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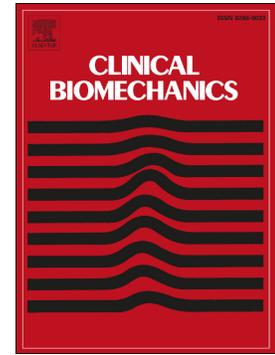
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Comparison of acromiohumeral distance in symptomatic and asymptomatic patient shoulders and those of healthy controls

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TITLE PAGE

Title: Comparison of acromiohumeral distance in symptomatic and asymptomatic patient shoulders and those of healthy controls.

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ABSTRACT

Background: The reduction of the subacromial space has traditionally been linked to rotator cuff pathology. The contribution of this narrowing, both in the development and maintenance of rotator cuff tendinopathy, is still under debate. The objective of the present study was compare the acromiohumeral distance at 0 and 60 degrees of active shoulder abduction in scapular plane, static position, in both symptomatic and contralateral shoulders, between participants with unilateral rotator cuff related shoulder pain, and in asymptomatic participants.

Method: This was a cross-sectional observational study. Seventy-six participants with chronic shoulder pain were assessed. Forty participants without shoulder pain were also recruited to compare the acromiohumeral distance with symptomatic participants. The acromiohumeral distance was measured at 0 and 60 degrees of active shoulder abduction in all the groups by ultrasound imaging. Mean differences between symptomatic versus contralateral shoulders, and versus healthy controls, were calculated.

Findings: There were no statistical significant differences ($p > .05$) in the acromiohumeral distance at 0 degrees of shoulder elevation between the groups. However, significant differences were found at 60 degrees between symptomatic and contralateral shoulder groups (0,51mm; 95%CI: -0,90 to -0,12).

Interpretations: Differences in shoulder pain perception at 0 degrees are not attributable to acromiohumeral distance differences. However, treatments focused on increasing AHD at 60 degrees could be prescribed, as a significantly reduced AHD was found in symptomatic

shoulders when compared with contralateral shoulders. Further research is needed to determine, not only static differences in AHD, but also dynamic differences.

Keywords: rehabilitation ultrasound imaging; musculoskeletal pain; shoulder pain

ACCEPTED MANUSCRIPT

Comparison of acromiohumeral distance in symptomatic and asymptomatic patient shoulders and those of healthy controls.

INTRODUCTION

Shoulder pain is currently considered as one of the most common musculoskeletal conditions in the general population, and it reaches 66.7% for lifetime prevalence (Luime et al., 2004), being higher in women (Bergman et al., 2010) and also increasing with age (Linsell et al., 2006). Rotator cuff related shoulder pain (RCRSP) is the most common cause of shoulder pain (Lewis, 2016). It is defined as an over-arching term that encompasses a spectrum of shoulder conditions including: subacromial pain (impingement) syndrome, rotator cuff (RC) tendinopathy, and symptomatic partial and full thickness rotator cuff tears (Lewis, 2016).

Narrowing of the subacromial space (SAS) has traditionally been linked to the development of RC pathology (Girometti et al., 2006; Roy et al., 2009). The quantification of the SAS has been carried out using the acromiohumeral distance (AHD), defined as the shortest linear distance between the most inferior aspect of the acromion and the adjacent humeral head (Hébert et al., 2003). It has been related to the presence and severity of some shoulder disorders, such as subacromial impingement syndrome (SIS) and RC tendinopathy (Kibler et al., 2013; Timmons et al., 2015). Radiographs, magnetic resonance and ultrasound imaging (US) have been traditionally used to quantify the AHD (Bailey et al., 2015). Due to its good reliability and validity, non-ionizing effects, and low-cost, ultrasound imaging has shown to be the best option to assess the AHD (McCreech et al., 2013). Acromiohumeral distance, measured by US, has been used as an outcome measure after the application of different physiotherapy procedures, such as exercise (Desmeules et al., 2004; Savoie et al., 2015), taping (Luque-Suarez et al., 2013), upper quadrant posture modifications (Kalra et al., 2010)

and intervention on scapular motion (Seitz et al., 2012), with the intention of guiding and establishing a treatment prognosis or outcome based on AHD findings.

