Article

Changes in Muscle Hardness from Resting to Mid-Range Lengthened Positions Detected by Shear Wave Elastography (SWE) with a Novel Protocol of Ultrasound Probe Placement

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Abstract: Muscle hardness and its relationship with different muscle lengths/positions are important for understanding its underlying physiological status, and yet remained unclear. This study aimed to detect the local muscle hardness at different muscle lengths and identify the influence of muscle position on muscle hardness in healthy adults. A total of 26 healthy adults participated in this study. Shear wave elastography (SWE) was used to measure the muscle hardness of the Rectus Femoris (RF), Tibialis Anterior (TA) and Gastrocnemius Medialis (GM). Each muscle was tested at both resting (RST) and mid-range lengthened (MRL) positions. A novel ultrasound probe placing method was introduced, applied, and evaluated in this study. Moderate to excellent intra-/inter-rater reliability (Intraclass Correlation Coefficient, ICC $\geq$ 0.70) was found for muscle hardness measurements. The muscle hardness significantly increased from the RST to MRL position for all three muscles ($p < 0.001$). This study found that the muscle hardness increased at its mid-range lengthened position from the resting position. The mid-range lengthened muscle position of TA and GM could also be sensitive enough to reflect the age-related changes in local muscle hardness. This study also highlights the importance of placing the assessed extremities in an appropriate and consistent position when assessing muscle qualities by ultrasonics in clinical practice.

Keywords: muscle hardness; elastography; shear wave elastography (SWE); ageing muscle; slack length

1. Introduction

Muscle hardness, defined as a mechanical property that represents the transverse muscle stiffness [1], exhibits an intrinsic viscoelastic tension [2]. At the molecular level, it has been shown that muscle fiber stiffness is proportional to the number of attached cross-bridges and changes linearly as the muscle fiber develops the force [3]. In the network of the tensional tissues (biotensegrity) [4], passive tension is independent of the central nervous system's contribution (non-neural), resulting from the intrinsic molecular interaction of the actomyosin filaments in sarcomeric units and myofibroblast cells of the skeletal muscle [2]. However, the neural component of the muscle tension, which is mostly defined as the active muscle tension, has a physiological implication to the muscle's tone at rest [5]. The active and passive tension, made up of the passive elements within and surrounding the muscle, together with the active elements of the muscle, need to act in accordance in order to maintain the dynamic behavior of the musculoskeletal stability [6].

When the joint angle changes, the amount of lengthening (elongation) of the muscle becomes important for understanding the intrinsic properties of the muscle. The lengthening of the skeletal muscle, accompanied with an angular motion, exhibits a resistance caused by the passive stiffness when its motor neuron is quiescent and myofibrils are not actively contracting [7]. This resistance becomes measurable and increases with increasing tension when the muscle goes beyond its slack length, which is usually assumed to be the

length measured with the joint in middle-range position and when the net joint torque is minimized [8]. With aging, the intrinsic properties of the muscle and tendon also change and hence alters the muscle stiffness and musculoskeletal stability [6]. On the other hand, even though the muscle morphology remains similar for six decades, the aging factor could begin to affect the characteristics of skeletal muscle as early as at 30 years of age [9,10].

To investigate the hardness of the muscular tissue, a non-invasive assessment of the localized muscle mechanical properties has been increasingly conducted using the ultrasound shear wave elastography (SWE) technology [11,12]. The ultrasound probe generated a radiation force followed by tissue deformation, resulting in propagation of a transient shear wave. This propagation was captured by the ultra-fast ultrasound image to calculate the tissue hardness [13]. This technology makes it possible to assess the stiffness of both superficial and deep muscles [10]. The ultrasound SWE has opened the window towards a better understanding of the alterations in muscle hardness, and has thus inspired researchers to find out the effect of the stretching treatment on muscle stiffness under various conditions. However, there have been variations in the revealed results in shear modulus, such as 9.24 ± 1.98 kPa in Gastrocnemius Medialis (GM) muscle with knee extended [11], and 16 ± 5 kPa in lateral gastrocnemius during ankle plantar-flexion and 26 ± 9 kPa during ankle dorsi-flexion while the muscle is isometrically contracting [12]. The application of the measurement technique is another important factor that influences muscle shear modulus, which is already sensitive to (i) specific tension, (ii) degree of anisotropy, (iii) pennation angle, and (iv) angle between muscle fibers and transducer direction [14].

