

# Influence of transducer pressure and examiner experience on muscle active shear modulus measured by shear wave elastography



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## ABSTRACT

**Introduction:** This study examined the effects of ultrasound transducer pressure and examiner experience on the biceps femoris long head and semitendinosus muscle active shear modulus in healthy individuals ( $n = 28$ ).

**Methods:** Active shear modulus was assessed using shear wave elastography at 20% of knee flexor maximal voluntary isometric contraction. Examiners with different experience levels measured the muscles' shear modulus with three pressure levels: mild, moderate, and hard.

**Results:** A main effect of transducer pressure was found for both biceps femoris long head ( $p < 0.001$ ;  $\eta^2_p = 0.314$ ) and semitendinosus muscles ( $p < 0.001$ ;  $\eta^2_p = 0.280$ ), whereas differences were found between mild-moderate (biceps femoris long head:  $p = 0.013$ ,  $d = 0.23$ ; semitendinosus:  $p = 0.024$ ,  $d = 0.25$ ), and mild-hard pressures (biceps femoris long head:  $p = 0.001$ ,  $d = 0.47$ ; semitendinosus:  $p = 0.002$ ,  $d = 0.47$ ). Examiners performed similar shear modulus measurements in the biceps femoris long head ( $p = 0.299$ ;  $\eta^2_p = 0.041$ ) and semitendinosus ( $p = 0.177$ ;  $\eta^2_p = 0.066$ ), although the experienced examiner showed a higher measurement repeatability (biceps femoris long head: ICC = 0.86–0.95, semitendinosus: ICC = 0.89–0.96; vs. biceps femoris long head: ICC = 0.78–0.87, semitendinosus: ICC = 0.66–0.87).

**Conclusion:** Transducer pressure influences the active shear modulus measurement between mild and moderate or hard pressures. Additionally, examiner experience seems to have no influence on muscle active shear modulus measurement when assessed at the same site (using casts).

**Implications for practice:** Future studies assessing active muscle shear modulus should use mild transducer pressure and having experienced examiners in order to improve measurement reliability.

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## Introduction

In the past two decades, ultrasound has increasingly been used in the diagnosis of various musculoskeletal injuries and diseases,<sup>1</sup> and to better understand skeletal muscle physiology.<sup>2</sup> One of the ultrasound application methods is shear wave elastography (SWE). This technique quantifies the velocity with which induced shear waves propagate in the tissue of interest by applying an acoustic

“pushing” beam.<sup>3</sup> Measuring shear wave velocity along the muscle fascicle direction is considered as a valid and reliable method to estimate the muscles' passive (i.e., at rest) and active (i.e., during contraction) shear modulus.<sup>4–6</sup> Particularly, faster shear wave velocity has been associated with higher shear modulus. However, it has been reported that certain methodological aspects of equipment usage must be followed in order to obtain an accurate and reliable muscle shear modulus measurement.<sup>7,8</sup>

One methodological aspect is transducer pressure, as higher pressures tend to increase tissue shear modulus.<sup>9–11</sup> For instance, Kot et al. (2012) reported a significant increase in the rectus femoris muscle shear modulus across three transducer pressures (i.e., light, moderate, and hard).<sup>10</sup> Also, Harvey et al. (2018) reported that the shear wave measurement reliability is affected by

**Abbreviations:** BFlh, Biceps femoris long head; SWE, Shear Wave Elastography; ST, Semitendinosus.

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transducer pressure.<sup>12</sup> However, the aforementioned studies analyzed muscle in a resting condition (i.e., passive condition), where the muscle shows lowest shear modulus value. In the assessment of muscle shear modulus during contraction (i.e., active shear modulus), a higher level of shear modulus is manifested,<sup>13</sup> which makes the muscle more resistant to extrinsic sources of deformation; thus, the effect of transducer pressure should be less significant in comparison with rest. Nevertheless, it remains unclear how transducer pressure influences the active muscle shear modulus.

Another methodological aspect is examiner experience.<sup>14–16</sup> Previous studies have reported better reliability outcomes for elastographic measurements in examiners with higher experience.<sup>14–16</sup> However, previous elastographic findings were obtained in the supraspinatus tendon<sup>15</sup> and on thyroid and salivary glands.<sup>16</sup> As skeletal muscles have distinct mechanical and architectural properties compared to the aforementioned structures,<sup>17</sup> and shear wave velocity measurement depends on the transducer alignment in respect to the fascicles' orientation<sup>18</sup> and the exerted pressure,<sup>19</sup> which theoretically may vary depending on examiners' experience, it is important to examine if the examiners' experience affects the measurement of muscle shear modulus using SWE. This is especially relevant during muscle contraction (i.e. active), as muscle shear modulus and fascicle alignment change with the level of contraction.<sup>18</sup> Importantly, one of the most common beginner mistakes is the excessive applied pressure on the skin, which will impact the shear modulus values; therefore, it is valuable to understand the behavior of an experienced versus inexperienced examiners regarding their applied transducer pressure at different levels using their intuition and visual sonogram feedback.

