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The shear modulus of lower-leg muscles correlates to intramuscular pressure

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ABSTRACT

Shear wave elastography (SWE) is emerging as an innovative tool to evaluate muscle properties and function. It has been shown to correlate with both passive and active muscle forces, and is sensitive to physiological processes and pathological conditions. Similarly, intramuscular pressure (IMP) is an important parameter that changes with passive and active muscle contraction, body position, exercise, blood pressure, and several pathologies. Therefore, the objective of this study was to quantify the dependency of shear modulus within the lower-leg muscles on IMP in healthy individuals. Nineteen healthy individuals (age: Mean age ± SD, 23.84 ± 6.64 years) were recruited. Shear modulus was measured using ultrasound SWE on the tibialis anterior (TA) and peroneus longus (PL) muscles using pressure cuff inflation around the thigh at 40 mmHg, 80 mmHg, and 120 mmHg. Changes in IMP were verified using a catheter connected to a blood pressure monitor. It was found that IMP was correlated to TA and PL shear modulus (spearman's rank correlation = 0.99 and 0.99, respectively). Applying a gradual increase of cuff pressure from 0 to 120 mmHg increased the shear modulus of the TA and PL muscles from 15.83 (2.46) kPa to 21.88 (4.33) kPa and from 9.64 (1.97) kPa to 12.88 (5.99) kPa, respectively. These results demonstrate that changes of muscle mechanical properties are dependent on IMP. This observation is important to improve interpretation of ultrasound elastograms and to potentially use it as a biomarker for more accurate diagnosis of pathologies related to increased IMP.

1. Introduction

Intramuscular pressure (IMP) is the hydrostatic regional stress in a muscle (Ates et al., 2018; Baumann et al., 1979; Hargens et al., 1982; Nakhostine et al., 1993; Sejersted et al., 1984). IMP has been studied to understand normal muscle function and several muscle pathologies. IMP is linearly correlated to electromyography (EMG) collected by fine wire electrodes (Körner et al., 1984) and estimated muscle force during isometric exercise (Parker et al., 1984; Sadamoto et al., 1983; Sejersted et al., 1984), concentric and eccentric isokinetic exercises (Aratow et al., 1993), as well as dynamic exercises such as running and walking (Ballard et al., 1998). There is also a positive linear relationship ($r^2 = 0.95$) between IMP and passive muscle force measured in the rabbit tibialis anterior (TA), particularly at muscle lengths longer than optimal (Davis et al., 2003). Therefore, IMP is a good estimator of individual muscle force. Increased IMP could contribute to some pathologies such as chronic exertional compartment syndrome, in which increased IMP during exercise causes decreased blood flow distally, leading to clinical symptoms and impaired function (Van Den Brand et al., 2005; Wilder and Sethi, 2004). Van Den Brand et al. (2005) studied the effectiveness of using IMP for diagnosis of chronic exertional compartment syndrome on 50 patients before and after fasciotomy surgery and reported a sensitivity of 77% (95 CI = 67%–86%). However, a disadvantage of the current IMP techniques is that the measurements are invasive, which introduces the risk of infection. Therefore, the risk of infection, along with the associated pain and discomfort reduce the practicality of this approach.

Shear wave elastography (SWE) is an ultrasound-based imaging technique developed to non-invasively provide information about the shear modulus of soft tissues (Bercoff et al., 2004; Cortes et al., 2015; Eby et al., 2013; Palmeri and Nightingale, 2011). SWE has been successfully applied to evaluate the mechanical properties of muscles in the limb and characterize their changes following injuries (Andonian et al., 2016; Cortez et al., 2016; Eby

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et al., 2015; Yoshida et al., 2017). It has been used to directly measure individual muscle shear modulus during muscle contraction, showing increasing shear modulus with increasing force (Gennisson et al., 2005; Nightingale et al., 2002; Shinohara et al., 2010) over the full range of 0–100% of maximum voluntary contraction (Ateş et al., 2015). SWE has also been used for measuring shear modulus during passive stretching in various muscles (Arda et al., 2011; Kuo et al., 2013; Shinohara et al., 2010), demonstrating that the resistance to passive stretch correlates with muscle shear modulus (Maïsetti et al., 2012; Nordez et al., 2008).