The muscle shear modulus can be affected by the placement of the ultrasound probe, which merits further study to introduce a reliable ultrasonic assessment protocol. The muscle shear modulus was reported to be significantly different when an ultrasound probe was positioned at the proximal one third, the distal one third and in the middle, along with the contraction direction of the GM muscle [15]. The previous studies described the probe location with reference to the recommended placements of the electrodes of electromyographic (EMG) recording [16,17]. The muscle fiber orientation [14] and the non-fiber tissue's (epimysium or aponeurosis) distribution across the contraction direction also contribute to the anisotropic properties of the muscle, which affect the shear modulus value that researchers are not able to control. Therefore, it is important to precisely locate the probe along the muscle contraction direction.

This study consists of two cohesive objectives: (1) to determine the reproducibility of a newly proposed probe placement technique for TA, GM and RF muscles; and (2) to determine the difference in shear modulus when muscles are elongated from the relaxed resting condition to the mid-range lengthened (not maximally stretched) position. It is hypothesized that when the muscle is at its resting position, it has a lower shear modulus in comparison to the mid-range lengthened position.

2. Materials and Methods

2.1. Subjects

A total of twenty-six heathy subjects, with no history of any muscular or neurological disease, were recruited for the study. The subjects were asked not to perform any vigorous exercise at least 2 days prior to the testing, as exercise can induce muscle fatigue and alter the muscle hardness [9,18]. All subjects gave their written informed consent for inclusion before they participated in the study. This study was approved by the Human Subject Ethics Subcommittee of The Hong Kong Polytechnic University (Reference Number: HSEARS20170525002).

2.2. Ultrasound Shear-Wave Elastography

An Aixplorer multiwave ultrasound scanner (version 10.0; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer array (4–15 MHz, Super Linear 15-4, Vermon, Tours, France) was used. The scanner was set in shear-wave elastography (SWE)

mode, with the musculoskeletal muscle (MSK) preset [19]. The muscle shear modulus (μ) was calculated with shear wave speed (*v*) as follows (Equation (1)):

$$\mu = \rho v^2 \tag{1}$$

where ρ is the muscle mass density (1000 kg/m^3).

Muscle shear modulus was measured with Aixplorer scanner software (Q-Box^{TM}, version 10.0; Supersonic Imagine, Aix-en-Provence, France). For each of the three muscles and two muscle positions, a 10 s video (three values were recorded at 3.3 s, 6.6 s, and 10 s) was taken, while the SWE acquisition box was maintained as homogeneously as possible. The location of the region of interest (ROI) and the diameter of Q-Box was adjusted to avoid the artificial part (e.g., avoiding areas of aponeurosis or no measurement in the image [20,21]), with a minimum diameter of 6 mm [22]. The mean value within Q-Box was recorded, and the mean of the three values recorded from the 10 s video (recorded at 3.3 s, 6.6 s, and 10 s) was used for statistical analysis. Figure 1 gives an example of a typical TA muscle image at the resting position.
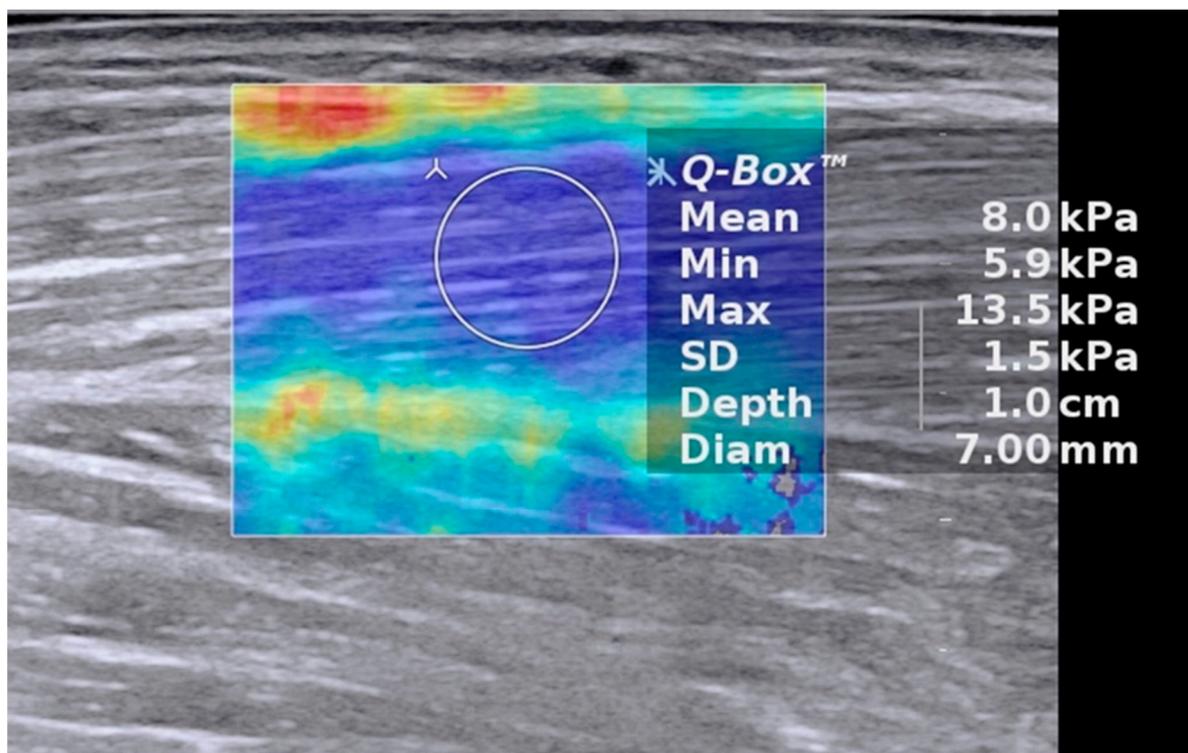