In recent years, the hamstrings have received an increased interest by the sports science and medicine community due to the high injury incidence when exposed to repeated sprint running activities.<sup>20</sup> The biceps femoris long head (BFLh) is the most affected hamstring muscle.<sup>21</sup> It has been suggested that changes in the active shear modulus of BFLh and semitendinosus muscle (ST) also seem to have clinical relevance.<sup>22,23</sup> Indeed, it seems that a lower contribution of ST muscle is compensated with an increase of the BFLh muscle after a knee flexion fatigue task in healthy individuals after a knee flexion fatigue task,<sup>24</sup> and football players with previous injury showed higher BFLh active shear modulus prior to a knee flexion fatigue task.<sup>23</sup> However, no differences were found on both BFLh and ST active shear modulus in football players with and without hamstring injury after a repeated sprint task.<sup>25</sup> It should be noted that BFLh and ST muscles are two synergistic muscles with different architectural properties (e.g., pennate vs. fusiform, respectively), and display different active shear modulus values for a given level of joint torque [i.e., the ST muscle shows higher shear modulus<sup>26</sup>]. However, it is still not clear if transducer pressure (as well as examiner experience) affects the active shear modulus measurement of these two muscles differently.

The aim of the present study was to determine the effect of (i) transducer's pressure (i.e., mild, moderate, and hard) and (ii) examiner experience (i.e., low vs. high) in assessing the active shear modulus of BFLh and ST muscles. In addition, the intra-examiner measurement repeatability was determined. We hypothesized that (i) the muscle shear modulus values would be greater for higher transducer pressures, in particular for BFLh muscle (i.e. less stiff muscle); (ii) there would be muscle shear modulus differences between examiners; and (iii) there would be significant intra- and inter-examiner differences for each level of pressure.

## Materials and methods

### Participants

Twenty-eight recreational active individuals (14 males and 14 females; age:  $28.0 \pm 10.0$  years; height:  $170.2 \pm 8.6$  cm; body mass:  $66.1 \pm 10.5$  kg) without a history of hamstring strain injury and any limitation in the knee joint that could compromise the task, participated in this study.

Participants were instructed to avoid any strenuous activities 24 h before the test to minimize confounding factors. All participants read and signed an informed consent prior to participating in the study. The Ethical Committee at the Faculty of Human Kinetics at the University of Lisbon approved the study (#31/2020).

### Procedures

#### Dynamometry

An isokinetic dynamometer (System 3, Biodex Medical Systems, Shirley, NY) was used to assess the joint torque with a sampling rate of 1000 Hz. Testing was performed only in one randomly chosen lower limb. Participants were positioned in the prone position with the hip in neutral position, the measured knee flexed at 30°, and with an orthosis positioning the ankle at neutral position to restrain dorsiflexion movements during contraction.<sup>24</sup> Previous elastography studies have demonstrated that the plantar flexor muscles are minimally stretched when the ankle is in neutral position.<sup>27,28</sup>

#### Shear wave elastography

Muscle shear modulus was assessed using an ultrasound scanner (Aixplorer, v12; Supersonic Imagine, Aixen-Provence, France) in SWE mode (musculoskeletal preset, penetrate mode, smoothing level 5, opacity 100%, scale of 0–800 kPa), coupled with a linear transducer array (SL10-2, 2–10 MHz, Super Linear, Vermon, Tours, France). The push frequency that generated the elastogram window was set automatically by the ultrasound equipment to approximately 1 Hz (range 0.8–1.4 Hz).<sup>3</sup> The velocity of shear waves generated by an ultrasonic beam (i.e. 'pushing beam' emitted by the probe) within the muscle are measured by a time-of-flight algorithm in each pixel of the elastogram map in ultrafast ultrasound sequences. It should be noted that the machine output is Young's modulus, which corresponds to a linear displacement, whereas the shear modulus corresponds to an angular displacement. Therefore to estimate the shear modulus it is necessary to have some considerations. First, the ultrasound scanner estimates Young's modulus through shear wave speed using the equation<sup>3</sup>:

$$E = 3\rho V^2$$

where  $E$  the Young's modulus,  $\rho$  is the muscle mass density ( $1000 \text{ kg/m}^3$ ) and  $V$  is the shear wave velocity. The ultrasound scanner also provides the corresponding Young's modulus which is related to shear modulus ( $G$ ), since  $G = \rho V^2$ , therefore shear modulus can be estimated using the following equation<sup>3</sup>:

$$E \approx 3G$$

being  $E$  the Young's modulus and  $G$  the shear modulus.<sup>29,30</sup> The shear modulus is the ratio of shear stress over shear strain. Since muscle strains occur due to a shear force on the muscle fibers, shear modulus is often used in muscle rigidity analysis and consequent injury. The muscles' region of interest where the muscle shear modulus was assessed corresponded to the largest cross-sectional area where the hyperechoic lines delineating the muscle fascicles (i.e. perimysial membranes) were well visualized. This

corresponded to ~55% of the distal-to-proximal femur length (measured from the lateral femoral condyle to the greater trochanter).<sup>26</sup> To ensure a stable measure of the muscle shear modulus during the contractions, a plastic cast was fixed to the skin using bi-adhesive tape on the cutaneous projection of each muscle's mid-cross-sectional area (Fig. 1). This also allowed for both examiners to perform the measurements always at the same site.

