Intra-rater reliability and smallest detectable change of compression sonoelastography in quantifying the material properties of the musculoskeletal system

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Title page

Title:

Intra-rater reliability and smallest detectable change of sonoelastography in quantifying the material properties of the musculoskeletal system

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Reliability of sonoelastography in quantifying the material properties of the musculoskeletal system.

Abstract

Musculoskeletal conditions can change tissue elasticity. Knowledge of musculoskeletal elasticity could therefore aid clinical diagnosis and management. Sonoelastography is an ultrasound-based system that examines the material properties of tissues, and it may be useful in musculoskeletal practice. Therefore, it is important to establish its clinimetric properties. This study aimed to explore the intra-rater reliability and the smallest detectable changes of sonoelastography in examining musculoskeletal structures. A quantitative reliability design was used to examine 22 healthy participants using a compression sonoelastography system that produces color-coded images. The deltoid, biceps brachii, brachioradialis, rectus femoris, gastrocnemius medius muscles, and Achilles tendon were examined twice at one-hour intervals to assess the intra-rater reliability. The sonoelastography images were analyzed using the strain index, strain ratio, and color pixels. The intra-rater reliability and the smallest detectable changes of each outcome variable were determined. Intra class correlation coefficient was used to quantify the repeatability of the measurements, and the smallest detectable changes were calculated to determine clinically important differences above the error of measurement. The intra-rater reliability for the strain index, strain ratio, and color pixel analysis ranged from moderate to excellent (intra-class correlation coefficients: 0.734–0.950, 0.776–0.921, and 0.754–0.990, respectively), with color pixel analysis demonstrating the highest reliability. The smallest detectable changes were determined for all structures, including the Achilles tendon (0.11 for the higher boundary of the strain index, 1.80 for the strain ratio, and 2.90% for red pixels, representing soft tissues). Color pixel analysis may be more reliable for sonoelastography interpretation than the strain index and strain ratio. The calculated smallest detectable changes could be used to identify clinically important differences.

Keywords:

Sonoelastography, reliability, muscle, tendon, strain, elasticity.

Reliability of sonoelastography in quantifying the material properties of the musculoskeletal system.

Introduction:

Knowledge of the material properties of human tissues can be important for understanding the underlying pathology and functional changes and thereby optimizing clinical diagnosis and management. For example, knowledge of the stiffness of breast masses aids in the differentiation of malignant and benign tumors (Nariya et al., 2010; Regini et al., 2010), and liver stiffness has been correlated with liver fibrosis, aiding diagnosis as well as determination of the fibrosis grade (Yeh et al., 2002; Tatsumi et al., 2008). The pathological course of tendinopathy can cause tissue softening, degeneration, or calcification, all of which significantly change the elasticity of the affected tissues (Arya and Kulig, 2919; De Zordo et al., 2010; Chard et al., 1994). Generalized laxity of the musculoskeletal system, with a significant reduction in musculotendinous stiffness, is a dominant feature of connective tissue disorders and is associated with mutations in genes encoding collagen (Simmonds and Keer, 2007; Hakim and Grahame, 2003; Rombaut et al., 2012). Moreover, musculotendinous elasticity is essential for normal functioning. Elasticity is needed for energy storage and for force transmission and release in the musculotendinous unit (Rabita et al., 2008). Changes in musculotendinous elasticity can cause deficiencies in the production and transmission of the forces needed to function (Arya and Kulig, 2010; Rabita et al., 2008; Bojsen-Moller et al., 2005).

Compression sonoelastography (SEG), an ultrasound-based technology that aims to quantify tissue elasticity by providing color-coded images, has been recently introduced into musculoskeletal practice (Garra, 2007; Wu et al., 2012; Alsiri et al., 2019). Compression SEG creates sonographic images of strain by exploiting the principle that soft tissues display greater deformation and hard tissues display less deformation when subjected to similar forces (Garra, 2007; Wu et al., 2012). The application of SEG in musculoskeletal practice is currently under exploration. Promising results have been reported, suggesting the potential value of SEG in detecting subclinical manifestations in the Achilles tendon (De Zordo et al., 2010; Drakonaki et al., 2009). We have demonstrated the potential of SEG to distinguish between patients with hypermobility spectrum disorders and healthy controls (Alsiri et al., 2019). However, several clinimetric properties of SEG, including its reliability and the smallest detectable change, need to be determined for various regions of the musculoskeletal system. The reliability of SEG has previously been explored in relation to a small number of specific structures. SEG has shown good inter- and intra-rater reliability for examining the Achilles tendon, good inter-rater reliability for examining the plantar fascia, and excellent intra-rater reliability for examining the biceps brachii muscle (Drakonaki et al., 2009; Wu et al., 2011; Yanagisawa et al., 2011). However, most of the previous studies used qualitative methods for SEG image analysis. Only a few studies have introduced more quantitative analytical approaches for specific structures such as the biceps brachii muscle and the plantar fascia (Wu et al., 2012; Yanagisawa et al., 2011). We have previously explored the deltoid, biceps brachii, brachioradialis, rectus femoris, and gastrocnemius muscles, and the patellar and Achilles tendon using semi-quantitative methods related to the strain index, strain ratio, and color pixel analysis (Alsiri et al., 2019). Such methods of analysis have the potential to overcome the subjectivity in SEG assessments, thereby enhancing its procedural reliability and optimizing its clinical use for purposes such as differential diagnosis (Wu et al., 2012). However, the additional clinimetric properties of such SEG outcome variables need to be established.