The contribution of the SAS narrowing as an extrinsic mechanism in the development and maintenance of RC tendinopathy and/or subacromial space, is still under debate. In a systematic review, Seitz et al (Seitz and Michener, 2010) concluded that AHD is greater in people with full thickness RC tear compared with healthy individuals and patients with subacromial impingement, whereas AHD is also greater when the arm is actively elevated in patients with less severe RC tendinopathy compared with healthy individuals. On the contrary, in a recent study Michener et al (Michener et al., 2013) did not find any statistically significant differences in AHD at rest between patients with subacromial impingement syndrome and healthy controls. Kalra et al (Kalra et al., 2010) also concluded the same, finding an absence of statistical significant differences between participants with RC disease and healthy controls, at both rest and 45 degrees of abduction, after a modification of the upper quadrant posture. Seitz et al (Seitz et al., 2012) did not find any significant difference in AHD between symptomatic and healthy shoulders after the application of the scapular assistant test. In a recent study (Navarro-Ledesma et al., 2017) the role of the AHD in the explanation of pain, disability, and shoulder range of movement, has been investigated. The authors found, a poor correlation between AHD and the shoulder pain and disability index (SPADI), as well as with shoulder range of movement, in a population of patients with chronic RCRSP. Hence, it is crucial to establish the real role of the AHD in RC tendinopathy.

Alternatively, other factors have recently been proposed contributing to the symptoms associated to RC pathology, such as intrinsic failure of the RC tendons, primarily due to excessive and mal-adaptive loading and potentially influenced by age, lifestyle, hormonal status and genetics. (Lewis, 2014). Should the AHD be significantly reduced in comparison to that in asymptomatic contralateral shoulder, and compared with healthy shoulders, it could

be thought that directing treatments towards correcting or ameliorating a reduced AHD, with the expectation that this will improve shoulder symptoms and function, would be an adequate choice. On the other hand, should the AHD in painful shoulder remain similar to that in asymptomatic contralateral and healthy shoulders, other factors should be taken into consideration to assist clinicians in clinical decision making, and to guide future research. First, intrinsic failure of the RC tendons (RC tendinopathy) primarily due to excessive and mal-adaptive loading may be considered.(Lewis, 2014; McCreesh et al., 2014) Second, there is some evidence on the presence of peripheral and/or central sensitization and cortical changes in chronic shoulder pain that can justify the perpetuation of symptoms.(Borstad and Woeste, 2015; Sanchis et al., 2015) Third, the influence of psychological factors on the prognosis of shoulder pain, such as catastrophizing,(Reilingh et al., 2008) depressive symptoms,(Gill et al., 2013) expectancies and self-efficacy.(Chester et al., 2016) The investigation of the potential differences in AHD between patients with shoulder pain, their asymptomatic contralateral shoulders and healthy individuals will contribute to the clarification of this uncertainty and will help to increase the body of knowledge in the field.

The aim of this study is to compare the AHD at 0 and 60 degrees of shoulder abduction, in both symptomatic and contralateral shoulders, in patients with unilateral RCRSP, and in healthy controls.

METHOD

Study design

The present study was a cross-sectional, case-control design. It was developed in three different primary care centers, and conducted according to the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Malaga, Spain (PI9/012014).

Participants

Participants had to meet at least three of the following inclusion criteria: i) positive Neer test; ii) positive Hawkins-Kennedy test; iii) positive Jobe test; iv) painful arc present during flexion or abduction; v) pain during resisted lateral rotation and/or abduction. Furthermore, other inclusion criteria had to be met: vi) both men and women aged between 18 and 55 years; (vii) no history of significant shoulder trauma, such as fracture; (viii) no clinically (positive drop-arm test) and/or ultrasonography-suspected partial (Figure 1) or full thickness (Figure 2) cuff tear (Wiener and Seitz classification)(Wiener and Seitz, 1993). Participants were ineligible to participate in this study if any of these conditions were present: (i) recent shoulder dislocation, systemic illnesses such as rheumatoid arthritis, and evidence of adhesive capsulitis as indicated by passive range of motion loss $> 50\%$ in 2 planes of shoulder motion when compared to the contralateral side; (ii) shoulder pain that was deemed to be originating from any passive and/or neck movement or if there was a neurological impairment, osteoporosis, hemophilia and/or malignancies; (iii) corticoid injections during the six months prior to the study; (iv) analgesic-anti-inflammatory medication intake during the assessment stage of the study; (v) participants involved in shoulder overhead activities.