The similarities of the relationships between IMP and shear modulus to muscle mechanical properties and pathologies strongly suggest that these parameters may be related. Ultrasound SWE also shows promise for identifying abnormal muscle shear modulus in neuromuscular and musculoskeletal disorders (Drakonaki and Allen, 2010; Kwon and Park, 2012; Kwon et al., 2012). Passive measurements of shear modulus have also been found to be sensitive to muscle stiffness caused by different pathologies, including tennis leg (Yoshida et al., 2017), cerebral palsy (Bilgici et al., 2018), Parkinson's disease (Du et al., 2016) and chronic neck pain (Kuo et al., 2013). Shear modulus also may be a potential biomarker for measuring abnormal muscular fibrosis, since normal intramuscular connective tissue is found to have different shear modulus compared to muscular fibrosis (Ferraioli et al., 2012). These results show that shear modulus is sensitive to physiological processes and pathological conditions. Therefore, the objective of this study was to quantify the dependency of shear modulus within the lower-leg muscles on IMP in healthy individuals. We hypothesized that estimated muscle shear modulus using ultrasound SWE would be positively related to the amount of IMP.

2. Methods

2.1. Subjects

The Institutional Review Board (IRB) of the Pennsylvania State University approved the study (STUDY00007849) and all participants gave informed consent prior to any evaluation. Nineteen healthy participants (eight males and eleven females; Mean age ± SD, 23.84 ± 6.64; Mean BMI ± SD, 23.00 ± 2.89) were recruited in this study. Participants were excluded if they had cardiovascular conditions; were easily bruised; had been diagnosed with any injury in their leg muscles; had a history of any neurological disease; or had a terminal illness.

2.2. Experimental setup

A Verasonic ultrasound system (Verasonic Inc., Redmond, WA, USA) with a L7−4 transducer (128 elements, beamwidth = 4–7 MHz, center frequency = 5.2080 MHz) was used in this study. A custom implementation of the supersonic SWE method proposed by Bercoff et al. (2004) was used to measure muscle shear modulus. Acoustic output intensity was measured to ensure the method satisfied FDA limits for intensity for use in human subjects. 64 central elements of the transducer emitted seven focused ultrasound pushing beams (500 push cycles at 5.2080 MHz frequency; push duration = 96 μs) at seven focal points with the time interval of 200 μs at different depths to create quasi-plane shear waves propagating through the tissue. The reason for choosing these parameters was to ensure shear waves are produced with a high amplitude, leading to the accurate estimation of the muscle shear modulus. The propagation speed of the shear wave was calculated within the region of interest (ROI) using a frame rate of 10,000 frames/s. The ROI size = 7.39 × 7.39 mm^2 was chosen for measurement of shear modulus in lower leg compartments (Sadeghi et al., 2018). The corresponding shear modulus map could be constructed based on the equation $\mu = \rho c^2$, where ρ is the tissue density typically assumed as 1000 kg/m^3, c is the shear wave speed and μ is the shear modulus. The technique was validated using calibrated homogeneous elasticity phantom with different shear modulus values (Model 040GSE, CIRS, Norfolk, Virginia, USA) (Lin et al., 2018).