The aim of the current study was to explore the intra-rater reliability and the smallest detectable change of SEG in examining a range of musculoskeletal structures in the upper and lower limbs by using an objective image analysis approach.

Methods:

Ethics:

The research was conducted with the approval of the Research Ethics Committee of X Ministry of Health (ref: 571/2017). Informed consent was obtained from all participants. Voluntary participation, confidentiality, privacy, and dignity were explained and maintained.

Design and sample size:

A quantitative reliability research design was employed by conducting two trial examinations (Walter et al., 1998). The sample size for the intra-rater reliability study was determined using the functional approximation proposed by Walter, Eliasziw, and Donner (Walter et al., 1998). The number of participants required to estimate the intra-class correlation coefficient (ICC) was determined to be 15 at $\alpha = 0.05$ and $ß = 0.20$, assuming the measurements were repeated twice (Walter et al., 1998). Twenty-two participants were recruited to allow for potential attrition.

Recruitment and eligibility criteria:

Healthy participants were recruited from among the staff at Al-Razi orthopedic hospital, Kuwait. An advertising email was sent which detailed the eligibility criteria for participating. Participants who responded to indicate they were willing to participate were contacted by the chief investigator to check their eligibility criteria and to arrange an examination appointment. The data collection started in June 2017 and ended in August 2017.

Healthy women and men aged ≥ 18 years were included. The exclusion criteria were as follows: injury in the upper or lower limbs during the previous three months, surgery on the upper or lower limbs during the previous 12 months; fracture in the upper or lower limbs during the previous 12 months; pregnancy and parturition during the previous 12 months (due to postpartum ligament laxity); connective tissue disorder; and conditions that might cause weakness in the upper or lower limbs. Three female potential participants were excluded, one because of pregnancy and two because of parturition within the previous 12 months.

Instrumentation:

Compression SEG (Voluson E8, General Electric Company, Milwaukee, WI, USA) was used to assess the perpendicular resting elasticity of a range of musculoskeletal structures. As all the examined structures were superficial, a high frequency transducer was used (6 - 15 MHz) to penetrate 4 - 5 cm below the skin. B-mode ultrasound image brightness was standardized using the default gain setting for musculoskeletal imaging. SEG is an ultrasonography-based system that measures tissue displacement (strain) in the direction of the applied force by applying external mild compressions (stress) using a SEG probe (Sconfienza et al., 2010; Turan et al., 2013; Klauser et al., 2014). The resulting tissue displacement is calculated and then converted in real-time into color-coded images, where each color represents a different degree of elasticity (Figure 1) (Turan et al., 2013; Klauser et al., 2014). The elasticity index and ratio are strain measures generated by SEG to reflect the mechanical properties of the tissues semi-quantitatively (Bamber et al., 2014; Tan et al., 2013). The strain ratio can be calculated from the elasticities of an area of interest and a reference area, usually subcutaneous fat (Klauser et al., 2014; Bamber et al., 2014). SEG use is quick and practical (Turan et al., 2013; Hoyt et al., 2008).

Figure 1 will be inserted here---------------------------------

Data collection:

Data collection began with measurement of the participants' height and weight. The dominant limbs (self-declared by participants) were scanned in a resting position, starting with the upper limb and followed by the lower limb structures. Real-time SEG was conducted to obtain real-time SEG images by inducing local strain through compression and decompression with the SEG transducer. The color scale for the display was red for soft tissues, green for intermittent elasticity, and blue for hard tissues. The SEG system is equipped with a visual quality indicator to allow adjustment of the applied strain and to tell the user if the applied compression is sufficient to measure tissue elasticity (Figure 1) (Klauser et al., 2014). The SEG examination yielded a color map that was superimposed on a B-mode ultrasound image (Figure 1). The examiner had four years of experience in using SEG.

The dominant deltoid, biceps brachii, brachioradialis, rectus femoris, and gastrocnemius medius muscles, and Achilles tendon were assessed using the higher and lower boundaries of the strain index and the strain ratio (Figure 1). The examined structures of the upper and lower limbs were selected to be both easily accessible and to aid in formulating a generalizable concept regarding the intra-rater reliability of SEG in examining the musculoskeletal system. The higher boundary of the strain index refers to the elasticity of the area of interest (muscle or tendon), and the lower boundary of the strain index refers to the elasticity of the reference area (subcutaneous fat), which was selected to be as superficial as possible (Bamber et al., 2014). SEG examination was conducted in accordance with the guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) (Bamber et al., 2014).