Figure1# A partial thickness RC tear for exclusion criteria

Figure2# A total thickness RC tear for exclusion criteria

A sample of 90 participants with unilateral chronic RCRSP lasting more than three months in the dominant arm, was recruited. General practitioners (GPs) carried out the recruitment. Then, participants were assessed for eligibility by research assistants (two physiotherapists with 20 years of experience). Four participants declined to participate, and ten participants did not meet the inclusion criteria, hence, a sample comprised of 76 participants was assessed. All participants signed a consent form. Confidentiality of the participants'

information and informed consent were password protected and stored at the University of Malaga, Spain.

A sample of 40 participants with the dominant shoulder free of pain was recruited in order to compare with participants with shoulder pain. These participants were recruited from the same three primary care centres by the GPs, being men-women aged between 18 and 55 years, without any complaints of arm, neck and shoulder (CANS) during the last year.

Sample size was determined based on preliminary studies.(Kalra et al., 2010; Seitz et al., 2012) Based on these data, we calculated our desired sample size using a desired minimal difference of 2,5 mm in AHD between groups. The average standard deviation was conservatively estimated at 4 mm. For a power of 90% and an alpha of .05, sample size calculations indicated 27 subjects per group.

Outcome measure

A diagnostic ultrasound unit, Sonosite M-turbo (GE Healthcare, Wauwatosa, WI) with a dynamic range up to 165 dB, was used. Furthermore, a 6–13-MHz linear transducer with 196 piezoelectric crystals with a specific ultrasound system called “SonoMB® multi-beam imaging”, to increase resolution and improve visualization of physiological and subtle tissue differences, was used to capture images in a grey scale of 256 shades. All patients had standardized ultrasonography in both shoulders(Teefy et al., 2000)(Corazza et al., 2015), whereas only the dominant arm was measured in healthy subjects. Ultrasound images were obtained by a single examiner (SN), who was a licensed physiotherapist with advanced training in rehabilitative ultrasound imaging (RUSI), and 5-years of experience, and who carried out the ultrasound shoulder evaluation during the assessment phase. The ultrasound

examiner was blind to all measurements (values were obscured by placing a shield on the ultrasound screen, meanwhile a research assistant registered the data), and was blind to the previous condition of each patient (shoulder function and pain severity), as well as to the affected side and dominance upper limb. All the ultrasound measures were expressed in millimeters. During the ultrasound shoulder assessment for eligibility of participants, the modified 5-grade Wiener and Seitz classification was followed (Wiener and Seitz, 1993), in which grades III, IV and V were reasons of exclusion. Furthermore, a full-thickness of RC was defined as the inability to visualize the rotator cuff due to complete avulsion and retraction under the acromion, or as a focal defect in the rotator cuff created by a variable degree of retraction of the torn tendon edges. (Teefy et al., 2000)

Acromiohumeral distance

AHD was measured at 0 (Figure 3) and 60 degrees of active shoulder elevation in the scapular plane (Figure 4), with the participant seated in an upright position. Sixty degrees of shoulder elevation was selected because, first, Bailey et al (Bailey et al., 2015) has pointed out that humeral elevation at which rotator cuff becomes vulnerable to extrinsic impingement is much lower than previously believed, thus, US assessment of AHD might be considered during active contraction and for up to 60 degrees of shoulder elevation, and, second, there is a difficulty to visualise the AHD in a shoulder elevation greater than 60 degrees due to the acoustic shadow of the acromion. The process followed to evaluate AHD has been previously used in different populations, such as healthy volunteers (Luque-Suarez et al., 2013) and patients with shoulder pain. (Desmeules et al., 2004) (Kalra et al., 2010) Patients were seated in an upright position without back support and with their feet flat on the ground, pulling their shoulders back and look straight ahead, to achieve retracted shoulders and extension in the thoracic and cervical spine, similar to the upright position described by Kalra et al. (Kalra et al., 2010) A hydro-goniometer was placed on the patient's arm to determine 60° of active

shoulder elevation, with thumb pointing up. To assist in positioning arm elevation in the scapular plane, a room divider was positioned at an angle of 30 degrees forward from the subject's frontal plane, which was marked with tape on the floor.(Theodoridis and Ruston, 2002) The ultrasound transducer was placed on the most anterior aspect of the acromion edge, with the long axis of the transducer placed in the plane of the scapula and parallel to the flat surface of the acromion. AHD was measured in millimeters, using the calipers on the ultrasounds' screen. When AHD was visualised, the screen was frozen and the measurement was conducted in the moment. AHD was defined as the shortest linear distance between the most inferior aspect of the acromion and the adjacent humeral head.(Desmeules et al., 2004) (Figure 5)