Figure 1. An example of typical elastography images of tibial anterior muscle at resting position. Q-Box^{TM}: the Aixplorer scanner software for measuring the shear wave modulus in the region of interest (white circle in the upper middle of the figure); SD: standard deviation.

2.3. Experimental Procedure

The three target muscles of the lower extremity were Rectus Femoris (RF), Tibialis Anterior (TA) and Gastrocnemius Medialis (GM) of right side. All three muscles were tested under two positions: resting (RST) and mid-range lengthened (MRL). Under both positions, subjects were asked to maintain the still and relaxed state, to avoid any movements that would induce muscle contraction and consequently change the read-out values. Since no active muscle movement was involved, under both positions, the muscular architectural change was induced by changing the muscle tension from the resting state to the mid-range lengthened state (see Figure 2) for the probe positioning on the reference muscles under two testing positions).
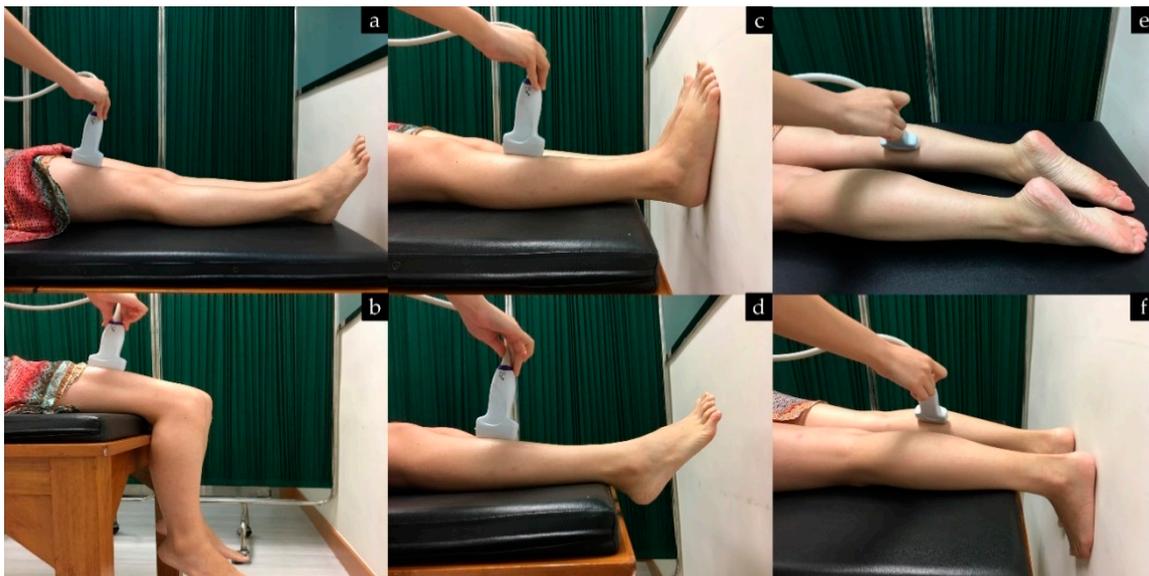
Figure 2. Probe locations for each muscle and position. (**a**) Rectus femoris at resting (RST) condition; (**b**) rectus femoris at mid-range lengthened (MRL) condition; (**c**) tibialis anterior at RST condition; (**d**) tibialis anterior at MRL condition; (**e**) gastrocnemius medial at RST condition; (**f**) gastrocnemius medial at MRL condition.