The middle fibers of the deltoid muscle were longitudinally scanned with the participant in a sitting position, shoulder in a neutral position, elbow joint at 90^0 of flexion, with the forearm supinated on the participant's thigh (Backhaus et al., 2001). The biceps brachii and brachioradialis muscles were longitudinally scanned with the participant in a supine position, the shoulder positioned by the participant's trunk, and the forearm in a supinated position. The biceps brachii was scanned 5 cm above the elbow at the biceps common muscle belly, and the brachioradialis was scanned 5 cm below the elbow (Chen et al., 2017). The rectus femoris was scanned in a supine position, with the knee extended. Longitudinal images were obtained for the rectus femoris muscle 10 cm above the knee joint line. The gastrocnemius medius (longitudinal) and Achilles tendon (transverse) were examined while the participant was in a prone position with the knee extended and the foot hanging over the plinth edge in a relaxed position (De Zordo et al., 2009). The gastrocnemius medius was scanned at 30% of its proximal length, calculated from the midpoint of the popliteal fossa to the midpoint between the ankle malleoli (Kawakami et al., 1998; Chino et al., 2012). The examination position could affect the elasticity of the examined muscles and tendons, so the examination positions were carefully standardized to be as clinically replicable as possible.

The examination procedure was repeated on the same day by the same examiner to assess intra-rater reliability, with an interval of one hour between the two examinations. Two to three participants were examined per day, with one to two participants being examined in the one-hour interval between the two examinations for each participant. This examination schedule offered the advantage of making it difficult for the examiner to remember the earlier results when performing the second examination on a given participant. A longer time interval between the examinations was not practical and repeating the examination on another day might have introduced the risk of changes in the results because of external factors such as the effects of exercises or fatigue. The same researcher conducted the data collection and analysis, but analyses were performed only after all images were acquired. Each structure was examined two to three times, and one image for each structure was saved for further analysis, selected according to the sufficiency of the compression magnitude assessed by the SEG's visual quality indicator.

Statistical analysis:

Statistical Package for Social Sciences (SPSS 23, IBM Corp., Armonk, USA) was used for data analysis. SEG measures tissue elasticity semi-quantitatively, in terms of strain index and strain ratio (Garra, 2007). The higher boundary of the strain index was calculated by tracing the area of interest, and the lower boundary of the strain index was calculated by tracing the subcutaneous fat. The two boundaries of the strain index are strain measures obtained automatically by the SEG during the examination after tracing the area of interest and subcutaneous fat. These boundaries are then used to calculate the strain index (Alsiri et al., 2019). The strain ratio is the ratio of elasticity of the area of interest and the elasticity of the subcutaneous fat, which was estimated from the ratio of the higher and lower boundaries of the strain index (Alsiri et al., 2019; Bamber et al., 2014; Tan et al., 2013; Griffith, 2015) Color pixels were analyzed using ImageJ, a Java-based processor (U. S. National Institute of Health, Maryland, USA) with a downloaded plugin (color pixel counter) (Figure 2) (Pichette, 2010). Image J is an adaptable image analysis system, that can be used for diverse purposes including medical imaging (Schneider et al., 2012). It was used to count the color pixels of each color of the SEG images (Pichette, 2010). As the cross-sectional area of the examined structures varies between participants, the percentage of the color was calculated using the following formulae used by Richardson et al. (2007) (Richardson et al., 2007).

Total pixels = red mean pixels + green mean pixels + blue mean pixels

Red pixel percentage = red mean pixels / total pixels * 100

Green pixel percentage = green mean pixels / total pixels * 100

Blue pixel percentage = blue mean pixels / total pixels * 100

Figure 2 to be inserted here ---

ICC (3.1 type) (two-way mixed effect model with absolute agreement) were used to assess the repeatability of the measurements (Koo and Li, 2016; Cicchetti et al., 2015). Smallest detectable changes were calculated for the strain index, strain ratio and color pixels (Polit, 2014). The quality of the data was checked and successfully enhanced following good clinical practice and data management guidelines (Broeck et al., 2005). Before data analysis, all SEG images were screened for the presence of artifacts and insufficient compression as suggested with the quality visual indicator, after which erroneous data were excluded before analysis (Broeck et al., 2005).

Results:

The demographic characteristics of the 22 participants are summarized in Table 1. Images with artifacts were excluded, as well as images with insufficient compression, as determined by the visual quality indicator supplied with the SEG system (Figure 1). Table 1 shows the number of participants examined for each anatomical structure. The exclusions did not cause the study to be underpowered. The areas with the largest number of exclusions were the biceps brachii and brachioradialis muscles, but even for these, the sample size was 15 following exclusions, which is equal to the essential sample size determined by the sample size calculation (Table 1).

