#### **Short title:** Sonoelastography in healthy knee tendon

**Title:** Reliability of strain sonoelastography in the assessment of the quadriceps and patellar tendon in healthy adults.

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### **Abstract**

**Objective:** To report the intra- and inter-operator reliability of ultrasound strain elastography measures in the assessment of quadriceps and patellar tendons.

**Materials and methods:** Forty tendons were investigated of 20 healthy volunteers. Five anatomical sites were examined and analysed by three operators of differing levels of experience. Ultrasound was performed over two measures, employing three sonoelastography measurement techniques using a GE S8 with linear probe (L6–15 MHz). The percentage of exact agreement, Cohen's kappa and  $_{ICC2,1}$ were performed to assess intra-and inter-operator reliability.

**Results:** The patellar tendon is more reliably measured across all techniques compared to the quadriceps tendon, particularly the distal region. Colour scoring was the most reliable method of sonoelastography. Colour scoring intra- and inter-operator reliability was better for patella tendon sites across all operators (60–95% agreement range), and greatest for experienced operators. Elasticity index intra-operator reliability was greatest for the most experienced operator compared with the least (ICC range 0.35–0.72 and ICC 0.17–0.60). Elasticity ratio intraoperator reliability of the patella tendon was fair–excellent for the experienced operator (ICC range 0.43–0.91), excluding the mid patellar region (ICC 0.13). Poor–fair inter-operator reliability was observed for elasticity ratio (ICC range 0.0–0.54) and elasticity index (ICC range 0.0–0.57).

**Conclusions:** Strain elastography of the patellar tendon is more reliable than the quadriceps tendon. Intra-and inter-operator reliability was better when undertaken by more experienced operators. Colour scoring was more reliable than elasticity ratio and index methods. Poor–fair intra- and inter-operator reliability of the elasticity ratio and elasticity index was observed.

**Keywords:** Ultrasound; elastrography; musculoskeletal; reliability; knee; tendon

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## Introduction

Ultrasound elastography is a relatively new ultrasound (US) application that permits assessment of tissue elasticity. The two types of elastography are strain and shear wave. Real-time strain elastography (SE) was the first method to be introduced and establishes elasticity from tissue distortion under a manually applied orce, where large strain indicates softer tissue and small strain indicates harder tissue.<sup>1</sup> Employed as an adjunct to traditional B-mode US, SE provides opportunity to yield tissue elasticity measures<sup>2</sup> through qualitative colour map scoring (CS), and semi-quantitative measurements including the elasticity ratio (ER) and elasticity index (EI).<sup>3</sup> Shear wave elastography (SWE) utilises shear wave propagation speed to quantify tissue proprieties with faster propagation through hard tissue compared with soft tissue. Quantification via EI directly translates to tissue shear modulus. Unlike SE, SWE does not require manual operator movement to produce strain, hence reducing operator dependence. Despite improved quantification and reproducibility, SWE is subject to artefacts as a result of non-uniformity of tissues and tissue boundaries.<sup>4,5</sup> Both techniques continue to evolve and current breast imaging research proposes they are complementary, with similar diagnostic performance superior to that of B-mode US alone. $6-8$ 

SE is widely performed in breast and thyroid imaging, providing improved ability to characterise mass lesions.<sup>2,9–11</sup> There has been an increase in SE studies of the musculoskeletal (MSK) system, with a focus on tendon examinations.<sup>12–21</sup> SE values derived from healthy participants of the Achilles and patellar tendons have been reported.<sup>12,15,18,20</sup> Furthermore, the validity of the technique has been demonstrated via cadaveric study (100% sensitivity and specificity correlated to histopath-ology).<sup>13</sup> Application of SE in this area is appealing because non-invasive and accurate measurements of tendon stiffness may allow for greater understanding of tendon pathology and targeted rehabilitation strategies. However, the reliability of this technique is underreported and prior to the widespread use in the clinical practice and/or specific pathological groups, it is essential that the reliability is demonstrated.