Figure3# Transducer and patient position in the measurement of the acromiohumeral distance at 0 degrees of shoulder abduction

Figure4# Transducer and patient position in the measurement of the acromiohumeral distance at 60 degrees of shoulder abduction

Figure5# AHD distance at 0 (left) and 60 (right) degrees of shoulder elevation

The Shoulder Pain and Disability Index (SPADI)(Roach et al., 1991) was assessed in the symptomatic sample. The SPADI has shown a good internal consistency with a Cronbach's alpha of 0.95 for the total score, 0.92 for the pain subscale and 0.93 for the disability subscale as well as the ability to detect change over time.(MacDermid et al., 2006)

Data analysis

Normality for all US variables was explored using the Kolmogorov Smirnov test for the two groups of participants with shoulder pain (affected and non-affected), and the Shapiro-Wilk test for healthy group (controls). Comparisons for all the variables between the affected and

non affected groups were calculated using paired sample t-tests. Comparisons between affected group with controls were calculated using independent sample t-tests. A p-value < 0.05 was considered statistically significant. To calculate the intra-rater reliability of all the US variables, three measurements of each one were collected, and a two-way mixed (3,1), consistency, intraclass correlation coefficient (ICC) was, then, calculated. A reliability coefficient less than 0.50 was an indication of “poor” reliability; “moderate” between 0.50 and 0.75, “good” between 0.76 and 0.90; and “excellent” over 0.90.(Portney and Watkins, 2000) The standard error of measurement (SEM) and the minimal detectable change at 95% confidence of interval (MCD95) were also obtained. For all the US calculations, three measurements were taken for each position by the examiner, three for 0 degrees and, afterwards, three for 60 degrees, and an average of three was used for the statistical analysis for each angle. An interval of one minute was provided between measures, and the patient was encouraged to move freely. Patients were then repositioned and the second and third sets of measurements were successively taken. For data analysis, a new outcome measure was created, as the percentage of reduction in AHD from 0 to 60 degrees of shoulder elevation.

RESULTS

Sample characteristics

Demographic characteristics are shown in Table 1. No between-group differences were found for gender, age and height.

TABLE1* Sample characteristics (Standard Deviation)

The ICC was 0,88 (0,83-0,92) for AHD measurements at 0 degrees, and 0,98 (0,97-0,99) at 60 degrees. The SEM was 0,05 at 0 degrees and 0,003 at 60 degrees. The MDC95 was 0,13 at 0 degrees and 0,01 at 60 degrees.

Mean values for AHD in different groups

Mean values for AHD at both 0 and 60 degrees of shoulder elevation in the different groups are shown in Table 2, as well as the percentage of reduction in AHD from 0 to 60 degrees of shoulder elevation.

TABLE2* Mean values (95% CI) for ultrasonography measurements and mean differences between groups, expressed in millimeters

Mean differences in AHD between groups

Mean differences in AHD between groups are shown in Table 2. Comparisons between affected and non-affected shoulders were calculated using paired-sample t-tests for dependent samples. Comparisons between affected and healthy controls were calculated using independent sample t-tests. There was an absence of significant differences between all groups regarding AHD at 0, and at 60 degrees for the comparison between affected shoulders and healthy controls. Nevertheless, there was a statistically significant difference in AHD at 60 degrees between affected and contralateral shoulder groups of -0,51 (95%CI: -0,90 to -0,12; $p < .05$), which surpassed the MDC95.

DISCUSSION

Main findings

The aim of this study was to determine the differences in AHD at 0 and 60 degrees of shoulder abduction in both symptomatic and asymptomatic shoulders, in patients with chronic RCRSP, and in individuals with shoulder free of pain. Our findings suggest no significant differences in ultrasound measurements when comparing all the groups, except for AHD at 60 degrees when affected and contralateral non-affected shoulders were compared.

