Relationship between shear wave velocity and muscle activation is inconsistent across different muscle types

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ABSTRACT

There is an increasing use of shear wave ultrasound elastography to quantify properties of muscles under various conditions such as muscle length, levels of activation, in healthy and pathological muscle. However, little is known about the variability in shear wave velocity (SWV) among muscles as most studies investigate one specific muscle. The purpose of this study was to assess the variability of the relationship between SWV and activation across muscles with different architecture, specifically pennation angle: biceps brachii (parallel), tibialis anterior (moderately pennate), gastrocnemius (highly pennate). We tested these muscles at matching levels of activation and similar muscle lengths, relative to optimal length, to limit differences in force across muscles. Shear wave velocity-squared increased with higher levels of muscle activation ($p<0.001$), but the relationship between SWV^2 and activation was different across all three muscles ($p<0.001$) with the slope of the SWV^2- EMG relationship greater in the tibialis anterior than in the medial gastrocnemius and biceps brachii (both $p<0.001$). This indicates that SWV^2 was inconsistent across muscle types, even when considering similar levels of activation and torque production. Under passive conditions, SWV^2 was different across muscles ($p<0.001$), even at shortest lengths, when differences due to passive force were minimal. We found that the probe-fascicle angle did not alter the relationship between muscle activation and SWV^2. Across all muscles, we found no difference in predicting SWV^2 with model included probe-fascicle angle ($r^2 = 0.90$) compared to one that did not include probe-fascicle angle ($r^2 = 0.90$, $p = 0.96$). Our findings suggest that the SWV varies across muscles under passive and active conditions even when relative length is considered and that SWV may be dependent on other muscle properties, other than pennation angle, that are different between the three muscles.

INTRODUCTION

Evaluating muscle properties associated with various pathologies and how these changes are influenced by therapeutic interventions, has driven much ultrasound development. Characterizing muscle architecture using B-mode ultrasound has yielded insight into fascicle lengths and pennation angle in basic muscle architecture and mechanics in addition to changes in muscles in populations such as cerebral palsy and stroke. Shear wave ultrasound elastography emerged as potential tool to estimate mechanical properties in muscle, though recent work demonstrates shear wave velocity (SWV) is sensitive to both stress and stiffness (Bernabei et al., 2019).

Previous research has shown high inter-session and inter-operator repeatability of SW measurements in passive (Koo et al. 2013; Lacourpaille et al. 2012) and active (Lapole et al. 2014; Yoshitake et al. 2014) conditions when controlling for muscle level of activation and length, justifying a systematic assessment using shear wave ultrasound elastography of human muscles already underway (Le Sant et al. 2017, 2018). However, the remarkable differences in anisotropy of muscle tissues and the muscle-specific characteristics of activation and length-dependent tensional changes challenge the ability of ultrasound to produce consistent and accurate estimates of SW velocity (SWV) across muscles.

We have previously shown that short-range stiffness of cat muscles vary linearly with force and in a similar fashion across muscles with different architectures (Cui et al. 2008).

This suggests that SWV might be constant across muscles if activation and sarcomere length are the same, as a change in passively or actively generated forces would alter SWV proportionally (Bernabei et al. 2017; Eby et al. 2013). In contrast, measures obtained across different muscle types seem to vary substantially under passive conditions (Dubois et al. 2015; Le Sant et al. 2017; Xu et al. 2016), suggesting a muscle-specific relationship between SWV and muscles' mechanical properties.

We hypothesize here that such variability of SWV could be explained by architectural and morphological differences between muscle types. First, architectural variations caused by differences in pennation angle, fascicle length and the presence of stiffer aponeuroses can lead to muscle-specific differences in the stress-strain relationship during active contractions (Azizi and Deslauriers 2014; Blemker et al. 2005). Supporting this idea, Koo et al. (2013) showed a partial correlation between SWV and architectural parameters, such as cross-sectional area and muscle mass, in passive muscle. Second, estimates of SWV can be related to a unique descriptor of mechanical properties of tissues, such as shear modulus, if measurements are made along the fibre direction (Gennisson et al. 2010; Royer et al. 2011). Measurements made with small deviations from the fiber direction, described here as probe-fascicle angle which result from pennation angles and changes in pennation angle upon activation, have been suggested to be potentially inaccurate (Herzog 2017). In addition, an interesting complication is given by differences across muscles in the relative use of recruitment in the force generation process (Hodson-Tole and Wakeling 2007; Lawrence and De Luca 1983); for similar levels of force within a muscle compartment can be obtained by recruiting some fibers at high stiffness or many

at lower stiffness. In conclusion, comparing SWV across different muscles is non-trivial and may lead to substantial, unexplained variability which may mask the effects of changes in a muscle' mechanical properties.

This study aims at assessing the variability of the relationship between SWV and activation across muscles with different architecture, specifically pennation angle: biceps brachii (parallel), tibialis anterior (moderately pennate), gastrocnemius (highly pennate). We tested these muscles at matching levels of activation and similar muscle lengths, relative to optimal length, to limit differences in force across muscles. We hypothesized that the dependency of SWV on these parameters will be independent of muscle. In testing this hypothesis, we also expect to reveal what can be considered normal inter-muscle variability when using SWE to estimate muscle mechanical properties in humans.

