaged as a straight

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segment and no single image can capture the tendon in total. It is also difficult to assess mechanical parameters such as strain using ultrasound. Improved shoulder ultrasound imaging techniques will provide more information for the clinician and researcher ultimately improving the assessment and treatment of shoulder injuries.

To perform thorough assessments of the shoulder, quantitative values beyond dimensions of tissue need to be obtained; specifically, the assessment of strain within a tendon during a muscle contraction could improve the evaluation of patients with tendon injury. Diagnostic ultrasound can be used with a method called speckle tracking to make new quantitative *in vivo* measurements of muscle and tendon strain. The values that are obtained from speckle tracking can then be used to determine important clinical factors that can be used to assess the shoulder, such as risk for muscle or tendon injury. Having actual numbers from the speckle tracking method will allow researchers and clinicians to make conclusions that are based on objective measurement rather than subjective information.

Speckle tracking is a method that uses changes in the position of greyscale color pixels of ultrasound images to measure changes in tissue position over time [7, 8]. More recently, speckle tracking has been used to make measurements on tendon and non-cardiac muscle tissue. Speckle tracking is an ultrasound-based quantitative method that might aid in the assessment of shoulder injury. The focus of previous muscle and tendons studies has been on the strain and strain rate of muscle and tendon of the wrist, knee, and lower leg; which is due to the ease at which a researcher or clinician can use ultrasound to capture an image or a video of muscle and tendon of the wrist, knee, and lower leg since each are superficial and straight [9-12]. Speckle tracking with ultrasound could be used to assess the strain of supraspinatus tendon, which may help researchers and clinicians in the study of mechanisms and treatment of shoulder injury.

Speckle tracking, in the measurement of tendon strain, is an emergent method that is used with ultrasound to make new *in vivo* measurements of tendon and muscle, such as the Achilles tendon [9-12]. Methods must be precise, or have low error, and reliable, or have high consistency, to give researchers and clinicians confidence in measurements. The precision and reliability of supraspinatus tendon strain measurements has not been explored. The purpose of this study was to examine the reliability and precision of speckle tracking to measure supraspinatus tendon strain. The current investigation tested the hypothesis that Speckle tracking produces a reliable measurement of strain within the supraspinatus tendon during a maximal voluntary muscle contraction.

#### Methods

## I Participants

## Participant Information

Forty-one (41) healthy individuals participated in this repeated measures study. Participant demographic information can be found in (Table 1). All participants were between 18 and 30 years, no participants had a history of shoulder injury and none reported that they had a history of significant upper extremity athletic activity. No participants were excluded from the study, 17 participants were male, and 24 participants

were female. Thirty-seven (37) participants were right hand dominant, 2 participants were left hand dominant, and 2 participants were ambidextrous. Participant demographic data can be found in (Table 1). This investigation was approved by the Marshall IRB (IRBNet #1399964). All participants provided written informed consent prior to the start of data collection.

**Table 1:** Participant Demographic Data. Participant information (mean  $\pm$  standard deviation) including age, weight, height, PENN scores, shoulder range of motion, and shoulder strength measures.

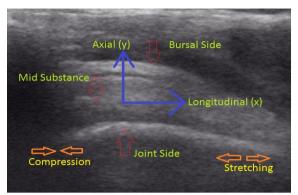
Age (years)	$22.2 \pm 2.3$
Weight (kg)	$76.9 \pm 18.6$
Height (cm)	$170.0 \pm 9.5$
PENN pain score	$29.5 \pm 1.2$
PENN SAT	$9.9 \pm 0.4$
PENN function	$59.0 \pm 1.7$
PENN total	$98.4 \pm 2.4$
Shoulder External Rotation (°)	$101.7\pm14.3$
Shoulder Internal Rotation (°)	$50.8 \pm 9.0$
Shoulder Abduction (°)	$152.0 \pm 9.3$
Shoulder Flexion (°)	$160.4 \pm 6.6$
Shoulder External Rotation Strength (lbs.)	$19.9 \pm 4.7$
Shoulder Internal Rotation Strength (lbs.)	$19.3 \pm 7.5$
Shoulder Abduction Strength (lbs.)	$21.3 \pm 8.0$

#### II Instrumentation

A diagnostic ultrasound unit, (Mindray M5; Mindray Ltd., National Ultrasound, Inc., Duluth, GA USA) with an adjustable 6.0-12.0 MHz frequency linear array transducer was used to capture all images. MATLAB R2017b (The MathWorks, Inc., Natick, MA) and Ncorr (Ncorr, Blaber and Antoniou, Georgia Institute of Technology) were used for speckle tracking analysis [13]. Measurements of force will be made using a handheld dynamometer (microFET2, Hoggan Scientific LLC, Salt Lake City, UT).