2.3. Measurement procedure

2.3.1. Shear modulus recording

The proposed protocol evaluated changes of shear modulus within the lower leg muscles (Tibialis Anterior, TA; Peroneus Longus, PL) at different levels of IMP using blood pressure cuff inflation. Participants were asked to rest in a supine position for 15 min and ultrasound images of the left TA muscle were taken before applying a blood pressure cuff. Subjects laid supine with the hip and knee in full extension and the ankle was constrained in its resting position by a fixture made of foam. The fixture was also positioned to avoid contact of the calf muscles with the examination table due to inducing potential deformations to the TA and PL. An initial shear modulus measurement was performed by placing the ultrasound transducer in a longitudinal view along the length of the muscle fibers and minimizing contact pressure through the use of an adjustable fixture. Images were taken at 30% of the distance between the head of the fibula and the tip of the lateral malleolus. The location of the ultrasound transducer was marked on the skin for accurate placement throughout testing. During SWE measurements, the examiner monitored the real time B-mode ultrasound images to ensure that there was no noticeable muscle movement. Once the transducer was kept stationary for a few seconds and the image of the fascicles inside the muscle were clearly identified, the elastography mode was then activated to measure the shear modulus of the muscle. An initial elastography was performed in order to obtain a baseline level for the superficial part of TA and PL shear modulus. The measurement depth (from the transducer surface to the bottom of the ROI) was approximately 3 cm in all participants. The elastography data were discarded if muscle movement was detected, the transducer and the skin were not in full contact or the shear wave propagation was not observed in the ROI. The IMP of TA and PL was then increased using a blood pressure cuff placed approximately 10 cm above the left knee, and inflated to 40, 80 and 120 mmHg (Fig. 1(a)). Elastography measurements were taken after keeping the thigh pressurized for 2 min at each cuff pressure level (40, 80 or 120 mmHg). The shear modulus was measured in five successive trials at each cuff pressure level by an individual examiner and the median value was used in the analyses. The custom time-of-flight process of used a distance between points of 1.77 mm and a time between acquisition frames of 100 μs, which imposes a maximum speed that can be detected of 17.74 m/s. For our current study, it was highly unlikely that saturation would affect the results in this analysis. Due to the between-participant variation in the baseline TA and PL shear modulus values, the change in shear modulus (relative to baseline shear modulus) for the superficial part of TA and PL muscles as a function of IMP was calculated.

2.3.2. Intramuscular pressure recordings

To directly measure the IMP corresponding to each cuff pressure level, an intramuscular pressure system was used (Fig. 1(b)). The intramuscular pressure system was comprised of a patient monitor (Edan IM50), a JELCO® IV Catheter (22G × 1″), and a BD connector compatible pressure transducer. An experienced clinician inserted the catheter into the TA muscle following local skin anesthesia at the same location of the elastography measurement. The catheter, which itself is plastic, was inserted into the skin using

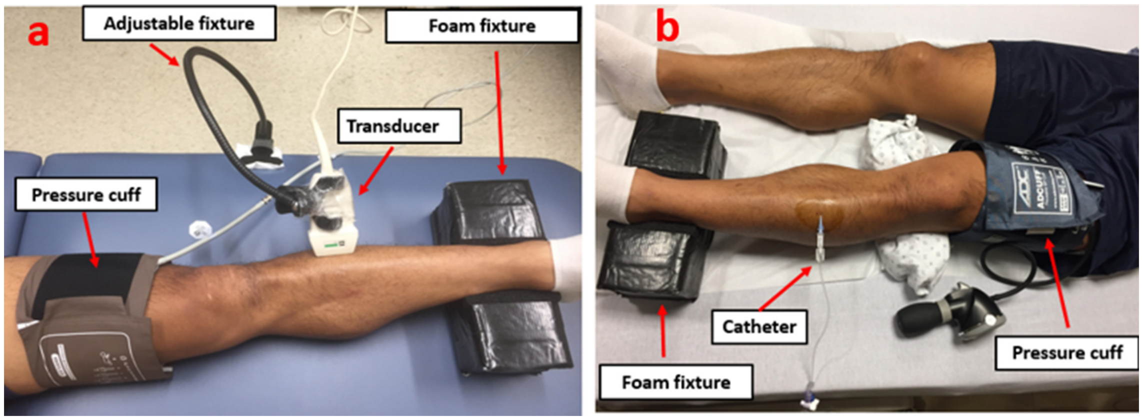

Fig. 1. The experimental setup for: (a) the elastography while the TA muscle was pressurized with the pressure cuff around the thigh (b) the IMP measurement inside the TA muscle.