### Protocol

A single testing session was performed. The examiner with more experience had used ultrasound for three years, with one year of SWE experience. The examiners with low experience only had ~60 h to be familiarized with both ultrasound techniques. Examiners were trained for three sessions before the testing session to perform shear wave measurements with different transducer pressures according to their perception, with the muscles at a resting condition. Transducer pressure was categorized into three levels: mild, moderate, and hard, according to the perception of each examiner while visualizing the B-mode image. Mild pressure was defined as placing the transducer very lightly on top of a slight amount of coupling gel on the surface of the skin without affecting

BFlh and ST muscle thickness. Hard pressure was performed by placing the transducer with a great force that could deform the thickness of the BFlh and ST muscles, whereas moderate pressure was performed by placing the transducer with a gentle force that could just barely deform the BFlh and ST muscles.

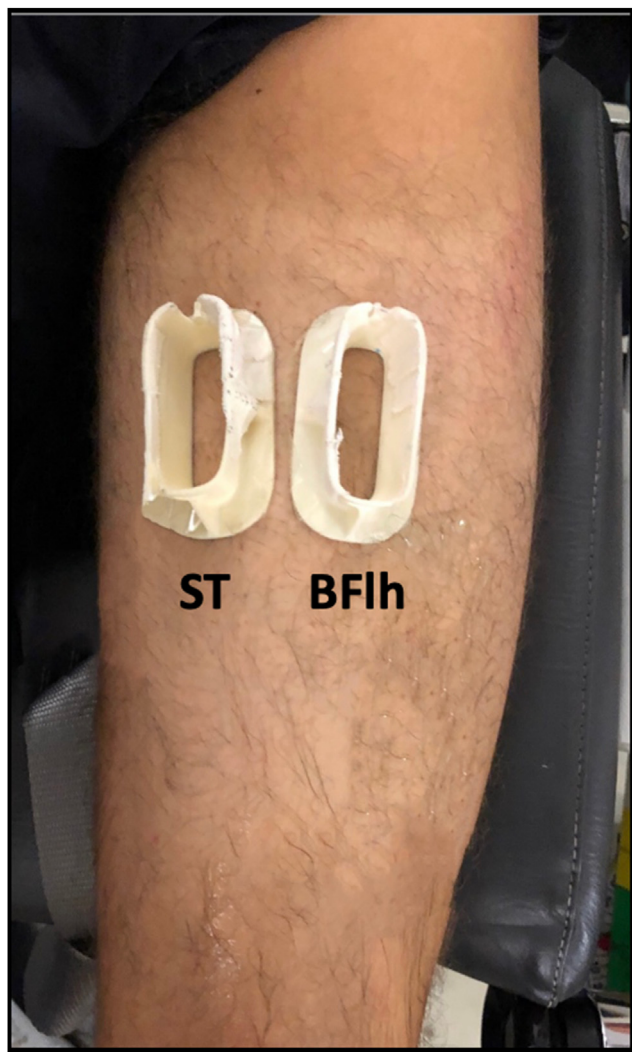
The session started by determining the region of interest for each muscle and positioning the casts, both of which were done by the more experienced examiner. Participants then performed 20 sub-maximal contractions at their own perceived exertion to familiarize themselves with the dynamometer, followed by two maximal voluntary isometric contractions (MVIC) with 3-s duration and 1-min rest between trials. Based on the MVIC highest peak torque, individuals familiarized themselves with the 20% of MVIC trials using visual feedback of torque production. For testing, each examiner performed two measurements for each pressure level, resulting in a total of 24 trials per participant. One examiner performed the first six measurements (two for each pressure level) in one muscle, followed by six measurements by the other examiner. This procedure was repeated for the other muscle. Each muscle contraction trial lasted 30-s, with approximately 30-s of rest between measurements. The transducer device, pressures, muscle order, and examiner sequence were randomized. Male participants were evaluated by one of the low experience examiners as females were by the other. The high experience examiner rated all participants.