SE is considered operator dependent due to the freehand application of stress to produce an elastogram.16,22,23 Additionally, the interpretation of examinations can introduce subjectivity, where image reporting is generally based on one individual's analyses of findings. There are currently no published studies demonstrating the intra- and inter-operator reliability of SE measurements of the quadriceps tendon, and only one study to date, which reports intra- and inter-operator reliability of two SE measurement methods (CS and ER) of the healthy patellar tendon.<sup>18</sup> Porta et al.<sup>18</sup> reported high reliability of SE of the patellar tendon from two experienced operators, suggesting potential utility for serial measurements in clinical practice and prospective research. Other tendon SE reliability studies are largely limited to results based on multiple rater's review of video clips or static images acquired by a single operator.<sup>12,14–16,18,20,24</sup> These studies lack external validity in that they address the reliability of image interpretation without accounting for variations in operators' image acquisition. Furthermore, studies $13,15,16,18,25$  have adopted different methods, making comparisons challenging.

The purpose of this study is to address the lack of evidence concerning reliability of SE examinations through assessing the intra- and inter-operator reliability of SE measures (CS, ER and EI) for the quadriceps and patellar tendons

# Material and Methods

The research protocol was approved by the institutional ethics committee (HLS/PSWAHP/16/203) and was carried out within the imaging suite of the research institution. Written informed consent was obtained from all individual participants included in the study.

# Study population

Participants were included if they met all of the following conditions: were aged between 18 and <40 years, had no history of knee osteoarthritis or history of knee pain (in last three months), no history of knee injury or surgery and a body fat content considered within normal limits (male <25%, female < 39%).<sup>26</sup> Participants were excluded if they were unable to provide informed consent, had a condition affecting the quadriceps or patellar tendon including tendinopathy, rheumatological/MSK condition, knee pain/instability, and if there were any abnormalities including altered shape, fibrillar pattern or echo texture, demonstrated on B-Mode US during screening for eligibility. Participants were excluded if they had a history of auto-immune or connective tissue disorder, or if they were in receipt of oestrogen or steroid medication due to previous association with tendon abnormalities.<sup>21</sup>

## Examination protocol

Equipment

Participants were examined using US equipment, GE Logic S8, software version R2, revision 1.1, with multi-frequency linear array transducer (L6– 15MHz). Body fat percentage (%) was calculated using a Tanita Body composition analyser, TBF-300MA.

#### **Operators**

All participants were scanned consecutively and independently by three operators with differing levels of US experience (operator 1, MSc award and 12 years' clinical experience including three years of SE experience (*>*50 examinations), operator 2, PgC award and seven years' clinical experience with one day of SE application training (*<*5 examinations), operator 3, no previous US training and one day SE application training (*<*5 examinations). Following one-day group training, an examination protocol was developed to facilitate a standardised protocol for anatomical and technical assessment.

### Ultrasound protocol

Participants dominant leg was scanned in a supine lying or seated position with knee supported at 30<sup>°</sup> of flexion in line with current clinical practice guidance.<sup>27</sup> Initial B-mode US screening for eligibility was completed by operator 1 for all participants. The quadriceps tendon was located with B-mode US in the perpendicular longitudinal plane, and employing the equipment elastography package, an SE map was performed at standardised anatomical sites (see Figure 1).