METHODS

We set up our experiments to compare measures of ultrasound SWV across muscles by isolating factors that are known to impact the propagation of sound waves in muscle tissues. We looked first at increasing levels of muscle activation as well as various muscle lengths in passive state, which will control tissues tension. Second, we measured probe-pennation angles to exclude potential confounding effects due to probe orientation together with changes in pennation angle during activation. We excluded potential discrepancies in SWV across muscles that might be due to different operating points on the length-force relationship by comparing these muscles only at lengths yielding the highest rate of force generation.

Subjects

Twelve healthy young adults took part in this study (7 females and 5 males, mean (SD) age: 29 (5) years; height: 1.72 (0.11) m; body mass: 73.7 (14.2) kg). Subjects had no history of major orthopedic diagnosis, musculoskeletal trauma, or persistent joint pain at the targeted body locations. All experimental procedures were approved by Northwestern University's Institutional Review Board (#0020-33-96), in compliance with the Helsinki Declaration. All subjects received and signed informed consent prior to collecting data.

Setup

The goal of this study was to assess the relationship between SWV and activation across muscles with different architecture. We chose three muscles: i) the long head of the BB, ii) MG, and iii) TA because of their remarkably different architectural features (Table 1).

Participants sat upright on a Biodex (Biodex Medical Systems Inc., Shirley, USA) chair. Their dominant forearm or lower leg was secured in a neutral position with a fiberglass cast. The cast was then secured to a metal plate attached to the shaft of the Biodex motor and aligned to its rotational axis (e.g. Fig. 1A). The Biodex motor was set to position control, maintaining the relevant joint at the desired angle. Joint torques were measured with a six degrees-of-freedom load cell (JR3 Inc., Woodland, USA), attached to the Biodex motor shaft. Surface EMG (Bagnoli EMG system, Delsys Inc., Boston, MA; 20-450Hz bandpass) was recorded using bipolar electrodes (#272, Noraxon, USA) placed on the targeted muscles as well as synergistic and antagonistic muscles (triceps brachii,

MG, and soleus) to account for effects of co-activation. EMG, load cell and ultrasound trigger signals were sampled (2 kHz) and digitized with a 16-bit data acquisition board (USB-6343, National Instruments, Austin, US).

Ultrasound measurements

The ultrasound transducer was secured on the muscle of interest with a neoprene custom-molded holder and positioned at the mid-belly region of BB and at the proximal region of MG and TA muscle bellies. The imaging plane was aligned with the fascicle plane as viewed from the B-mode image. The region for elastography was a 25x10 mm rectangle between the superficial and deep aponeuroses (Fig. 2A). For each isometric contraction a B-mode ultrasound image and a SW propagation video were simultaneously captured using a research grade ultrasonography system (Aixplorer SuperSonic Imagine, Aix-en-Provence, France) with a linear transducer array (4-15 MHz, SuperLinear 15-4, Vermon, France). The parameters of the system were set to i) mode: MSK – foot and ankle ii) opt: std iii) persist: no, to maximize the sampling rate and avoid bias due to locked proprietary data post-processing. The software on our system provides SW movie data sampled at 8 kHz in sequences of 42 frames for each acoustic radiation pulse, recorded simultaneously with a single B-mode ultrasound image.

Protocol

We assessed the relationship between SWV and muscle activation at three different lengths for each muscle by changing the joint angle. Each muscle was tested in a separate session. Three different angles were selected based on literature ranges for the muscles of interest (Leedham and Dowling 1995; Maganaris 2001, 2003): 100-110-120°

for BB; 35-45-55° for TA; maximum dorsiflexion to +5° and +10° plantarflexion for MG (Fig. 1B) (Maganaris 2001, 2003; Kawakami et al., 2002). Adjustment to the angles were made if subjects reported discomfort. Angles at adjacent joints (knee, hip, shoulder) affecting the muscle of interest were recorded and held constant throughout data collection.

To assess the relationship between SWV and muscle activation, we instructed subjects to volitionally contract the relevant muscles. For each muscle at each joint angle, passive and maximum voluntary contraction (MVC) trials were performed at the beginning of each session to normalize EMG data (Besomi et al., 2020). Trials were then conducted at increasing levels of muscle activation from 0% to 50%MVC in 5%MVC steps. EMG feedback was provided to the subject to control the level of muscle activation. The EMG feedback signal was digitally rectified and low-pass filtered (1Hz low-pass, II-order Bessel) before being displayed to the subject. Trials were randomized across levels of muscle activation and imposed joint angles.

Data analysis

Torque and EMG data. Signals were averaged over a 450-ms time-window prior to the onset of ultrasound data recording. The mean electromyographical data were then normalized and expressed as a percentage of the MVC value recorded in each measurement session.

To ensure an accurate comparison of the SWV-EMG relationship across the different muscles, we attempted to place the muscle as close to their optimal length as possible. For each joint angle tested, we found the slope of the EMG-torque relationship (Fig. 2B). We chose the angle with the highest slope as the angle where the muscle was closest to its optimal length. We used this joint angle for further analysis.

