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Supraspinatus tendon micromorphology in individuals with subacromial pain syndrome

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Peak spatial frequency radius
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Study Design: Cross-sectional cohort.

Introduction:
Shoulder pain affects approximately 16 per 1000 persons per year, and the 1-year prevalence of shoulder pain has been estimated to be between 7% and 27% in adults younger than 70 years. Injuries to the rotator cuff tendons within the subacromial space can give rise to shoulder pain. Specifically, subacromial pain syndrome (SPS) and rotator cuff dysfunction are frequently diagnosed conditions in individuals with shoulder pain.

Tendons are organized as a parallel array of collagen bundles. In ultrasound imaging, this organization is manifested as a speckle pattern that shows a banded pattern and directional dependency, commonly described as fibrillar echotexture on ultrasound. Tendon

Conclusions:
Collagen disruption (PSFR) measured via ultrasound images of the supraspinatus tendon was not different between participants with SPS or in those with visually rated tendon defects. PSFR is not related to shoulder pain, function, and strength, suggesting that supraspinatus tendon collagen disorganization may not be a contributing factor to shoulder SPS. However, collagen disruption may not be isolated to a single region of interest.

Level of Evidence: 3b: case-control study.

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injuries can alter the collagen fibers distribution and organization, resulting in a more isotropic and disorganized speckle pattern (i.e., the pattern contains less of a banded structure). Qualiﬁed clinicians qualitatively interpret ultrasound images for the assessment and diagnosis of tendon disorders. In recent years, computational analysis of ultrasound images has been used to objectively quantify the degree of collagen ﬁber organization. Bashford et al. analyzed Achilles tendon ultrasound images using parameters derived from the Fast Fourier transform. When applied to an image, this analysis mathematically converts the original domain of the image (space) to a 2-dimensional (2D) spatial frequency spectrum. Healthy participants have a 2D spatial frequency spectrum characterized by a pair of bright spots, which correspond to the dominant spatial frequencies in the image. The brightness (magnitude) and size (spatial bandwidth) of these spots have been shown to correlate to the healthy tissue. The location of the bright spot indicates the dominant spacing of the speckle bands. The distance from the spatial frequency origin to the location of the peak spatial frequency was denoted the peak spatial frequency radius (PSFR). Greater PSFR values indicate a more structured and organized collagen network (and thus healthier tendon tissue), up to the spatial resolution limit of the imaging system. In contrast, pathological and painful tendons exhibit smaller PSFR along with a less-bright and broader pair of spots in the spatial frequency spectrum, suggesting disorganization of the speckle pattern and thus disorganized collagen ﬁbers. In pathological Achilles tendons, PSFR is related to mechanical properties with higher PSFR associated with greater tendon stiffness and elastic modulus.

Prior research using ultrasound imaging has shown macro-morphological changes in the supraspinatus tendon of greater cross-sectional thickness in individuals with SPS compared with controls. Tendon thickening at the shoulder may be problematic, as the supraspinatus tendon in individuals with SPS and manual wheelchair users has been shown to occupy a greater proportion of the subacromial space. Mechanistically, tendon thickening may lead to the development of shoulder pain and tendon degeneration via compression in the subacromial space. Another means of injury is abnormal tendon loading that contributes to tendon degeneration and tears, which may be the predominant mechanism for most supraspinatus tears that occurs on the articular side of the tendon. Micromorphological analysis via spatial frequency analysis of the supraspinatus tendon may provide a quantitative measure of tendon degeneration by assessing alterations of the collagen ﬁber distribution and organization of the supraspinatus tendon. In addition, it is not clear whether shoulder pain and tendon degeneration (as measured using PSFR) are related to pain, functional loss, and weakness, which are commonly reported impairments of patients with SPS. This foundational knowledge is necessary to determine the clinical utility of PSFR. Should spatial frequency analysis serve as a marker of supraspinatus tendon degeneration, it would have great potential to be used in the clinic to guide treatment dosage, prognosis, and monitor tendon recovery for return to sport or activity participation decisions.