Longitudinal tendon scans were performed based on a previous recommendation for higher reproducibil-ity.<sup>20</sup> To minimise the operator dependency where free hand stress is required, manufacturers display an onscreen quality indicator to guide operators in achieving sufficient stress to produce an elastogram. Each map was generated by applying light transducer compression guided by on screen compression quality indi-cator.<sup>28</sup> As recommended by previous studies, 13, 17, 20, 28 a minimum 5 seconds cine loop for each anatomical site was recorded to facilitate retrospective analysis and appropriate image selection over the compression cycle. Elastograms were produced using the optimal settings as outlined by Havre et al.<sup>29</sup> (elasticity dynamic range 4; persistence 5; smoothing 2; rejection 1; frame rate high). A fixed SE map box size of full sector width was employed with 3cm depth to adequately assess knee tendons whilst maintaining 25–50% of colour map box region of interest (ROI).<sup>16,20,30</sup> The patellar tendon was then located with B-mode US in perpendicular longitudinal plane and an elastogra-phy map was performed and recorded at proximal enthesis, mid portion and distal enthesis (Figure 1). This scan sequence was repeated by each operator to obtain two scans for independent analysis for assessment of intra- and inter-operator reliability. Image acquisition for each participant was undertaken consecutively and independently on the same day by each operator.

### Image analysis

Each operator selected a representative SE static image from their previously captured cine loop during the middle of the compression–relaxation cycle, which demonstrated sufficient stress denoted by onscreen green quality indication bar. Analysis was performed directly from the US equipment ensuring standardisation of viewing conditions. All analyses were undertaken independently by each operator. To eliminate recall bias, scan 1 and scan 2 scoring analysis was performed over multiple dates, commencing at least one month following scan series

### Elastography colour map score

Using a similar three-point scale employed in previous studies,13,31,32 (Grade 1, no strain/hard = blue (no green colour evident); Grade 2, average strain/intermediate = green/ yellow or green (no red colour evident); and Grade 3, greatest strain/soft=red (positive for red colour)), grading of respective SE colour maps were performed and recorded by each operator.

#### Elasticity ratio

The Q-analysis ratio measurement option within the GE Logiq S8 equipment elastography package was selected to perform the ER measurement. Using the corresponding reference site (Table 1), a fixed size reference ROI of 5mm was positioned within homogenous fat pad tissue. Based on previous findings, variable size of reference ROI did not significantly affect results in either a laboratory-based or clinically applied study (liver).<sup>33,34</sup> However, we consider that the use of a fixed size reference ROI delivers enhanced protocol standardisation. An anatomical site (Table 1) ROI was freehand traced with US machine tracker-ball,<sup>28</sup> and an ER value was automatically calculated by the elastography package, and recorded.

#### Elasticity Index

Within the Q-analysis measurement tool, each operator traced a freehand ROI for each anatomical site (Table 1). The tendon elasticity value (EI) of each anatomical area was generated by the application, and recorded.

### Statistical analysis

Statistical analysis were performed using SPSS, version 24 (IBM Corp., Released 2013. IBM SPSS Statistics for Windows, Version 24.0. Armonk).<sup>35</sup> To assess intra- and inter-operator reliability, we used two independent scan series yielding two SE measurements (CS, ER and EI) for each anatomical site, from each operator. Reliability of elastography CS (grades 1–3) were estimated using the percentage of exact agreement calculated from raw data and Cohen's quadratic weighted kappa. Intra class coefficient's  $_{\{ICC2,1\}}$ <sup>and</sup>  $_{ICC3,1)}$  were performed to assess the reliability of ER and EI, determined by the test of variation of measurements from two scans, of three operators measuring the same subjects. Twotailed statistical significance was defined as p 0.05. The minimum accepted kappa agreement and ICC threshold levels were defined as > 0.40 (poor **<** 0.40, fair 0.40–0.59, good 0.60–0.75, excellent 0.75–

1.00) with the level of clinical significance determined as fair.36

## Results

### Participant characteristics

Twenty healthy adult volunteers (5 males, 15 females; mean(range) age 29.3 (21-39) years; Body Mass Index 23.2 (17.9-29) kg/m<sup>2</sup>; body fat percentage 23.8 (13-39%)) were included in this study. Dominant lower limbs were self-reported (Left n=5, Right n=15). 40 tendons were examined twice by three operators; a total of 200 SE (five anatomical sites performed twice for 2 participants) examinations and measurements were performed by each operator.