## III Protocol

Longitudinal ultrasound images of the supraspinatus tendon (Figure 1) were collected during 5 maximal isometric arm abduction contractions. All images were ultrasound images were collected from the participant's right shoulder. Scapular and humeral motion was controlled during the maximal abduction contraction using external stabilization techniques (Figure 2). Ultrasound cine loops files were saved to the ultrasound unit hard drive and later exported as AVI files. The AVI files were imported into the Ncorr software for speckle tracking analysis. Strain measurements were made at the bursal side (top), mid-substance, and joint side (bottom) of the widest visualized point of the supraspinatus tendon. Strain was measured in an axis parallel to, and perpendicular to the long axis of the tendon (Figure 1). Strain parallel to the long axis of the tendon will be referred to as longitudinal strain. Strain orthogonal to the long axis of the tendon will be referred to as axial strain.



**Figure 1:** Strain Direction and Tendon Position Labels. A greyscale ultrasound image of the right-side supraspinatus tendon. The top of the image is where the ultrasound transducer is placed. The two dark blue arrows represent the direction in which the longitudinal (x) and axial (y) strains occur. The longitudinal strain is along the length of the tendon while the axial strain is orthogonal to the longitudinal strain, vertically. Positive strain values represent expansion or stretching while negative strain values represent compression or shrinking. The arrows do not represent positive or negative directions since strain is not a vector. The inward, bottom left, and outward, bottom right, facing orange arrows represent compression and stretching, respectively.

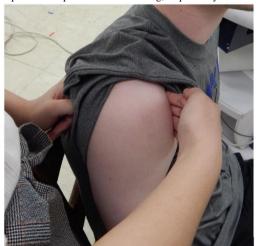


Figure 2: Scapula Stabilization, the right scapula of the participant was stabilized by placing a fist against the inferior angle and placing a hand against the corticoid process.

### **IV Procedures**

## i Ultrasound Imaging

A comprehensive ultrasound imaging evaluation of the shoulder was performed, evaluating the shoulder for tendon or muscle tears, tendinosis, muscle atrophy, joint and bursal effusions, calcified tendon, impingement syndrome, as described by Jacobson [14]. The participant was seated with their shoulder and upper arm exposed. The participant's arm was positioned at their side with the elbow in full extension and the shoulder in maximum internal rotation. For measurements of strain, the ultrasound transducer was placed flat on the most anterior aspect of the lateral acromion (Figure 3). The linear array transducer frequency was set to 10 MHz. A handheld force transducer was held against the participant's wrist and the participant's scapula was stabilized by placing

a fist at the inferior angle of the participant's right scapula and anterior portions of the shoulder in place (Figure 2). The participant's scapula was stabilized to prevent movement of the scapula during the maximal abduction contraction. Stabilizing the participant's scapula prevented the ultrasound probe or the participant's shoulder from moving during the MVIC Movement of the ultrasound probe or the participants shoulder could have led to the ultrasound image moving out of frame of the supraspinatus tendon. Diagnostic ultrasound video collection started approximately one second before participants were asked to start arm elevation, and video collection ended after participants reached maximal contraction.



**Figure 3:** Ultrasound Transducer Placement. The ultrasound transducer is placed flat on the most anterior aspect of the lateral acromion in line with the tendon, with the arm against the participant's side and in internal rotation

## ii Speckle Tracking

Ultrasound AVI files were imported into Ncorr for analysis [13]. Ncorr is a speckle tracking system based in MATLAB that allows the user to track movement from a reference image to subsequent images [13]. The system is first opened by calling it within MATLAB. A reference image is set into the program, which is the first ultrasound image before movement occurs. Current images, at least one but as many as required are then set into the program, the current images are subsequent images to the reference image, the images that the speckle identified in the reference image is track to. The region of interest, which is the area of the images that you want to identify for speckle tracking and strain analysis, is set. Digital Image Correlation (DIC) parameters, including subset radius and spacing, are then set. The subsets are the specific points in the image that are tracked, so the radius and spacing determine how big and how dense the subsets appear. The DIC analysis is then run, to determine the displacements inside the region of interest. The displacements are then formatted, which allows the user to set units and determine the correlation coefficient minimum. Strain analysis is then performed, which determines the strains inside the region of interest from the displacements. The user can then plot and observe data present in the displacement or strain plots.

