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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 twenty 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% CI= 0.92, 1.10)]. Reporting readings in m/s resulted in higher ICC of .83 (.65, .93) in comparison to .77 (.52, .90) for kPa 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 .63 (.20, .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; Ultrasound; Muscles; Reliability.

Introduction

Shear wave elastography (SWE) of skeletal muscle has recently started to gain interest in the field of musculoskeletal medicine. It provides a non-invasive quantitative measure of local tissue elasticity with less operator dependency when compared to strain (compression) elastography¹. The feasibility and value of SWE has previously been tested in breast², liver³, thyroid⁴ 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 non-invasive and relatively inexpensive imaging biomarker for diagnosis and disease monitoring. Skeletal muscle imaging however has to overcome various 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 towards 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 two 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 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 tissues⁷⁻¹⁰. However, none have investigated its effect on reliability. Although shear wave propagation is known to be depth dependent⁸, 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 non-dominant thigh muscles. As muscular development and loading could cause a difference in muscle elasticity, assessing this could help understand differences which need to be taken into account when conducting research studies.

An understanding of factors which 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.

Materials and methods

Participants

Twenty healthy participants (13 males: 7 females) from various ethnicities, volunteered for this cross-sectional study. The mean \pm SD age and BMI 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.

Shear wave elastography

SWE acquisitions were performed by a board-certified sonographer (AMA) with more than four years ultrasound experience (two years with SWE). The SWE software package on the General Electric

LOGIQ-E9 system (GE Healthcare, Buckinghamshire, UK) employing a linear 9-5 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 SWV¹³. This technology allows a free selection of large ROI with frame rates close to 1 frame/sec. A circular ROI with an area of 75mm², equivalent to 1cm 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 1cm × 1.2cm shear wave elastography box. Due to 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 method¹⁴.

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 five minutes. For vastus lateralis, participants laid 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. Lastly, for biceps femoris (long head), participants laid prone, bent knees (90°) and rested legs 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.

Units

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

$$E = 3 \rho V_s^2 \quad (1)$$

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 m/s squared 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.

Depth

For vastus lateralis only, three SWE acquisitions were recorded, each containing three 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 75mm². Readings were repeated three times to test for reliability.

Probe load

For vastus lateralis only, readings were acquired using two probe load techniques. Firstly, 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. Secondly, without contacting the skin using 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 trade-off. These two acquisition techniques were chosen as 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.

Leg dominance

Participants were asked about their leg dominance at the beginning of each exam. When unsure, they were asked, “which leg would you kick a ball with?”¹⁵. The same acquisition methods and location were applied when scanning the non-dominant side. This investigation was performed on the vastus lateralis only.

Statistical analysis

Repeated measures ANOVA 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 non-dominant leg as well as between using normal probe load and standoff gel. A two-sided p-value of less than 0.05 was considered significant. Reliability was quantified using one-way random (average measure) intra-class correlation coefficients (ICC) of the three repeated measures for each muscle and acquisition method. The reliability coefficients are interpreted as follows: .00-.20 ‘poor agreement’, .21-.40 ‘fair agreement’, .41-.60 ‘moderate agreement’, .61-.80 ‘substantial agreement’ and >.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 gel¹⁷. Within participants coefficient of variance (WSCV) was calculated as a measure of variability by calculating within-subject standard deviation¹⁸ 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. Log-likelihood 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, N.Y., USA) and MLWin 3.00 (Centre for Multilevel Modelling, University of Bristol)¹⁹ were utilized to perform statistical analysis.

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% CI) 1.01 (0.92, 1.10)]. Table 1 lists the means for each muscle in addition to variability and reliability results using the two reporting units. Using SWV (m/s), reliability coefficients were almost perfect ($ICC > .80$) 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) amongst 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 the units were identical. Association between them for the four muscles is plotted in Figure 3.

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

$$SWV \text{ variance} = -0.009 + (0.004 \times \text{depth}) + (0.004 \times \text{depth}^2) \quad (2)$$

Approximately 95% of measurements are expected to lie between -2 and +2 SD around the mean; estimated variance (SD^2) of 0.07 at 4cm depth equated to an interval of ± 0.53 m/s, whilst at 6cm (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 ($ICC=.83$) to the lower margin of substantial agreement ($ICC=.62$) 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 agreement were ± 0.37 (95% CI 0.29, 0.45). No significant mean SWV difference was found between the dominant and non-dominant vastus lateralis [-0.04 (-0.09, 0.01), $p = .082$]. ICC (95% CI) for the non-dominant vastus lateralis was .80 (.59, .91), which is similar to ICC for the dominant side reported in Table 1.