The purpose of this study was to (1) characterize the micromorphological structure of the supraspinatus tendon in participants with SPS and healthy controls using the PSFR; (2) characterize the PSFR between participants grouped on a tendon visual quality score; and (3) assess the relationship between shoulder pain and PSFR of the supraspinatus tendon with participant reported function and shoulder strength. We hypothesized that (1) participants with SPS would have lower PSFR (greater collagen disorganization) compared with healthy controls; (2) participants with visualized altered tendon quality would have lower supraspinatus PSFR compared with participants with no visual alterations in tendon quality; and (3) greater shoulder pain and lower supraspinatus PSFR would be related to greater loss of shoulder function and weakness.

### Methods

#### Participants

This study is a secondary analysis of data collected for a cross-sectional study of participants with SPS (shoulder pain group, n = 20) and without shoulder pain (control group, n = 20) who were matched by age (±5 years), sex, and laterality of arm tested (Table 1). Participants with SPS were recruited from local physical therapy clinics with a diagnosis consistent with SPS. Participants with SPS also had to have 3/5 positive ﬁndings of impingement: positive Neer or HawkinsKennedy test, painful arc during active arm elevation, and pain or weakness with resisted isometric external rotation, or resisted scapular plane abduction with humeral internal rotation (empty can test). Participants in the control group were recruited from the local community and had: (1) no complaints of shoulder or spine pain within 6 months and (2) no known shoulder pathology. Exclusion criteria for both groups included (1) the history of upper arm fracture; (2) systemic musculoskeletal disease; (3) shoulder pain with active or passive cervical spine motion; (4) previous shoulder surgery; (5) positive apprehension test; (6) evidence of adhesive capsulitis as indicated by passive range of motion loss >50% in 2 planes of shoulder motion; and (7) evidence of a full-thickness rotator cuff tear detected during ultrasound imaging. All participants completed the informed consent, which was approved by the Virginia Commonwealth University Institutional Review Board.

#### Procedures

Each participant completed the Pennsylvania Shoulder Score (PENN), which is a valid and reliable questionnaire developed to

| Table 1 | Demographic characteristics, patient-reported outcome, and strength value for the control and shoulder pain group |
|-----------------|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Control group | Shoulder pain group | Mean (SD) | Mean difference | 95% confidence interval | P value |
| Age, y          | 45.20 (11.13) | 44.50 (11.12) | 0.70 | 6.37 to 7.87 | .844 |
| Height, m       | 1.72 (0.07)  | 1.70 (0.10)  | 0.02 | –0.04 to 0.07 | .496 |
| Weight, kg      | 72.47 (15.71) | 81.49 (17.37) | –9.00 | –19.61 to 1.61 | .093 |
| PENN pain, 0-30 (30, no pain) | 29.30 (1.34) | 19.45 (4.36) | 9.85 | 7.79 to 11.91 | <.001 |
| PENN function, 0-60 (60, completely satisfied) | 9.70 (1.13) | 4.95 (2.54) | 4.75 | 3.49 to 6.01 | <.001 |
| PENN total, 0-100 (100, no deficits) | 59.24 (2.82) | 39.47 (8.57) | 19.76 | 15.68 to 23.85 | <.001 |
| PENN tend, 0-100 (100, no deficits) | 98.24 (4.91) | 63.87 (13.97) | 34.36 | 27.66 to 41.07 | <.001 |
| Scapion strength, %BM | 0.10 (0.03) | 0.06 (0.02) | 0.04 | 0.02 to 0.06 | <.001 |
| External rotation strength, %BM | 0.11 (0.04) | 0.08 (0.03) | 0.03 | 0.01 to 0.05 | <.001 |

BM = body mass; PENN = Pennsylvania Shoulder Score; SD = standard deviation.
measure shoulder pain, satisfaction with shoulder use, and function. Each domain is scored separately and added to obtain a total score (0-100; 100: no pain, maximum satisfaction, and full function).