a 22 gauge needle inside of the catheter. The Catheter depth was approximately 3 cm inside the compartment of the muscle (similar to the ultrasound measurement depth). The thigh muscle was then pressurized using the blood pressure cuff and it was kept for 2 min at 40, 80 and 120 mmHg, respectively, to ensure stable changes in lower-leg muscle pressure. The IMP of the TA muscle was then recorded at each cuff pressure level (40, 80 and 120 mmHg), respectively.

2.4. Statistical analysis

The normality of data distribution was analyzed by the Shapiro–Wilks test. For the statistical analysis, a linear mixed effects model followed by post-hoc Bonferroni correction was used, with IMP as a dependent variable and the cuff pressure condition as a fixed variable to find possible significant differences between various cuff pressure conditions. Additionally, a linear mixed effects model followed by post-hoc Bonferroni correction was used, with the cuff pressure condition (0, 40, 80 and 120 mmHg), muscle type (TA and PL) and gender as fixed variables, while the shear modulus was used as the dependent variable. The spearman's rank correlation coefficient was applied to analyze the correlation between the TA muscle shear modulus and IMP. For all analyses, the level of statistical significance was set as $p < 0.05$. All statistical analyses were performed using IBM SPSS statistics software (v24, IBM, Chicago, IL, USA).

3. Results

From the normality test, it was observed that the IMP data were not normally distributed ($P < 0.01$) and therefore non-parametric statistics were used. IMP increased as a function of cuff pressure for the TA muscle (Fig. 2). Specifically, the IMP values (median and interquartile range) for the TA muscle at 0 mmHg, 40 mmHg, 80 mmHg and 120 mmHg cuff pressure were 13 (3.5) mmHg, 17 (5.5) mmHg, 22 (8.5) mmHg and 25 (12.5) mmHg, respectively. The results of the linear mixed effects model indicated that cuff pressure has a significant effect on the IMP of TA muscle ($p < 0.01$). Additionally, from the Bonferroni post-hoc analysis, a significant difference was obtained between the IMP at 0 and 120 mmHg cuff pressure (Table 1). IMP returned to normal value (zero cuff pressure) within five minutes after release of cuff pressure in all participants.

The change in shear modulus (relative to the shear modulus at zero pressure) for TA and PL muscles as a function of IMP are shown in Fig. 3. Specifically, the change in shear modulus (median and interquartile range) at 40 mmHg, 80 mmHg and 120 mmHg cuff pressure was 1.22 (2.09) kPa, 3.02 (4.94) kPa and 5.10 (5.02) kPa, respectively, for the TA muscle; and 1.12 (1.64) kPa, 3.02 (3.74) kPa and 3.22 (4.96) kPa for the PL muscle. Figs. 4 and 5 show representative shear modulus maps of the TA and PL muscles at each cuff pressure level. Normality test demonstrated that the shear modulus data was not normally distributed ($P < 0.01$) and therefore non-parametric statistics were performed. From the linear mixed effects model, it was observed that muscle and cuff pressure had a significant effect on muscle shear modulus ($P < 0.01$ and $P < 0.01$, respectively), while gender did not. Additionally, from the Bonferroni post-hoc analysis, shear modulus was significantly different between the 0 cuff pressure and the cuff pressure at both 80 and 120 mmHg. Significant differences for shear modulus were also found between cuff pressure at 40 mmHg and cuff pressure at both 80 and 120 mmHg (Table 2). Additionally, the shear modulus for both the TA and PL muscles were found to increase as a function of IMP (Figs. 6 and 7). Specifically, there were significant positive correlations found between the median shear modulus and IMP for the TA muscle (rho = 0.99, $p < 0.01$), and the PL muscle (rho = 0.99, $p < 0.01$).