Comparison with other studies

To our knowledge this is the first study to investigate the presence of differences in AHD between these three different groups. Similar studies have been carried out in participants with SIS compared with healthy controls. They showed an absence of statistical significant differences between groups in AHD at 0 degrees of shoulder elevation.(Desmeules et al., 2004; Kalra et al., 2010; Michener et al., 2013) On the other hand, our results are not in accordance with those obtained by Cholewinski et al.(Cholewinski et al., 2008), who found significant differences between affected shoulder and healthy shoulder. This discrepancy could be explained by the fact that the method of measurement for the AHD was different when compared with our study. At 60 degrees, no statistical significant differences were found between symptomatic and healthy groups. This does concur with other studies which compared AHD measured at 45 (Kalra et al., 2010; Seitz et al., 2012) and 90 degrees(Seitz et al., 2012) in participants with RC disease and SIS compared with healthy controls. However, their comparisons were made after a scapular assistant test(Seitz et al., 2012) and changes in posture.(Kalra et al., 2010) Interestingly, we found statistical significant differences between symptomatic and asymptomatic shoulders at 60 degrees of shoulder elevation, and these differences (0,51 mm greater in asymptomatic group) surpassed the MDC95 (0,01).

Likewise, we found a greater decrease in AHD from 0 to 60 degrees in the symptomatic group compared with the asymptomatic group (3,92%), which was extremely close to being statistically significant ($p=0.08$). Both findings are in consonance with those attained in previous studies which investigated the differences in AHD between dominant and non-dominant arms at both 0 and 60 degrees of shoulder abduction, in either injured(A. Maenhout et al., 2012) or uninjured(Annelies Maenhout et al., 2012) shoulder, but their findings were obtained in overhead athletes.

Several underlying reasons could exist to explain the absence of differences in AHD between these three different groups at 0 degrees. Reduced AHD has traditionally been matched to RC tendinopathy.(Lewis, 2014; McCreesh et al., 2013) A lack of RC muscle function, leading to a superior migration of the humeral head, alterations in acromion morphology, and abnormal scapular posture (typically anterior tilt and downward rotation), have resulted in a reduced AHD and RC pathology. Accordingly, many surgical(Judge et al., 2010) (subacromial decompression) and physical therapy interventions(Desmeules et al., 2004; Savoie et al., 2015) (exercise therapy with an emphasis on RC muscles to improve their depressor function) have been focused on ameliorating this in an attempt to relieve shoulder symptoms. Nevertheless, a recent theory has suggested the possibility that other factors could be involved in the explanation of symptoms associated with RC tendinopathy.(Lewis, 2014) Intrinsic failure of the RC tendons primarily due to excessive and mal-adaptive loading and potentially influenced by age, lifestyle, hormonal status and genetics, would be factors to take into account. As these factors were not measured in this study, we can only speculate whether any of them would have a real implication in the present study. On the other hand, a recent study (Navarro-Ledesma et al., 2017) has found a poor correlation between AHD and shoulder pain and range of movement, in subjects with chronic RCRSP, which strengthens the aforementioned theory. Additionally, other areas should capture the attention of the both

clinicians and researchers when an explanation of shoulder pain is needed, as pointed out by Lewis. (Lewis, 2014) Psychological factors, the role of the central sensitization and cortical changes would contribute to explain why the shoulder hurts. Likewise, a recent study (Michener et al., 2013) has found that the percentage of supraspinatus thickness that occupies the AHD at 0 degrees was greater in participants with SIS than in healthy controls. This could be a key factor to consider in future investigations rather than the assessment of the AHD in isolation.

On the other hand, we found significant differences in AHD at 60 degrees of shoulder elevation between symptomatic and contralateral shoulders. This could be attributed to an insufficient scapular upward rotation during shoulder elevation in the symptomatic group, since this has been demonstrated as a potential mechanism that reduces AHD. (Seitz et al., 2012) This may be evaluated in further investigation, using a dynamic evaluation of AHD and scapular positioning during different arm elevation angles.

Strengths and limitations

This study has a number of strengths including similar demographic characteristics of different groups. Moreover, a careful screening for exclusion criteria was carried out using US imaging assessment following recognized guidelines (Corazza et al., 2015) (Wiener and Seitz, 1993) and recommended patient positioning. (Navarro-Ledesma et al., 2017) (Desmeules et al., 2004) (Kalra et al., 2010) However, the present study has some limitations that should be recognized. First, the person taking the US measurements was aware that the subject was either a patient or an asymptomatic control. Second, the relatively small sample size of this study indicates the need to interpret the results with caution. Third, participants with chronic RCRSP did present AHD mean values greater than 7 mm, which is not considered a reduced value for subacromial space, hence the findings should not be

extrapolated to other populations. Four, only static differences in AHD at both 0 and 60 degrees were assessed, so we do not know whether there could have been differences during a dynamic assessment. Five, although there was not a statistically significant difference between symptomatic and healthy controls ($p=0.11$) in gender distribution, conclusions should be taken with caution since there was not a balanced distribution for men and women in both groups. Six, this study was focused on the analysis of AHD and its influence on RC tendons. However, this could introduce bias when the results are interpreted because it is well-known that other subtypes of impingement can affect RC tendons (supraspinatus and infraspinatus), e.g. posterior-internal impingement.(Braman et al., 2013) This impingement involves the undersurface or articular surface of the rotator cuff tendons (supraspinatus and infraspinatus) becoming entrapped or stressed between their humeral attachment and the glenoid-labral complex. Seven, the SPADI was not assessed in healthy controls, so this could indicate bias. Finally, participants with a swollen supraspinatus tendon were not excluded for this study, so this could introduce bias.