Ultrasound data

Shear wave ultrasound data were processed using a custom-written MATLAB program where SWV was estimated using a time-to-peak method (Bernabei et al. 2017) Our results are reported in terms of SWV instead of the more commonly reported shear or Young's moduli because muscle anisotropy violates the assumption made on the theoretical relationship between shear and Young's moduli for simple materials (Royer et al. 2011). In addition, there is evidence to suspect that SW propagation depends not only on tissues' intrinsic stiffness, but also on the stress imposed on the tissue, which in muscle and tendons might be the dominant factor (Bernabei et al. 2017, 2020; Martin et al. 2018). However, since there is a known proportionality between muscle stress, shear modulus and the square of SWV, we reported most of our results in terms of SWV^2 squared.

Muscle architecture data

In each position, probe-fascicle angle and fascicle length were extracted from ultrasonic images of the targeted muscles. Typically, pennation angle, the angle between the fascicle and aponeurosis, is measured as an architectural parameter. In this study, we quantify probe-fascicle angle instead; probe-fascicle angle (α) was defined as the angle

between a fascicle and the horizontal line of the region of interest for elastography provided by the Aixplorer in the DICOM image (Fig. 2A, color map rectangle). This angle is useful to assess if fascicle orientation affects shear wave propagation as the shear waves travel parallel to the probe direction; thus, the horizontal line of the region interest is used for reference. Fascicle length was estimated by tracing the same fascicle from the superficial aponeurosis to the deep one. Images were pre-processed to enhance contrast of the features of interest. All architectural measurements were completed by a single operator; the known reproducibility of this measure (Kawakami et al. 1998) was confirmed here by a correlation coefficient of 0.997 across three repeated measurement sessions over subsequent days, on all probe-fascicle angle data from the same muscle (30 samples x 3 days).

Statistics

Linear mixed-effects models were used for all hypothesis tests. Our primary hypothesis was that the SWV-muscle activation relationship is consistent across muscle types. We tested this with the squared SWV as the dependent factor; EMG amplitude as a continuous factor, the three muscles as fixed factors, and each subject as a random factor. In addition to our primary hypothesis, we tested two additional hypotheses. The first was that SWV-muscle length relationship under passive conditions would be consistent across muscle types. We tested this with the squared SWV as dependent factor, muscle length and muscle type as fixed factors and subject as random factor. The final hypothesis tested was that any differences in SWV-muscle activation relationship could be explained by probe-fascicle angle; fascicle orientation relative to the horizontal

direction of the probe. We tested this with the squared SWV as dependent factor, EMG and probe-muscle fascicle angle as continuous factors and subject as random factor. Post-hoc ANOVAs with Satterthwaite corrections were used to test model significance and the overall significance of each modeled predictor. Significance was assessed at the $p < 0.05$ level and fixed effects estimates reported as average values with 95% confidence interval. All statistical tests, as well as all signal and image processing, were performed in MATLAB (Mathworks, Natick, USA). Complete details of all linear mixed-effects model can be found in supplementary material.

RESULTS

Effects of activation on SW velocity across muscles

The relationship between muscle activation and shear-wave velocity differed drastically between muscle types. Similar to previous measurements in contracting muscles (Lapole et al. 2014; Yoshitake et al. 2014), SWV^2 increased linearly with higher levels of muscle activation from 5 % to 50 %MVC, as shown in Fig. 3A for one subject. However, the relationship between SWV^2 and activation differed across muscles, with BB and MG showing a lower slope compared to TA. For all subjects, the linear mixed-effects model accurately modeled the data ($R^2 = 0.87$). Contrary to our null hypothesis, there was a significant difference between SWV^2 – EMG relationships measured across muscles ($F_{2,31} = 17.8$, $p<0.001$) The SWV^2 – EMG relationship was significantly greater in the TA (4.8 [4.2, 5.4] $(m^2/s^2)*(\%MVC)^{-1}$) than in the MG (2.5 [1.8, 3.1] $(m^2/s^2)*(\%MVC)^{-1}$, $t_{31} = 5.6$, $p<0.001$) or the BB (3.0 [2.4, 3.5], $(m^2/s^2)*(\%MVC)^{-1}$, $t_{31} = 4.6$, $p<0.001$). The

difference between MG and BB did not reach significance levels (t_{31} = 1.2, p = 0.23). This indicates that SWV measurement is inconsistent across muscle types

Difference in SW velocity at short muscle lengths across muscles under passive conditions

The results of different SWV^2-activation slope among the three muscles were limited to muscle lengths approximately at the optimal length; this length, modified with ankle angle, was determined as when the slope was the greatest. However, SWV^2 increases as a function of muscle length, so we wanted to explore if there were differences in SWV^2 at very short muscle lengths where the muscle was under minimum tension. This would provide evidence that SWV^2 varied across muscles with different archiecture even when tension was not a confounding factor. Previous direct measures performed *in vivo* on an animal model showed a linear dependency between SWV^2 and passive tension, as determined by manipulating muscle length (Bernabei et al. 2020). We made additional SWV^2 at the shortest possible muscle lengths; at the minimum/maximum joint angles where placement of the ultrasound probe was still possible. The values of SWV^2 were substantially different across muscles in resting conditions even at their shortest lengths (Fig. 4A 'short', $F_{1,16}$ = 18.5, p < 0.001), where we expected differences due to passive muscle tension to be minimal. The BB had the lowest SWV^2 (3.1 [1.4 4.9] m^2/s^2), significantly lower than MG (5.5 [3.8 7.2] m^2/s^2, t_{16}=2.1, p=.05) and the TA (9.8 [8.1 11.6] m^2/s^2, t_{16}=6.0, p<0.001). Shear wave squared in the MG was also significantly lower than the SWV^2 in the TA (t_{16}=4.0, p=.0012).

