Title: Can patient characteristics explain variance in ultrasound strain elastography measures of the quadratus femoris and patellar tendons?

Manuscript category: Original research

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Running title: Exploration of strain elastography variance in knee tendon.

1.1 Introduction

Ultrasound elastography is a non-invasive imaging method which evaluates tissue elasticity. An extension to conventional B-mode ultrasound (US), elastography provides an inexpensive opportunity to evaluate mechanical tissue properties, with potential for greater understanding of tendon pathology and clinical utility for targeted rehabilitation strategies. Ultrasound elastography estimates tissue stiffness from either an applied stress (strain elastography (SE)), where a large distortion is attributed to softer tissue, and small distortion to stiffer tissue), or lateral shear wave propagation speed (shear wave elastography (SWE))[1].

Recent clinical studies report SE and SWE to have similar diagnostic performances, superior to that of B-mode US alone [2,3]. Therefore, elastography techniques could play an important role in the detection, follow up, and treatment response of mechanical tendon alterations, where previous Achilles tendon studies identify elastic alteration in response to pathology [4,5]. The quadriceps and patellar tendons are important peri-articular structures supporting the function, stability and range of movement of the knee joint. With significant extensor mechanism and tensile loading roles [6], any changes in the morphology and elasticity of the quadriceps and/or patellar tendon may contribute to knee joint pain and dysfunction.

SE is the most common elastography technique [7] with established clinical applications for breast, lymph node, thyroid and prostate imaging [8–11]. Common measurement methods include colour scoring (CS); where a colour coded elastogram represents different magnitudes of relative tissue strain, elasticity ratio (ER); semi-quantitative assessment through comparison of two tissue types within defined regions of interest (ROI) to demonstrate the elastic contrast, and elasticity index (EI) which is a numerical value applied to the average elasticity of tissue within an ROI; where stiffer than average tissue are represented >1 [12–14]. There has been continued interest in musculoskeletal (MSK) SE over the past decade, with studies exploring reliability, measurement methods, and correlation with B-mode US and histopathology [4,15–18]. Findings suggest that experienced

operators employing a standardised protocol can reliably perform SE knee tendon examinations [15,18]. Furthermore, validation of the SE technique has been achieved with perfect positive correlation in Achilles tendon histopathology [4]. Whilst SE is regarded as reliable under standardised conditions, and valid in specific clinical areas, variability within SE remains evident [17,19].

Exploration of SE in pathological MSK groups is a vital step in developing this imaging tool, however identifying and addressing sources of variability and establishing normative patterns must first be achieved. Few previous studies have described knee tendon pattern of SE measures; colour map scoring (CS) [20] and elasticity ratio's (ER), in healthy [15,21] and athletic populations [21,22], yet different methods and equipment make comparison of results challenging. Common methodological considerations contributing to potential SE variation include: operator experience, tendon examination sites, examination protocols, measurement techniques and equipment. Whilst operator dependency, protocols and measurement techniques are somewhat considered in contemporary literature, no direct comparison of clinical SE outputs from 2 US systems has been reported. This is an important step to enhance widespread use in the clinical environment; often hosting a variety of different ultrasound equipment.

Additionally, participant characteristics have the potential to impact SE outcomes. Increased body habitus is a general limitation affecting B-mode and Doppler US imaging. Deeper structures can be more difficult to visualise due to increased penetration and attenuation, leading to reduced image resolution. Currently, the role of participant characteristics such as body mass index (BMI) and leg circumference as potential sources of error in SE have yet to be explored. The purpose of this study is to 1) explore the associations between participant characteristics and magnitudes of difference in paired SE measures obtained from 2 US systems 2) compare the SE pattern description of the quadriceps and patellar tendons using 2 different US systems.

Materials and methods

The study was approved by the local institutional ethics committee (HLS/PSWAHP/18/159) and was carried out within the imaging suite of research institution. All participants provided written informed consent.

1.1.1 Population

Participants were included if they met all of the following; were able to provide written informed consent, were aged between 18 and \leq 40 years, had no history of: knee osteoarthritis, knee pain (in last 3 months), knee injury or surgery, and had a body fat content considered within normal limits (male <25%, female <39%) [23]. Participants were excluded if they met any one of the following: the presence of a condition affecting the quadriceps or patellar tendon, including tendinopathy, a rheumatologic/MSK condition, knee pain/instability, or history of knee surgery; the presence of any abnormalities, including an altered shape, fibrillary pattern, or echo texture, detected by B-Mode US during screening for eligibility; a self-reported presence or history of autoimmune or connective tissue disorders; and current receipt of oestrogen or steroid medication for a previous association with tendon abnormalities [24].

