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*Research Article*

# **Evaluation of Gender Differences in Shear-Wave Ultrasound Elastography of the Shoulder Joint in Kwazulu-Natal, South Africa**

**Ramdev. H.<sup>1</sup>, Matthews. G.<sup>2</sup>, Dludla. Z.<sup>1</sup>, Naidoo. M.<sup>3</sup>, and Govender. N.<sup>4</sup>**

<sup>1</sup>*Dept of Radiography, Faculty of Health Sciences, Durban University of Technology, Durban, South Africa, 4000*

<sup>2</sup>*Dept of Statistics, Faculty of Applied Sciences, Durban University of Technology, Durban, South Africa, 4000*

<sup>3</sup>*Specialist radiologist, Jackpersad & Partners Inc., Mount Edgecombe Hospital, Durban, South Africa, 4000*

<sup>4</sup>*Dept of Basic Medical Sciences, Faculty of Health Sciences, Durban University of Technology, Durban, South Africa, 4000*

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## **ABSTRACT**

Shear wave data for the rotator cuff and biceps tendons in asymptomatic individuals is limited in KwaZulu-Natal, South Africa. This study aimed to investigate the gender differences in tendon elasticity of the rotator cuff and biceps tendons using shear wave ultrasound elastography (SWUE). Rotator cuff and biceps tendons (sagittal and axial planes) of 260 patients (21-45 years), were evaluated using SWUE. An independent samples t-test was used to investigate whether differences in the tendon elasticity measurements for males and females exist. Gender based reference ranges for the mean elasticity of each tendon were found by constructing a 95% confidence interval. Gender differences for tendon elasticity were observed for the sagittal plane of the biceps tendon (proximal, middle and distal), the axial plane of the biceps tendon (middle) and the sagittal plane of the subscapularis tendon (middle). The sagittal supraspinatus (distal) and axial supraspinatus (middle) revealed a significant gender difference in tendon elasticity. A significant gender difference in tendon elasticity was noted for the teres minor tendon (axial and sagittal). Only the axial (proximal) region of the Infraspinatus tendon showed a significant gender difference in elasticity. Notably, males had higher mean elasticity values than the females in all the tendons that demonstrated significant elasticity differences. A non-invasive gender estimate of the elasticity of the rotator cuff and biceps tendons (kPa), is provided which may complement B-Mode ultrasound in the screening of rotator cuff and biceps tendon pathology.

**Keywords:** *rotator cuff tendon, biceps tendon, tissue elasticity, shear wave ultrasound elastography, gender differences, B-mode*

\*Author for correspondence: Email: [nalinip@dut.ac.za](mailto:nalinip@dut.ac.za); Tel: + 27-31 373 2796

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## **INTRODUCTION**

Shoulder pain/dysfunction is associated with rotator cuff tendinopathy and tears (Chepeha *et al.*, 2015, Weinreb *et al.*, 2014, Trent *et al.*, 2013, Hou *et al.*, 2017). Current diagnosis of tendon pathology is based on physical examination and plain film radiography of the affected area (Gennisson *et al.*, 2015), however, plain film radiography is unable to identify inflammation or early soft tissue changes (Kehl *et al.*, 2016). Despite its wide use in evaluating rotator cuff and biceps tendons (Oliveira *et al.*, 2017), B-Mode is limited in clinically distinguishing between tendinopathy and tendon tears thereby precluding early diagnosis (Lee *et al.*, 2016, Ooi *et al.*, 2014, Dirrichs *et al.*, 2016). While Magnetic Resonance Imaging (MRI) and Magnetic Resonance Arthrography (MRA) are accurate predictors of rotator cuff tears, challenges such as high costs and invasiveness remain (Ooi *et al.*, 2014, Trent *et al.*, 2013).