Effect of the angle between ultrasound probe and fascicle on SWV

We investigated whether architecture such as pennation angle, which varied across the three muscles, affected SWV^2; specifically, we examined whether the angle between the fascicles and horizontal direction of the probe, as the shear waves travel parallel to the probe direction. We found that the probe-fascicle angle did not alter the relationship between muscle activation and SWV^2. Across all muscles, we found no difference in predicting SWV^2 with model included probe-fascicle angle ($r^2 = 0.90$) compared to one that did not include probe-fascicle angle ($r^2 = 0.90$, $\chi^2_{63} = 44.6$, $p = 0.96$). To ensure that this effect of probe-fascicle angle was not overshadowed by the differences in SWV between the muscles, we also ran the likelihood ratio test on all three muscles independently. In any individual muscle, there was no significant difference in the prediction of SWV^2 when probe-fascicle angle was included (BB: r2 = 0.85; MG: r2 = 0.88; TA= 0.90) compared to when it was not included (BB: $r^2 = 0.85$, $\chi^2_9 = 5.0$, $p = 0.83$; MG: $r^2 = 0.87$, $\chi^2_9 = 8.3$, $p = 0.50$; TA: $r^2 = 0.89$, $\chi^2_9 = 14.3$, $p = 0.11$).

DISCUSSION

This study examined if SWV was different across muscles with different architecture, specifically pennation angle. We found that the relationship between SWV^2 and activation was different across the BB, TA, and MG. Even at very short lengths under passive conditions where muscle force would be at a minimum, SWV^2 was different across the three muscles. We investigated whether architecture such as pennation angle, which varied across the three muscles, affected SWV^2; specifically, we examined whether the angle between the fascicles and horizontal direction of the probe, as the shear waves travel parallel to the probe direction. We found that the probe-fascicle angle did not alter the relationship between muscle activation and SWV^2.

Previous experiments in humans have assessed SWV in muscle tissues by controlling the joint angle spanned by the target muscles (Maïsetti et al. 2012; Le Sant et al. 2017), the level of joint torque (Lapole et al. 2014) or passive muscle force (Koo et al. 2013). These studies have observed that SWV in individual muscles vary with the extent of expected passive and active muscle tension. However, when comparing different human muscles with similar levels of passive and active tension, we found substantial differences in the average estimates of SWV. While it is not surprising that SWV increased with higher levels of activation, the average SWV and its rate of increase varied drastically between BB, MG and TA in active conditions. Because we compared SWV^2 across muscle length ranges that corresponded to the maximum rate of change for joint torque, we are confident in excluding the possibility that these inconsistencies may arise as a consequence of differences in the EMG-torque relationship specific to each muscle. In

addition, it was interesting to notice how the range of SWV^2 values collected from 5 to 50% MVC, were drastically lower in MG (13.20 to 84.95 m^2/s^2) with respect to BB (67.37 to 168.01 m^2/s^2) and TA (79.12 to 391.88 m^2/s^2), considering the representative subject of Fig. 3A. Without knowing the optimal length of the muscles, we were only able to make measurements at a length at which the three muscles could be compared; we used the length, modified by the joint angle, at which the slope of the SWV^2 -activation as the greatest. To further investigate whether our results were confounded by muscle length, we made SWV^2 measurements under passive conditions at the shortest length possible (minimum and maximum joint angles at which the ultrasound probe could be placed). Shear wave velocity squared was still different when comparing these muscles at small joint angles yielding virtually no joint torque. A possible explanation for the differences in SWV^2 under active and passive conditions is pennation angle which is highest in the MG and lowest in the BB. However, our results demonstrate that the probe-fascicle angle did not alter the relationship between muscle activation and SWV^2.

