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The effect of unit, depth, and probe load on the reliability of muscle shear wave elastography: Variables affecting reliability of SWE

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Abstract

Purpose: There is currently no standardized method for muscle shear wave elastography (SWE). The objective of this study was to investigate the effect of unit of measurement, depth, and probe load on the reliability of muscle SWE.

Methods: The vastus lateralis, biceps femoris, biceps brachii, and abductor digiti minimi muscles were scanned on 20 healthy participants. The SWE readings were measured in shear wave velocity (m/s) and Young's modulus (kPa). Three acquisitions of varying depths were acquired from vastus lateralis. Minimal probe load was compared with the use of a standoff gel layer. Three repeated measurements were acquired to assess reliability using intraclass correlations (ICC).

Results: The mean elasticity varied across muscle groups and ranged from 1.54 m/s for biceps femoris to 2.55 m/s for abductor digiti minimi (difference = 1.01 m/s [95% confidence interval, CI = 0.92, 1.10]). Reporting readings in meters per second resulted in higher ICC of 0.83 (0.65, 0.93) in comparison to 0.77 (0.52, 0.90) for kilopascal for the vastus lateralis muscle only. Variance increased proportionally with depth reaching 0.17 (equivalent to ± 0.82 m/s) at 6 cm. Using a standoff gel decreased ICC to 0.63 (0.20, 0.84) despite similar mean elasticity readings to minimal probe load.

Conclusions: Different acquisition and technical factors may significantly affect the reliability of SWE in skeletal muscles. Readings acquired in the unit of shear wave velocity (m/s) from depths less than 4 cm using a minimal probe load without a standoff gel yielded the best reliability.

KEYWORDS

elastography, muscles, reliability, ultrasound

1 | INTRODUCTION

Shear wave elastography (SWE) of skeletal muscle has recently started to gain interest in the field of musculoskeletal medicine. It provides a noninvasive quantitative measure of local tissue elasticity with less operator dependency when compared with strain (compression) elastography. The feasibility and value of SWE has previously been tested

in breast,² liver,³ thyroid,⁴ and prostate⁴ imaging; however, its role in the evaluation of muscle is less established and generally considered to be in the technical validation phase.

Muscle disorders may alter the biomechanical properties of muscle, and therefore, SWE has the potential to be a useful noninvasive and relatively inexpensive imaging biomarker for diagnosis and disease monitoring. Skeletal muscle imaging however has to overcome various

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anatomical challenges, such as anisotropy, contraction, and position-related changes due to structure and tissue heterogeneity due to myotendinous and aponeurotic structures. All of these features have been shown to influence SWE readings in recent papers. ^{5,6} In order to work toward the development of a standardized procedure, we report on the effects of further factors such as the unit of measurement, depth, and probe load on the reliability of skeletal muscle.

SWE machines track the propagation of shear waves to estimate shear wave velocity (SWV) by calculating the difference in the shear wave arrival time between 2 or more locations of known distances. Several commercially available SWE systems offer the option to report readings in SWV (m/s) and Young's modulus (kPa). In our practice, we have observed that we frequently encounter repeated consecutive acquisitions that have the same SWV but slightly different Young's modulus. Such occurrences suggest that the original SWV reading could potentially be more reliable than the Young's modulus. The SWE systems also allow placing acquisition sample boxes at different depths extending to approximately 75% of the corresponding maximum depth of B mode. Several articles have reported on the effect of depth in different tissues.⁷⁻¹⁰ However, none have investigated its effect on reliability. Although shear wave propagation is known to be depth dependent,⁸ there is no standardized protocol or recommendation regarding measurement depth in muscle. SWE is less operator dependent than strain elastography; however, it is still dependent on the pressure applied by the operator. Different degrees of probe load (precompression force) have been shown to result in significantly different SWE readings on breast and thyroid tissues. 11,12 Previous studies investigating probe load and depth applied statistical inferences to test for difference without testing for reliability. No previous studies have reported on the elasticity of the dominant versus nondominant thigh muscles. As muscular development and loading could cause a difference in muscle elasticity, assessing this could help understand differences that need to be taken into account when conducting research studies.