Shoulder strength was measured using a MicroFet2 (Hoggin Health Industries Inc., West Jordan, UT) handheld dynamometer. To limit variability in the strength measurements, the handheld dynamometer was attached to a stabilization device mounted on a door frame. The participant was positioned seated in a chair for all strength measurements. Shoulder strength in scaption and external rotation were assessed. Scaption was performed with the arm extended to 90° of abduction, neutral rotation of the forearm, and the elbow flexed to 90°. A rolled towel was placed under the elbow to maintain proper shoulder positioning and to limit abduction. Participants performed 2 trials of a maximal isometric contraction against the dynamometer for 5 seconds. Strength was measured in kgf and normalized to body mass (kg) and reported as a percentage of body mass.

A diagnostic ultrasound unit, LOGIQe (GE Healthcare, Wauwatosa, WI) with a 4-12 MHz linear array transducer was used to acquire B-mode images of the supraspinatus tendon. The ultrasound acquisition parameters remained constant between participants and included: transducer center frequency 8 MHz, depth 5cm, and 1 transmit focus point. An investigator with advanced training in musculoskeletal ultrasound obtained all the images.

Ultrasound images of the supraspinatus were captured according to standardized procedures established by the European Society of Musculoskeletal Radiology. Participants were seated with neutral trunk posture and head facing forward for all measurements. Participants were then asked to place their involved hand on the ipsilateral posterior hip with the humerus in extension and external rotation (modified Crass position). The ultrasound transducer was placed on the anterior aspect of the shoulder to capture longitudinal and transverse views of the supraspinatus tendon. For the longitudinal view, the transducer was placed parallel to the supraspinatus tendon to capture the shape of the tendon below the acromion to the footprint on the greater tuberosity of the humerus. For the transverse view, the transducer was oriented perpendicular to the supraspinatus tendon to collect the cross-sectional aspect of the tendon and the landmark of the biceps tendon. Two cine loops of 5 seconds were saved in both cross-sectional and longitudinal views and were used for analysis.

Data analysis

A musculoskeletal radiologist with expertise in musculoskeletal ultrasound of the rotator cuff qualitatively assessed the supraspinatus tendon structure in both cross-sectional and longitudinal cine loops. The radiologist was blinded to the group assignments. Each supraspinatus tendon was scored based on the echogenicity appearance. Specifically, the tendon score 0 = normal, if there was normal architecture comprised convex contour and echogenic fibrillar pattern; 1 = diffuse abnormality if (1) irregular tendon contour and (2) heterogeneous echogenicity/loss of fibrillar echo-texture and 2 = focal anechoic defect indicative of partial thickness tear with these defects not differentiated based on the size or location.

For the spatial frequency analysis, 3 static longitudinal images were extracted for each participant. These images were processed using a custom-made MATLAB program (Mathworks, Natick, MA) for the PSFR analysis. First, the supraspinatus tendon area was identified by measuring 15 mm medial to the most lateral visible of the supraspinatus footprint on the superior aspect of the greater tubercle of the humerus (Fig. 1). Within this region, a 32 × 32 pixel kernel, which corresponded to a square with 3.21 mm sides, defined the region of interest. This square was placed in the most visually echogenic area of the tendon, and the PSFR was then calculated. The kernel was zero padded to 128 × 128 samples to increase frequency resolution. A 2D high-pass filter (−3dB cutoff about 1.0 mm−1) was then applied to attenuate low-spatial frequency artifacts. The PSFR (mm−1) obtained in the 3 images was then averaged and used for further analysis. The investigator who conducted the PSFR analysis was blinded to group assignment.

Statistical analysis

Intrarater reliability of the method used to calculate the PSFR was assessed by analyzing the same image from 10 random participants in the study (irrespective of group assignment). Intraclass correlation coefficient (ICC3,3) and pooled standard deviations were calculated for the PSFR measurement. These variables were then used to calculate the standard error of the measurement ( SEM = pooled standard deviation × /ICC ) and the minimal detectable change at the 95% confidence interval (MDC95) ( MDC95 = SEM × / 1.96 ) for PSFR.

The radiologist was asked to assign a score twice to the supraspinatus tendon of 10 randomly participants (irrespective of group assignment). Supraspinatus cine loops were coded differently and graded at least 3 days apart. Cohen’s k coefficient was then calculated to establish reliability of the qualitatively grading.