The intra-rater reliability of SEG for the lower boundary of the strain index ranged from moderate to excellent (ICCs ranged from 0.734 to 0.950) (Table 2). The reliability for the higher boundary of the strain index ranged from good (ICC 0.852 for the deltoid muscle) to excellent (ICC 0.943 for the brachioradialis muscle) (Table 2). The intra-rater reliability for the strain ratio ranged from moderate (ICC 0.776 for the rectus femoris muscle) to excellent (ICC 0.921 for the deltoid muscle) (Table 2). The smallest detectable change ranged from 0.01 to 0.03 for the lower boundary of the strain index, 0.09 to 0.19 for the higher boundary of the strain index, and 1.80 to 4.93 for the strain ratio (Table 2). Table 2 summarizes the intra-rater reliability and smallest detectable change values for all the examined structures.

Table 1 to be inserted here ------------------------

Table 2 to be inserted here ------------------------

The intra-rater reliability of the color pixel analysis ranged from moderate to excellent for the three colors (Table 3). For the analysis of red pixels (soft tissues), the ICC ranged from 0.839 to 0.990, indicating good to excellent reliability. The lowest reliability was observed for the biceps brachii muscle, and the highest reliability was observed for the Achilles tendon. The ICC for green pixels (intermediate elasticity) ranged from 0.754 to 0.933, indicating moderate to excellent reliability (Table 3). The ICC for green pixels was lowest for the deltoid muscle and highest for the rectus femoris muscle. The blue pixel analysis (hard tissues) showed good to excellent intra-rater reliability, with ICC values ranging from 0.844 to 0.942 (Table 3). The lowest reliability was observed for the biceps brachii muscle and the highest reliability was observed for the Achilles tendon (Table 3). The smallest detectable changes for color pixel analysis ranged from 2.90% to 22.95% (Table 3).

Table 3 to be inserted here ----------

Discussion:

The intra-rater reliability of SEG in examining the musculoskeletal system was variable and dependent on the method of analysis and anatomical site. Analysis of the SEG images using the strain index, strain ratio, and color pixel analysis showed moderate to excellent intra-rater reliability. Higher intra-rater reliability was identified with color pixel analysis when compared to the strain ratio for all the examined structures. These findings highlight the impact of the image analysis approach on the intra-rater reliability of SEG. Color pixel analysis can provide more accurate results as it takes into account the entire area of interest, whereas calculation of strain index and ratio is limited to a selected area within the area of interest. Thus, the subjectivity in selecting the area for use in calculating the strain index and ratio might explain its slightly reduced intra-rater reliability when compared to that of color pixel analysis. Color pixel quantification could enhance the clinical benefits of SEG for assessing the compression material properties. The smallest detectable changes could be used to determine clinically useful differences, above the level of measurement error, and ranged from 0.09 to 0.19 for the strain index, 1.80 to 4.93 for the strain ratio, 2.90 to 9.27% for the proportion of red pixels, 3.84% to 22.14% for green pixels, and 6.99% to 22.95% for blue pixels.

The current study introduced a semi-quantitative analysis approach that considers the entire area of interest by using ImageJ software. This software counted the pixels of each color (red, green, and blue) in the SEG images, and the percentages of each color could then be calculated to compensate for between-participant differences in the cross-sectional areas of structures. The strain index and ratio are quick to determine and clinically accessible, but they might not provide precise quantification for the entire area of interest. Color pixel quantification using easily available software could enhance the clinical benefits of SEG.

The reliability of SEG was examined previously in relation to specific musculotendinous structures, such as the biceps brachii muscle and Achilles tendon. High intra-rater reliability was shown for SEG examination of the biceps brachii muscle: the ICC ranged from 0.939 to 0.971 using the strain ratio, compared to 0.776 to 0.882 using a tissue hardness meter (Yanagisawa et al., 2011). In the current study, the intra-rater reliability was relatively similar for examination of the biceps brachii muscle using the strain ratio (0.839 ICCs) and by color pixel analysis (0.839, 0.878, and 0.844 ICCs for the red, green, and blue pixels, respectively). A reference hydrogel material was used by Yanagisawa et al. (2011) to obtain the strain ratio, which could explain their higher intra-rater reliability when compared to the current study. The current study used subcutaneous fat tissue as a reference to obtain the strain index and strain ratio. High test-retest reliability has been reported previously for Achilles tendon examination; ICCs 0.83-0.95 (Schneebeli et al., 2016), which is consistent with the ICC of 0.824 obtained in the current study.