An understanding of factors that determine the reliability of SWE is imperative before examining pathological cases in clinical practice. Our hypothesis for this study is that SWE reliability is dependent on unit, depth, and probe load. The objective of this study is to test the effect of using different reporting units, acquisition depth, and probe load on the reliability of SWE in healthy skeletal muscle. A secondary objective is to determine if leg dominance has an impact on muscle elasticity.

2 | MATERIALS AND METHODS

2.1 | Participants

Twenty healthy participants (13 males: 7 females), from various ethnicities, volunteered for this cross-sectional study. The mean \pm SD age and body mass index were 36 ± 11.8 and 23 ± 3.1 , respectively. All participants were drug free and had no history of joint or muscle problems. None of the participants was considered athletic or engaged in competitive exercise programs. Participants were instructed to avoid

any strenuous activities 24 hours before the test to minimize confounding factors. Written informed consent was obtained from all participants. The study had been approved by a UK research ethics committee and was conducted according to good clinical practice guidelines.

2.2 | Shear wave elastography

SWE acquisitions were performed by a board-certified sonographer (A. M.A.) with more than 4 years ultrasound experience (2 years with SWE). The SWE software package on the General Electric LOGIQ-E9 system (GE Healthcare, Buckinghamshire, UK) employing a linear 9- to 5-MHz probe was utilized for this study. Briefly, this system quantifies the velocity of shear wave propagation using the comp-push excitation method and applying time-interleaved shear wave tracking to detect the SWV.13 This technology allows a free selection of large region of interest (ROI) with frame rates close to 1 frame per second. A circular ROI with an area of 75 mm² equivalent to 1 cm in diameter, was chosen for all SWE acquisitions with the exception of the small abductor digiti minimi muscle, for which a smaller ROI was used to cover a 1 cm × 1.2 cm SWE box. Because of the anisotropic nature of skeletal muscles, all acquisitions were performed with the probe oriented longitudinally to muscle fibers. This is determined when multiple fibers were continuously visible on the B-mode image. The probe was placed approximately at the midportion between the proximal and distal myotendinous junctions of each muscle. Measurements were obtained from the muscle belly away from any myoaponeurotic or myotendinous structures. Three consecutive measurements were recorded for each muscle and acquisition method.¹⁴

2.3 | Muscles and positioning

Four muscles were investigated in a resting state: vastus lateralis, biceps femoris, biceps brachii, and abductor digiti mini. The selection was based on choosing muscles from various depths, architectures, and sizes. All participants were asked to relax their muscles before the examination for 5 minutes. For vastus lateralis, participants were positioned supine with knees fully extended and feet slightly everted. For biceps brachii, participants remained in the same previous position and were then asked to bend their elbow (90°), relax their shoulder, and rest the supinated forearm on their torso. Next, for abductor digiti minimi, the dominant hand was pronated and rested on a cushion with the fifth finger being maintained in a slight abduction by the operator's hand. Last, for biceps femoris (long head), participants were positioned prone, with knees bent (90°) and legs rested against a wall. These positions allowed the investigation of the muscles in a resting state, ensuring no passive stretching or active contraction could affect the readings. The same order was followed when acquiring SWE images.

2.4 Units

After each acquisition, mean reading of the ROI was displayed and recorded in SWV (m/s) and Young's modulus (kPa). The latter is measured from the SWV using the following equation



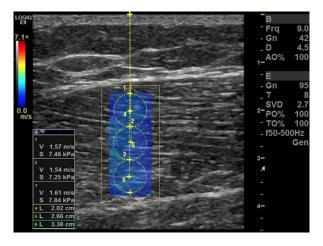


FIGURE 1 SWE of the vastus lateralis muscle demonstrating region of interest (ROI) placement at 3 different depths

$$E=3 \rho V_s^2, \tag{1}$$

where E is Young's modulus of elasticity, 3 is a constant related to Poisson's ratio for strain, ρ is tissue density (assumed to be 1 g/cm³), and V_S is the velocity of shear waves. The system's software calculates the sum of the value of each pixel in squared meters per second and multiplies it by 3. Two decimal places were reported by the machine and used in the analyses for each unit. Depth, probe load, and leg dominance analyses were performed using SWV.