Clinical implications

Our findings could have clinical implications. Steering treatments towards increasing subacromial space would be useful in patients with RC symptomatology in order to increase AHD between 0 and 60 degrees of shoulder elevation. Other therapeutic possibilities which focus on the treatment of intrinsic alterations of the RC tendons, and/or other coexisting factors (central sensitization, psychological factors) need to be considered, since we found that AHD at 0 degrees can not explain differences in pain perception between different groups of this study. Nevertheless, as AHD measurements were taken in static positions and dynamic changes were not assessed, results should be taken with caution and further investigation is needed. Also, the role of the long head of the biceps as a humeral head

depressor and its relationship with the AHD needs more attention in future investigations, as well as the implication of the posterior-internal impingement in RCRSP.

CONCLUSIONS

Differences in shoulder pain perception at 0 degrees are not attributable to AHD differences. However, treatments focused on increasing AHD at 60 degrees could be indicated, since a significantly reduced AHD was found in symptomatic shoulders compared with contralateral shoulders. Further research is needed to investigate, not only static differences in AHD, but also dynamic differences.

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TABLE 1

Table 1: sample characteristics (Standard Deviation)

		AFFECTED	HEALTHY	p
AGE		45,72(10.32)	46,42 (7.02)	0.92
GENDER	FEMALE	50 (65.79%)	19 (47%)	0.11
	MALE	26 (34.21%)	21 (53%)	
SPADI		57,75 (19.45)	-	-
HEIGHT (cms)		166,05 (8.47)	168,40 (1.33)	0.16
DURATION OF SYMPTOMS		12-24 weeks= 21 24-48 weeks= 10 +48 weeks= 44		

p: statistical significance between groups. A p-value less than 0.05 was considered significant.

TABLE 2

Table 2: mean values (95% CI) for ultrasonography measurements and mean differences between groups, expressed in millimeters

	Affected shoulder	Non-affected shoulder	Healthy shoulder	Affected-non affected ^a	p	Affected-Healthy ^a	p
AHD 0 (mm)	9,46 (9,12 to 9,79)	9,65 (9,33 to 9,97)	9,52 (9,15 to 9,89)	0,19 (-0,55 to 0,16)	.28	-0,07 (-0,59 to 0,46)	.80
AHD 60 (mm)	6,38 (6,01 to 6,75)	6,89 (6,62 to 7,17)	6,71 (6,33 to 7,09)	-0,51 (-0,90 to -0,12)	.01	-0,33 (-0,85 to 0,19)	.25
Percentage of reduction in AHD from 0 to 60 degrees	31,58 (27,63 to 35,53)	27,66 (-7,60 to 55,43)	29,27(25,82 to 32,72)	3,92 (-0,60 to 8,44)	.08	2,30 (-3,64 to 8,25)	.44

^a: between group mean differences (95% CI)

*: $p < .05$

HIGHLIGHTS

- No differences in acromiohumeral at 0 degrees exist among different shoulders.
- Differences at 60 degrees were found between symptomatic and contralateral shoulder.
- Acromiohumeral distance at 0 degrees can not explain shoulder pain perception.
- Increasing acromiohumeral distance at 60 degrees may benefit patients.