### Data acquisition and processing

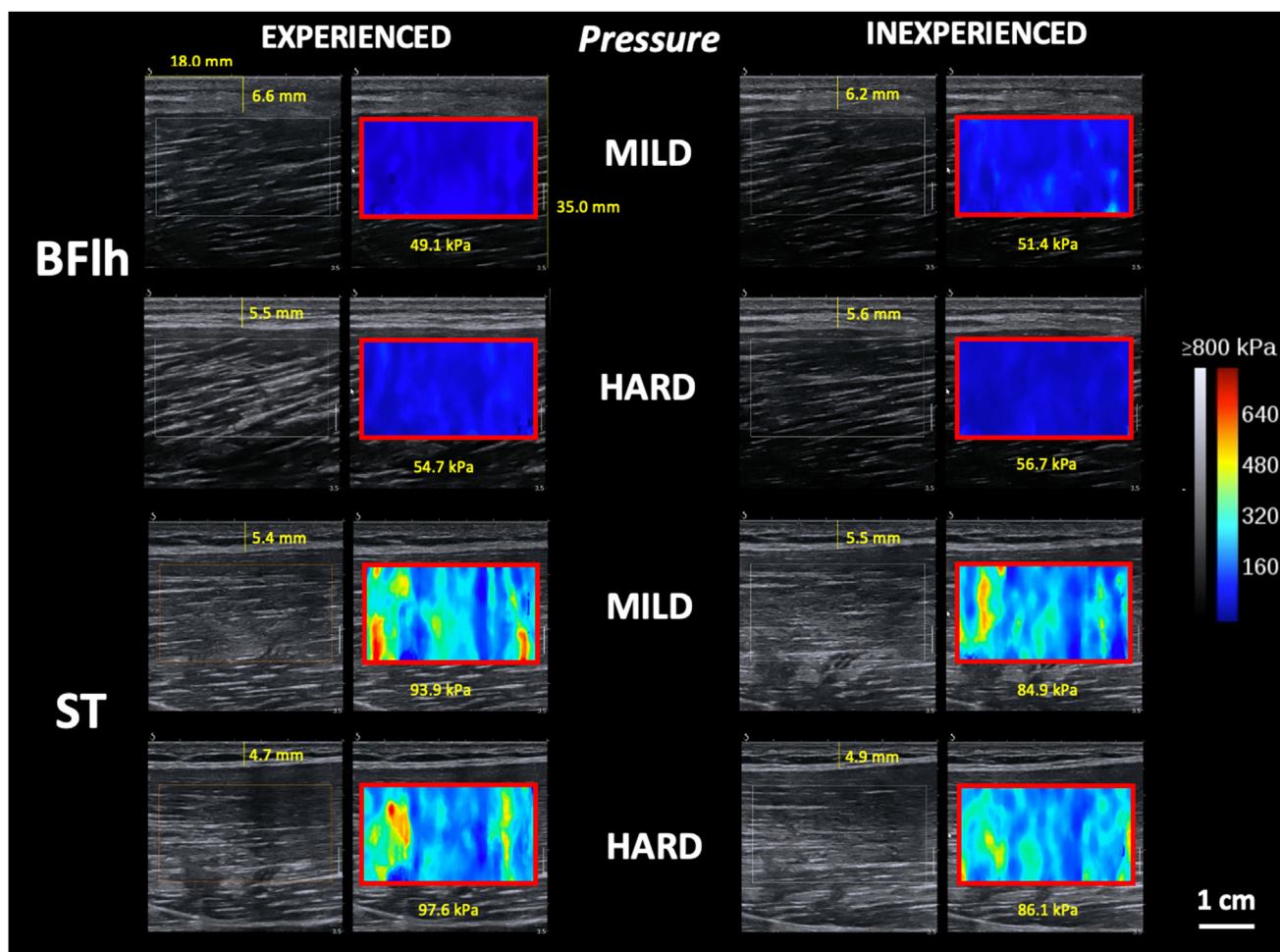
Dynamometry and elastography data were synchronized using an external switch that simultaneously started data collection for both devices, and acquired using a Biopac MP100 acquisition system (Biopac, Santa Barbara, CA). Shear wave data were processed using automated MATLAB routines (The Mathworks Inc., Natick, MA). For the shear modulus calculation, each clip exported from Aixplorer's software was sequenced in.jpeg images. Image processing converted each pixel of the color map into a value of the Young modulus based on the recorded color scale. The largest region of interest in the elastogram window was determined by avoiding aponeuroses and tissue artifacts (e.g., vessels) and the values were averaged to obtain a representative muscle value. Within each trial, the most stable Young's modulus values observed with ~15-s were averaged and divided by 3 to better represent the muscle shear elastic modulus.<sup>3</sup> The shear modulus of each muscle was considered for statistical analysis. The B-mode images were processed using Image J software (v1.47, NIH, Bethesda, MD), whereas the subcutaneous tissue thickness was measured (in millimeters) from the most superficial point of the skin to the deepest visible point on the superficial aponeurosis of each muscle, at the center of the B-mode images (Fig. 2).

### Statistical analysis

Data analysis was performed using IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, NY). Normality of data distribution was assessed using the Shapiro–Wilk test. The effect of transducer pressure and examiner experience on muscle active shear modulus and subcutaneous tissue thickness was determined using a repeated measures ANOVA [2 (experience: low, high) × 3 (pressure: mild, moderate, hard)] for each muscle, using the average value of the two measurements. Post-hoc analysis was conducted using Bonferroni correction to determine the differences between transducer pressures when a significant main effect was observed. The agreement between the examiners' measurements was examined using Bland–Altman analysis. The effect size of the differences found in these tests was measured using Cohen's *d* and classified as small ( $d = 0.2$ ), medium ( $d = 0.5$ ), and large ( $d = 0.8$ );



**Figure 1.** Examples of plastic casts (fixed to the skin with bi-adhesive tape) were used on Biceps femoris long head (BFlh) and Semitendinosus (ST) muscles to ensure that repeated muscle shear modulus measurements would be performed at the same site.