Ultrasound elastography has recently gained popularity in evaluating the elastic properties of soft tissues to confirm biomechanical changes associated with pathology (Ryu and Jeong, 2017, Tudisco *et al.*, 2015). It is fast, non-invasive, cost-effective, safe, highly reproducible and may increase the diagnostic sensitivity of tendon pathology prior to its detection on B-Mode ultrasound (Prado-Costa *et al.*, 2018, Hatta *et al.*, 2016, Tudisco *et al.*, 2015, Wu *et al.*, 2012, Arda *et al.*, 2011). Currently, tissue elasticity is qualitatively and quantitatively assessed by strain ultrasound elastography (SUE) and shear wave ultrasound elastography (SWUE) respectively (Garra, 2015). Manual compression is inessential when evaluating tissue stiffness using SWUE, since it quantitatively measures the biomechanical properties of soft tissue (using a numerical data) and concurrently transmits acoustic radiation force impulse (ARFI) pulses (Baumer *et al.*, 2018, Qiu *et al.*, 2015, Rahman *et al.*, 2013, Hou *et al.*, 2017).

Ultrasound elastographic evaluation of musculoskeletal tissues is limited (Roskopf *et al.*, 2015, Wu *et al.*, 2012, Hou *et al.*, 2017), with the Achilles tendon being the earliest and most studied structure (De Zordo *et al.*, 2010, Cosgrove *et al.*, 2013). Whilst rotator cuff tendons have been extensively studied using ultrasound elastography, the focus was mainly on the supraspinatus tendon (Muraki *et al.*, 2015). Limited shear wave data exists on the rotator cuff and biceps tendons in both asymptomatic and symptomatic individuals (Baumer *et al.*, 2018, Hou *et al.*, 2017, Muraki *et al.*, 2015, Tudisco *et al.*, 2015, Ooi *et al.*, 2014). This study therefore aimed to measure the elasticity of the rotator cuff and biceps tendons in a South African asymptomatic population using shear wave ultrasound elastography. We report all elasticity values in kilopascals, and provide a shear wave ultrasound elastographic gender-based reference range of the rotator cuff and biceps tendons.

## MATERIALS AND METHODS

**Study population:** This prospective and quantitative study was conducted in a private radiology practice, situated in a regional hospital, in Durban, KwaZulu-Natal. Institutional ethical approval (REC73/17) and hospital permission was obtained. Two hundred and sixty (n=260) asymptomatic participants with no history of shoulder pain were recruited. All participants were aged between 21-45 years and not on any steroids or anti-inflammatory medication for a period of 24 hours. Demographic and ultrasound elastographic data of the rotator cuff and biceps tendons were collected during examination using the General Electric Logiq E9 ultrasound equipment (GE Logiq E9). Both B-Mode and shear wave ultrasound elastography data was evaluated with a 10-14 MHz linear transducer.



**Figure 1:** Transducer position for ultrasound assessment of supraspinatus tendon

**B-Mode ultrasound collection:** Participants were seated in an upright and relaxed position, facing the sonographer. All anatomical regions were examined in the sagittal and axial planes based on a previously standardized protocol (Sahan *et al.*, 2018, Gupta and Robinson, 2015). The proximal, middle and distal sites of the tendons were scanned in both planes using the multi frequency linear, hand-held transducer with coupling medium. Imaging of the biceps tendon was

performed with participant's hands placed on the lap, palms facing up and elbows flexed at 90 degrees. The subscapularis tendon was then surveyed with abduction of the forearm. The acromio-clavicular joint and coraco-acromio ligament were thereafter assessed. Imaging of the supraspinatus and rotator cuff interval followed, with internal rotation of the arm and the palm of the hand placed behind the participant's back (Fig. 1). Dynamic assessment was performed to evaluate subacromial impingement. The infraspinatus and teres minor tendons, posterior labrum and spino glenoid notch were imaged by placing the hand across the chest to the contralateral side of the shoulder and placing the transducer inferior to the spine of the scapula, posterolaterally on the humeral head.