The current findings suggest that SEG could be more reliable in some structures than in others. The analysis using the strain index showed the lowest intra-rater reliability (moderate to good) for examining the deltoid and biceps brachii muscles. In contrast, excellent intra-rater reliability was demonstrated for examining the brachioradialis muscle and the lower limb structures using the strain index. Similarly, the intra-rater reliability of SEG using color pixel analysis showed the lowest values for the deltoid (green pixel analysis), biceps brachii (red, green, and blue pixel analysis), and brachioradialis (green pixel analysis). However, color pixel analysis for the lower limb structures showed predominantly excellent intra-rater reliability, except for the gastrocnemius medius' green pixel analysis data, which showed good intra-rater reliability. This could be related to differences in the structure size and morphology. One of the confounding factors in compression SEG is the lack of usable anatomical planes in the examined areas, which might result in slippage of the transducer during the examination, either anteriorly or within the area of interest (Bamber et al., 2014). Moreover, 82% of the participants in the current study were female, and the sizes of the examined structures in their upper limbs were relatively small. Thus, the applied vertical compression might not have been highly reproducible due to the small size of the structures, which might have made it slightly more difficult to apply uniform compressions. However, for the lower limb structures, the calculated reliability was higher. This could be related to the larger size of the examined areas and the presence of more usable anatomical planes, allowing uniformly perpendicular compressions.

Analysis of the red pixels showed the highest reliability (though only marginally), with ICCs ranging from 0.839 to 0.990, followed by the blue pixels (ICCs ranging from 0.844 to 0.942), and then green pixels (ICCs ranging from 0.754 to 0.933). The red colors in the current study indicated soft tissues that could easily be deformed by the compressions applied, which might explain their high reported reliability. The green and blue colors indicated intermediate and hard tissues, which were not so easily deformed. Color pixel analysis for all three colors showed predominantly good to excellent reliability, although the red pixels were the most reliable indicators of tissue elasticity.

Intra-rater reliability studies can identify the capacity of the same rater to provide reproducible and consistent measurements, which is clinically important (Gwet, 2008). The SEG is a highly operator-dependent system, so it is important to explore the consistency of the same rater for clinical follow-up purposes. However, future studies should examine other clinimetric properties. The methodology and the findings of the current exploration can form a solid foundation for future studies.

One of the main strengths of the current research is introducing a software which can convert the subjective SEG images into objective numbers, allowing determination of the consistency of this image analysis approach. ImageJ has been used previously to quantify color-coded SEG images, where this analysis method has successfully differentiated between normal and pathologic tissues (Alsiri et al., 2019; Elyas et al., 2017) The established smallest detectable changes can be used to identify the smallest amount of change that needs to be observed before determining an actual condition-related change free from measurement error (Terwee et al., 2009). The established smallest detectable changes can help clinicians interpret results by having a benchmark of the expected measurement error and system variability (Terwee et al., 2009). Clinically, condition-related differences can be only considered if the changes are larger than the measurement error estimated with the smallest detectable changes, which is also known as the minimal clinically important difference (Terwee et al., 2009). For example, the SDC for red pixel analysis for the Achilles tendon was established as 2.90% in the present investigation. In a separate investigation, we found a difference of 12.54% in the proportion of red pixels between people with hypermobility spectrum disorders and healthy controls, a difference that is much higher than might be expected through measurement errors (Alsiri et al., 2019). Future studies should examine the capacity of SEG to distinguish between health and other pathologic tissues.

All the structures examined in the current exploration were superficial. Thus, caution should be exercised when examining deeper musculoskeletal structures, for which the reliability of SEG has not yet been examined. Presenting the higher and lower boundary of the strain index together with the strain ratio could provide more comprehensive information on tissue elasticity in cases of discrepancies in the force being absorbed by the subcutaneous fat and the area of interest. Additionally, force mal-absorption by the examined areas can be detected as artifacts in the SEG images; therefore, artifacts in SEG images should be carefully excluded before analyzing them. SEG is a highly operator-dependent device, and the use of very low or high compression can substantially change the results (Wu et al., 2012). This issue was minimized by using an on-screen visual quality indicator. Increasing the compression magnitude changed the indicator from red (indicating insufficient compression) to green (indicating sufficient compression), but the indicator does not warn if excessive compression is applied. A numerical indicator might solve this issue. The established smallest detectable changes reflect the marginal error for examining normal musculoskeletal structures, which could be employed for clinical scenarios such as examination of pathological structures. However, future studies on pathological structures will be more clinically applicable.

The current study has several limitations. First, the participants were recruited from a governmental hospital, and their body mass indexes showed that, on average, they were overweight to obese. This factor might have produced the artifacts observed in some images, resulting in an inability to obtain eligible elastograms and consequent exclusion from analysis. In overweight/obese patients with thick subcutaneous fat layers, the probe compression force could have been absorbed mainly by the subcutaneous fat before reaching the area of interest, putting the reliability of SEG at risk. Data for between two (for the gastrocnemius medius muscle), and seven (for the biceps brachii and brachioradialis muscles) of the 22 participants were excluded for each musculoskeletal structure due image artifacts and suboptimal elastograms. This could be controlled for in future research by adding body mass index to the inclusion or exclusion criteria. However, this approach could jeopardize the generalizability of the results. Second, the sitting position for examining the deltoid muscle might not be the optimal position to stabilize the participant for the examination, as there was movement in some cases. It is recommended to use a side-lying position for deltoid muscle examination.