## Colour Map scoring

Intra-operator reliability, expressed as percentage agreement for CS, ranged from a mean of 97% for the most experienced operator to 73% for the least experienced across two measurements (Table 2). Kappa values were better for the experienced operator (*k*, 0.79–1.0), compared to the inexperienced SE operators (*k*, 0–0.46; Table 2). For all three operators (from most experienced to least), tendon site reliability was greatest at the distal patellar tendon (mean percentage of 95%, 90%, 95% respectively. The distal patellar site also demonstrated greatest inter- operator reliability (mean percentage agreement of operator pairings over two scans and measures, 90%). Intra-operator reliability was poorest at the proximal quadriceps tendon site (percentage agreement operator 1, 100%. Operator 2, 50%, operator 3, 60%) and inter-operator reliability was poorest at the distal quadriceps site (mean percentage agreement of operator pairings over two measures, 49%). Inter-operator reliability was similar for both measures (70% and 72%, *k <*0.50). Based on measure 2, mean percentage agreement of CS ranged from most reliable operator pairing 74% (operators 1 and 3), to least reliable, 70% (operators 2 and 3).

### Elasticity ratio

Operator 1 demonstrated excellent intra-operator reliability in two anatomical sites within the patellar tendon (distal, ICC, 0.91; *p* = 0.000 and proximal, ICC, 0.85; *p*= 0.000, Table 3), with fair reliability in both quadriceps tendon sites (proximal, ICC, 0.49 *p*= 0.012 and distal, ICC, 0.43 *p*= 0.025) and poor reliability in the mid patellar tendon portion (ICC, 0.13; *p* = 0.295). Fair reliability was achieved by operator 2 in all three patellar tendon sites (proximal, ICC, 0.49; *p* = 0.012, mid portion, ICC, 0.44; *p* = 0.022, distal, ICC, 0.41; *p* = 0.031). There was poor reliability within the quadriceps tendon elasticity ratio measurements (proximal, ICC, 0.30; *p* = 0.097 and distal, ICC, *<*0; *p*= 0.803). Operator 3 demonstrated fair intra-operator reliability (ICC, 0.40; *p* = 0.028) at the proximal patellar tendon portion. Reliability at all other tendon sites was poor (ICC, *<*0.40). For measure 2, fair inter-operator reliability was determined at the distal patellar region for the most experienced pairing (ICC, 0.54; *p* = 0.005). No further reliability was observed.

### Elasticity Index

Intra-operator reliability was greatest for operator 1, followed by operator 2 and poorest for operator 3. Intra-operator reliability was fair to good (ICC, 0.56– 0.72; *p<* 0.004; Table 4) for operator 1 in four of the five anatomical sites. The proximal quadriceps tendon demonstrated poor reliability (ICC, 0.35;  $p = 0.062$ ). Operator 2 demonstrated reliability from fair to good (ICC, 0.47–0.68;  $p < 0.04$ ) in all tendon sites excluding the distal quadriceps (ICC, 0.17; p = 0.234). Operator 3 generated the least reliable results with the distal quadriceps and proximal patellar tendon rating good to fair, respectively (ICC, 0.60–0.42; p <0.03). All remaining sites yielded ICC values less than the accepted level of 0.40. Fair inter-operator reliability was observed at distal patellar region over scan measures 1 and 2 by the most experienced pairing (ICC, 0.50 and 0.48; p = 0.01 respectively). No other sites, operator pairings or group values demonstrated reliability over both measures.

## Discussion

Emerging evidence suggests SE may have a clinical role in the assessment of tendon structures; $^{13,15}$ however, inconsistent methods in measurement and assessment have been employed. This study is the first to report reliability of three common SE measurements: CS, ER and EI, applied to the assessment of the quadriceps and patellar tendons of healthy adults and performed by a range of operators. Unique to previous studies, this study provides data from scans performed and interpreted by three operators of differing levels of experience.