**Figure 2.** Biceps femoris long head (BFLh) and semitendinosus (ST) active shear modulus (at 20% of maximal isometric voluntary contraction) maps with mild and hard transducer pressures obtained by examiners with low (i.e. inexperienced) and high (i.e. experienced) ultrasound experience. The red box corresponds to the window used to calculate the shear modulus average (pointed in yellow) in the Matlab routine.

as well as by calculating the partial eta square ( $\eta^2_p$ ) values obtained from ANOVA's findings, which were classified as small ( $\eta^2_p = 0.01$ ), medium ( $\eta^2_p = 0.06$ ), and large ( $\eta^2_p = 0.14$ ).<sup>31</sup> The intraclass coefficient correlation (ICC) was used to determine the intra-examiner repeatability ( $ICC_{2,1}$ ) of active shear modulus assessment, and the standard error of measurement (SEM) was determined as a methodological error outcome. The ICCs were classified as poor (<0.5), moderate (0.5–0.75), good (0.75–0.9) and excellent (>0.9).<sup>32</sup> Pearson's correlation coefficient was used to determine the association between the deformation of subcutaneous tissues and shear modulus values. The magnitudes of Pearson's coefficients were classified as weak (<0.3), moderate (0.3–0.7), and strong (>0.7).<sup>33</sup> Data are presented as mean  $\pm$  standard deviation. Statistical significance was set at  $p < 0.05$ .

## Results

Fig. 2 shows representative active shear modulus maps taken by both examiners from both muscles at the three levels of transducer pressure. The depicted shear modulus represents the instantaneous value when the image was taken. Table 1 shows the results for the BFLh and ST muscle active shear modulus measurements between examiners and pressures, the intra-day repeatability outcomes for both examiners. Regarding active shear modulus analysis, no

significant experience  $\times$  pressure interaction was found for the BFLh ( $p = 0.826$ ;  $\eta^2_p = 0.007$ ) and ST muscles ( $p = 0.744$ ;  $\eta^2_p = 0.011$ ); as well as a nonsignificant effect of the experience factor for the BFLh (experienced:  $36.9 \pm 15.0$  kPa; inexperienced:  $35.4 \pm 13.4$  kPa;  $p = 0.299$ ;  $\eta^2_p = 0.041$ ) and ST muscles (experienced:  $63.2 \pm 21.2$  kPa; inexperienced:  $66.0 \pm 22.8$  kPa;  $p = 0.177$ ;  $\eta^2_p = 0.066$ ). However, a significant effect of transducer pressure was seen for the BFLh ( $p < 0.001$ ;  $\eta^2_p = 0.314$ ) and ST muscles ( $p < 0.001$ ;  $\eta^2_p = 0.280$ ). The post hoc analysis showed a significant difference between mild ( $32.7 \pm 15.8$  kPa) and moderate ( $36.2 \pm 14.4$  kPa;  $p = 0.013$ ,  $d = 0.23$ ) and between mild and hard ( $39.56 \pm 13.43$  kPa;  $p = 0.001$ ,  $d = 0.47$ ) pressures for the BFLh muscle (Fig. 3). For the ST muscle, significant differences were detected between mild ( $59.2 \pm 23.64$  kPa) and (moderate:  $65.1 \pm 23.7$  kPa;  $p = 0.024$ ,  $d = 0.25$ ) pressures, and between mild and hard ( $69.5 \pm 19.8$  kPa;  $p = 0.002$ ,  $d = 0.47$ ) pressures. For both muscles, the experienced examiner showed good to excellent repeatability, compared to the inexperienced examiner which showed moderate to good repeatability.

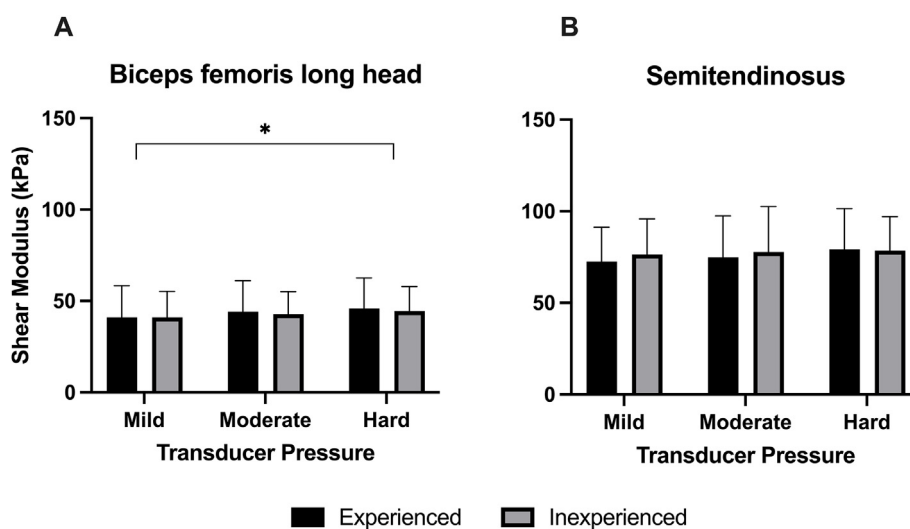
The Bland–Altman plots of absolute values for transducer pressures on BFLh and ST muscles are presented in Fig. 4; whereas marginal bias was observed and no significant associations were seen between the absolute differences (between examiners) and average shear modulus measurements.