Discussion

This study set out to evaluate factors which 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 since 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. Ewerson 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.

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 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 three acquisitions is necessary to provide reliable readings in clinical practice²³. Reliability appeared to be higher for the superficial muscles in comparison to the deeper muscles. Although the abductor digiti minimi muscle resulted in the highest ICC and lowest WSCV, the 95% CI, as seen on 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 then reports the average of all pixels in kPa. This conversion method produces two problems. Firstly, 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 kPa but not in m/s rendering it less reliable. This is evidenced when looking at the ICC in Table 1 for vastus lateralis. The remarkably greater WSCV in kPa is expected due to the larger range of the results. Moreover, kPa will overestimate elasticity in heterogenous (high standard deviation) acquisitions due to the effect of squaring in equation 1. The difference in reliability between the two 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 as shear wave maps tend to be even more

heterogeneous. Figure 3 illustrates that the two units are not synonymous as they did not fit the line in all observations. The second problem is when kPa value is manually calculated from mean m/s; 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 m/s. 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, as Young's modulus assumes density is constant and equals 1 g/cm^3 . This is inaccurate, as the density differs and is higher for muscles (1.06 g/cm^3) than fat (0.90 g/cm^3) for example ^{24,25}. Young's modulus assumes that tissues are isotropic and homogeneous; both assumptions are not the case when investigating muscles. Only one previous study by Youk et al. has compared SWE units ²⁶. They tested the diagnostic performance of the two units on 130 breast masses. Although the diagnostic performance indices were not identical, there was no significant difference between mean m/s and kPa. 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 two units. We recommend using SWV as a surrogate for tissue elasticity instead of Young's modulus. This will help both with study result reliability and allow more accurate comparison between studies.

Investigating depth is of particular importance, as 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. Ewerson et al. found SWV decreasing marginally with depth ($R^2=.019$) 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 ($R^2=.30$, $p<.001$) for the rectus femoris and negligible increase ($R^2=.03$ $p=.057$) for the gastrocnemius ⁷. Both studies had a small sample size of ten and five 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 two 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 as 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 4-5 cm²⁷. 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 accurately²⁸. Other probes with lower frequencies may result in different findings. The SWE mode on the machine we utilized is only available on the linear 9-5 MHz probe. Further research on higher BMI 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 muscles²⁰.

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.⁷ investigated the effect of probe load on muscle tissues over five healthy participants testing normal probe contact versus slight axial stress. The same investigation was performed previously by Kot et al.²⁹ 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 a 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^{11,30}. We sought to investigate the reliability of probe load for two 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, as it could be useful and reliability may be higher. Despite no significant differences between the mean SWV for the two methods, the 95% CI of the limits of agreement indicates that reading variability ranges between 16.5% – 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 non-dominant 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 myopathies³¹. 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 inter-operator 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 m/s and kPa are not synonymous. Readings in kPa are affected by tissue heterogeneity and are less reliable in comparison to m/s. 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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Table 1

Table 1 Mean, variability and reliability of the different muscles for the two SWE units.

Muscle	Shear wave velocity (m/s)			Young's modulus (kPa)		
	Mean (95% CI)	WSCV	ICC (95% CI)	Mean (95% CI)	WSCV	ICC (95% CI)
Vastus lateralis	1.76 (1.71, 1.81)	4.4%	.83 (.65, .93)	9.61 (9.02, 10.20)	11.0%	.77 (.52, .90)
Vastus lateralis (standoff gel)	1.73 (1.66, 1.80)	9.0%	.62 (.20, .84)	9.56 (8.75, 10.37)	20.0%	.56 (.08, .82)
Biceps brachii	1.76 (1.71, 1.81)	3.1%	.91 (.82, .96)	9.40 (8.81, 9.98)	6.6%	.91 (.82, .96)
Abductor digiti minimi	2.55 (2.46, 2.65)	2.1%	.97 (.94, .99)	19.90 (18.37, 21.43)	4.6%	.97 (.94, .99)
Biceps femoris	1.54 (1.48, 1.60)	4.6%	.90 (.79, .96)	7.59 (6.99, 8.19)	9.0%	.90 (.79, .96)

WSCV= Within Subjects Coefficient of Variation. ICC=Intraclass Correlation Coefficient