The positioning of the ultrasound probe was standardized and applied consistently throughout all experiments. The general procedure for the correct probe positioning were as follows: (i) find the body landmarks, and draw a line in between (Figure 3a); (ii) mark the point on the line which is corresponding to the target muscle, and draw a transverse line on it (Figure 3b); (iii) place the probe along the transverse line, and observe the target muscle with the conventional B-mode image; (iv) mark the medial and lateral border of the target muscle on the skin (Figure 3c); (v) change the probe orientation to align with muscle' contraction direction, while the probe remaining in the middle of the medial and the lateral border; and (vi) ask subject to contract the muscle, to verify that the position is positioned on the right muscle. These probe positioning steps were consistent for all three muscles, while the landmark points differed accordingly to the specific tested muscle.

For RF muscle, the body landmarks were anterior superior iliac spine and the superior border of the patella, with the transverse line drawn in the middle of the line between landmarks [16]. The subject was lying in supine position on the examination table. For the RST position, the subject was in supine position, with the knee in the neutral position on the examination table (Figure 2a). For the MRL condition, the subject maintained supine position, with the knee off the table at 90 degrees flexion (Figure 2b).

For TA muscle, the body landmarks were fibula head and lateral malleolus, with the transverse line drawn on the upper one-third of the line between landmarks [23]. The subject was lying in supine position on the examination table. For the RST condition, the foot was positioned against the wall at 90 degrees dorsiflexion (Figure 2c). For the MRL condition, the subject's ankle was off the table from the above malleoli, and the food dropped without extra force applied (Figure 2d).

For GM muscle, the body landmarks were the popliteal fossa and the cross point of Achilles tendon and two malleoli, with the transverse line drawn on the upper one-third of the line between landmarks [24]. The subject was lying in prone position on the examination table. For the RST condition, the foot was positioned on the bed and maintained relaxed (Figure 2e). For MRL condition, the ankle was off the table, with the foot kept against the wall at 90 degrees (Figure 2f).
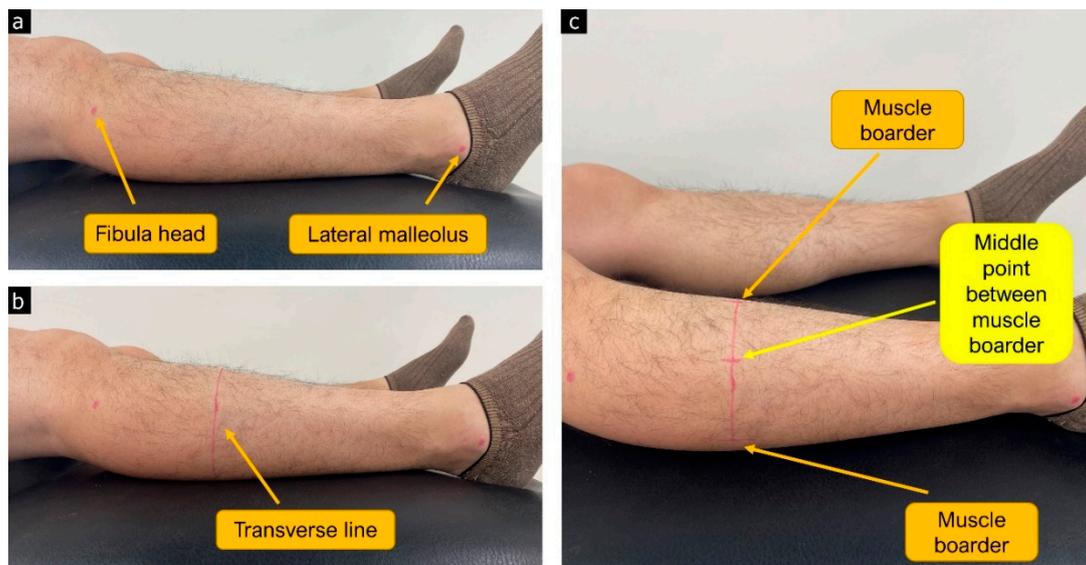
Figure 3. Illustration of applying the novel probe placement procedure on tibialis anterior muscle. (**a**) Mark the body landmarks of the fibular head and lateral malleolus for TA; (**b**) draw a transverse line on the upper one-third of the line between landmarks for TA; and (**c**) locate the middle point between the muscle boarder, which was confirmed with ultrasound B-mode image.