## iii Statistical Analysis

The interclass correlation coefficient (ICC) was determined using the strain measures from the 3 location in the supraspinatus tendon during the 5 maximal isometric contractions, in order to determine the inter image consistency of the strain measures. Higher the ICC values represent greater consistency and reliability of the strain measures. Strain was measured using image 1 twice, ICC's were calculated using the repeated strain measures in order to determine the Intra image consistency of strain measurements. Intraclass correlation coefficients [ICC (2-way random)] were used to determine the inter-rater reliability of all strain measurements [15]. ICC values were considered very good for values 0.81-1.00, good for 0.61-0.80, moderate for 0.41-0.60, fair for 0.21-0.40, and poor for values below 0.20 [16]. The standard error of the measure (SEM) and minimal detectable change (MDC) for the strain measures were calculated. Measurement error was calculated with the standard error of measure SEM = standard deviation x [ $\sqrt{(1-ICC)}$ ], which estimates the error about a single measure of a variable. The lower the error, or MDC, the higher the precision. The MDC represents the error when a measure is taken twice (change over time) and was calculated by multiplying the SEM by the  $\sqrt{2}$  [17, 18]. All statistical calculations were performed using SPSS 24.0 (IBM, Chicago, IL).

#### Results

Four participants were excluded from the intra image testing, because the images could not be processed in Ncorr due to an error of seed placement. The mean longitudinal direction strains across the imaged tendon locations ranged from -2.12 to 1.791%. (Table 2) The ICC of the longitudinal strain across the imaged tendon locations ranged between 0.982 and 0.992. The SEM 95% CI of the longitudinal strain across the imaged tendon locations ranged from 0.319% to 0.327%. The MDC 95% CI of the longitudinal strain across the imaged tendon locations ranged 0.451% to 0.463%. The mean axial strains across the imaged tendon locations ranged -1.129% to 0.342% (Table 3). The ICC of the axial direction strain across the imaged tendon locations ranged from 0.984 to 0.99. The SEM 95% CI of the axial strain ranged 0.052% to 0.235%. The MDC 95% CI of the axial strain ranged 0.074% to 0.333%.

**Table 2:** Intra Image Results, Longitudinal-Strain. The values are for the longitudinal direction component. The values are comparisons within multiple strain measurements of the same image. For the bursal side, mid-substance, and joint side section of the widest portion of the tendon, the mean, as a percent change in length, ICC, SEM with 95% confidence interval and MDC with 95% confidence interval in the same units as the mean

Longitudinal	<b>Bursal Side</b>	Mid-substance	Joint side
Mean (%)	1.791	-1.405	-2.12
ICC	0.984	0.982	0.992
SEM 95% C. I. (%)	0.327	0.327	0.319
MDC 95% C. I. (%)	0.463	0.463	0.451

Nine participants were excluded from the inter image testing. Eight participant's images could not be processed in Ncorr due to an error of seed placement. One participant had 4 images that were collected, which did not meet the required 5 images. The mean longitudinal strain across the tendon locations ranged -1.941 to 1.765% (Table 4). The ICC of the longitudinal strain across the imaged tendon locations ranged 0.957 to 0.970. The SEM 95% CI of the longitudinal strain across the imaged tendon locations ranged 0.436% to 0.631%. The MDC at 95% CI of the longitudinal strain across the imaged tendon locations ranged 0.616% to

0.892%. The mean of the axial direction strain across the imaged tendon locations ranged -1.053% to 0.387% (Table 5). The ICC of the axial strain ranged 0.955 to 0.986. The SEM at 95% CI of the axial strain across the imaged tendon locations ranged 0.045% to 0.449%. The MDC at 95% CI of the axial strain across the imaged tendon locations ranged 0.064% to 0.635%.