4. Discussion

This study quantified the ability of ultrasound SWE as a non-invasive measure of IMP in the lower-leg muscles of healthy individuals. The proposed protocol consisted of estimating shear modulus using SWE for the TA and PL muscles at different levels of IMP induced with a blood pressure cuff around the thigh. As the increase of IMP, in this study, was caused by the restriction of venous blood flow, it was expected that other muscles in the lower leg would experience a similar increase in IMP. The results indicated that muscle shear modulus is correlated to IMP. Therefore, this observation suggests that the shear modulus can provide a non-invasive measurement of IMP. Shear modulus could be a potential biomarker for evaluating physiological and pathological changes in IMP.

Ultrasound imaging has been employed for measurement of IMP in several previous studies. Wiemann et al. (2006) inflated a thigh tourniquet in a stepwise fashion from 40 to 100 mmHg in a group of healthy individuals to increase IMP in the TA muscle transiently. They employed an analog non-invasive ultrasound device using fast Fourier transform analysis of the fascial displacement waveform to measure a voltage potential associated with fascial displacement. They reported that the shape of the ratio of the fundamental harmonic to the second harmonic waveform was linearly

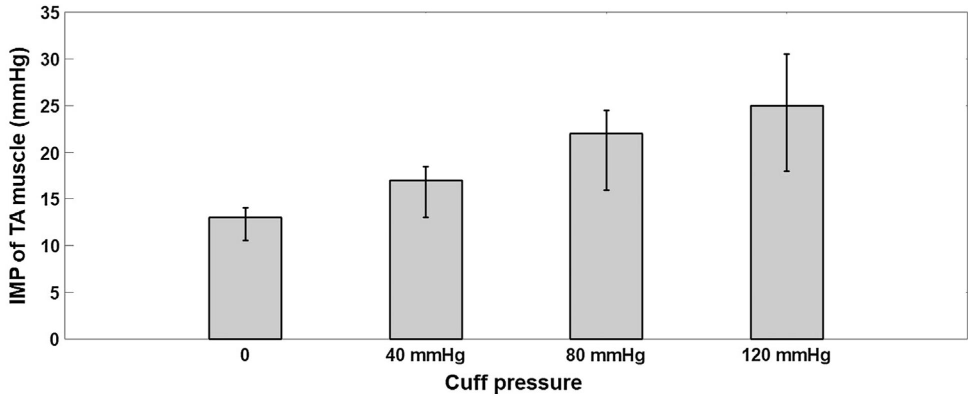

Fig. 2. IMP measured in the TA muscle increased as function of the cuff pressure ($p < 0.01$) (median, interquartile range).

Table 1
Post hoc Bonferroni test results for IMP (mmHg) between different pressure cuff conditions.

Pressure cuff (mmHg)	Mean difference	Standard deviation	95% CI		Significance
			Lower bound	Upper bound	
0–120	−12	5.34	−20.56	−3.44	<0.001
0–40	−3.6	1.14	−12.16	4.96	1
0–80	−8.2	2.95	−16.76	0.36	0.07
40–80	−4.6	2.19	−13.16	3.96	0.76
40–120	−8.4	4.77	−16.96	0.16	0.06
80–120	−3.8	2.68	−12.36	4.76	1