**Shear Wave Ultrasound Elastography (SWUE) data collection:** The shear wave ultrasound elastography window size was selected based on the tendon size being examined. Sagittal and axial planes of three standardised sites (proximal, middle and distal) were scanned within each tendon. Six measurements were obtained for each tendon, except for the teres minor tendon (2), due to its small length. The shear wave ultrasound elastogram mode was activated using the Logiq E9 linear transducer, which utilises the comb-push excitation method that concurrently transmits acoustic radiation force impulse (ARFI) pulses. Time-interleaved shear wave tracking was used to estimate shear wave velocity, and the Young's modulus measurement in kilopascals (kPa), was applied. Both B-Mode and SWUE images were displayed side by side through a split screen (Fig. 2), with SWUE superimposed on a B-Mode image as a colour-coded, real-time picture. The colour scale, representative of relative tissue stiffness, ranged from red (soft tissue), to yellow/green (intermediate stiffness), to blue (hard tissue). The elastograms superimposed over B-Mode images were evaluated based on their colour displayed. Dark blue denoted stiff elasticity (hard tendons); dark to light blue depicted intermediate stiffness of elasticity, whilst a mixture of colours depicted tendon softening.

A circle was used to denote the region of interest (ROI) on the rotator cuff and biceps tendons, and ROI measurement of elastic modulus was placed centrally in the tendon, within the acquisition box. Various diameter sizes (range 1.8-4mm) of the ROI, were placed within the examined tendon. The most representative SWUE image of at least three concordant cycles was chosen, and the quantitative estimates of the mean stiffness of each tendon of the rotator cuff and biceps tendon stiffness were measured from the shear wave propagation velocity and recorded in kPa. The parametric estimates were derived from the software implemented in the ultrasound equipment. Verification of ultrasound data for both study groups were confirmed by a senior radiologist and subsequently recorded on printed paper and stored on Picture Archiving and Communications System workstation (PACS).

**Statistical analysis:** All data was analysed using IBM SPSS version 25. A p-value < 0.05 was considered as statistically significant. Data was tested for normality and continuous data was summarised as means, standard deviation, and minimum and maximum. All categorical data was summarised as frequency tables. Independent Student t-tests were used to

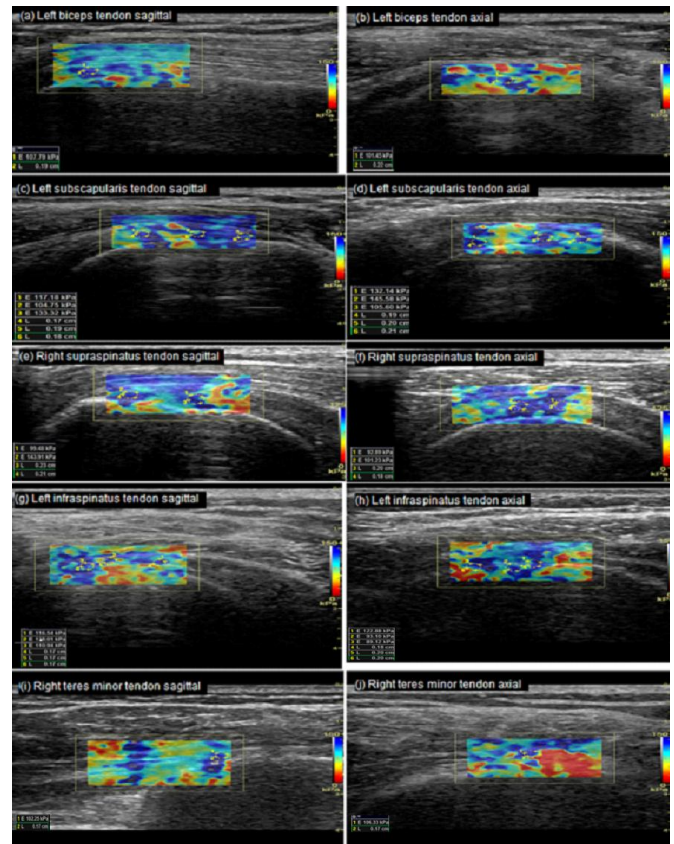
compare the means of elasticity of tendons for males and females. Reference ranges for all the rotator cuff and biceps tendons for males and females were calculated based on a 95% confidence interval for the mean.

**RESULTS**

**Demographic characteristics:** The demographic profile is summarized in Table 1. Of the total population recruited, 61% were females. The mean age was 34.52±7.75 years, whilst the mean BMI was 26.70±5.10kg/m<sup>2</sup>. None of the participants reported the use of pain medication.