1.1.2 Examination protocol

1.1.2.1 Equipment

Participants were examined using 2 US systems: LOGIQ S8 (software version R2, revision 1.1, with GE Healthcare, Waukesha, WI; L6-15MHz) and Mylab 70 XVG (version EVO 13.60M; Esaote SpA, Genoa, Italy; LA523, L4-13MHz). Body fat percentage (%) was calculated by scales based method using a Tanita Body composition analyser, TBF-300MA. Leg circumference was measured in centimetre (cm) using a standard measurement tape, at the level of mid-pole patella. Redcap [25], a data capture application, was employed to record data on participant physical and body composition

characteristics including height, weight, BMI, body fat %, leg circumference and self-reported physical activity levels (using the International Physical Activity Questionnaire (IPAQ) long form, and scored as high, moderate and low physical activity level) [26].

1.1.2.2 Operators

All scans and image analyses were performed by a single, trained operator with 12 years of US experience, and SE experience of >50 examinations.

1.1.2.3 Scan protocol

A standardised scan protocol was adopted to minimise variance of SE outcomes from scan parameters. Participants' self-reported dominant lower limbs (defined as leg used to kick a ball) were scanned in a seated or lying position with the knee supported in 30 degrees of flexion using a standardised pad, in line with current imaging guidance [27]. Initial B-mode US screening of the quadriceps and patellar tendon was performed for study eligibility. An SE map was performed at 5 defined tendon areas (proximal quadriceps [PQT], distal quadriceps [DQT], proximal patellar [PPT], mid patellar [MPT] and distal patellar tendon [DPT] and details of the protocol and image analysis are described in full elsewhere [28]. The scan sequence was repeated to obtain two scans series for analysis of the difference between two measures, for each US system. To minimise anatomical confounders such as changes to tendon status and to address the study aims, participant's examinations were completed on the same day, performed on the GE then Esaote US system, successively.

1.1.2.3.1Colour map score

Using a similar three-point scale employed in previous studies [4,18,28–31] (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)), visual grading of respective elastography colour maps were performed and recorded.

1.1.2.3.2Elasticity ratio

To perform ER measurements, the Q-analysis ratio option within the elastography package (GE) and elastography measurement tab, option ElaXto Ratio (ELX-T-RAT; Esaote) were selected. Reference tissue to perform the ER measurement was identified as homogenous fat pad inferior to the tendon site. Participants were excluded if there was no homogeneous fat pad. Full detail of the measurement method is described elsewhere [28]. In line with manufacturer ER applications, the GE reference tissue was assigned as tissue ROI 1 whereas the Esaote reference tissue was appointed ROI 2 [32,33].

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GE ER = \frac{ROI 1 (reference tissue)}{ ROI 2 (tissue of interest)}
$$

$$
e\,set ER = \frac{ROI 1 (tissue of interest)}{ ROI 2 (reference tissue)}
$$

1.1.2.3.3Elasticity Index

The EI measurement method was available on the GE system only. Within the Q-analysis measurement tool, a freehand ROI was traced for each individual tendon site. The tendon elasticity value (EI) of each ROI was generated and recorded.

1.1.3 Statistical analysis

Descriptive statistics were used to present participant characteristics and were expressed as means, range and percentages. CS elastography values were presented as mean percentages and ER and EI elasticity values as medians and interquartile ranges. Pearson/Spearman's correlations were performed to evaluate association between ER and participant characteristics. Paired-sample *t* test or Wilcoxon signed rank test were performed to compare ER and EI measures between scan measure 1 and scan measure 2 for individual US systems. The standard error of measurement (SEM) was calculated by dividing the standard deviation of the mean differences between two measures by $\sqrt{2}$. With a confidence interval of 90%, minimal detectable change (MDC₉₀) values were calculated

using the formula: 1.65* $\sqrt{2}$ *SEM. Interquartile ranges (IQR) and (MDC₉₀) were analysed for measurement variance to determine the most vulnerable tendon sites. Two-tailed statistical significance was defined as $p \le 0.05$. Correlation threshold levels were defined as poor <0.40, fair 0.40-0.59, good 0.60-0.75 and excellent 0.75-1.00 [34]. Statistical analyses were performed with SPSS, version 24 software (IBM corporation, Armonk NY) [35].

1.2 Results

1.2.1 Participant demographics

A total of forty tendons were eligible for evaluation from twenty volunteers (5 males, 15 females); mean (range) age 29.3 (21-39) years; BMI 23.2 (17.9-29) kg/m²; body fat 23.8 (13-39) %; leg circumference 37.5 (33-42.5) cm and activity level (high 37%, moderate 58%, low 5%).