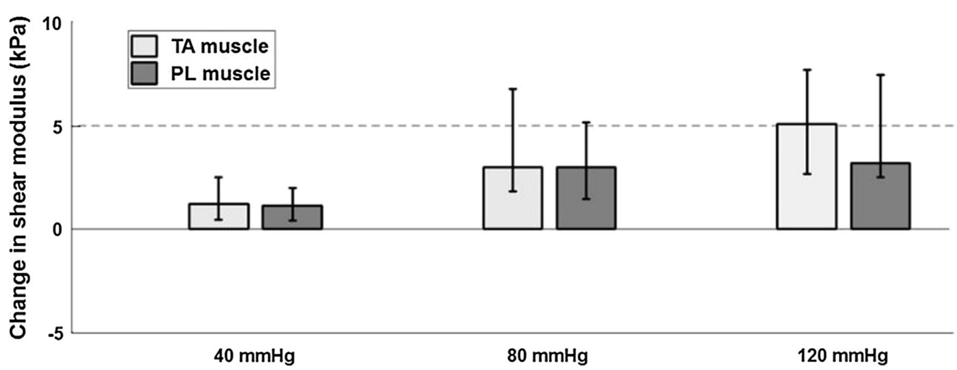

Fig. 3. The change in shear modulus of TA and PL muscles as function of cuff pressure (values at each cuff pressure level represent changes with respect to shear modulus at zero cuff pressure) (median, interquartile range).

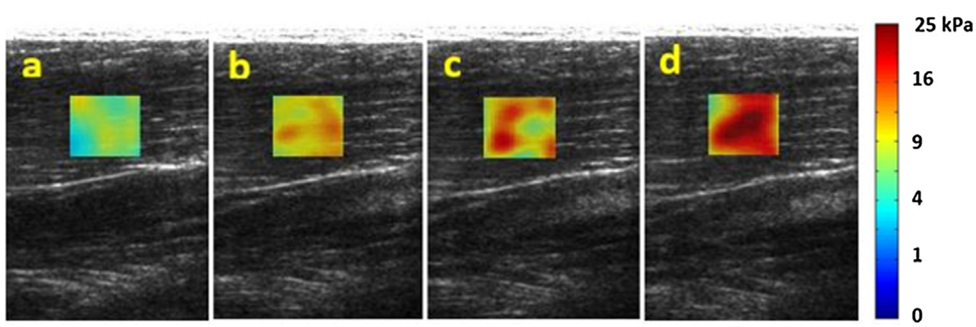

Fig. 4. The shear modulus maps of the TA muscle at each cuff pressure level: (a) 0 (b) 40 mmHg (c) 80 mm Hg and (d) 120 mmHg.

correlated to IMP. This suggested that fascial displacement could be used as a diagnostic indicator of elevated IMP. Garabekyan et al. (2009) tested a digital non-invasive ultrasound device for measuring fascial displacement in a controlled porcine model of acute compartment syndrome using a broader range of IMPs. They reported increased fascial displacement in right TA muscle with elevated IMP induced by injection of 0.045% albumin compared to contralateral compartment with normal IMP. However, facial

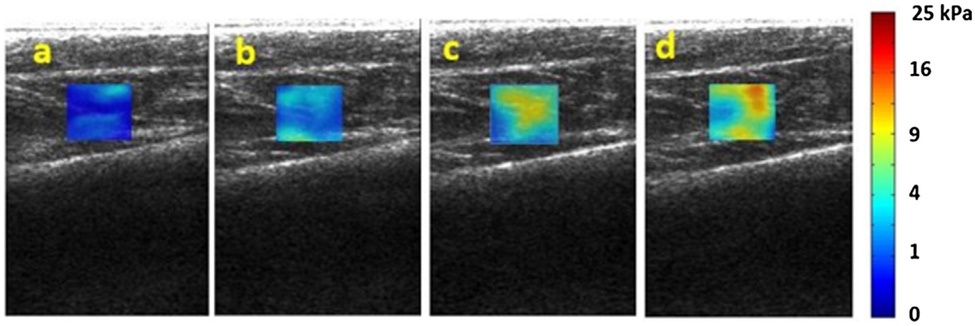

Fig. 5. The shear modulus maps of the PL muscle at each cuff pressure level: (a) 0 (b) 40 mmHg (c) 80 mm Hg and (d) 120 mmHg.

Table 2
Post hoc Bonferroni test results for shear modulus (kPa) between different pressure cuff conditions.