**B-Mode Ultrasound Analysis:** The B-Mode examination revealed a normal, homogenous, and fibrillary medium grey echo pattern in all tendons. Closely spaced echogenic parallel lines were observed in the sagittal planes in contrast to the multiple echogenic dots in axial planes.

**Elastography Colour Map and Shear Wave Analysis**  
The qualitative colour map analysis of investigated tendons are shown (Fig. 2; Table 2).



**Figure 2:** Shear wave colour mapping and kPa values of left biceps tendon (a-b), left subscapularis tendon (c-d), right supraspinatus tendon (e-f), left infraspinatus tendon (g-h) and right teres minor tendon (i-j) in both the sagittal and axial planes. The numbers to the left of images depict the shear wave velocity in kPa values, represented by ‘E’. The yellow circles on the colour mapped tendon represent the region of interest (ROI) delineated by arrows (white). ‘L’ represents the width of the tendon examined.

**Table 1:**  
Demographic characteristics of study population

Gender	n (%)
Female	159 (61.2)
Male	101 (38.8)
BMI (kg/m <sup>2</sup> )	
15-25	118 (45.4)
26-35	128 (49.2)
36-45	14 (5.4)
Age (years/mean±SD)	34.52±7.75

**Table 2.**  
Means and standard deviations of SWUE for Colour maps of rotator cuff and biceps tendons at various insertion sites

Tendon	Colour Map Categories:		
	$\bar{x} \pm s$ (n, (% of total))		
	Dark blue	Dark to light blue	Mixture of colours
<b>Biceps - sagittal</b>	110.2±16.6	81.5±8.3	58.8± 11.1
Prox	(211, 81.2%)	(45, 18.1%)	(2, 0.8%)
<b>Biceps - axial</b>	106.1±13.4	79.4±9.7	70.9±15.1
Prox	(194, 74.6%)	(57, 21.9%)	(9, 3.5%)
<b>Subscapularis - sagittal</b>	110.3±14.3	80.2±9.4	75.6±20
Dist	(219, 84.2%)	(39, 15%)	(2, 0.8%)
<b>Subscapularis - axial</b>	110.5±14.4	79.8±9.2	57.6±19.4
Dist	(205, 74.8%)	(45, 17.3%)	(7, 2.7%)
<b>Supraspinatus - sagittal</b>	113.9±14.6	81.7±8.3	53.8±10.3
Dist	(224, 86.2%)	(33, 12.7%)	(3, 1.2%)
<b>Supraspinatus - axial</b>	110.5±14.6	80.9±11.4	72.5±27
Dist	(211, 81.2%)	(35, 13.5%)	(13, 5%)
<b>Infraspinatus - sagittal</b>	110.4±15.3	79.7±10.5	61.6±14.4
Dist	(217, 83.5%)	(35, 13.5%)	(8, 3.1%)
<b>Infraspinatus - axial</b>	107.9±13.5	80.1±12.9	64.4±14.6
Dist	(207, 79.6%)	(46, 17.7%)	(7, 2.7%)
<b>Teres minor - sagittal</b>	105.6±12.4	79.0±8.6	54.5±6.7
	(204, 78.5%)	(47, 18.1%)	(9, 3.5%)
<b>Teres minor – axial</b>	106.5±11.8	81.2±9.6	55.9±14.9
	(194, 74.6%)	(54, 20.8%)	(12, 4.6%)

**Table 3:**

Quantitative Shear wave elastography (kPa) of rotator cuff and biceps tendons (n=260)