1.2.2 Colour map scoring pattern

There were no tendon sites classified within the hard CS category for both machines. 82% of the PQT sites were scored as intermediate, and 18% as soft over the 2 machines (Table 1). 52% of the DQT sites were scored intermediate, and 48% soft. A slightly larger proportion of GE measurements were categorised as intermediate and the Esaote system soft, for the DQT (Table 1). The patellar tendon sites (n=3) scores were predominately soft. The PPT was categorised as soft for all participants, using both US systems.

Location for Table 1

1.2.3 Elasticity Ratio

For the GE system, quadriceps tendon sites had higher ER values (range, 9.25 – 9.75; greatest, PQT, 9.75; Table 2) indicating greater stiffness, compared to the patellar tendon sites (range, 2.08-3.33; lowest, PPT, 2.08). Similarly, for the Esaote system, ER values were also greater for the quadriceps tendon (quadriceps tendon range, 2.79-2.81; greatest PQT, 2.81; patellar tendon range, 1.16-2.29; lowest, PPT, 1.16).

Location for Table 2

1.2.4 Elasticity Index

Greater GE EI values indicate the quadriceps tendon sites were stiffer (range, 3.23-3.50; greatest PQT, 3.50; Table 2) compared to the softer and more elastic patellar tendon sites (range, 0.88-1.30; lowest, PPT, 0.88).

1.2.5 Difference between measurements

1.2.5.1 Magnitude of difference between measurements using different US systems

Greater magnitude of differences between ER scan 1 and ER scan 2 were observed at tendon sites PQT, DQT and MPT (Table 3, Figure 1), however differences were non-significant. Significant difference was observed between ER scan 1 and scan 2 of the PQT using the Esaote system (Cohen's d; 0.48, *p*=0.04; Table 3). Greater differences were observed for EI measures at the quadriceps tendon sites compared to the patellar tendon sites (Table 3, Figure 2); however, the differences were not significant.

Location for Table 3, Figure 1 and Figure 2.

1.2.5.2 Association of the magnitude of difference between elasticity measures and participant characteristics

The difference between scan 1 and scan 2 continuous measures (ER and EI) of the most vulnerable sites (determined as the greatest magnitude of difference (IQR) between scans; ER: PQT, DQT and MPT for each US system and EI: PQT and DQT), were correlated against participant demographics (BMI, body fat % and leg circumference, Figures 3-5). Good associations were observed for the GE EI values, and activity level at the PQT (*r*=0.71, CI: -0.38-0.5, *p*=0.01). For GE ER, fair association was observed for body fat % and BMI at the DQT (*r*=0.43, CI: -0.02-0.73, *p*=0.05 and *r*=0.49, CI: 0.05-0.76, *p*=0.03 Figures 3 and 4). Esaote ER measurements at the DQT were also observed with significant (fair) association for BMI (*r*=0.49, CI:0.06-0.77, *p*=0.028) but were non-significant for body fat % (*r*=0.43, CI:0.007-0.743, *p*=0.056, Figure 4). There was no relationship between participant leg circumference and the difference between measurements. Outlier analysis was performed and extreme outliers (n=2) were identified as participants with greater extremes of body fat %, BMI and leg circumference compared within the population sample. Outliers were not removed as considered a reflective sample of the study population.

Location for Figures 3, 4, and 5.

1.3 Discussion

This study describes elastography measures of healthy knee tendon using 2 US systems and explores the associations between magnitude of measurement difference and participant's characteristics. The esaote system produced marginally softer tendon CS values compared to the GE, ER values were markedly different, however determined the patellar tendon to be softer than the quadriceps tendon, and that both US systems demonstrated greater measurement variability at the quadriceps and mid-patellar tendon regions. Additionally, BMI and body fat % were significantly associated with ER measurement differences of the DQT.

1.3.1 Sonoelastography pattern description

The elastic pattern of the quadriceps tendon was characteristically stiffer than the patellar tendon and consistent with a previous SE study using a GE US system [21]. The different anatomical arrangement of the quadriceps and patellar tendon can be considered responsible for their respective stiffness' [15]. Despite equipment standardisation, scan data were interpreted using a

different CS scale [21], which makes direct comparison with our results difficult. Emerging imaging techniques require rigorous validation with histopathology [36]. As such, the three-point scale within our study was adopted on the basis of being the only tendon CS scale, to date, to be validated via histopathology correlation (100% for Achilles tendon) [4]. Our methodology states clear coding determination (e.g. no green evident within stiff category and red evident in soft), and our strict parameters enhance future replication, where we have previously reported high inter-machine agreement of CS measures when performed by a trained operator [28].