Pressure cuff (mmHg)	Mean difference	Standard deviation	95% CI		Significance
			Lower bound	Upper bound	
0–120	−5.56	4.33	−7.87	−3.24	<0.01
0–80	−4.24	2.84	−6.56	−1.93	<0.01
40–80	−2.57	1.92	−4.89	−0.26	0.02
40–120	−3.89	3.78	−6.20	−1.57	<0.01

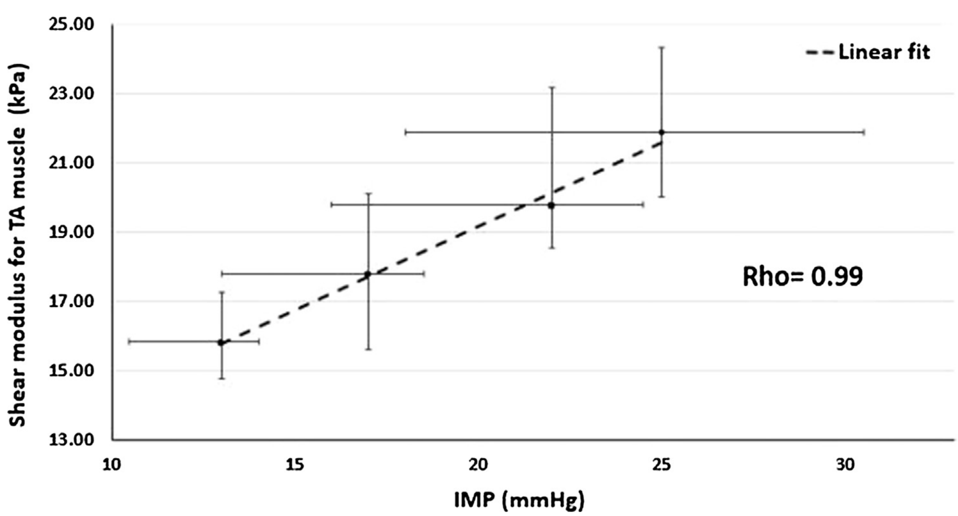

Fig. 6. The shear modulus of TA increased as function of IMP (median, interquartile range) ($p < 0.01$). The horizontal bars represent the interquartile range in IMP and the vertical bars represent the interquartile range of shear modulus.

displacements (or changes in muscle thickness) do not always correlate to estimated muscle force in the same way it has been shown for IMP (Muraki et al., 2013). The novelty of our paper is that we provided information about the relationship between IMP and shear modulus of lower leg compartments using SWE.

IMP measurements require invasive techniques, which may produce a pain response and introduce infection risk due to the required needle insertion. Therefore, an estimate of shear modulus measured via SWE can be used to overcome limitations related to the invasive measurement of IMP. Our results indicated there was a linear correlation between IMP and shear modulus, which is in a range relevant to pathological conditions like compartment syndrome. Previous research has demonstrated that SWE is a reliable method for measuring an estimate of shear modulus of muscles (Alfuraih et al., 2017; Song et al., 2013). Therefore, measurement of shear modulus using SWE could serve as both an accurate and reliable non-invasive measurement for IMP.

The observed correlation between shear modulus and IMP may have several potential clinical applications. The results of this study suggests that shear modulus can potentially monitor pathologies related to abnormal IMP. Shear modulus may also help clinicians diagnose or monitor the recovery process of compartment syndrome disease, instead of currently used methods, such as needle manometry, which suffers from significant variability depending on the depth of needle insertion, amount of fluid introduced, and soft tissue occlusion of the needle (Pedowitz et al., 1990). The blood pressure cuff inflation around the thigh may simulate compartment syndrome with reversible neuromuscular dysfunction. Therefore, the results of this study may be beneficial for practitioners to consider the contribution of shear modulus changes on the diagnosis of compartment syndrome using ultrasound SWE, which could provide better insight into compartment function at different levels of blood pressure.