As an alternative explanation, architectural differences across muscles could affect SW propagation, specifically the SWV measured with ultrasound. , We have discussed above the effect of the probe-fascicle angle where the orientation of the fascicles is related to the parallel direction of probe. The orientation of the robe relative to the line of action of the muscle can also affect shear wave propagation. Previous work in humans in vivo (Gennisson et al. 2010; Maïsetti et al. 2012) and in animal specimens (Eby et al. 2013), show that the effect of the orientation of the probe, relative to the line of action of the muscle,on SWV within a muscle was substantial when the insonification plane was not

aligned with the plane of the muscle fascicles. The higher the degree of misalignment, the lower the average SWV estimate. In this skewed configuration, only portion of the muscle fascicle is visible in the b-mode image. Because pennation angle varies across muscles and during activation, investigators are aware that the orientation of the probe in respect to muscle fascicles can change between different muscles and even between different regions of the same muscle. Online adjustments of probe orientation allow to correct for such changes, but optimal alignment in muscles with complex architecture, such as MG and TA, is complicated by out of plane fiber curvature. A titling angle within the plane of the fascicles, mimicking the effects of changes in fibers pennation in a single plane, showed small effects (<1.3 % for an angle of max 20°; Miyamoto et al. 2015) in the parallel-fiber BB. In our experiment, we observed that some residual variability of SWV was associated with changes in pennation angle upon activation in TA, but not in MG. Note that we limited our analysis to angles in the insonification plane, so that this result might underestimate the effect of 3D misalignment on wave propagation, especially in muscles where pennation angle changes extend over multiple planes, such as TA and MG.

Other explanations of why SWV^2 may be different in these three muscles include differences in internal structures and composition. We have shown that SWV depends on muscle stress and stiffness (Bernabei et al., 2019). Although direct measurements of stress in muscle is not possible, local variations in strain (Blemker et al., 2005; Jensen et al., 2016) occur in muscles with complex internal architectures such as internal tendons of the TA and BB, respectively, under passive and active conditions. These non-uniform

strains may lead to nonuniform stresses within the muscle such that depending on where SWV is measured, SWV values will vary. Muscle composition may also influence shear wave propagation. There are differences in the collagen content of the BB, TA, and MG (10.0 mg/mg, 10.7 mg/mg, and 13.4 mg/mg; Lin, 2011), though the effect on SWV requires further investigation. Other notable muscle properties that vary among the muscles investigated in the study include physiological cross-sectional area (BB, 2.1cm^2; TA, 10 cm^2; MG, 21 cm^2; Murray et al., 2000; Fukunaga et al., 1992) which influences force generation.

CONCLUSIONS

Our results demonstrate that the variation of SWV across different muscle types cannot be accounted for by passive and active changes in muscle tension alone. We also showed that the angle between probe orientation and muscle fascicles given by pennation cannot explain such discrepancy. The factors determining SWV variation should be clarified before comparing estimates of muscle mechanical properties provided by ultrasound across different muscle types. Note that we calculated an indirect estimate of active tension by measuring EMG and by characterizing torque-EMG relationship for each target muscles. We also assumed that the relationship between passive tension and joint angle would be similar across muscle types. Future studies providing direct measures of tension and stiffness as well as a measure of 3D probe-fascicle angle would be helpful to clarify SWV dependency on mechanical and architectural factors that differ across muscle types.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.S.M.L., E.J.P., and T.G.S. conceived and designed research; M.B. and S.S.M.L. performed experiments; M.B. analyzed data; M.B., S.S.M.L., D.L., E.J.P., and T.G.S. interpreted results of experiments; M.B. and D.L. prepared figures; M.B. drafted manuscript; M.B., S.S.M.L., D.L., E.J.P., and T.G.S. edited and revised manuscript; M.B., S.S.M.L., D.L., E.J.P., and T.G.S. approved final version of manuscript.

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TABLE

Table 1. Architectural characteristics of target muscles | mean (SD)

Muscle	Fascicle length (cm)		Pennation angle (deg)		PCSA (cm^2)
Biceps brachii	12.8 (3.2)*	NA	~0*	0.5 (0.0)§	3.1 (1.0)‡
Tibialis anterior	6.8 (0.8)†	NA	9.6 (3.1)†	7.7 (0.9)§	10.9 (3.0)†
Medial Gastrocnemius	5.1 (0.9)†	5.4 (0.6)§	23.4 ± 2.3**	18.6 (3.1)§	21.1 (5.7)†

§ Present study (n=12), fascicle length and pennation angle with muscle at rest, obtained at the joint angle with steepest torque-EMG slope.
* Murray et al., 2000 (n=10)
**Chino et al., 2018 (n=15)
‡ Veeger et al. 1997 (n=14)
† Ward et al., 2009 (TA: n=21, MG: n=20)