Intra-operator reliability was greater for patellar tendon assessment, compared to that of the quadriceps tendon, across measurement methods (CS and EI). We suspect that this is due to the challenging delineation of the quadriceps tendon myotendinous junction as opposed to the enthesis of the patellar tendon. Martinoli et al.<sup>37</sup> described a gradual muscle to tendon attachment of the proximal quadriceps tendon on B-mode US, in comparison to the abrupt and defined patellar tendon attachment to bone. Our findings indicate identifiable anatomical landmarks of the patellar tendon may enhance intra-operator reliability relative to the quadriceps tendon.

US imaging is known to be highly operator dependent as demonstrated by variable reliability of other MSK US techniques, such as detection of B-mode and Doppler rheumatological features<sup>38,39</sup>. Within the SE context, our findings do not reach the same comparable intra-operator reliability as Porta et al.'s<sup>18</sup> patellar tendon study who employed two experienced radiologist operators (operator 1 with one month SE experience, and operator 2 with one day of SE experience). High intra-operator, and fair– good inter-operator reliability was reported despite the one-month variation in SE experience. In contrast, we found that the most experienced US/SE operator achieved the greatest reliability, which is likely the result of a wider SE experiential gap between our operators. Inter-operator reliability of ER was not achieved to the same degree as the previous study within our experienced operator pairing, and variability in the ER measurement technique may contribute to this.

Porta et al. measured ER using a freehand drawn, and similarly sized to tendon, reference ROI of overlying subcutaneous tissue<sup>18</sup>. Alternatively, we employed a small and standardised homogenous reference area of posteriorly positioned fat pad. Commonly used reference sites from previous SE studies include the pre-femoral and Hoffa fat pads.<sup>15,24</sup> These sites are accessible and facilitate easy placement of ROI's within one sector width, thus minimizing placement error. The inclusion of only homogenous reference tissue within the ROI's for our study was justified on the basis that relative strain would be affected by the inclusion of different tissue types.<sup>33,40</sup> Furthermore, fat pad reference ROI's were reportedly superior to skin reference ROI's in ER intra-operator reliability measurements of the relaxed Achilles tendon (ICC 0.95 and 0.30 respect-ively), $17$  further rationalising our methodology for future practice.

There is an increasing body of evidence across other MSK US reliability studies to suggest that with suitable training, good inter-operator reliability between inexperienced and experienced operators can be achieved.<sup>41,42</sup> However, our study reveals that operator 3's reliability was poorer compared to operator 2 despite the same SE training. The elastogram overlays the standard B-mode image, therefore both image applications should be optimised to provide accurate imaging data.<sup>29</sup> A lack of understanding of US science and technology, tissue characteristics and inherent artefacts may contribute to this finding. In addition, Carlsen et al.<sup>30</sup> found that experience of observers significantly affected ability to accurately characterise stiffness of tissue mimicking lesions using CS. The impact of inexperience is demonstrated through reduced intra-operator reliability within our CS data set where operator 1 ranged from 90% to 100% observed agreement and operator 3, 60–95%.

Operator 1 demonstrated fair ER reliability of the quadriceps tendon sites compared to poor reliability of the remaining operators, an outcome considered consistent with enhanced operator expertise. Intra-operator reliability for ER were in the poor–fair range with the exception of the proximal and distal patellar sites for the most experienced operator, where reliability was excellent. Operator 2 also demonstrated clinically significant reliability, suggesting ER as a reliable measurement for suitably trained operators at these anatomical areas. As anticipated, group ICC's are poorer owing to skill variation across the three operators.

EI intra-operator reliability was better compared to the ER method for our inexperienced operator, with ICCs ranging from fair–good in the patellar tendon (mid and distal), and distal quadriceps tendon segments respectively. Similarly, for the more experienced operators, patellar tendon EI measures had greater intra-operator reliability compared to the ER method and again the distal patellar measurement was found to be reliable. EI measurement involves calculation of the relative hardness of the ROI tissue within an elastogra-phy image. Despite standardisation, the ER method requires placement of an additional reference ROI, which introduces further opportunity for operator error. Indeed, our findings suggest that compared to ER, EI may be a more reliable method of measurement. An in vitro study similarly reported slightly better reliability for EI measurement compared to ER measurements obtained by three operators.<sup>43</sup> Nevertheless, this study reported excellent reliability for both measurement methods but findings are limited by the non-clinical setting, where human factors can be more rigorously controlled and where there is limited clinical applicability.