2.5 Depth

For vastus lateralis only, 3 SWE acquisitions were recorded, each containing 3 ROIs (superficial/moderate/deep) positioned serially along the axial beam axis (Figure 1). Depth from the skin to the center of each ROI sample was recorded. The superficial and deep ROIs were placed away from the muscle edges (epimysium) to avoid the potential effect on elasticity. All ROIs had the same area of 75 mm². Readings were repeated 3 times to test for reliability.

2.6 | Probe load

For vastus lateralis only, readings were acquired using 2 probe load techniques: first, with the probe in light direct contact with the skin using only a minimum layer of gel without causing flattening or deformation of the superficial epimysium layer; and second, without contacting the skin using a copious amount of "standoff gel" clearly visible on top of the images. Approximately 5 mm of gel was utilized as a standoff layer, which was checked on the B-mode image prior to the acquisitions. This selected thickness was considered feasible without a significant depth tradeoff. These 2 acquisition techniques were chosen because they are the easiest to reproduce in clinical situations, in our opinion. They are also the most reasonable to be tested in terms of applying the lightest pressure on the skin. Three measurements were acquired successively for each technique.

2.7 | Leg dominance

Participants were asked about their leg dominance at the beginning of each examination. When unsure, they were asked, "which leg would you kick a ball with?" The same acquisition methods and location were applied when scanning the nondominant side. This investigation was performed on the vastus lateralis only.

2.8 | Statistical analysis

Repeated measures analysis of variance with post-hoc Bonferroni-corrected pairwise comparisons was used to compare mean SWV between muscles; terms were included for muscle (4 levels) and repeated measurement (3 levels). The same test was used to compare the vastus lateralis elasticity between dominant and nondominant leg as well as between using normal probe load and standoff gel. A 2-sided P value of less than .05 was considered significant. Reliability was quantified using 1-way random (average measure) intraclass correlation coefficients (ICC) of the 3 repeated measures for each muscle and acquisition method. The reliability coefficients are interpreted as follows: 0.00-0.20 "poor agreement," 0.21-0.40 "fair agreement," 0.41-0.60 "moderate agreement," 0.61-0.80 "substantial agreement," and >0.80 "almost perfect agreement." 16 Bland-Altman mean bias and 95% limits of agreement were used to evaluate probe load with and without a standoff gel.¹⁷ Within participants coefficient of variance (WSCV) was calculated as a measure of variability by calculating within-subject standard deviation¹⁸ and then dividing it by the mean. To investigate whether depth of assessment affected reliability, a multilevel linear regression model was constructed that included random terms for participants (level 3), relative depth of assessment (superficial/moderate/ deep; level 2), and repeated measurement (level 1). Measured depth of assessment (cm) was included as an explanatory variable. Loglikelihood values from models with and without an additional term that modeled the variability of level 1 SWE measurements as a function of measured depth of assessment were compared. SPSS version 24 (IBM Corp, Armonk, NY) and MLWin 3.00 (Centre for Multilevel Modelling, University of Bristol)¹⁹ were utilized to perform statistical analysis.

3 | RESULTS

Pairwise comparisons revealed that SWV differed between all muscles (P<.001) with the exception of vastus lateralis and biceps brachii, where the mean SWVs were both 1.76 m/s (P = 1). The largest difference was between the abductor digiti minimi and biceps femoris (mean difference [95% confidence interval, CI] 1.01 [0.92, 1.10]). Table 1 lists the means for each muscle in addition to variability and reliability results using the 2 reporting units. Using SWV (m/s), reliability coefficients were almost perfect (ICC > 0.80) across all muscles. Although within-subject variability, demonstrated as WSCV, was lowest for the abductor digiti minimi, Figure 2 shows relatively large between-subject variability (wide 95% CI) among the readings. The difference in reliability between the units was only noticeable for the vastus lateralis muscle with and without standoff gel. Otherwise, ICC coefficients between

TABLE 1 Mean, variability, and reliability of the different muscles for the 2 SWE units