<b>Tendon</b>		<b>Means ± SD (kPa)</b>	<b>Min - Max</b>	<b>95% Confidence limits</b>	
<b>Biceps - sagittal</b>	<i>Proximal</i>	104.83±18.89	53.25-164.90	102.52	107.14
	<i>Middle</i>	105.43±18.32	43.01-174.35	103.19	107.66
	<i>Distal</i>	106.16±17.91	58.25-170.22	103.97	108.34
<b>Biceps - axial</b>	<i>Proximal</i>	98.91±17.70	47.93-155.25	96.75	101.08
	<i>Middle</i>	99.04±16.14	47.87-149.32	97.07	101.01
	<i>Distal</i>	98.32±15.36	56.49-144.65	96.44	100.20
<b>Subscapularis - sagittal</b>	<i>Proximal</i>	105.33±20.42	34.79-163.07	102.84	107.82
	<i>Middle</i>	108.05±20.86	55.04-190.18	105.50	110.60
	<i>Distal</i>	105.06±18.02	42.17-161.78	102.86	107.26
<b>Subscapularis - axial</b>	<i>Proximal</i>	105.67±19.20	38.88-167.81	103.33	108.02
	<i>Middle</i>	104.68±19.90	29.83-169.61	102.25	107.11
	<i>distal</i>	103.65±19.44	36.39-169.89	101.28	106.03
<b>Supraspinatus- sagittal</b>	<i>Proximal</i>	109.78±22.50	23.65-173.05	107.03	112.52
	<i>Middle</i>	112.00±20.76	35.38-175.04	109.47	114.54
	<i>Distal</i>	109.10±18.57	41.91-162.11	106.83	111.37
<b>Supraspinatus - axial</b>	<i>Proximal</i>	105.98±19.95	51.26-166.63	103.54	108.41
	<i>Middle</i>	107.08±20.60	52.46-156.71	104.56	109.59
	<i>Distal</i>	104.67±19.52	44.48-156.17	102.29	107.06
<b>Infraspinatus - sagittal</b>	<i>Proximal</i>	106.80±19.55	27.01-178.22	104.41	109.19
	<i>Middle</i>	109.52±19.26	48.93-170.16	107.17	111.87
	<i>Distal</i>	104.79±19.64	31.51-175.21	102.39	107.19
<b>Infraspinatus - axial</b>	<i>Proximal</i>	103.58±17.40	41.99-159.85	101.46	105.71
	<i>Middle</i>	105.54±18.23	44.97-153.63	103.32	107.77
	<i>Distal</i>	101.70±17.12	44.71-150.69	99.61	103.79
<b>Teres minor - sagittal</b>		99.05±17.62	39.12-152.63	96.90	101.20
<b>Teres minor - axial</b>		98.88±18.06	34.98-148.95	96.68	101.09

**Table 4:**

Means, standard deviations for SWUE for males and females, p-values and 95% confidence limits for SWUE for biceps and tendons of the rotator cuff