All operators scored CS tendon sites within the intermediate (green/yellow) or soft (red) tissue categories resulting in only two of three scale categories being presented within our data. Consequently, low or non-calculated kappa values are a result of low prevalence within CS categories,<sup>44</sup> therefore we report strength of agreement using the percentage of observed agreement. Inter-operator reliability of CS method was greater for the patellar tendon (mean 78% agreement) compared to the quadriceps tendon sites (mean 53% agreement). Inter-operator reliability within the patellar tendon was greater for the experienced operator pairing with excellent inter-operator agreement (88%) achieved within the patellar tendon compared to fair agreement (50%) within the quadriceps tendon. Wang et al.<sup>45</sup> describe the depth of structure as a potential for observer error within their study, which revealed reduced diagnostic performance in deeper positioned thyroid nodules for CS. A reduction in pressure at greater depths within the quadriceps tendon compared to the

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more superficially located patellar tendon may contribute to our findings. This theory is further strengthened by our findings where greatest inter-operator reliability was demonstrated at the superficially located distal patellar tendon region; however, further investigation is required to substantiate this finding.

With the exception of the experienced pairing distal patellar measurements, we found poor ER reliability across operator pairings and all other anatomical sites (maximum inter ICC 0.38). This finding is inconsistent with a previous SE Achilles tendon study, <sup>20</sup> which reported 'good' reliability for the ER measurements of two experienced radiologists. Using Fleiss<sup>46</sup> categories for the ICC range, inter-ICC's of 0.51 (good) were reported, yet would only be considered fair within our study parameters (0.40–0.59). Our study investigates different tendons but also includes operators of differing levels of experience, which may contribute to reduced inter-operator reliability of the ER method. However, our study includes data based on each operator performing, measuring and interpreting their tendon SE scans, and can be considered reflective of the true clinical setting.

There are a few limitations to this study, including the relatively small sample size and lack of prevalence across all three CS criteria resulting in non-calculated kappa values. Equipment variations may impact SE reliability and warrants investigation in future studies. Histopathological validation of SE was not performed and out with the scope of this study.

# Conclusion

This study reports important findings demonstrating the reliability of measurement methods for SE of the quadriceps and patellar tendons in healthy subjects. Whilst intra- and interoperator reliability of CS is encouraging, EI and ER methods are less reliable, particularly across operators. EI of the patella tendon demonstrates fair to good reliability for trained oper ators (intra); however, only the distal patellar demonstrates inter-operator reliability. ER is a reliable repeat measure of the proximal and distal patellar regions by trained operators only. EI has better reliability compared to the ER method, which introduces an additional ROI and increased opportunity for operator error. We conclude that ER and EI are not reliable SE measures of the quadriceps tendon in healthy adults and emphasis must be placed on operator training and standardisation of image acquisition and analysis for this tool to be used for repeated measurement in the patellar tendon for future research or clinical practice.

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**Figure 1:** Elastrography map sites. From top to bottom; proximal quadriceps tendon, distal quadriceps tendon, proximal patellar tendon, mid patellar tendon, distal patellar tendon.





\* *PQT; proximal quadriceps tendon, DQT; distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, op; operator, %; observed agreement, k; kappa, c; constant, nc; not calculated.*



\* *PQT; proximal quadriceps tendon, DQT; distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, ICC; intra class coefficient, sig; significance, op; operator.*



\* *PQT; proximal quadriceps tendon; DQT, distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, ICC; intra class coefficient, sig; significance, op; operator.*