**Table 3:** Intra Image Results, Axial-Strain. The values are for the axial direction component. The values are comparisons within multiple strain measurements of the same image. For the bursal side, mid-substance, and joint side section of the widest portion of the tendon, the mean, as a percent change in length, ICC, SEM with 95% confidence interval and MDC with 95% confidence interval in the same units as the mean.

Axial	Bursal side	Mid-substance	Joint side
Mean (%)	-1.129	-0.035	0.342
ICC	0.988	0.984	0.990
SEM 95% C. I. (%)	0.235	0.052	0.184
MDC 95% C. I. (%)	0.333	0.074	0.261

Table 4: Inter Image Results, Longitudinal-Strain. The values are for the longitudinal direction component. The values are comparisons within multiple strain measurements of the same image. For the bursal side, mid-substance, and joint side section of the widest portion of the tendon, the mean, as a percent change in length, ICC, SEM with 95% confidence interval and MDC with 95% confidence interval in the same units as the mean

Longitudinal	Bursal side	Mid-substance	Joint side
Mean (%)	1.765	-1.326	-1.941
ICC	0.957	0.970	0.962
SEM 95% C. I. (%)	0.575	0.436	0.631
MDC 95% C. I. (%)	0.813	0.616	0.892

**Table 5:** Inter Image Results, Axial-Strain. The values are for the axial direction component. The values are comparisons within multiple strain measurements of the same image. For the bursal side, mid-substance, and joint side section of the widest portion of the tendon, the mean, as a percent change in length, ICC, SEM with 95% confidence interval and MDC with 95% confidence interval in the same units as the mean.

Axial	Bursal side	Mid-substance	Joint side
Mean (%)	-1.053	-0.030	0.387
ICC	0.955	0.986	0.974
SEM 95% C. I. (%)	0.449	0.045	0.286
MDC 95% C. I. (%)	0.635	0.064	0.405

#### Discussion

The purpose of the current study was to examine the reliability of strain measurement of the supraspinatus tendon during an isometric contraction using ultrasound speckle tracking. The investigation tested the hypothesis that ultrasound speckle tracking would provide consistent measures of strain in the supraspinatus tendon during maximal isometric contraction. The very good ICC values, no evidence of systematic error, and low measurement error found in this investigation provides evidence to support the hypothesis that speckle tracking of ultrasound images produces reliable and precise measurements of strain of the supraspinatus tendon during an isometric contraction.

The ICC values for the longitudinal and axial strains of both within (repeated measures on the same image) and between (different trials) images were high (>0.9) for all locations within the tendon (Bursal side, mid-substance, and Joint side). The high between trial and within trial ICC values suggests that speckle tracking of ultrasound images produce reliable measures of strain within the supraspinatus tendon during an isometric contraction. Large loss of data could have a negative effect on the current study's outcomes. The data from 9 participants (21%) of the 41 participants involved in the current study was not used in the analysis. The data from one participant was excluded because only four useable images were collected from the participant. The data from another 8 participants were excluded because of problems associated with the Ncorr speckle tracking processing.

The speckle could either not be identified in the initial or final ultrasound image. Possible explanations could be that the investigator collecting the ultrasound image moved the probe during collection, the participant's shoulder was not properly stabilized, or that the speckle tracking analysis was not performed appropriately. Seven (7) of the nine excluded participants were among the first 12 participants in the study, the remaining two participants were among the first 19 participants in the study. The 9 excluded participants were among the first half of participants in the study, so there was likely a learning effect of the investigators with the procedures. The excluded data of this current study could have an effect the analysis of this current study, since a large percentage (21.4%) of all subjects were either excluded or their data was not used. The results of the current study support the reliability of the strain measurements however there is a need to improve the image collection procedures for future investigations.