Figures legends

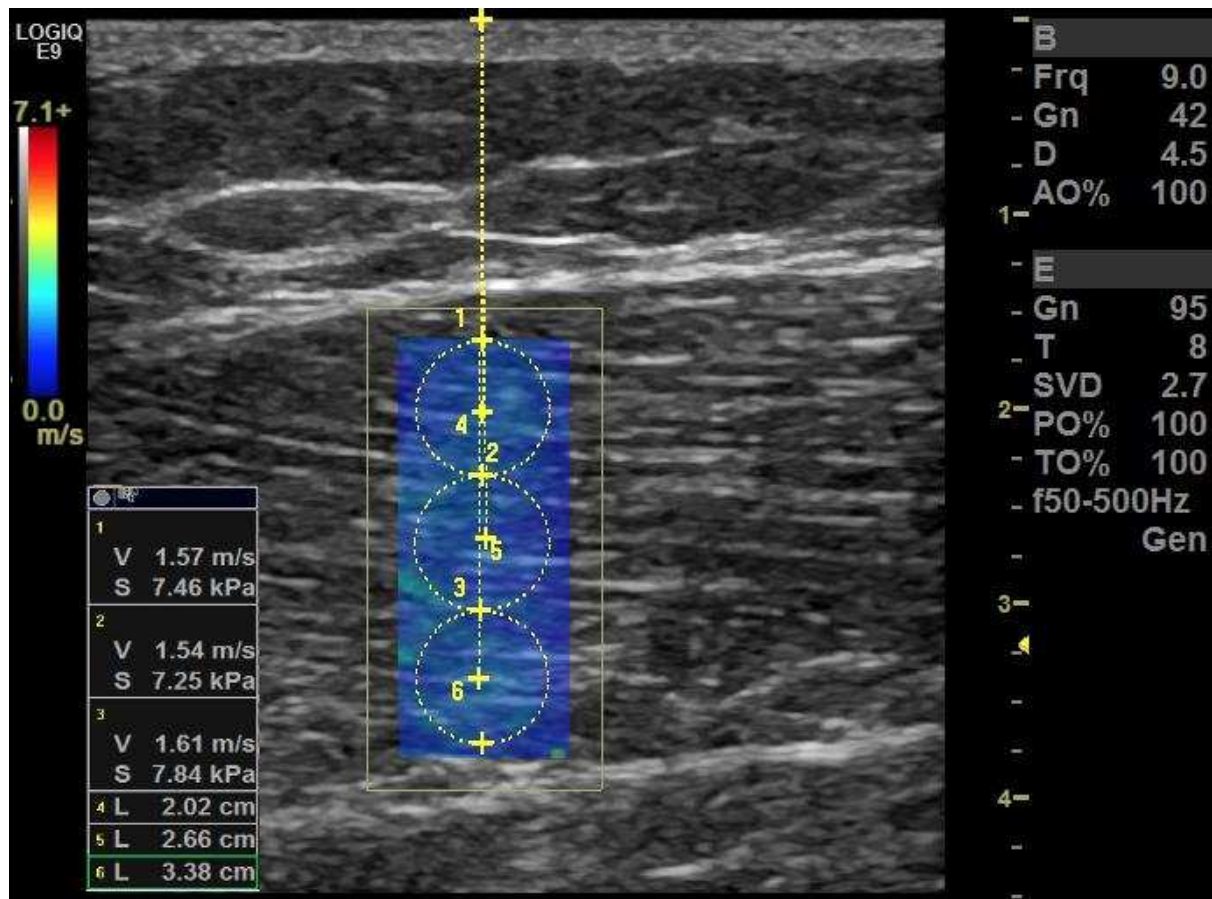


Figure 1 SWE of the vastus lateralis muscle demonstrating ROI placement at three different depths.