ACCEPTED MANUSCRIPT

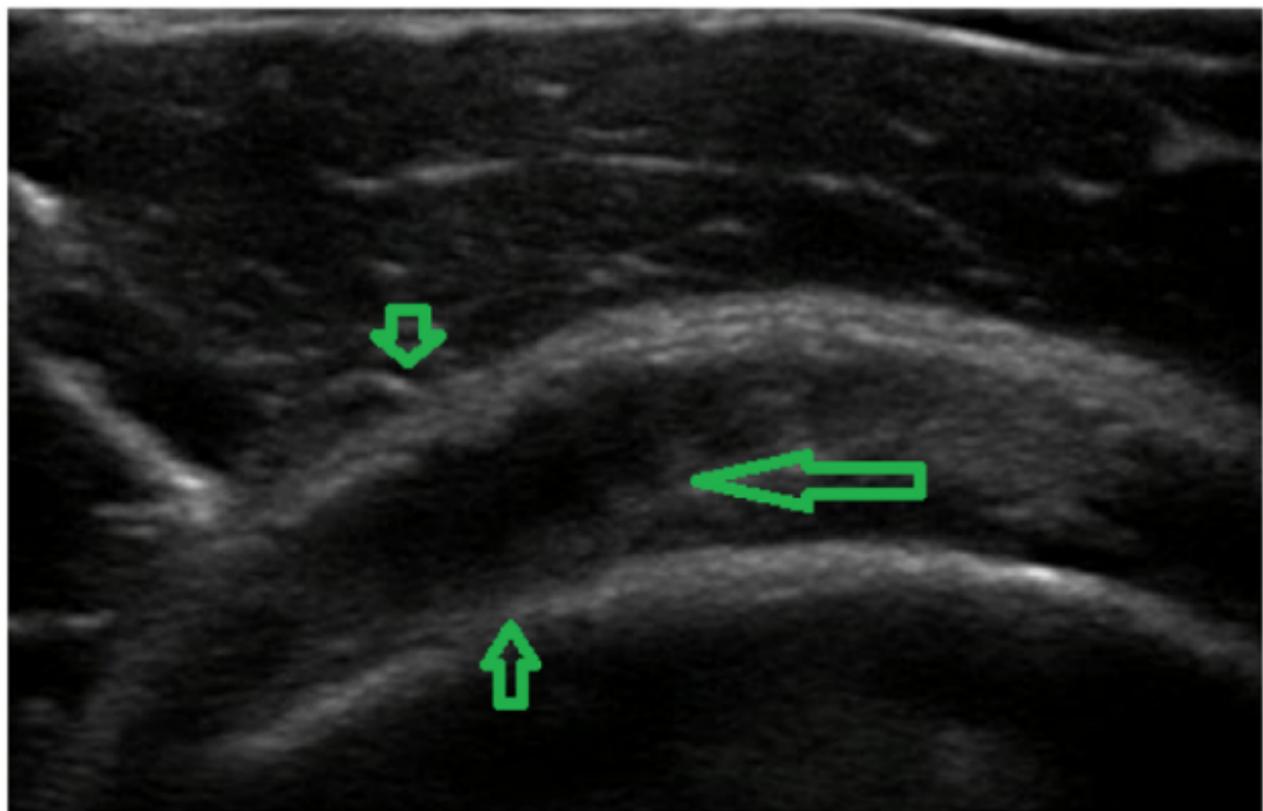


Figure 1

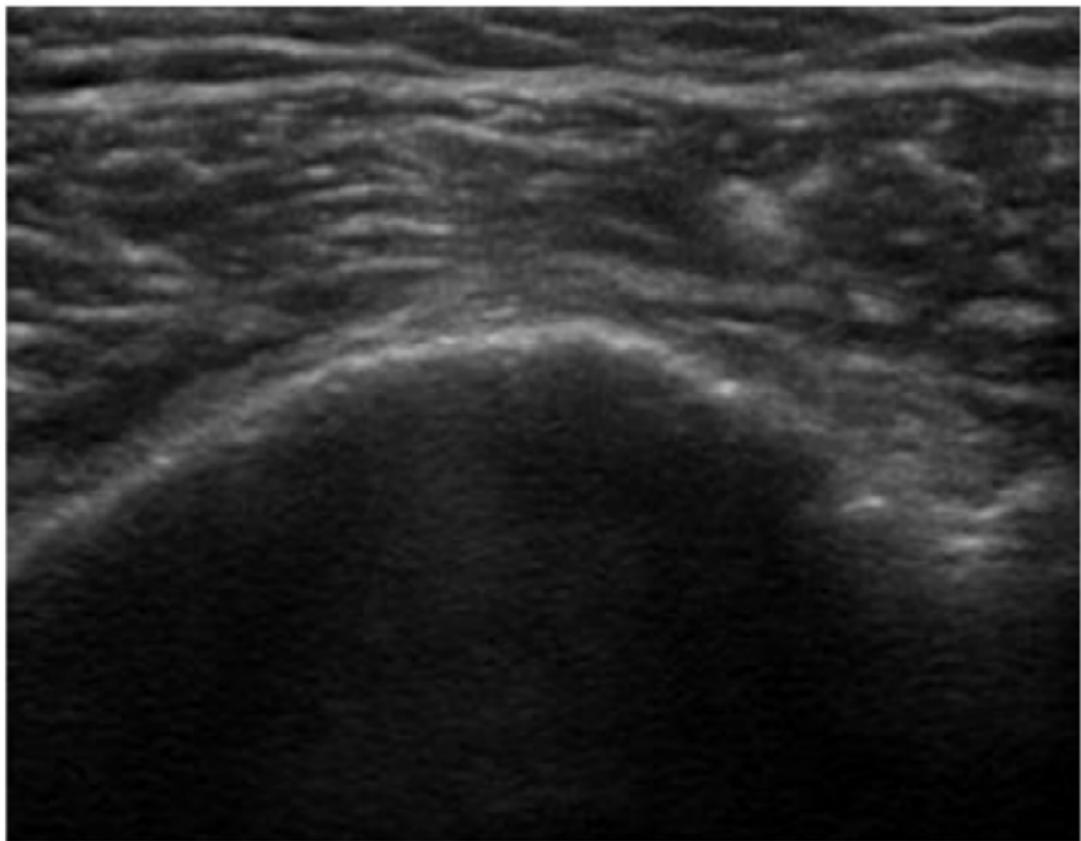