Demographic information (age, height, weight, and body mass index), patient-reported outcomes (PENN pain, function, satisfaction, and total score), and strength were compared between groups using an independent sample t-test. Average PSFR was compared between groups using an independent sample, 1-tailed t-test. Participants were also stratified based on the musculoskeletal radiologist supraspinatus qualitative assessment (0, 1, 2), and average PSFR was then compared between these groups using a 1-way analysis of variance. In case of a significant group main effect, Tukey post hoc test was used to assess between-group differences. In the SPS group, the relationship between PENN pain and function subscales, supraspinatus average PSFR, and shoulder scaption and external rotation strength was assessed using Pearson’s correlation coefficient (directional, 1-tail). Alpha level was set at 0.05 for all analyses.

Fig. 1. Methods used to identify the region of interest (yellow square) used for the calculation of the PSFR of the supraspinatus. The most lateral visible point of the supraspinatus footprint on the greater tubercle (A) was identified. The red line represents the 15-mm medial distance from point A. The region of interest (yellow square) was placed in the most echogenic area within the supraspinatus tendon area (green outline). This picture is not scaled to actual measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Results

Groups were similar in terms of age, height, and weight (Table 1). In the SPS group, 4 participants reported symptoms of shoulder pain and loss of function lasting between 6 and 12 weeks, whereas 16 participants reported symptoms lasting more than 12 weeks. Participants with SPS had lower PENN subscales and total scores (P < .001) than the control group. Participants with SPS had weaker scaption (P < .001) and external rotation (P = .002) strength than the control group.

The radiologist demonstrated substantial reliability in qualitatively grading the ultrasound images (κ = 0.661). Furthermore, the method of calculating PSFR demonstrated good intrarater reliability (ICC = 0.977; 95% CI: 0.914-0.994). Variability between measurements was calculated with a pooled standard deviation of 0.38 peak/mm, and the error metrics were calculated as the standard error of the measurement = 0.06 peak/mm and a MDC95 = 0.18 peak/mm.

The musculoskeletal radiologist assigned a score of 2 to n = 10 tendons, a score of 1 to n = 19 tendons, and a score of 0 to n = 11 tendons. The between-group distribution of the supraspinatus tendon quality scores is reported in Table 2.

PSFR was not significantly different between groups: SPS group = 1.08 ± 0.15 and control group = 1.12 ± 0.16 (mean difference = 0.04; 95% CI: −0.05 to 0.15; P = 0.19), depicted in Figure 2. PSFR was also not significantly different between the 3 tendon quality rankings, irrespective of group assignment (P = .556) depicted in Figure 3.

In participants with SPS, there was a significant positive linear relationship between the PENN pain subscale and PENN functional subscale (r = 0.805, P < .001), and shoulder strength in both scap tion (r = 0.578, P = .004), and external rotation (r = 0.623, P = .002). Greater scores on the PENN pain subscale (less shoulder pain) was related to greater perceived function as measured using the PENN functional subscale and greater shoulder strength. PSFR of the supraspinatus tendon was not significantly related to the PENN pain (r = −0.012, P = .480) and function subscale (r = −0.022, P = .463), and scaption (r = 0.098, P = .341), and external rotation strength (r = −0.329, P = .078).

Discussion

The overall goal of this study was to examine the supraspinatus tendon collagen organization via ultrasound-based PSFR measures and visually qualitative supraspinatus tendon structure, and the potential contributions to patient-reported shoulder function and pain in individuals with SPS. Our findings indicate that despite pain and functional loss, supraspinatus collagen organization (PSFR) was similar between the supraspinatus tendons of participants with SPS and healthy controls. Moreover, tendons visually rated with tendon focal or diffuse abnormalities presented similar PSFR, regardless of participant group. In our study, PSFR and tendon visual quality do not appear to be associated with the presence of SPS. In addition, shoulder pain is related to perceived function and strength in participants with shoulder pain, but supraspinatus PSFR does not appear to be related to shoulder pain, functional loss, and weakness.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Normal (0)</th>
<th>Diffuse abnormalities (1)</th>
<th>Focal defects (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Shoulder pain</td>
<td>3</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>19</td>
<td>10</td>
</tr>
</tbody>
</table>