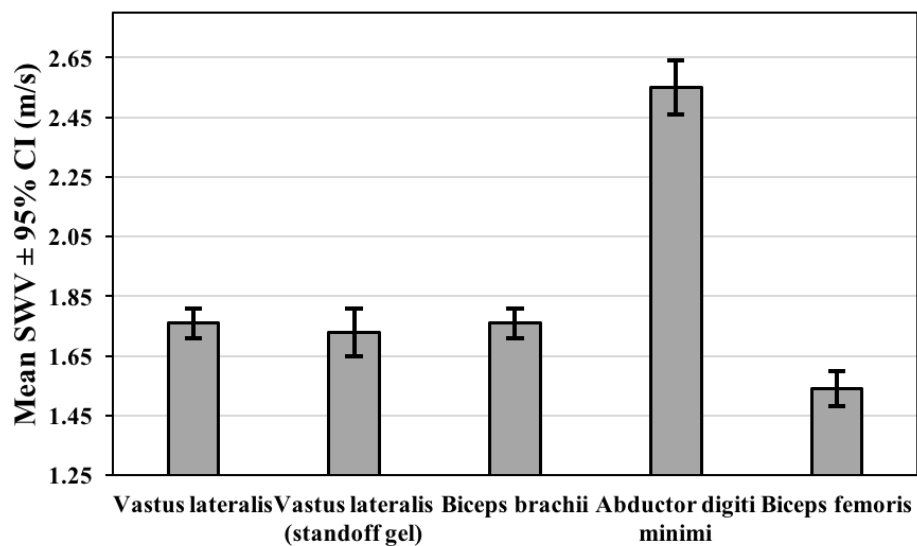


Figure 2 Bar chart demonstrating the distribution of the acquired mean SWV for the different muscles. The standoff gel acquisition method is also included showing larger between-participants variability (wider 95% CI) in comparison to the normal acquisition despite the relatively similar mean SWV.

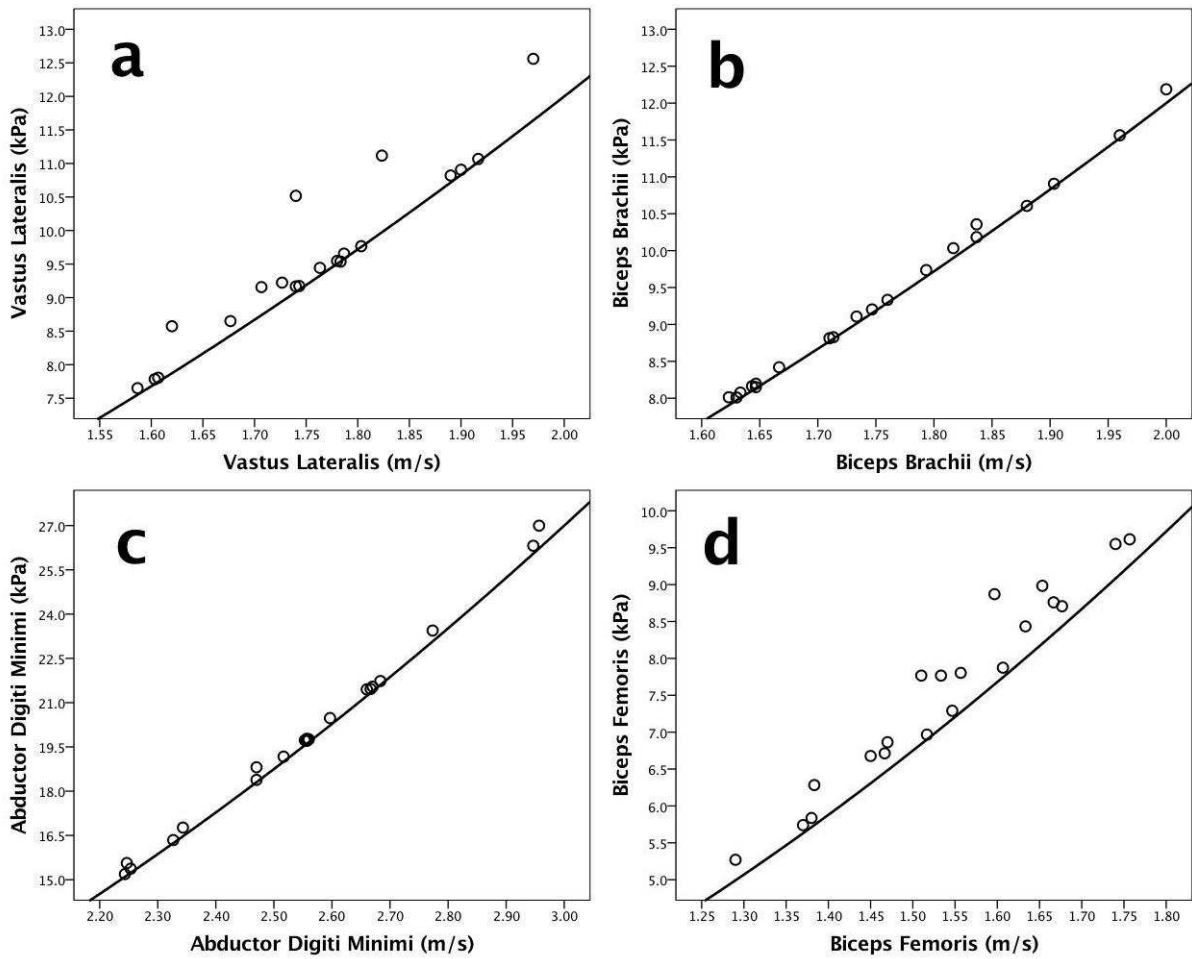


Figure 3 Association between means kPa and m/s units for the vastus lateralis (a), biceps brachii (b), abductor digiti minimi (c) and biceps femoris (d). In each case, the plotted line represents the direct transformation $kPa = 3(SWV)^2$. The degree of association decreased for vastus lateralis and biceps femoris where, on multiple occurrences, kPa overestimates m/s.