<b>Tendon</b>		<b>Females Means ±SD</b>	<b>Males Means ±SD</b>	<b>p - value</b>	<b>Females 95% Conf. Limits</b>		<b>Males 95% Conf. Limits</b>	
<b>Biceps - sagittal</b>	<i>Proximal</i>	101.93±18.84	109.40±18.15	0.002*	98.98,	104.88	105.82,	112.99
	<i>Middle</i>	103.13±17.59	109.04±18.94	0.001*	100.38,	105.89	105.31,	112.79
	<i>Distal</i>	104.09±16.86	109.41±19.07	0.019*	101.46,	106.74	105.64,	113.18
<b>Biceps - axial</b>	<i>Proximal</i>	97.46±17.95	102.21±17.13	0.096	94.65,	100.27	97.83,	104.60
	<i>Middle</i>	97.31±15.82	101.77±16.33	0.030*	94.84,	99.19	98.55,	105.00
	<i>Distal</i>	97.41±14.84	99.75±16.11	0.232	95.09,	99.74	96.57,	102.94
<b>Subscapularis - sagittal</b>	<i>Proximal</i>	103.68±20.02	107.93±20.87	0.102	100.55,	106.82	103.81,	112.05
	<i>Middle</i>	105.48±18.44	112.11±23.73	0.018*	102.59,	108.37	107.43,	116.80
	<i>Distal</i>	103.36±18.10	107.72±17.65	0.057	100.53,	106.20	104.24,	111.21
<b>Subscapularis - axial</b>	<i>Proximal</i>	105.33±19.40	106.21±18.98	0.717	102.29,	108.37	102.47,	109.97
	<i>Middle</i>	103.58±20.59	106.40±18.74	0.267	100.36,	106.81	102.70,	110.10
	<i>Distal</i>	102.65±20.55	105.23±17.52	0.298	99.41,	105.89	101.78,	108.70
<b>Supraspinatus - sagittal</b>	<i>Proximal</i>	110.41±23.59	106.21±18.98	0.717	106.72,	114.11	104.69,	112.88
	<i>Middle</i>	110.96±20.92	113.65±20.51	0.309	107.68,	114.24	109.61,	117.70
	<i>Distal</i>	106.60±18.23	113.04±18.52	0.006*	103.75,	109.46	109.39,	116.70
<b>Supraspinatus - axial</b>	<i>Proximal</i>	104.44±20.59	108.40±18.73	0.118	101.22,	107.67	104.71,	112.11
	<i>Middle</i>	104.32±20.38	111.42±20.29	0.007*	101.13,	107.52	107.42,	115.43
	<i>Distal</i>	103.39±19.77	106.70±19.04	0.183	100.30,	106.49	102.95,	110.46
<b>Infraspinatus - sagittal</b>	<i>Proximal</i>	105.16±18.77	109.38±20.55	0.090	102.23,	108.10	105.33,	113.44
	<i>Middle</i>	109.32±18.92	109.84±19.87	0.832	106.36,	112.28	105.92,	113.77
	<i>Distal</i>	104.85±19.66	104.69±19.72	0.949	101.77,	107.93	100.80,	108.59
<b>Infraspinatus - axial</b>	<i>Proximal</i>	101.79±16.34	106.41±18.69	0.037*	99.23,	104.36	102.72,	110.10
	<i>Middle</i>	105.35±16.81	105.84±20.35	0.834	102.72,	107.99	101.83,	109.87
	<i>Distal</i>	100.64±16.07	103.37±18.61	0.201	98.12,	103.16	99.70,	107.05
<b>Teres minor - sagittal</b>		96.71±16.49	102.74±18.76	0.007*	94.13,	99.29	99.04,	106.45
<b>Teres minor - axial</b>		97.03±18.29	101.79±17.39	0.038*	93.31,	99.52	98.37,	105.23

\* p-value<0.05 was considered statistically significant

The elastograms depicted as “dark blue” indicates harder elasticity and greater strength. The “dark blue” category occurs in 74-86.15% of this cohort. “Dark blue” elastograms were also more concentrated at the distal supraspinatus tendon in the sagittal plane, in contrast to the axial planes of the proximal biceps, and teres minor tendon. Similarly, the ‘dark to light blue’ elastograms occurred in 12.69-21.92% of our cohort whilst a ‘mixture of colours’ only occurred in 0.8-5% of participants. Higher mean elasticity values ( $113.9 \pm 14.6$  kPa) were observed for the sagittal plane of the distal supraspinatus tendon versus the axial plane (Table 3). A statistically significant difference was noted between the elasticity means of the tendons categorised by “dark blue”, “dark to light blue” and “mixture of colours” ( $p=0.001$ ; Table 3). Notably “dark blue” exhibits the highest means across all tendons, followed by “dark to light blue”, whereas the “mixture of colours” demonstrates the lowest elasticity means. This colour grading with corresponding elasticity means provides a guide to the range of elasticity values for each tendon for each of the colour maps.

**Reference ranges for SWUE of the rotator cuff and biceps tendons:** The mean, standard deviation, minimum and maximum values were calculated for the entire sample of males and females. A 95% confidence interval for mean elasticity in sagittal and axial planes for the tendons for proximal, middle and distal are shown in Table 3. These confidence intervals can be used in practice as reference ranges for the elasticity of the respective tendons.