The pathological processes in the Achilles and patellar tendinopathy appear to disrupt the collagen fiber distribution, and this is reflected by a lower PSFR. Furthermore, spatial frequency parameters have classified the degree of collagen organization with an 80% accuracy in differentiating patients with tendinopathy from healthy controls and reaching 95% when using advanced discrimination techniques such as neural networks. The pathological process in the rotator cuff tendons alters the histological and molecular characteristics of the tendons, which generates isotropic changes on ultrasound images. Tendinopathy can also be associated with structural tendon changes. Despite the presence of pain and structural tendon changes in participants with SPS enrolled in the current study, collagen disruption, as measured by the PSFR of the supraspinatus tendon, was not significantly different from healthy control participants. The PSFR difference between matched pairs only exceeded the MDC95 in 7/20 (35%) of the sample. Finally, the high rate of supraspinatus abnormalities found in the control group suggests that imaging findings should not be used in isolation without
taking into consideration the participant’s clinical presentation, as imaging may not discriminate between individuals with and without SPS. The PSFR was calculated in the region of interest of a single 10.3 mm² area of the tendon. The absence of PSFR differences between groups may indicate that even in healthy shoulders, some areas of the supraspinatus may present with abnormal collagen fiber distribution. Animal model of rotator cuff tears have also shown that histopathological characteristics of the tendon are dependent upon the region sampled, as the tear produces area of increased and decreased stress within the tendon. Therefore, increasing the size of the region of interest or examining multiple areas of the tendon may potentially provide different PSFR results. Previous research reported that volleyball players with anterior knee pain demonstrated lower PSFR only in the proximal portion of the patellar tendon but not in the distal portion. The absence of PSFR differences when participants were grouped based on the qualitative scoring of tendon appearance by the musculoskeletal radiologist further support that the PSFR results may not be extended outside of the specific region of interest that was analyzed. Qualitative scoring was based on analysis of both cross-sectional and longitudinal cine loops, which reflects the diagnostic methodology used in clinical practice. Therefore, this assessment was not limited to the same specific small region of the supraspinatus tendon as the PSFR analysis.

The PSFRs of the supraspinatus tendon in the present study were approximately 35% lower in the shoulder pain group and 50% lower in the control group compared with previously reported values of tendons of the lower extremity. The differences between the lower extremity literature and the results of the present study may be indicative that both groups presented with collagen disorganization at the supraspinatus, whereas these differences did not occur in the lower extremity. Although participants that reported shoulder pain within 6 months of enrollment were excluded from the control group, 60% of the tendons in the control group were qualitatively classified as having abnormalities. It is possible that previous shoulder injury may have contributed to the lower PSFR measured in the control group compared with previous study in the lower extremity. The anatomy of the supraspinatus tendon may have also contributed to the difference in our results as compared with prior results from tendons of the lower extremity. As the distal supraspinatus approaches the footprint attachment, the tendon curves inferiorly along its lateral most and anterior most aspects. This curved shape differs from tendons like the patellar and Achilles tendons, whose straight course lends themselves more readily to analysis using placement of a single region of interest. In addition, the supraspinatus is characterized by as many as 5 different histologic layers, with the second and third layer being the thickest layers. Closely packed, parallel, large bundles of tendon fibers characterize the second layer. In contrast, the bundles of tendon fibers included in the third layer appear smaller, lack homogeneous orientation, and cross each other at a 45° angle. Not all the supraspinatus tendon fibers are oriented parallel to the ultrasound transducer, and it is likely that the region of interest for the PSFR calculation included bundles from both the second and third layers, which potentially affected the calculation of the supraspinatus PSFR. Finally, ultrasound images were acquired with a frequency of 8 MHz and a depth of 5 cm, which are appropriate settings to measure thickness changes of the supraspinatus. However, ultrasound images acquired with these parameters may lack the spatial resolution necessary to measure PSFR. Because the supraspinatus is a fairly superficial anatomical structure, using higher acquisition frequency and lower depth (between 3 and 4 cm) may be more appropriate for future studies.