There are several sources of errors associated with the SWE and IMP measurements. IMP variability may be caused by variations in

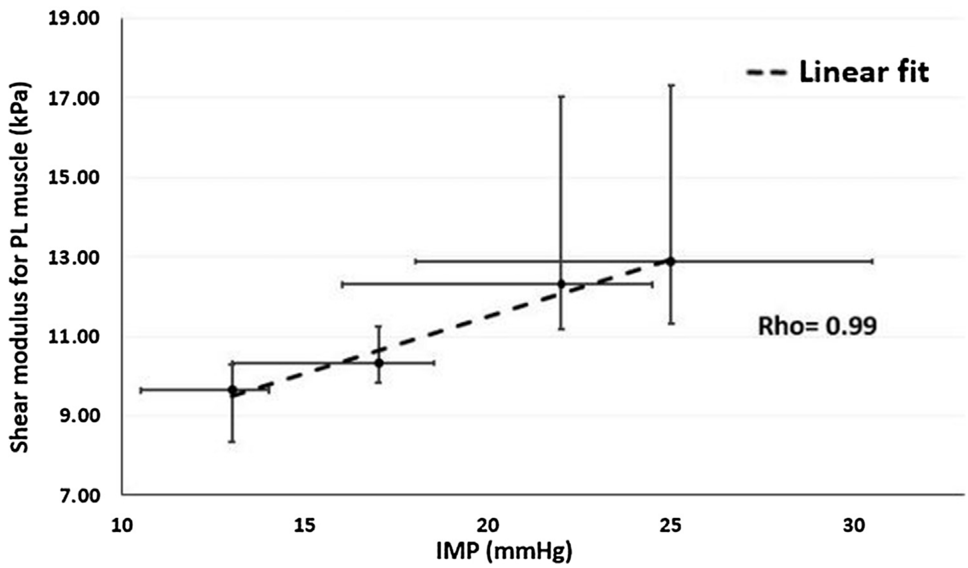

Fig. 7. The shear modulus of PL increased as function of IMP (median, interquartile range) ($p < 0.01$). The horizontal bars represent the IMP interquartile range and the vertical bars represent the shear modulus interquartile range.

the needle insertion angle relative to the direction of the muscle fascicles. Additionally, SWE variability may be caused by misalignment of the transducer relative to the direction of the muscle fascicles or slight muscle displacements due to swelling of the muscle. ROI location is also an important parameter in SWE measurement that affects the shear modulus. Mathevon et al. (2018) suggested performing the SWE measurements at the thickest part of the lower leg muscles. Therefore, anatomical mapping of the muscle in the B-mode image should be conducted to determine the optimum probe location. Future studies will focus on SWE measurement in muscles with different architectures to explore the IMP and shear modulus relationship further.

There are some limitations in this study. First, the majority of the subjects recruited were under age 30. Progressive muscular weakness linked to a shrinking of muscle mass and change in muscle cross-section is reported in the elderly (Merletti et al., 2002). Denervation of motor units also occurs with aging, which can transition type II muscle fibers into type I fibers (Merletti et al., 2002). Hence, these physiological changes and denervation of motor units with aging may be factors affecting muscle function and IMP. Another limitation is that the effect of pennation angle on the reliability of the results was not evaluated. Our future studies will investigate these effects on the shear modulus of the lower leg muscles at different IMP levels.

In conclusion, this study demonstrated that the shear modulus estimated using ultrasound SWE can measure variations in IMP within the lower-leg muscles in healthy individuals. The IMP level was found to correlate to shear modulus in both the TA and PL muscles. Overall, the preliminary results presented in this study suggest that changes in shear modulus could serve as a surrogate non-invasive measurement of IMP levels and could be a useful clinical biomarker for more accurate diagnosis of pathologies related to increased IMP, such as chronic exertional and acute compartment syndrome.

Conflict of interest

The authors of this paper have no financial or personal conflicts of interest related to this work.

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