	Shear wave velocity (m/s)			Young's modulus (kPa)		
Muscle	Mean (95% CI)	WSCV, %	ICC (95% CI)	Mean (95% CI)	WSCV, %	ICC (95% CI)
Vastus lateralis	1.76 (1.71, 1.81)	4.4	0.83 (0.65, 0.93)	9.61 (9.02, 10.20)	11.0	0.77 (0.52, 0.90)
Vastus lateralis (standoff gel)	1.73 (1.66, 1.80)	9.0	0.62 (0.20, 0.84)	9.56 (8.75, 10.37)	20.0	0.56 (0.08, 0.82)
Biceps brachii	1.76 (1.71, 1.81)	3.1	0.91 (0.82, 0.96)	9.40 (8.81, 9.98)	6.6	0.91 (0.82, 0.96)
Abductor digiti minimi	2.55 (2.46, 2.65)	2.1	0.97 (0.94, 0.99)	19.90 (18.37, 21.43)	4.6	0.97 (0.94, 0.99)
Biceps femoris	1.54 (1.48, 1.60)	4.6	0.90 (0.79, 0.96)	7.59 (6.99, 8.19)	9.0	0.90 (0.79, 0.96)

Abbreviations: ICC, intraclass correlation coefficient; WSCV, within subjects coefficient of variation.

the units were identical. Association between them for the 4 muscles is plotted in Figure 3.

As for depth, Figure 4 illustrates that mean SWV was not affected by depth (SWV per centimeter [standard error] = 0.013 [0.029]; likelihood ratio test $\chi^2(1)=0.65$, P=.421). However, there was strong evidence that at greater depths of assessment the repeated SWV measurements were more variable (likelihood ratio test $\varphi\chi^2(1)=41.4$, P<.001) (Figure 5). The equation for this association was estimated to be:

SWV variance=
$$-0.009+(0.004\times depth)+(0.004\times depth^2)$$
. (2)

Approximately 95% of measurements are expected to lie between -2 and +2 SD around the mean; estimated variance (SD²) of 0.07 at 4-cm depth equated to an interval of ± 0.53 m/s, while at 6 cm (variance = 0.17), this increased to ± 0.82 m/s.

Mean SWV (m/s) was not significantly different when using a standoff gel in comparison to normal probe load (mean difference [95% CI] 0.03 (-0.03, 0.09), P = .317]. Reliability decreased from almost perfect agreement (ICC = 0.83) to the lower margin of substantial agreement (ICC = 0.62) for normal probe load and standoff gel, respectively. WSCV doubled when using a standoff gel, increasing from 4.4% to 9.0%. Using the latter technique, mean SWV decreased by 0.03 m/s expressing negligible average bias (Figure 6); the 95% limits of

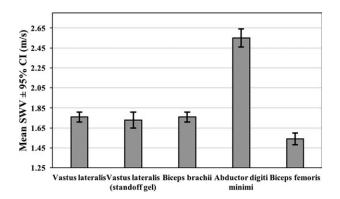


FIGURE 2 Bar chart demonstrates the distribution of the acquired mean shear wave velocity (SWV) for the different muscles. The standoff gel acquisition method is also included and shows larger between-participants variability (wider 95% CI) in comparison to the normal acquisition despite the relatively similar mean SWV

agreement were ± 0.37 (95% CI 0.29, 0.45). No significant mean SWV difference was found between the dominant and nondominant vastus lateralis (-0.04 [-0.09, 0.01], P = .082). ICC (95% CI) for the nondominant vastus lateralis was 0.80 (0.59, 0.91), which is similar to ICC for the dominant side reported in Table 1.

4 | DISCUSSION

This study set out to evaluate factors that may be important in the standardization of muscle assessment using SWE. To our knowledge, no previous studies have tested the same variables using the same technical and statistical methodology. There is also limited knowledge on the performance of newly introduced shear wave systems, such as the one we utilized (LOGIQ-E9). This is particularly important because each system applies its own technology, and variations regarding performance to other systems might be expected. The majority of previous SWE studies were designed to test diagnostic performance for various pathologies without specifically focusing on possible variations induced by acquisition methods. Our study has confirmed that the type of unit of measurement, depth of measurement, and overlying pressure from the probe may all influence the final SWE reading.