**Gender differences of SWUE for rotator cuff and biceps tendons:** An independent samples t-test was used to investigate whether gender differences in the tendon elasticity exist. The means, standard deviations, p-values and 95% confidence intervals for the mean elasticity of the tendon for males and females are shown in Table 4. A statistically significant difference in elasticity was observed between males and females for the proximal ( $p=0.002$ ), middle ( $p=0.011$ ), and distal ( $p=0.019$ ) biceps tendon in the sagittal plane, middle biceps tendon in the axial ( $p=0.030$ ), as well as the middle subscapularis tendon in the sagittal plane ( $p=0.018$ ). Moreover, a statistically significant gender difference in elasticity was observed for the distal supraspinatus tendon in the sagittal plane ( $p=0.006$ ), middle supraspinatus in the axial plane ( $p=0.007$ ), proximal infraspinatus tendon in the axial plane ( $p=0.037$ ), teres minor tendon in the sagittal ( $p=0.007$ ) and axial planes ( $p=0.038$ ). Higher mean elasticity values were noted amongst males compared to females for all tendons except for the sagittal supraspinatus (proximal), where for males  $\bar{x} = 108.79$  and for females  $\bar{x} = 110.41$ . This was however, not a significant difference since the  $p$ -value = 0.717

## DISCUSSION

Imaging modalities such as MRI and MRA are expensive and unable to provide real time data, hence limiting its use in establishing pathological and biomechanical properties of tendinopathies. Based on the paucity of biomechanical data

available on normal tendons in south Africa, we aimed to establish a reference range of the rotator cuff and biceps tendons using SWUE. Our B-Mode ultrasound findings of the rotator cuff and biceps tendons displayed parallel echogenic lines in the sagittal planes and echogenic dots in axial planes, similar to previous reports (Hou *et al.*, 2017, Hodgson *et al.*, 2012, Hackett *et al.*, 2019). Notwithstanding the nature of an asymptomatic population, we still observed minor pathological changes in 11 samples, of which 9 were over the age of 40 years. These changes included partial thickness tears and calcifications in 9 distal supraspinatus tendons and calcifications in 2 distal subscapularis tendons, which may be attributed to degenerative anomalies characteristic of ageing (Lee *et al.*, 2016, Matthewson *et al.*, 2015).

Variations in colour map pathologies exists widely (Hou *et al.*, 2017, Lalitha *et al.*, 2011, Ohuegbe, 2014), presumably due to varying manufacturer preset options. Our data is based on the GE Logiq E9 manufacturer presets, similar to a previous report (Lalitha *et al.*, 2011), an exception is their use of the GE E8 ultrasound equipment. In our study, tendon colour mapping at the humeral insertion were recorded as ‘dark blue’ colour maps, indicative of harder elasticity and higher kPa values. Ohuegbe (2014) reported an 86% predominance of blue/green colour maps in their 284 supraspinatus tendons sampled, grading them as hard tendons, however their ultrasound manufacturer details was unavailable to verify comparisons (Ohuegbe, 2014).

Pathological changes of the supraspinatus tendons at the distal insertion was noted in 4 of our participants, corroborating Lalitha and colleagues, who demonstrated tendinosis as a mixture of green and red colour maps, indicative of fluctuations in soft elasticity (Lalitha *et al.*, 2011). In contrast, Hou’s group reported blue colour maps in regions of tendinosis or tears (Hou *et al.*, 2017), whilst Ohuegbe (2014) reported a 14.1% tendinopathy in 40 asymptomatic tendons, as yellow colour maps, indicative of intermediate tissue stiffness (Ohuegbe, 2014). More recently, Sahan’s group reported a 10% prevalence of blue/green colour maps in the biceps tendons of asymptomatic participants aged  $47.95 \pm 11.19$  years, indicative of hard tissue (Sahan *et al.*, 2018), suggesting that hardness increased in tendinosis in symptomatic participants. Our study highlights soft elasticity as a “mixture of colours” (1.53%), whereas others report “green and yellow” (35%) as soft elasticity (Sahan *et al.*, 2018, Ohuegbe, 2014, Hou *et al.*, 2017). Variations in colours noted in other studies, depicting levels of elasticity may be attributed to the pre-sets applied on the specific ultrasound equipment used.