ER values are unit-less and dependent upon scan protocol and US system. Representation scales differ between manufacturers and have been developed using their own specific technologies [37,38]. Consequently, a spectrum of tendon study outcomes have been published [15,17,18,24,29,31,39–42]. Disparity in ER values are evident between those reported by Ozcan et al [18] demonstrating DQT GE ER values of 1.47-1.73, compared to the results of this study with a median value of 9.25 from the same site using the same equipment. Similar systematic differences were observed for the PPT and DPT sites between these studies. The difference in ER values may be due to disparity in reference tissue assignment. Adopting a reference region of similar size to the tendon of interest, Ozcan et al. included a mixed composition of tissue with varying stiffness'. In contrast, our small homogenous reference point elicits comparably elevated ER from a soft tissue baseline, and eliminates heterogeneous fat pad reference tissue sampling. Findings from a phantom based study report that *in vivo* reference tissue positioning may have a significant influence on ER calculation, since surrounding tissue is often heterogeneous [43]. Variable ER values as a consequence of reference tissue assignment is previously recognised [44] and our findings reinforce the requirement for the implementation of strict and reproducible methodological parameters, to minimise discrepancy in future SE studies and clinical applications.

Porta et al's patellar tendon SE study reported more comparable ER values to ours (mean; $1.47 \pm$ 0.64, 4.38 + 1.36, 3.32 + 1.20 for proximal, mid portion and distal tendon sites respectively) [15].

The skin reference ROI used for calculation of ER also corresponds more closely to the reference site adopted in our study, since skin is more elastic compared to fat pad. SE outcomes were also comparable, with the PPT site determined as most elastic (Table 2). To the contrary, Ozcan et al. found the PPT to be stiffer in ER than the DPT [21]. However, consistently and within our study, the EI method of elasticity quantification also found the PPT to be the most elastic tendon site, further significant association between EI and ER measures was observed (Table 3) [45]. In the context of differences with other studies, there is clear need for a standard approach to future SE examinations.

Healthy control data from Teber et al's renal dialysis quadriceps tendon study reports EI values from a GE system [31]. Slightly increased mean EI values of the DQT (right: 3.79; left: 3.69) compared to our study results (median; 3.23) were recorded. In addition to measurement errors and measurement method, agreement and reliability depend upon the population in which measurements are made [46]. Previous studies confirm that quadriceps and patellar tendon elasticity changes with knee position, due to quadriceps tendon elasticity increasing with flexion [47–49]. Whilst we supported the knee in 30 $^{\circ}$ of flexion as recommended [27], a 45 $^{\circ}$ knee flexion with parallel foot placement was employed by Teber et al. [31]. This, in conjunction with an older population, where increased tendon stiffness is recognised [50,51], may explain their slightly increased EI values. To minimise SE measurement error, future methods must be standardised.

1.3.2 Magnitude of difference between measures

Our previous work found no association for ER between US systems [28]. Additionally, lack of consistency between US systems ER scales reported in this study, presents possible clinical ambiguity, potentially impacting reported findings and patient management. Importantly, excluding the Esaote ER measures of the PQT (*p*=0.004, Table 3), there were no significant differences between 'within system' ER or EI measures. The significant difference of Esaote PQT ER measures could be a chance finding due to the number of statistical tests performed (Bonferroni correction for number of

tests *p*<0.01). Informed by our findings, we recommend that the same US system is employed in follow up ER examinations, with examinations performed by the same operator.

As determined by both US systems, the quadriceps and mid-patellar tendon sites were most variable over repeated measurements, and is supported by our previous findings of comparably reduced intra-operator reliability at these sites [18,28]. Yet, despite increased variability, differences between measures for each US system were not significant therefore may be considered clinically appropriate.

1.3.2.1 Association between the difference between measures and participant characteristics

Body habitus is a widely recognised influencing factor affecting US imaging [52]. Increased BMI and body fat % are participant features related to larger body habitus, contributing to reduced US image quality [53]. Despite our study including only participants with a healthy body fat % [23], findings indicate that greater magnitudes of difference between repeated measures (within-machine) were associated with increased participant BMI, and body fat % at the DQT. This is largely unsurprising as the thigh is an adipose storage site [54] therefore likely to be affected by these participant attributes.