The first part of the study evaluated whether SWE readings were influenced by the types of muscle. Our results confirmed that there were differences. For example, there was a significant difference in mean SWV between the quadriceps (vastus lateralis) and hamstring (biceps femoris) muscle. Dubois et al.⁶ reported stiffness readings of 4.5 kPa and 5.6 kPa for vastus lateralis and biceps femoris, respectively. Our mean elasticity readings for vastus lateralis and biceps femoris were almost twice as high (9.61 kPa and 7.59 kPa, respectively) and were in agreement with Lacourpaille et al.²⁰ The latter study also found a significantly higher stiffness in the abductor digiti minimi (13.5 kPa), although lower than our reported mean elasticity (19.9 kPa). As these studies by Dubois et al.⁶ and Lacourpaille et al.²⁰ utilized the same SWE system (SuperSonic Imagine, Aix-en-Provence, France), the discrepancy may be due to factors related to acquisition methods. Ewertsen et al.8 investigated the biceps brachii muscle and reported an SWV that is almost exactly the same as ours (1.76 m/s vs. 1.77 m/s). It should therefore be appreciated that variations in agreement occur depending on machines and technical and acquisition methods across studies.

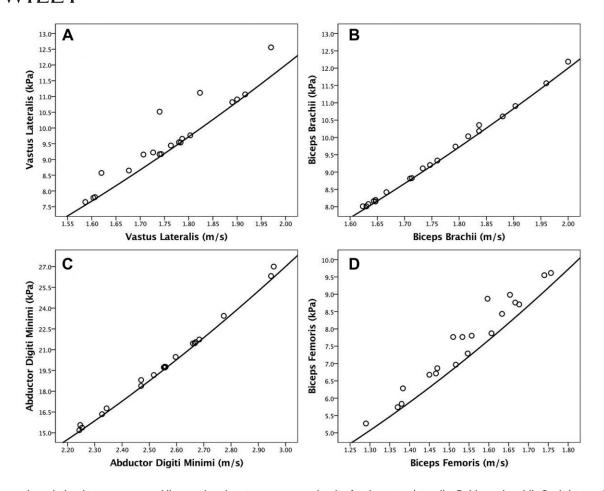


FIGURE 3 Association between means kilopascal and meters per second units for A, vastus lateralis; B, biceps brachii; C, abductor digiti minimi; and D, biceps femoris. In each case, the plotted line represents the direct transformation $kPa = 3(SWV)^2$. The degree of association decreased for vastus lateralis and biceps femoris where, on multiple occurrences, kPa overestimates m/s

The reliability of SWE, as presented by the ICC coefficients in Table 1, indicate that our measurements with the LOGIQ E9 can acquire repeated measurements with similar reliability to what others

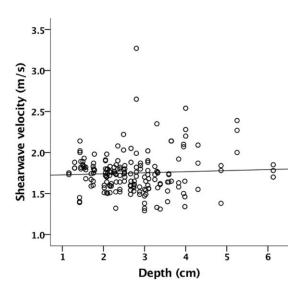


FIGURE 4 Scatterplot shows no substantial influence of depth on mean shear wave velocity (SWV)

have reported on similar healthy skeletal muscles using the same muscles but different systems. 5.20-22 To our knowledge, this is the first reported data with the LOGIQ E9 in muscles. We reported the ICC for the average of several measures instead of single measures considering that the average of at least 3 acquisitions is necessary to provide reliable readings in clinical practice. Reliability appeared to be higher for the superficial muscles in comparison to the deeper muscles. Although

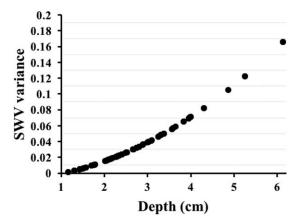


FIGURE 5 Estimated variance of shear wave velocity (SWV) measurements as a function of depth of acquisition

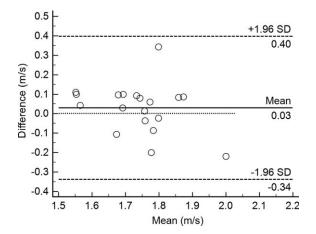


FIGURE 6 Bland-Altman plot demonstrating the difference against the mean between the measurements of the vastus lateralis with and without standoff gel. The central solid line is the mean SWV difference between the 2 methods displaying small, negligible bias (0.03 m/s). The 2 lines represent the upper and lower 95% limits of agreement at -0.34 m/s and 0.40 m/s. The width of the limits indicates that readings could vary by 22% between the 2 methods

the abductor digiti minimi muscle resulted in the highest ICC and lowest WSCV, the 95% CI, as seen in Figure 2, were wide, indicating large mean SWV variability between the subjects. The reason for this feature is unclear and could be related to anatomical factors like muscle size or technical acquisition factors like muscle relaxation upon positioning.