Higher elasticity values were noted in the sagittal compared to the axial planes of all tendons measured in both males and females. A statistically significant difference was noted in the teres minor tendon in both the sagittal and axial planes ( $p<0.05$ ). Overall, the lower elasticity values observed in the axial planes may be due to muscular skeletal orientation similarly reported by others (Davis *et al.*, 2019), whereas the higher values in the sagittal plane (proximal, middle and distal biceps tendon) contradicts the lower values reported by Sahan’s group (Sahan *et al.*, 2018). The lack of statistical significance observed in elastic values between the fluid and

non-fluid portions of the biceps tendon sheath was also similar to others, suggesting the non-existence of shear wave in pure fluids and its ineffectiveness in examining cystic structures (Winn *et al.*, 2016). We also report much lower mean elastic values of the distal insertion of the normal supraspinatus tendon in the sagittal plane in contrast to others (Hackett *et al.*, 2019, Arda *et al.*, 2011, Dischler *et al.*, 2018). Elasticity variations may be attributed to varying ultrasound equipments and algorithm programs used, patient positioning, the degree of transducer pressure and orientation in relation to the region of interest, the number of measurements and the depth of acquisition.

We ensured a minimal degree of tension in positioning techniques since increased tension used in musculoskeletal elastography has been associated with increased stiffness and higher kPa values (Eby *et al.*, 2013). Moreover, the speed of shear wave is more rapid through stiff contracted tissue especially along the long axis of tendons and muscle, when compared to the axial plane due to tendon anisotropy (Aubry *et al.*, 2014). Tendon stretching possibly leads to higher stiffness in asymptomatic participants whilst tendon positioning under passive and active conditions minimally affects SWUE (Aubry *et al.*, 2014). Lower mean elasticity values were reported in symptomatic supraspinatus tendons when exposed to tension ( $p < 0.024$ ), versus the relaxed symptomatic supraspinatus tendon, alluding to underlying rotator cuff pathologies or changes in the neuromuscular firing patterns linked to pathology (Baumer *et al.*, 2018).

Males demonstrated higher elasticity values of the supraspinatus tendons compared to females, corroborating Arda *et al.* (2011); however, lower values were noted for males ( $36.0 \pm 13.0$  kPa) and females ( $29.1 \pm 12.4$  kPa) compared to the values obtained in this study. The higher elasticity (kPa) values observed in our study may be attributed to technical method of locating the ROI at specific sites within all the tendons. In contrast, the ROI within the supraspinatus tendon was undefined by Arda and co-workers (Arda *et al.*, 2011). Several others failed to demonstrate any gender variations in elasticities (Baumer *et al.*, 2018, Hou *et al.*, 2017, Hackett *et al.*, 2019) in contrast to us. We highlight higher and significant differences for the proximal infraspinatus tendon in the axial plane, the teres minor tendon in the sagittal and axial planes, for males in comparison to female tendons. Gender variations for the biceps tendon was demonstrated as one mean elasticity value for both males and females, in which the reference range was acquired in the sagittal plane (1-4 cm from proximal insertion) and axial plane where the tendon was most prominent (Sahan *et al.*, 2018). Notably, lower elasticity values were recorded in the sagittal and axial planes in the proximal biceps tendon for females versus males, and may be due to anisotropic changes and the inability to align the tendon perpendicular to the ultrasound beam, commonly experienced occur during transducer angulation.

Despite our large sample number, there was an unequal distribution of male and female participants. Future studies should explore an equal gender distribution to provide a proportionate population assessment. Additionally, standardisation of the frame rate and dynamic range of shear wave ultrasound software and positioning techniques of the rotator cuff and biceps tendons must be ensured, considering

that technical parameters differ between vendors, resulting in variable data between studies.

In conclusion, we have provided a possible reference range of SWUE for the rotator cuff and biceps tendons, based on 95% confidence intervals, as an ancillary imaging support for clinical practice. To our knowledge, this is the first South African study to estimate a reference range value in a single examination of the shoulder joint, as well as report on gender variations for SWUE for the proximal, middle and distal sites for rotator cuff and biceps tendons. Our data provides evidence that SWUE based on its reproducible quantification of tissue elasticity, can supplement B-Mode ultrasound during clinical assessment of the rotator cuff and biceps tendons. The reference values presented can be used as a baseline for future studies, to influence the early screening of rotator cuff and biceps tendon pathology. Future studies should integrate shear wave ultrasound elastography with CT and MRI, and fusion imaging in order to strengthen their screening performance in treatment planning.

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