Increased self-reported activity level was positively associated with increased measure variability of the PQT (GE EI measures). It is unclear why increased variation is observed but speculatively may be a consequence of altered tendon composition after exercise, muscle stiffness or muscle bulk, which may affect the measurement protocol. Kubo et al [55] previously suggested that increased activity through long contraction training causes changes in the internal structure of the tendon resulting in increased stiffness. Stiffer tendon may affect variation and future studies are required to investigate the relationship between increased activity levels and measurement variability, and its clinical significance. Meanwhile, this association may be a significant limitation in the future application of SE in quadriceps tendon, particularly as extensor weakness has been indicated as risk factor in the

development of knee osteoarthritis [56], and previous association of increased activity levels and quadriceps tendon stiffness have been reported [55].

SE application in MSK imaging is currently restricted by limited pathological understanding and lack of standardisation [18]. Moreover, there is indication of ambiguous or inverted ER calculations within SE studies [15,17,42,47]. These anomalies may be considered a consequence of inconsistent manufacturer assignment of tissue 1 and tissue 2 for ER calculation and the relative infancy of this technique, particularly within the MSK field. Hence it is vital that any normative, pattern description studies methodologies are clearly understood and recognised prior to application within the clinical setting. Contributing further, we evidence participant characteristics as a source of SE measure variation.

1.4 Strengths and limitations

This study is the first to directly compare inter-machine elasticity outputs and the first to consider participant characteristics and their influence of US SE variance. This study is subject to a few limitations including the relatively small sample size with gender imbalance and general homogeneity of CS scores. Additionally, histological correlation of SE findings was not possible. EI measurement method was only available on the GE system and therefore could not be directly compared. Knee angle was supported using a standardised 30° pad, however was not directly measured.

1.5 Conclusion

The patellar tendon is more elastic compared to the quadriceps tendon, as demonstrated by both US systems included in this study, despite different representative ER scales. Rising BMI and body fat % are associated with greater magnitudes of difference between SE measures of the distal quadriceps tendon, and increased activity level is associated with greater variability of measures at the proximal quadriceps tendon. Leg circumference was not significantly associated with magnitudes of

difference between repeated measures. Existing knee tendon SE findings are largely variable due to mixed semi-quantification methods and lack of a standard methodology. Protocol and equipment standardisation must be addressed to minimise measurement variation in the clinical setting where participant characteristics (BMI, body fat % and activity level) also influence repeated elastography measures.

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Table 1. Description of elasticity colour score for each ultrasound system.

Site; tendon site, system; ultrasound system, PQT; proximal quadriceps tendon; DQT, distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, n=20.

Table 2. Description of tendon site elasticity ratio and elasticity index values for each ultrasound system.

Site; tendon site, US; ultrasound, PQT; proximal quadriceps tendon; DQT, distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, ER; elasticity ratio, EI; elasticity index, IQR; interquartile range, SEM; standard error of measurement, MDC90; minimal detectable change at the 90% confidence interval, n=20.

Table 3. Description of tendon site elasticity values between scan 1 and scan 2 for each ultrasound system

GE US system									Esaote US system				
Site	ER median (IQR)		р	r/d	El median (IQR)		р	r/d	ER median (IQR)		p	r/d	
PQT	1.45	(4.95)	0.25	0.26	-0.25	(1.38)	0.94	0.39	0.30	(0.78)	0.04	0.48	
DQT	-0.25	(4.05)	0.91	0.02	-0.45	(1.18)	0.42	0.49	0.16	(0.84)	0.42	0.18	
PPT	0.05	(1.15)	0.75	0.07	0.15	(0.40)	0.69	0.09	0.05	(0.47)	0.92	0.02	
MPT	0.50	(1.60)	0.92	0.02	0.00	(0.50)	0.52	0.10	0.12	(1.08)	0.91	0.39	
DPT	0.00	(0.58)	0.92	0.02	0.00	(0.53)	0.72	0.06	0.65	(0.23)	0.84	0.03	

Site; tendon site, US; ultrasound, PQT; proximal quadriceps tendon; DQT, distal quadriceps tendon, PPT; proximal patellar tendon, MPT; mid patellar tendon, DPT; distal patellar tendon, ER; elasticity ratio, EI; elasticity index, IQR; interquartile range (Q1-Q3), r/d; effect size, p values are based on Paired T-Test or Wilcoxon signed rank statistical tests; bold text indicates p<0.05, n=20.

Figure 1. Association between the difference in tendon site elasticity ratio measures 1 and 2.

Figure 2. Association between tendon site elasticity index measures scan 1 and 2 for GE system.

Figure 3. Association of the difference between elastography measures scan 1 and scan 2 and participant Body Mass Index (BMI).

Figure 4. Association of the difference between elastography measures scan 1 and scan 2 and participant body fat %.

Figure 5. Association of the difference between elastography measures scan 1 and scan 2 and participant leg circumference.