FIGURES AND CAPTIONS

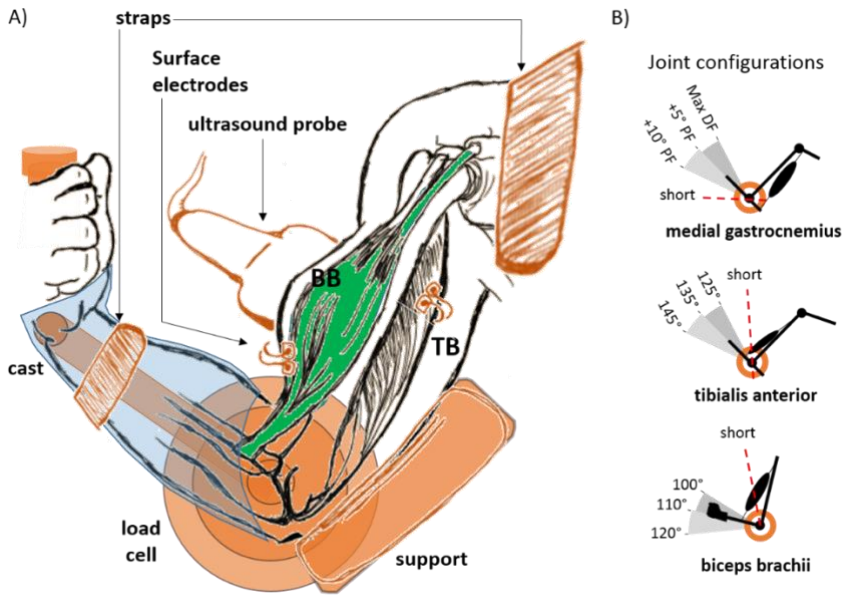

Figure 1. Schematic drawing of the experimental setup for measuring shear wave velocity (SWV), torque and activation on biceps brachii. The subject was secured to a Biodex chair by fixing shoulder joint to the chair and using a cast to fix the wrist to a load cell. Bipolar surface electrodes were placed on the target muscle (biceps brachii long head, green) and its main antagonist (triceps brachii). The ultrasound transducer was positioned on the mid-belly of the muscle, parallel to muscle fascicle direction. B) Different joint angle configurations were imposed for each of the three muscles, based on their specific physiological range of motion. An additional 'short' joint configuration corresponded to the smallest joint angle achievable in experimental conditions; this condition minimizes the effect of passive tension on shear wave measures. BB: biceps brachii; TB: triceps brachii; DF: dorsiflexion; PF: plantarflexion.

495

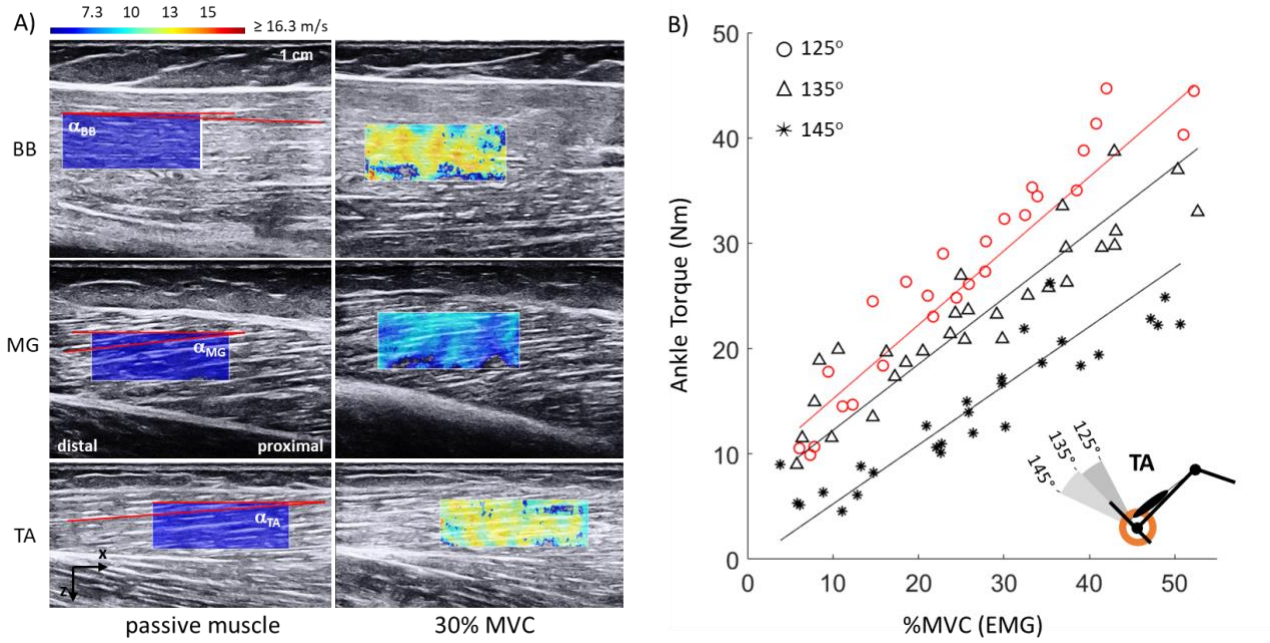

Figure 2. Ultrasound and torque data with different joint angle configurations and muscle activations. A) Heatmaps representing local shear wave velocity estimates on biceps brachii (BB), medial gastrocnemius (MG) and tibialis anterior (TA) for passive (left panels) and active muscle state (30% MVC, right panels). Red lines show an example of probe-fascicle angle measures (α) extracted for each muscle and condition. Note that MG heatmap shows qualitatively lower shear wave velocity, as calculated by the AIXplorer, compared to BB and TA at matching level of activation (30% MVC). B) Exemplar data of ankle torque measured in TA with increasing levels of activation and at three different joint angle configurations. Note that ankle torque covaried with muscle activation and its rate of change was different across ankle angles. The angle at which the highest slope occurred was used to estimate optimal length (red).