The magnitude of the mean strain ranged 0.35 to 2.12% for the intraimage measurements and was a range 0.030 to 1.941% for inter-image measurements. The 95% CI for the SEM ranged 0.052 to 0.327% for intra-image measurements and 0.045 to 0.631% for inter-image measurements. The 95% confidence interval for the MDC ranged 0.074 to 0.463% for intra-image measurements and 0.405 to 0.892% for interimage measurements. The 95% CI for the SEM, as a percentage of the mean strain, ranged 15.0 to 148.6% for intra-image and 32.5 to 150.0% for inter-image. The 95% CI for the MDC, as a percentage of the mean strain, ranged 21.3 to 211.4% for intra-image and 46.0 to 213.3% for inter-image. The 95% CI for the MDC, and therefore the SEM, was relatively large compared to the mean values of strain. The MDC being large relative to the mean strain values suggests there is a lack of precise measurements for all measurements. The reliability of the strain measurements in the current study are very good; however, the precision of the strain measurements needs to improve. The measurement error found in the current study is relatively high for all measurements, which can be improved by the ultrasound image collector gaining more experience and refining the procedures.

The locations of greatest strain within the tendon may be an indication of the region's most likely to be injured. For the longitudinal direction, the greatest stretching was on the bursal side (positive strain) and greatest compression was at the joint side (negative strain). The axial strain was greatest in compression at the bursal side (negative strain) and the greatest stretching at the joint side (positive strain). Longitudinal stretching occurred on the bursal side and longitudinal compression occurred on the joint side of the tendon, as indicated by the longitudinal

strain on the bursal side being positive and the joint side being negative. Axial compression occurred on the bursal side and axial stretching occurred on the joint side, as indicated by the axial strain on the bursal side being negative and the joint side being positive. In both directions, the mid-substance was compressing, but the strain was smaller at the mid-substance than at the bursal or joint sides of the tendon. The region of the supraspinatus tendon most likely to develop a tear is the joint side of the anterior border [19]. The region of the supraspinatus tendon most likely to develop a tear, the joint side of the anterior border, is the same region that was found to have the largest axial and longitudinal strain is this current study.

Though few studies have measured the strain of the supraspinatus tendon, the strain reported in the literature does not the strain magnitudes of the current study. Kim et al. Kim, Kim, utilized ultrasound speckle tracking to investigate supraspinatus tendon strain in vivo during an isometric contraction, tendon strain ranged from 3.4 to 17.0%, with greater strains being reported on the bursal side of the tendon [20]. The current study also found greater bursal side strains; however, the strains reported by Kim et al. were of greater magnitude. Slagmolen, Scheys identified several challenges to the Kim et al. study, such as how the shoulder and ultrasound image was stabilized to ensure a high quality and appropriate image, to internal and external validity of the Kim et al. paper [21]. Many of the challenges identified by Slagmolen et al. were addressed in the current study and are likely the explanation to the lower strain measured reported by the current study [22]. In a cadaveric study, Bey, Song found the strain of the supraspinatus tendon ranged from 0.9 to 2.5%, which are higher than the strains found in the current study except for the smallest strain, 0.9%, reported by Bey et al. applied a 34N load to the supraspinatus tendon, which is greater than the load the participants of the current study applied, resulting in greater strains reported by Bey et al. also found greater strain measures on the bursal side of the supraspinatus tendon [22]. Though the magnitudes of strain of previous studies do not match the magnitudes of strain of the current study, the location of largest strain of the supraspinatus tendon, which was the bursal side, was consistent.

During the ultrasound imaging procedure, the ultrasound probe was located over the anterior aspect of the supraspinatus tendon. The area of greatest strain measurements identified in the current study corresponds with the location of the highest prevalence of rotator cuff tears, reported by Ueda and Sugaya, and the bursal side of the anterior aspect of the supraspinatus tendon [19]. The bursal side of the anterior aspect of the supraspinatus tendon may experience the greatest strain and be the location of the highest prevalence of rotator cuff tears, because of tensile loading and compression of the tendon under the acromion during humeral abduction.

The strain within a tendon, resulting from muscle contraction, may vary amongst tendons, and the reason may be because of differences in tendon tissue composition or differences in maximum force of the movement or task. Arya and Kulig determined average strain values within the Achilles tendon were approximately 4.36%, which are greater than all strain values found in this current study [23]. Pearson, Ritchings determined mean strain values of the patellar tendon ranged from 3.7 to 7.9%, which are larger than all strain values found in this current study [9]. The magnitudes of the strain values reported for the Achilles and patellar tendons, in almost all cases, are larger than the magnitudes of

the supraspinatus strain values reported in the current study. The strain of the Achilles and patellar tendons may be larger than the strain of the supraspinatus because of the tensile load of the muscle contraction force on the Achilles tendon compared to the tensile and compressive forces on the supraspinatus tendon during the isometric contraction of the supraspinatus muscle. The loading differences may be results of muscle size, tendon composition, or tendon dimensions. From comparing measurements and results of this study to previous studies, measured strain does not result in similar magnitudes as the reported strain values of Achilles or patellar tendon studies; which may be the result of the different tissue composition of the tendons, such as collagen, elastin, or proteoglycans.