Several elastography systems offer the option to report SWE readings in SWV (m/s) and Young's modulus (kPa). The original measurement recorded by the machine is SWV; it then mathematically converts it to Young's modulus for each pixel and then reports the average of all pixels in kilopascal. This conversion method produces 2 problems. First, in consecutive acquisitions, the readings may have the same mean SWV but different standard deviations due to heterogeneity in the ROI pixels. In such instances, the acquisition with the higher standard deviation will have an artificially larger Young's modulus. This will induce a variability in kilopascals but not in meters per second, rendering it less reliable. This is evidenced when looking at the ICC in Table 1 for vastus lateralis. The remarkably greater WSCV in kilopascals is expected due to the larger range of the results. Moreover, kilopascals will overestimate elasticity in heterogonous (high standard deviation) acquisitions due to the effect of squaring in Equation 1. The difference in reliability between the 2 units was only noticeable in the vastus lateralis muscle due to the several occurrences of repeated measurements of similar SWV from heterogeneous acquisition samples having different standard deviations. This discrepancy problem and variation in reliability between the units might be greater in pathologies because shear wave maps tend to be even more heterogeneous. Figure 3 illustrates that the 2 units are not synonymous because they did not fit the line in all observations. The second problem is when kilopascal value is manually calculated from mean meters per second; the square root of the sum will be calculated instead of the sum of the square root of each pixel, generating an error. There would be no error if the acquired shear wave map is completely homogenous, with all pixels presenting the

same value in meters per second. The error will become greater if the shear wave map is heterogeneous. This conversion error is very common in the SWE literature when researchers compare their results to others

There are additional important inaccuracies associated with converting the velocity readings to Young's modulus. The variation in soft tissues densities is neglected, because Young's modulus assumes density is constant and equals 1 g/cm³. This is inaccurate, because the density differs and is higher for muscles (1.06 g/cm³) than for fat (0.90 g/cm³) for example.^{23,24} Young's modulus assumes that tissues are isotropic and homogeneous; both assumptions are not the case when investigating muscles. Only 1 previous study by Youk et al. has compared SWE units.²⁵ They tested the diagnostic performance of the 2 units on 130 breast masses. Although the diagnostic performance indices were not identical, there was no significant difference between mean meters per second and kilopascals. Nevertheless, they reported a significant difference in specificity and area under the curve when using the standard deviation of the entire lesion as a diagnostic method. Our result is the first to compare the reliability between the 2 units. We recommend using SWV as a surrogate for tissue elasticity instead of Young's modulus. This will help with study result reliability and allow more accurate comparison between studies.

Investigating depth is of particular importance, because reliability may diminish at greater depths due to the attenuation of the acoustic push pulses and tracking waves. In this study, mean SWV did not appear to be influenced by depth, in disagreement with previous studies, which reported conflicting results between each other. Ewertsen et al. found SWV decreasing marginally with depth ($R^2 = 0.019$) without P value significance; regardless, this is unlikely to be significant considering the weak R^{2.8} In contrast, Carpenter et al. reported substantive increase with depth ($R^2 = 0.30$, P < .001) for the rectus femoris and negligible increase ($R^2 = 0.03$, P = .057) for the gastrocnemius.⁷ Both studies had a small sample size of 10 and 5 subjects, respectively. None reported on the reliability of SWV at the different depths. Carpenter et al.⁷ attempted to study the effect of depth by testing for a difference between 2 random depths, named "superficial" and "deep." They reported a significant difference with the consideration that the depth readings did not exceed 2.5 cm. Their approach provides limited evidence on the effect of depth on the acquisitions integrity.