Conclusion

In conclusion, the current study indicates moderate to excellent intra-rater reliability of SEG for examining a range of musculoskeletal structures, dependent on the image analysis approach and anatomical structures. Color pixel analysis is a more precise and reliable analysis method for SEG color-coded images when compared with the strain ratio and strain index. The observed smallest detectable changes could be used to identify clinically important differences. Future studies are needed to establish the other clinimetric properties of SEG.

Conflict of interest:

The Authors declare no conflict of interest.

Author contributions:

Dr. Najla Alsiri: contributions to concept/design, acquisition of data, data analysis/interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article.

Prof. Saud Al-Obaidi: contributions to concept/design, critical revision of the manuscript and approval of the article.

Dr. Akram Asbeutah contributions to concept/design, acquisition of data, data analysis/interpretation, and approval of the article.

Prof: Shea Palmer contributions to concept/design, data analysis/interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article.

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Tables:

Table 1: Demographic characteristics of the examined participants; mean (standard deviation) (n =22).

	Men and women (n = 22)	Women (n = 18)	Men (n = 4)
Age (years)	34.72 (7.00)	33.38 (6.41)	40.75 (7.13)
Height (cm)	162.63 (7.80)	160.61 (6.68)	171.75 (6.13)
Weight (Kg)	74.72 (15.41)	70.50 (13.28)	93.75 (8.50)
Body mass index	27.97 (4.27)	27.26 (4.37)	31.17 (1.65)

Table 2: Reliability of the sonoelastography strain index and strain ratio for measuring the elasticity of a range of musculoskeletal structures.

Examined area	Lower Boundary of Strain Index					Higher Boundary of Strain Index					Strain Ratio*				
	Mean (SD) Trial 1	Mean (SD) Trial 2	ICC	95% CI	SDC	Mean (SD) Trial 1	Mean (SD) Trial 2	ICC	95% CI	SDC	Mean (SD) Trial 1	Mean (SD) Trial 2	ICC	95% CI	SDC
Deltoid muscle (n = 17)	0.05 (0.01)	0.05 (0.00)	0.734	0.279 – 0.903	0.01	0.49 (0.08)	0.48 (0.08)	0.852	0.600 – 0.946	0.11	9.05 (2.03)	8.81 (1.84)	0.921	0.780 – 0.972	2.72
Biceps brachii muscle (n = 15)	0.08 (0.02)	0.07 (0.01)	0.825	0.496 – 0.941	0.01	0.45 (0.08)	0.45 (0.11)	0.889	0.668 – 0.963	0.11	5.62 (1.05)	5.71 (1.08)	0.839	0.510 – 0.946	2.17
Brachioradialis muscle (n = 15)	0.08 (0.04)	0.08 (0.04)	0.911	0.732 – 0.970	0.03	0.64 (0.24)	0.60 (0.22)	0.943	0.833 – 0.981	0.19	8.39 (2.59)	8.87 (3.05)	0.827	0.476 – 0.942	3.84
Rectus femoris muscle (n = 16)	0.10 (0.03)	0.09 (0.03)	0.950	0.848 – 0.983	0.01	0.46 (0.09)	0.44 (0.08)	0.903	0.724 – 0.966	0.09	4.88 (1.14)	4.78 (1.26)	0.776	0.349 – 0.922	1.90
Gastrocnemius medius muscle (n = 20)	0.07 (0.03)	0.07 (0.03)	0.904	0.756 – 0.962	0.03	0.58 (0.19)	0.59 (0.18)	0.925	0.809-0.970	0.19	9.60 (4.00)	10.25 (4.88)	0.893	0.731 – 0.957	4.93
Achilles tendon (n = 18)	0.07 (0.02)	0.07 (0.02)	0.909	0.762 – 0.966	0.01	0.49 (0.12)	0.47 (0.11)	0.914	0.776 – 0.968	0.11	6.46 (1.79)	6.82 (2.10)	0.824	0.533 – 0.934	1.80

SD: Standard deviation, ICC: intra-class correlation coefficient, CI: confidence interval, SDC: smallest detectable change.

****Strain ratio is the ratio of the area of interest and adjacent subcutaneous fat.***

ICC < 0.5 indicates poor reliability, ICC between 0.5 and 0.75 indicates moderate reliability, ICC between 0.75 and 0.9 indicates good reliability, and ICC > 0.90 indicates excellent reliability (Portney and Watkins, 2008).