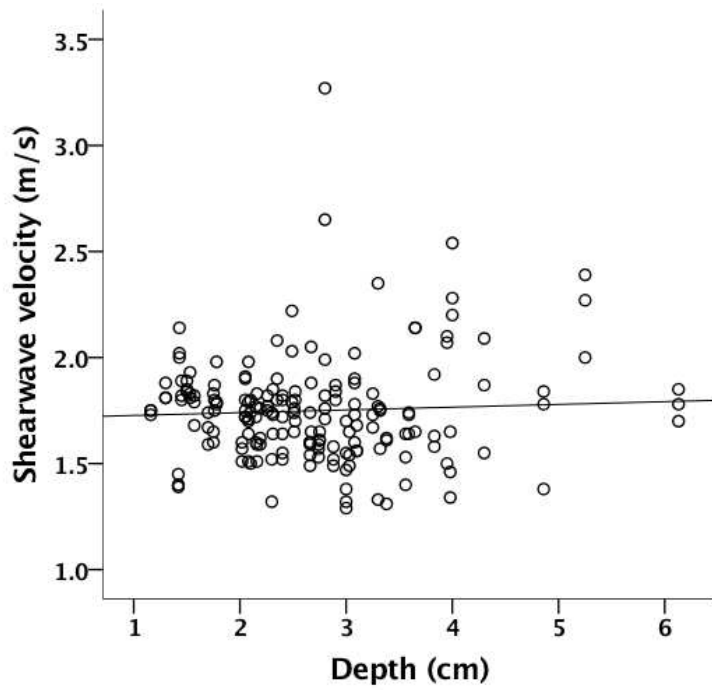


Figure 4 Scatterplot showing no substantial influence of depth on mean SWV.

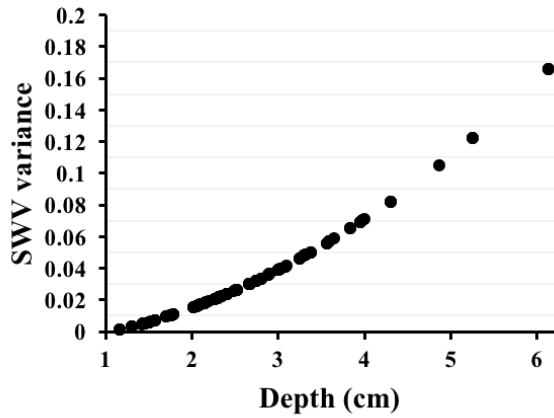


Figure 5 Estimated variance of SWV measurements as a function of depth of acquisition.

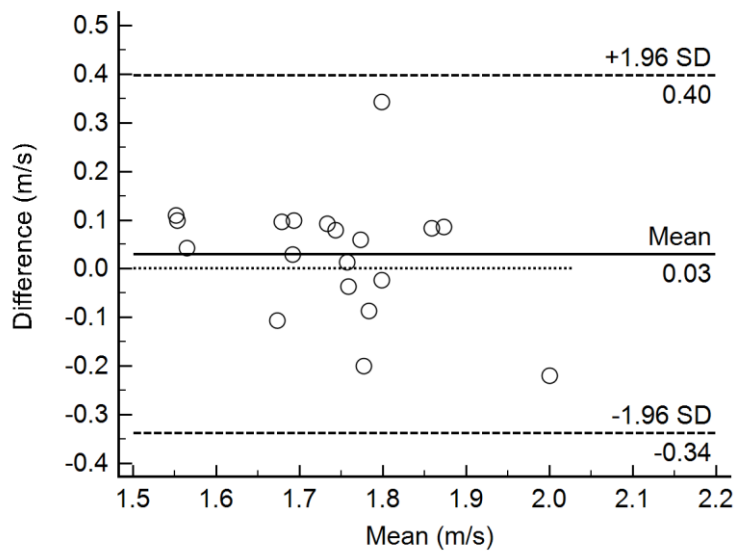


Figure 6 Bland-Altman plot demonstrating the difference against the mean between the measurements of the vastus lateralis with and without standoff gel. The central solid line is the mean SWV difference between the two methods displaying small, negligible bias (0.03 m/s). The two lines represent the 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 two methods.