No previous studies have analyzed the effect of depth as a continuous variable on muscle SWE as we did. We have shown that variability of the readings increases quadratically, as illustrated in Figure 5 and Equation 2. We would therefore not recommend acquiring readings deeper than 4 cm because the variability increases substantially reaching variance = 0.17 at 6 cm, equating to 95% of readings lying within ± 0.82 m/s. This is a wide interval given the mean reading was 1.76 m/s. To our knowledge, there is no known cutoff point for acceptable variability in SWE. However, considering depth feasibility, we consider the variance of 0.07 at 4 cm depth, equating to ± 0.53 m/s, to be the limit of acceptable variability. Likewise, recent guidelines on thyroid SWE recommend that acquisitions should not exceed depths of 4 to 5 cm. The strength of the acoustic radiation force impulse (push pulse) diminishes at higher depths (5.5cm), rendering the generated shear

waves too weak to be tracked accurately.²⁶ Other probes with lower frequencies may result in different findings. The SWE mode on the machine we utilized is only available on the linear 9- to 5-MHz probe. Further research on higher body mass index subject groups is necessary to validate our findings. Depth investigation results from phantoms may not be generalized to muscles because of anisotropy that may influence waves propagation in muscles.²⁰

Although SWE removes much of the operator dependency in comparison to strain elastography, probe load is one of the remaining operator-dependent factors. Carpenter et al. investigated the effect of probe load on muscle tissues over 5 healthy participants testing normal probe contact versus slight axial stress. The same investigation was performed previously by Kot et al.,²⁷ and both found a significant difference between the techniques but did not conduct any reliability analysis. The lone testing of difference is less informative and does not provide useful evidence on the most suitable method to recommend. Others investigated the effect of hard probe compression, which we consider is unreasonable and will most likely result in false, inconsistent readings due to impracticality and the high degree of stress influencing elasticity. 11,28 We sought to investigate the reliability of probe load for 2 reasonable, practical, and easy-to-replicate techniques. Our results support placing the probe in direct contact with the skin without any compressional force or standoff gel. The microbubbles in the gel layer may have potentially decreased the quality of the push pulse resulting in larger variance and lower reliability. Our finding for standoff gel may not be generalized to other organs, such as breast, where lesions are superficial, because it could be useful and reliability may be higher. Despite no significant differences between the mean SWV for the 2 methods, the 95% CI of the limits of agreement indicates that reading variability ranges between 16.5% and 25.5%. It suggests that results may not be accurately compared between studies utilizing different probe load acquisition techniques.

Leg dominance may relate to muscular development and potential variation. Reviewing the muscle SWE literature, we found that most research studies perform SWE on a single side because of the time limitations. To our knowledge, this study is the first to investigate the potential difference between sides. Our results show that the similarity assumption between dominant and nondominant side is valid for the vastus lateralis muscle on our subjects. This finding may not be directly generalizable to pathological cases because unilateral disease development is possible. Although many skeletal muscle pathologies may affect the thigh muscles symmetrically, such as idiopathic inflammatory myopathies.²⁹ Nevertheless, this finding is helpful to researchers in verifying that halving scanning time through scanning one side may be acceptable for healthy subjects.

We believe our study is original from several perspectives and discusses important considerations in SWE research and clinical applications. However, it has several limitations. No interoperator reproducibility was performed due to the feasibility to reduce scanning time for participants. Moreover, probe load, depth, and dominance were only tested on vastus lateralis because of time limitations also. Future research studies should examine our outcomes on pathological cases to confirm the findings. Nevertheless, the information we provided will be

helpful to future SWE studies on myopathies to ensure the acquisition of reliable readings.

In conclusion, the units of meters per second and kilopascals are not synonymous. Readings in kilopascals are affected by tissue heterogeneity and are less reliable in comparison to meters per second. SWV proportionally increase in variability as depth increases despite no significant change in the mean value. Placing the probe in direct contact with the skin using minimal pressure yields more reliable reading in comparison to utilizing a standoff gel between the probe and skin surface. Attention to these factors should assist in acquiring reliable readings and developing a standardized operating procedure.

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