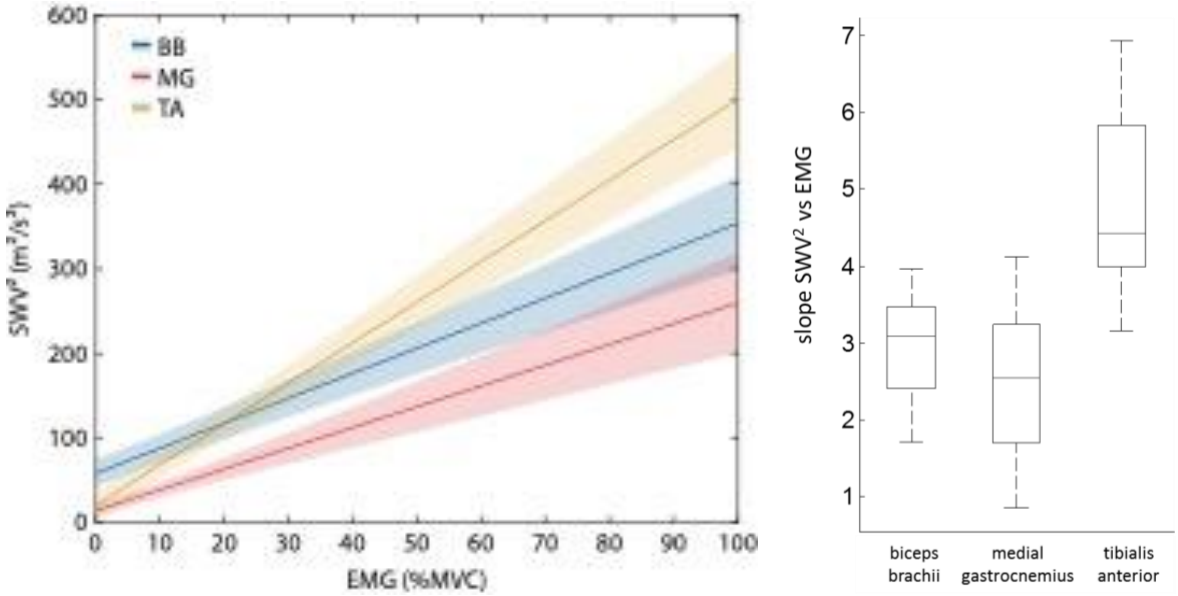

Figure 3. Variability of shear wave velocity measures across three different muscle types during isometric contraction. Left) Shear wave velocity squared as a function of activation for biceps brachii (blue), medial gastrocnemius (red), and tibialis anterior (yellow). Lines indicate the The solid lines indicates the estimated shear wave velocity-squared from their respective models, with the shaded region being the 95% confidence intervals. A linear mixed-effects model was used to test for differences in the relationship between SWV^2 and activation across muscles. Right) Median (midline), 75% and 25% percentile (box edges) for the slopes of the linear model across subjects (n=12). Whiskers represent the most extreme slopes. The model group estimates were 2.96 [2.25, 3.67], 2.48 [0.72, 4.24] and 4.32 [1.53, 7.11] $(m^2/s^2)*(\%MVC)^{-1}$ for BB, MG and TA, respectively (estimate [lower-upper confidence intervals]).