**Table 1**

Active shear modulus (i.e. shear modulus) values and intra-rater repeatability outcomes for different transducer pressures (i.e. mild, moderate, and hard) and examiners with low (i.e. inexperienced) and high (i.e. experienced) ultrasound experience.

Muscle	Pressure	Shear modulus (kPa)								Intra-rater ICC (95% CI)	
		Experienced				Inexperienced				Experienced	Inexperienced
		M1	M2	CV (%)	SEM	M1	M2	CV (%)	SEM		
BFlh	Mild	32.8 ± 17.7	32.7 ± 16.0	4.1	1.1	37.8 ± 14.0	44.2 ± 18.0	2.2	4.9	0.95 (0.90–0.98)	0.78 (0.57–0.89)
	Moderate	36.3 ± 14.5	37.3 ± 17.1	3.5	2.0	41.5 ± 14.7	44.1 ± 14.7	3.0	3.4	0.91 (0.82–0.96)	0.85 (0.70–0.93)
	Hard	38.9 ± 17.0	41.9 ± 13.9	2.9	2.9	41.7 ± 13.9	39.6 ± 14.2	3.3	2.5	0.86 (0.71–0.94)	0.87 (0.73–0.94)
ST	Mild	58.5 ± 22.2	56.6 ± 24.1	3.2	1.4	60.0 ± 24.6	61.8 ± 28.6	1.7	5.3	0.96 (0.91–0.98)	0.86 (0.73–0.93)
	Moderate	65.6 ± 23.5	62.8 ± 22.9	2.2	3.2	65.5 ± 26.9	66.3 ± 27.5	1.8	5.0	0.90 (0.80–0.95)	0.87 (0.75–0.94)
	Hard	68.3 ± 22.0	67.5 ± 23.0	2.1	3.6	70.6 ± 20.7	71.6 ± 23.6	1.3	10.9	0.89 (0.77–0.95)	0.66 (0.38–0.83)

**Abbreviation:** BFlh, Biceps femoris long head; ST, Semitendinosus; M1, measure 1; M2, measure 2; CV, Coefficient of Variation between measurements; SEM, Standard error of measurement; ICC, intraclass correlation coefficient; CI, Confidence Interval.



**Figure 3.** Shear modulus of the (A) biceps femoris long head (BFlh), (B) semitendinosus (ST) during isometric contractions at 20% of maximal isometric voluntary contraction, at different pressure levels (i.e., mild, moderate, and hard) for the experienced and inexperienced examiners.\* Significant difference between mild and hard pressure ( $p < 0.05$ ).

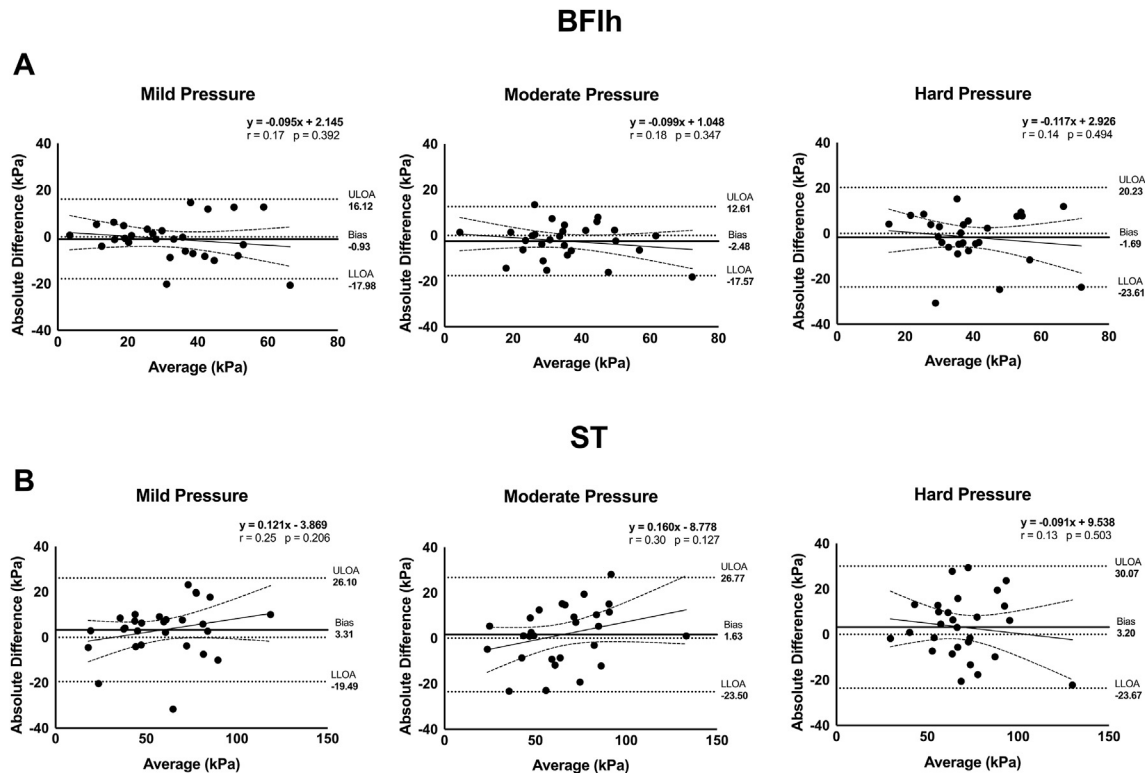
In respect to the analysis of subcutaneous tissue thickness, no significant pressure × experience interaction was found for the BFlh ( $p = 0.721$ ;  $\eta^2_p = 0.009$ ) and ST muscles ( $p = 0.056$ ;  $\eta^2_p = 0.127$ ); as well as a non significant effect of the experience factor for BFlh ( $p = 0.735$ ;  $\eta^2_p = 0.004$ ) and ST muscles ( $p = 0.466$ ;  $\eta^2_p = 0.021$ ). Conversely, a significant effect of transducer pressure was observed for the BFlh ( $p < 0.001$ ;  $\eta^2_p = 0.480$ ) and ST muscles ( $p < 0.001$ ;  $\eta^2_p = 0.405$ ). The post hoc analysis showed differences between mild and moderate pressures for BFlh (mild:  $11.7 \pm 4.8$  mm; moderate:  $10.7 \pm 4.2$  mm;  $p < 0.001$ ;  $d = 0.21$ ) and ST muscles (mild:  $9.5 \pm 4.2$  mm; moderate:  $8.9 \pm 3.6$  mm;  $p < 0.001$ ;  $d = 0.16$ ); and between mild and hard pressures on BFlh (hard:  $10.5 \pm 4.1$  mm;  $p < 0.001$ ;  $d = 0.27$ ) and ST muscles (hard:  $8.8 \pm 3.8$  mm;  $p < 0.001$ ;  $d = 0.19$ ). No significant differences were found between moderate-hard pressures in both muscles (BFlh:  $p = 0.062$ ; ST:  $p = 0.790$ ). Pearson’s analysis showed a negative moderate to strong correlation between subcutaneous thickness and shear modulus values in all transducer pressures of both examiners and muscles (Appendix A).