Figure 2



Figure 3



Figure 4

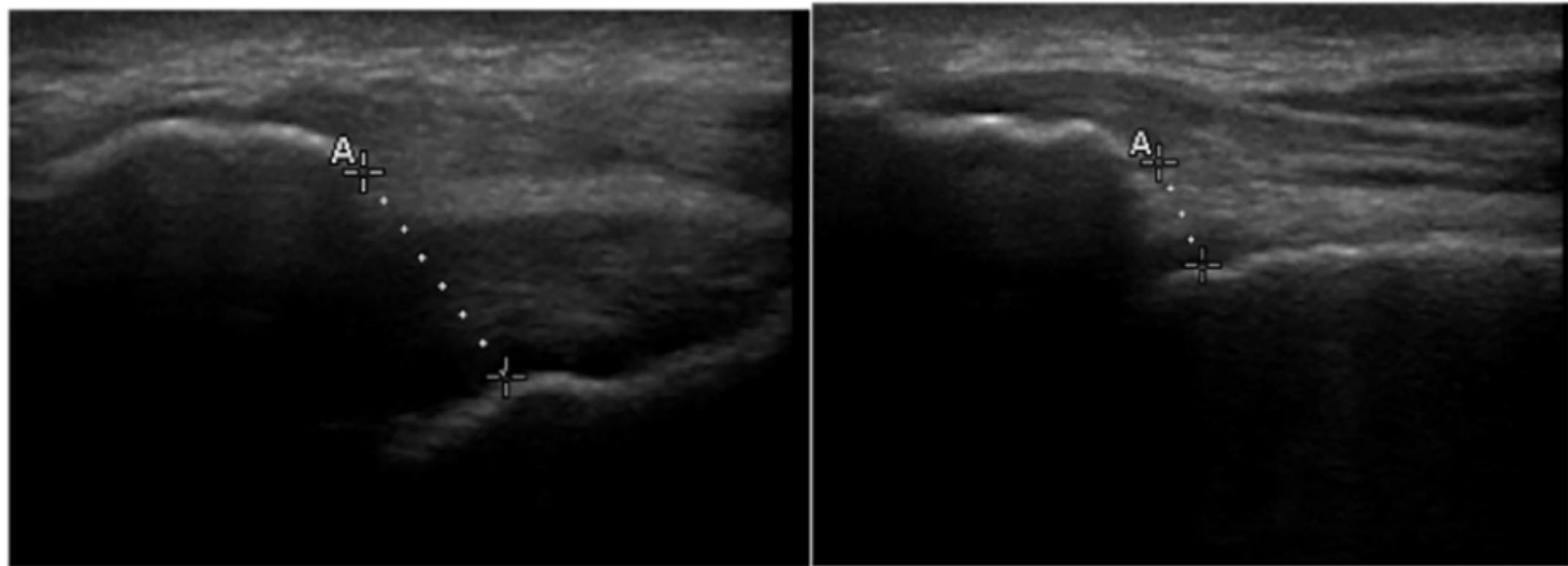


Figure 5