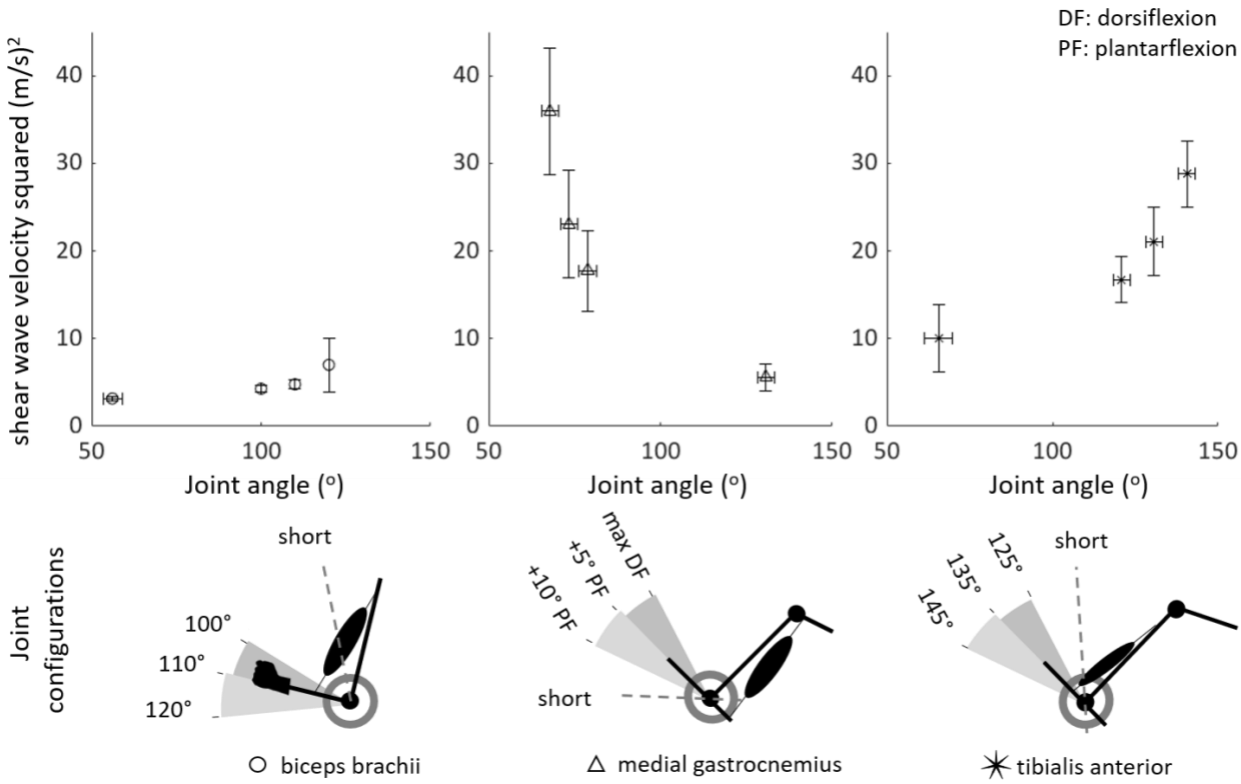

Figure 4. Dependency of shear wave velocity on passive muscle length across muscle types. A) Shear wave velocity squared (SWV^2) increased with lengthening the target muscles by imposing four different angles at the elbow and ankle. The 'short' condition, corresponding to the minimum measurable muscle length, shows differences in SWV^2 across muscles when the effect of passive tension is minimized. Repeated measures showed much lower variability in the biceps muscle (parallel) compared to tibialis (pennate) and gastrocnemius (highly pennate); mean ± SD, n = 12.

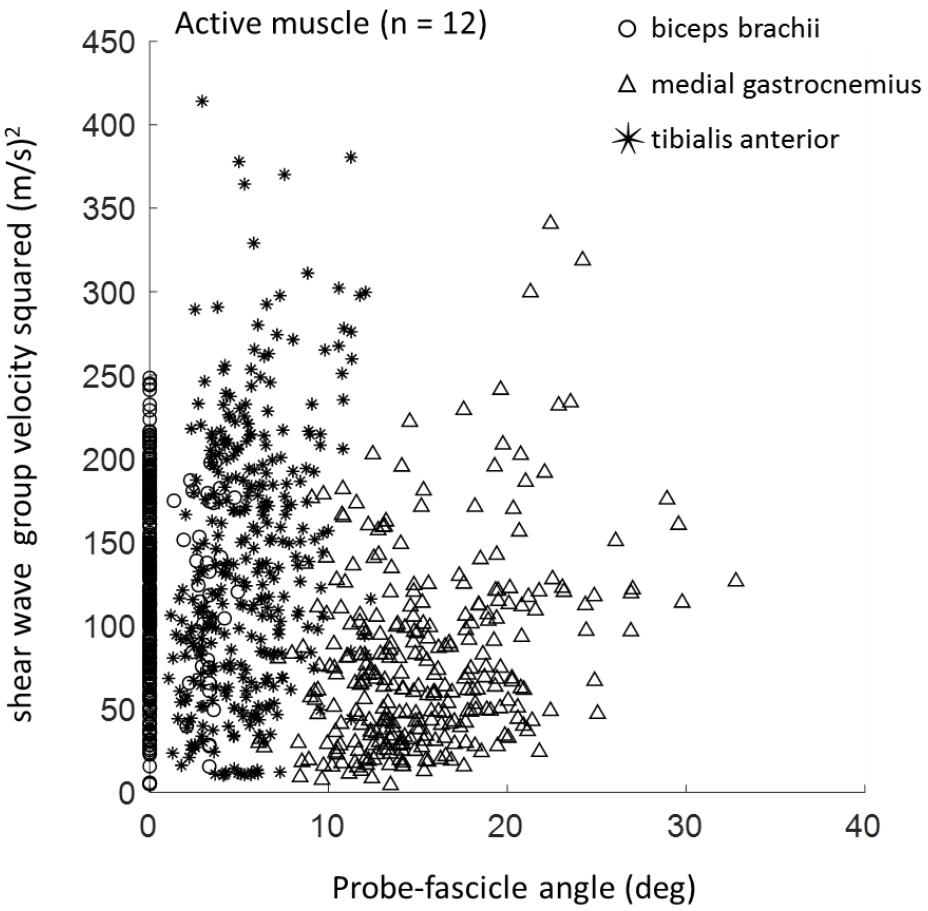

Figure 5. Effect of the angle between ultrasound transducer and fascicle orientation on SWV. Variation of SWV squared across different activation levels was partially associated with changes in the probe-fascicle angle in medial gastrocnemius (MG) and tibialis anterior (TA), while such angle was virtually zero for all applied conditions in biceps brachii (BB). The minimum probe-fascicle angle at 0 %MVC reflects the pennation angle for each muscle under passive conditions (mean ± SD, BB = 0.1 ± 0.43, MG = 12.1 ± 3.2, TA = 5.6 ± 1.9). Note that probe-fascicle angle will increase during activation due to pennation angle changes resulting from muscle contraction.