SDC = 1.96 x SDdiff; SDdiff refers to the standard deviation of the difference between the measurements attained at trial one and one hour later at trial two (Polit, 2014).

The "n" in column one adjacent to each structure shows the number of participants considered for each structure after exclusions due to image artifacts or insufficient compressions.

Table 3: Reliability of sonoelastography for measuring the elasticity of a range of musculoskeletal structures with color pixel analysis.

	Red pixel percentage*					**Green pixel percentage***					**Blue pixel percentage***				
	Mean (SD) Trial 1	**Mean (SD) Trial 2**	**ICC**	**95% CI**	**SDC**	**Mean (SD) Trial 1**	**Mean (SD) Trial 2**	**ICC**	**95% CI**	**SDC**	**Mean (SD) Trial 1**	**Mean (SD) Trial 2**	**ICC**	**95% CI**	**SDC**
Deltoid muscle (n = 16)	30.31 (7.32)	28.35 (7.36)	0.916	0.738 – 0.971	7.93	39.89 (3.72)	40.57 (4.55)	0.754	0.292 – 0.914	7.31	29.79 (8.44)	31.07 (6.85)	0.917	0.770 – 0.971	8.21
Biceps brachii muscle (n = 15)	26.88 (4.24)	28.25 (3.62)	0.839	0.520 – 0.946	5.52	42.97 (3.02)	43.26 (2.83)	0.878	0.639 – 0.959	3.84	30.13 (5.28)	28.48 (4.92)	0.844	0.540 – 0.947	6.99
Brachioradialis muscle (n = 15)	27.94 (10.64)	25.89 (8.99)	0.963	0.856 – 0.989	7.05	43.26 (5.99)	41.42 (5.25)	0.850	0.553 – 0.949	7.54	28.78 (11.42)	32.67 (10.88)	0.935	0.584 – 0.983	8.33
Rectus femoris muscle (n = 16)	30.91 (7.71)	30.01 (8.26)	0.912	0.753 – 0.969	9.27	43.78 (5.44)	42.96 (5.02)	0.933	0.812 – 0.976	5.05	25.29 (10.33)	27.01 (9.95)	0.917	0.769 – 0.971	10.83
Gastrocnemius medius muscle (n = 20)	30.02 (9.11)	28.96 (7.99)	0.960	0.899 – 0.984	6.76	46.71 (3.16)	46.50 (4.41)	0.800	0.488 - 0.921	6.23	23.26 (7.45)	24.54 (7.21)	0.911	0.780 – 0.965	8.03
Achilles tendon (n = 18)	5.64 (9.53)	5.98 (10.01)	0.990	0.974 – 0.996	2.90	38.88 (19.26)	40.96 (20.47)	0.914	0.773 – 0.968	22.14	55.46 (23.53)	53.05 (26.17)	0.942	0.848 – 0.978	22.95

SD: Standard deviation, ICC: intra-class correlation coefficient, CI: confidence interval, SDC: smallest detectable change.
ICC < 0.5 indicates poor reliability, ICC between 0.5 and 0.75 indicates moderate reliability, ICC between 0.75 and 0.9 indicates good reliability, and ICC > 0.90 indicates excellent reliability (Portney and Watkins, 2008).
****The SEG colored images were analyzed with ImageJ software by counting the pixels of red, green and blue colors. The percentage of each color pixel was then calculated.***
SDC = 1.96 x SDdiff; SDdiff refers to the standard deviation of the difference between the measurements attained at trial one and one hour later at trial two (Polit, 2014).
The "n" in column one adjacent to each structure shows the number of participants considered for each structure after exclusions due to image artifacts or insufficient compressions.

Figures:

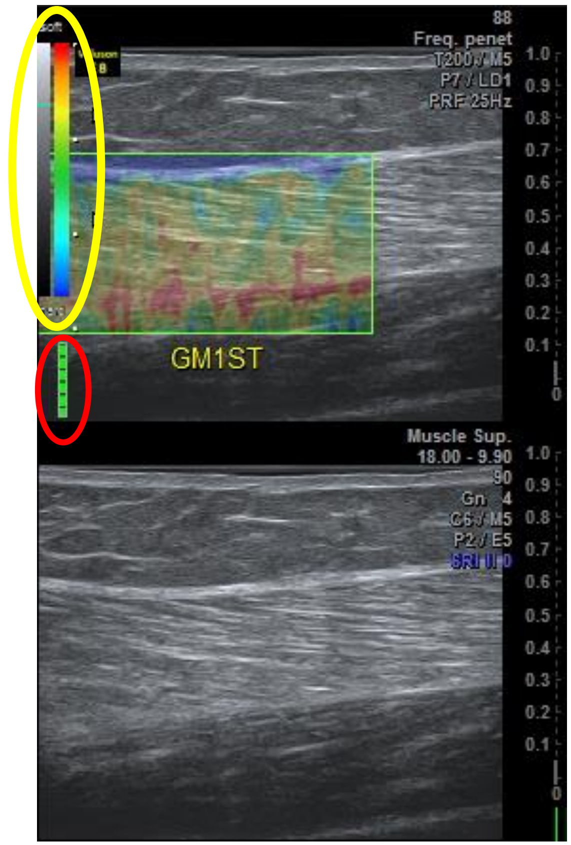

Fig. 1 *Longitudinal sonoelastography image of the gastrocnemius medius muscle (green rectangle) superimposed on a B-mode ultrasound image. The color scale (highlighted with the yellow oval) shows the color range; red indicates soft tissues, green indicates intermediate elasticity, and blue indicates hard tissues. The display shows a six-dot scale visual quality indicator to standardize the compression magnitude (highlighted with the red oval) [21]. With sufficient compressions, all six dots become green. Red dots, yellow dots, or less than six green dots indicate that compressions were insufficient to calculate tissue elasticity.*

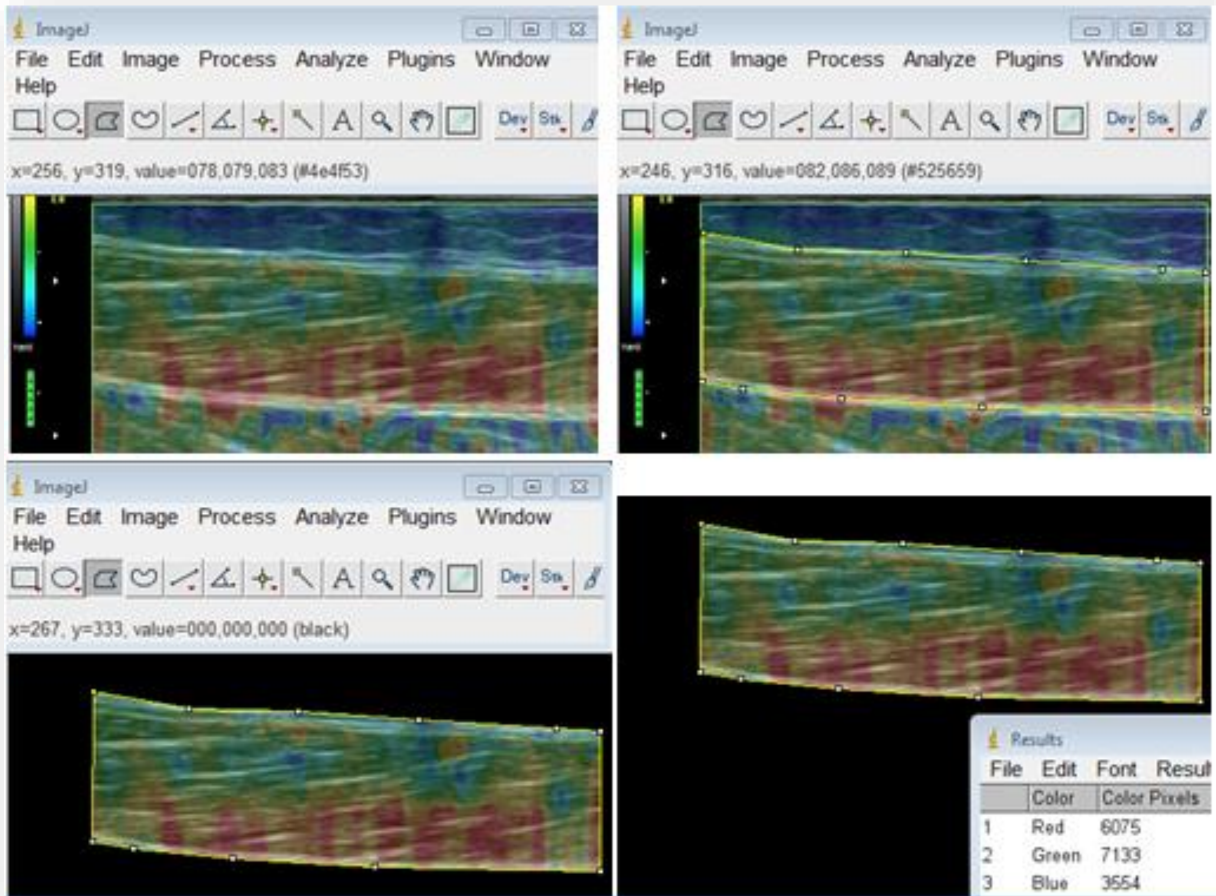

Fig 2: *Using ImageJ, the area of interest was cropped, including both the superficial and deep parts, and the surrounding structures were eliminated from the analysis using the "clear outside" option. ImageJ counted pixels of the red, green, and blue colors. The percentages of each color were calculated to compensate for differences in the cross-sectional area between participants.*