**Discussion**

To the best of our knowledge, this is the first study examining the effect of transducer pressure and examiner experience on the active shear modulus of two hamstring muscles in healthy individuals using ultrasound-based SWE. The main findings were: (i) differences were found on healthy adults for both ST and BFlh

muscles between mild and moderate, and mild and hard transducer pressures; (ii) no significant inter-examiner differences were seen for each level of transducer pressure; and (iii) the intra-day repeatability was higher for the experienced examiner.

Previous studies indicate that the muscle shear wave assessment in passive (i.e., resting) condition should be performed with the lowest possible pressure.<sup>8,10</sup> In fact, Kot et al. (2012) reported differences in both the maximum and mean value of the elastic modulus when different transducer pressures were applied. These authors found that the shear wave measurements in muscle increased with the increase in transducer pressure (with large effect sizes,  $d = 1.07–1.90$ ). In accordance with our initial hypothesis, significant differences in active shear modulus (with small effect sizes, i.e.  $d = 0.23–0.47$ ) were found between mild and moderate and mild and hard pressures for both BFlh and ST. However, a non-significant difference between moderate-hard pressure was noted for both muscles. This indicates that accurate active shear modulus measurement requires minimal transducer pressure (and visualizing the subcutaneous tissue thickness in b-mode images may help in this procedure), although its impact is lower compared to passive shear modulus measurements. It should be noted, however, that the BFlh muscle presents a lower active shear modulus compared to ST muscle for a given knee flexor contraction intensity.<sup>26,34</sup> This might be (partially) explained by muscle fascicle arrangement between BFlh muscle (i.e. pennate) and ST muscle (i.e. fusiform) (Kellis 2018), as the shear wave velocity tends to be higher when fascicles are parallel to the transducer plane.<sup>35–37</sup>



**Figure 4.** Bland–Altman analysis (i.e. agreement) of shear modulus measurements between examiners (i.e. negative bias values reflects tendency for the experienced examiner) for the three transducer pressures (i.e. mild, moderate, and hard) in both (A) biceps femoris long head (BFLh) and (B) semitendinosus (ST). Abbreviations. LLOA, lower limit of agreement; ULOA, upper limit of agreement; Bias, midpoint between the lower and upper limit of agreement.

Nevertheless, significant differences were seen between mild and moderate and mild and hard pressures for both muscles, which suggests that even considering the internal forces generated by the muscle contraction, the external force exerted by the transducer may still deform the subcutaneous tissues and increase the shear modulus values.

Our results also showed that applying moderate to hard transducer pressures did not lead to significant differences in subcutaneous tissue thickness or in active shear modulus values. Conversely, for both BFLh and ST muscles a significant difference was noted in subcutaneous tissue thickness between mild and moderate and mild and hard pressures, which was accompanied by a change in shear modulus values. Consequently, a negative (moderate to strong) correlation was found between subcutaneous tissue thickness and active shear modulus (i.e., compressing subcutaneous tissue thickness increases shear modulus values), as seen in [Appendix A](#). This indicates that visualizing the subcutaneous tissue thickness in the b-mode images during the active shear modulus assessment could be a good procedure to improve measurement accuracy. Thus, future researchers and clinicians should consider a minimal deformation of subcutaneous tissue to obtain a more accurate active shear modulus measure.