Participants of the current study performed 5 maximal isometric supraspinatus contractions. The contractions were performed with a minimum of 30-second rest between trials. However, minimal effect of repeated muscle contraction on tendon strain was found. Student t-tests were performed to tests the differences in supraspinatus tendon strain between trials. Out of the 12 t-tests, only one, the difference joint side axial strain between trials 1 and 5, was statistically different than zero. The increased compliance or stretching of the tendon between trial 1 and 5 may indicate a hysteresis, such that the mechanical characteristics of the tendon change during repeated movements. The lack of statistically significant differences between trials 1 and 5 suggests that the tendon did not experience a hysteresis.

Movement of the shoulder presented problems for ultrasound imaging and speckle tracking during the pilot study. This study investigated strain during an isometric contraction of the supraspinatus, to ensure the contraction was isometric, the participant's scapula and arm needed to be stabilized during the contraction in order to prevent movement. Movement of the shoulder could also prevent appropriate ultrasound images from being taken and prevent the speckle tracking process from being completed. For images of participants that did not have their shoulder stabilized, the seed placement, during the setting of the seed during the speckle tracking process, in the current image was either out of bounds of the region of interest or no placement could be made. In combination with holding the participant's arm down to prevent abduction of the shoulder, stabilizing a participant's shoulder reduced gross movement of the scapula and humerus. The reduction in movement of the scapula and humerus improved the ultrasound imaging and speckle tracking analysis by reducing error.

Limitations in the design of the current study persist, even though this study effectively determined the reliability and precision of strain measurements in the supraspinatus tendon of healthy participants. The potential misalignment between the orientation of the ultrasound images and the determined directions for strain, longitudinal (x) and axial (y), may lead to strain results that do not match perfectly with the true longitudinal and axial strains. This limitation was addressed by lining up the supraspinatus tendon image so that the fibers of the tendon were aligned with the longitudinal direction. The supraspinatus tendon may have become more compliant throughout the five isometric shoulder abductions, which could have led to strain results that might not be consistent for each measurement or would be different from the strain results of unfatigued, unstressed tendon.

The mean tendon strain of the fifth image was greater for all of the axial strain measurements and the mid-substance longitudinal strain measurement than the mean tendon strain of the first image. This difference in strain from the first to the fifth image suggests that the tendon was more compliant axially in the last measurement than compared to the first measurement. The change in the compliance of the tendon from the first image to the fifth image shows that there may have been changes in the supraspinatus tendon, such as: change in water content or make up or better motor unit recruitment. Changes in the supraspinatus tendon, such as the tendon stretching more after repeated movements, need to be controlled for, because those changes may affect the results or the interpretation of the results. Having the participant do warm-up stretches so that their tendon is more compliant could control changes in the supraspinatus tendon.

#### Conclusion

The ICC values for all measurements of strain in this study were very good (>0.9). The very good ICC values suggest that the strain measurements made in the current study are reliable for repeated measurement of supraspinatus tendon strain from a single image and for measuring strain of multiple images of a single participant. The SEM values for all measurements of strain were relatively high compared to the strain values. The relatively high SEM values suggest that the measurements of strain of this study are not precise for multiple measurements of the same image or for measurements of different images. Though the methods designed in this study do not provide a perfect candidate for making in vivo measurements of strain of the supraspinatus tendon, the methods in this study can be improved. Significant improvements need to be made in the procedures and the experience of the investigator collecting the images. The improvements that need to be made in the procedures include refining the technique of stabilizing a participant's scapula and improving ultrasound image quality through more experience in the ultrasound image collector. If these improvements are made, speckle tracking analysis may become a new tool to assess and study healthy and diseased rotator cuff.

## **Conflicts of Interest**

None.

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