Self-reported functional loss and shoulder weakness are common complaints of patients with SPS. In this study, greater shoulder pain (lower PENN pain subscale score) was related to greater loss of function (lower PENN function subscale score) and greater weakness in both scaption and external rotation. In contrast, PSFR of the supraspinatus was not significantly related to any of these measures. Therefore, other factors including the presence of shoulder pain or muscle function, rather than supraspinatus tendon degeneration, may be the primary contributors to the impairments of patients with SPS. Furthermore, pain relieving injection (lidocaine and bupivacaine solution) in the subacromial space increased shoulder abduction and external rotation strength in patients with subacromial impingement, whereas pain provoking injection (saline solution) in the subacromial space reduced infraspinatus activation and external rotation strength in healthy control. Miller et al. found that pain was associated with abduction strength loss in patients with shoulder pain and concomitant supraspinatus tear. In contrast, no associations were found between shoulder abduction strength and several supraspinatus muscle–related or tendon-related variables, such as tear size, fatty infiltration, and muscle atrophy. Therefore, shoulder pain may be the primary contributor to strength deficits. Tendon-related properties may be more prominent contributor of shoulder strength deficits at later stages of the degenerative process, as suggested by McCabe et al., who reported that only participants with large and massive rotator cuff tears exhibit strength deficits compared with participants with no or smaller size tears.

This study has some limitations. It must be noted that the images were acquired using standardized settings and are system dependent. Direct comparison is only possible within the same acquisition parameters. The lack of differences in PSFR in this study as compared to prior study by Kulig et al., may be explained by the differences in ultrasound parameters during image acquisition, especially potential lack of axial resolution in the current study. Furthermore, scanning a convex tendon, such as the supraspinatus, is challenging, and the quality of the images depends on the sonographer’s ability to maintain the longitudinal alignment between the ultrasound probe and tendon fibers. Ultrasound images are 2D and have inherent limitations when applied to 3D anatomical structures. The mechanistic nature of the study and novelty of the analysis limit the direct clinical applicability. Future studies are needed to further develop the methodology of the morphometry analysis as there is strong potential for clinical applications. Finally, ultrasound is a highly operator-dependent modality, and there can be tremendous variation between ultrasound technicians, interpreting radiologists and even ultrasound systems. Development of a standardized protocol for image acquisition and data analysis is a necessary step to decrease variation and advance widespread applications of musculoskeletal ultrasound.

Conclusion

In this study, PSFR of the supraspinatus tendon did not show significant difference between participants with SPS and healthy controls. When qualitatively assessing supraspinatus tendon as normal, diffusely abnormal, or having focal defects, similar results were observed between participants with SPS and healthy controls. These results may indicate that supraspinatus collagen organization and tendon quality are not contributing factors to shoulder pain in individuals with SPS, as healthy individuals exhibit similar tendon degeneration but do not report shoulder pain. However, further study with optimized scanning parameters for spatial frequency quantitation is needed to confirm this. Although shoulder pain is related to patient-reported outcome of function and
shoulder strength, PSFR has not yet been shown as a discriminator of differences. The results of this study implicate that treatments aimed at resolving pain may improve function and strength despite the presence of tendon degeneration.

References

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#1. The study design is best described as
   a. qualitative
   b. RCTs
   c. a case series
   d. a cross sectional cohort

#2. Tendinous collagen organization was studied by
   a. MRI
   b. biopsy
   c. estimates of PSFR utilizing an ultrasonic technique
   d. determining the PRSO with an electronic microscopic technique

#3. Strength testing was performed in
   a. scaption and external rotation
   b. elevation and internal rotation
   c. external rotation with the arm at the side
   d. internal rotation with the arm behind the back

#4. Statistical analysis included
   a. three ANOVAs
   b. an ANOVA and a Pearson correlation coefficient
   c. a student t-test and two standard deviations
   d. none of the above

#5. There was no significant correlation between pain and PSFR
   a. false
   b. true

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