The Bland–Altman analysis showed similar bias values (<3.21 kPa) for both BFLh and ST muscles at different pressures, with no association between the absolute differences between examiners and muscle shear modulus. This suggests that shear modulus differences between examiners do not depend on the muscle shear modulus. However, the examiner's experience in ultrasound can result in different repeatability outcomes. In the present study, we found higher repeatability outcomes for the experienced examiner when minimal transducer pressure (i.e.

mild) was applied, while for the examiner with less experience (i.e. ~60 h of ultrasound training) the repeatability outcomes did not show an association with the transducer pressure. As the amount of pressure depends on the examiner's subjective experience and transducer operating skills,<sup>16</sup> it is important that the examiner conducts training sessions (ideally with visual feedback on the muscle/subcutaneous tissues deformation caused) prior to performing the measurements. Thus, the present findings support the recommendation that transducer force should be kept as constant and minimal as possible throughout the active (and also passive) shear modulus measurement to lower the measurement error, when some experience is acquired by the examiner.<sup>38</sup> Finally, it is of paramount importance to consider these two factors when analyzing shear modulus values and relating them to muscle injuries, as they influence the SEM and could potentially lead to erroneous interpretations if SEM is not taken into account.

The present study also indicates a lack of differences in active shear modulus values between examiners with different transducer pressures at the same region of interest. This suggests that the expertise factor is not relevant when the transducer position is maintained between trials, for example, by the use of casts ([Fig. 1](#)), which ensure that the same region of interest is measured and only allows the transducer tilt (and pressure) to be changed. In fact, using such casts provides a significant help for clinicians and researchers when they have to assess the tissue shear modulus with repeated measures, decreasing time consumption. As shown by the present data, they may also be beneficial to examiners with less experience. On the other hand, higher repeatability outcomes were observed in the experienced examiner ([Table 1](#)). One possibility to explain this could be at the transducer tilt performed between examiners. It has been reported that a small tilting of the

ultrasound transducer between measurements can result in large changes in the mean and reliability of the echo-intensity value<sup>39</sup> and shear wave measurements.<sup>19</sup> Moreover, it has been suggested that examiners should maintain a precise transducer tilt during repeated ultrasound measurements in order to quantify minimal changes in echo intensity.<sup>40,41</sup> This can be also transferred to elastography measurements, since it has been reported that shear waves are sensitive not only to transducer pressure but also to the transducer orientation, with the muscle shear modulus depending on the orientation of the transducer relative to the examined structures.<sup>19</sup> For instance, Hirayama et al. (2015) demonstrated the importance of the examiner's skills in ultrasound shear wave imaging of the transversus abdominis by comparing a skilled and an unskilled examiner.<sup>42</sup> The skilled examiner had been using ultrasound elastography for 1 year, while the unskilled operator had only been familiarized with B-mode ultrasonography to some extent and was using elastography for the first time. Their results showed a greater reliability of the skilled examiner (ICC = 0.86) when compared to the unskilled examiner (ICC = 0.59). Also, a previous study also found good intra-rater repeatability measuring the BFLh passive shear modulus assessed with a constant force (4N) (ICC = 0.85); and reported good inter-rater reproducibility (ICC = 0.74) between two examiners who underwent two 1-h training sessions with an expert in ultrasound.<sup>38</sup> Nevertheless, the alignment between the transducer and the examined structures seems to be important to decrease the methodological error of measurement, which is presumably better and more stable in examiners with more experience.

This study has some limitations. First, considering the reduced sample size, our findings may not be generalizable to individuals in a different age range, with muscle pathology, or with different activity patterns. Second, transducer pressure was not determined with a reliable tool that could allow the measurement of the force exerted on the skin. However, the pressure was based on the examiner's perception, instead allowing for a faster, intuitive, and more practical maneuver, which was the goal of the study. Third, active shear modulus measurements were performed only at one level of knee flexor contraction intensity (i.e., 20%). As the muscle active shear modulus increases with the contraction intensity,<sup>13</sup> we do not know for instance if the differences found on active shear modulus disappear with greater contraction intensities when the active shear modulus is higher. Fourth, the muscle thickness during the active shear modulus measurements was not assessed. However, the subcutaneous tissue thickness showed to be associated with shear modulus values, which might be a good indicator of pressure level.

## Conclusion

The present study findings indicate that, at least in two hamstring muscles (i.e. BFLh and ST muscles) with distinct architectural and shear modulus properties, the transducer pressure influences the active shear modulus measurement between mild and moderate or hard pressures. Additionally, examiner experience seems to have no influence on muscle active shear modulus measurement when assessed at the same site (using casts), which could minimize the data collection duration. However, the active shear modulus measurement repeatability benefits from the examiner's experience, which allows a lower measurement error. In the future, researchers and clinicians when assessing muscle active shear modulus should consider the ultrasound transducer pressure [as it seems to be negatively correlated (i.e. moderate to strong) to the shear modulus values], as well as the examiners experience with ultrasound (to have lower methodological errors).

## Conflicts of interest

No potential conflict of interest was reported by the author(s).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.radi.2023.11.005>.

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