2.4. Reliability Test

A total of twelve subjects participated the reliability test [1]. The experimental procedure was conducted by two raters (R1 and R2) at the right side of the lower extremity to test the reliability. For each muscle, the first rater conducted the assessment (R1A) and marks the location of the transducer. Then the second rater executed the whole procedure at the right side (R2) for the inter-rater reliability testing. Thereafter, the first rater re-assessed the right side (R1B) for the intra-rater reliability testing.

2.5. Data Analysis

Statistical analysis was conducted using SPSS (Version 24 for Windows, SPSS Inc, Chicago, IL, USA). Intra- and inter-rater reliability were tested with the Intraclass Correlation Coefficient (ICC (3,1), two way random, absolute agreement) [25]. The following guideline was used to determine the strength of the ICC: <0.25 no correlation; 0.25–0.5 fair; 0.5–0.75 moderate to good; and >0.75 good to excellent correlation [26]. A mixed analysis of variance (ANOVA) was used to test the effect of muscle position [RST and MLR] on muscle shear modulus, followed by the post-hoc Bonferroni corrections. The significance level was set at 0.05.

3. Results

A total of 26 subjects participated in this study [24,27], with the subject demographic information shown in (Table 1).

Table 1. Demographic of the subjects (mean $\pm$ Standard Deviation).

Gender (Female:Male)	Age (year)	Height (m)	Weight (kg)	Body Mass Index (BMI)
12:14	33.5 ± 5.9	1.66 ± 0.10	60.3 ± 10.8	21.4 ± 2.6

Table 2 summarizes the results of the intra- and inter-rater reliability of each muscle. The intra-rater reliability was good to excellent (ICC: 0.781–0.938) for most of the muscles and positions with one exception for GM at RST position (ICC: 0.696). Inter-rater reliability ICC ranged from 0.707 to 0.895. In general, the intra-rater reliability was better than inter-rater reliability.

Table 2. Intra-rater and Inter-rater reliability at RST and MRL positions for TA, RF and GM muscles determined by Intraclass Correlation Coefficient, ICC (3,1) (n = 12).

		TA_RST	TA_MRL	RF_RST	RF_MRL	GM_RST	GM_MRL
Intra-rater	ICC (3,1)	0.920	0.938	0.786	0.781	0.696	0.862
	p-value	<0.001	<0.001	0.011	0.009	0.030	0.001
Inter-rater	ICC (3,1)	0.871	0.851	0.732	0.711	0.707	0.895
	p-value	0.001	0.002	0.019	0.025	0.026	<0.001

TA_RST: tibialis anterior at resting; TA_MRL: tibialis anterior at mid-range lengthened; RF_RST: rectus femoris at resting; RF_MRL: rectus femoris at mid-range lengthened; GM_RST: medial gastrocnemius at resting; GM_MRL: medial gastrocnemius at mid-range lengthened.

It was found that there was a significant dependence of the shear modulus on the muscle position for the three muscles ($p < 0.001$ for RF, TA and GM). Table 3 summarizes the shear modulus of both groups. There was significantly larger shear modulus at MRL position than that of at RST position for all the three muscles ($p < 0.001$).

Table 3. Shear modulus (kPa) of each muscle at both resting and mid-range lengthened positions (mean ± SD).

	RST (kPa)	MRL (kPa)
RF	3.6 ± 0.9	11.3 ± 2.4 **
TA	5.3 ± 0.8	12.0 ± 1.5 **
GM	3.5 ± 1.0	10.1 ± 1.5 **

** $p < 0.001$ compared to RST condition. RF: Rectus Femoris, TA: Tibialis Anterior, GM: Gastrocnemius Medialis, RST: resting position, MRL: mid-range lengthened position.

4. Discussion

This study introduced a newly developed probe placement on the GM, TA and RF muscles, and evaluated the reliability of this placement method, along with identifying whether significant difference in muscle stiffness existed between resting and mid-ranged lengthened positions. In the present study, we successfully demonstrated: (i) moderate to excellent inter- and intra-rater reliability of the introduced probe placement on the GM, TA and RF muscles, and (ii) shear modulus was higher under the MRL position than the RST position for all measured muscles in all subjects. This highlights the importance of placing assessed extremities in an appropriate position in clinical practice.

4.1. Reliability of the Muscle Hardness Measurement Using the Introduced Novel Probe Positioning

The present study introduced a novel probe placement protocol on the GM, TA and RF muscles, and revealed a moderate to excellent intra- and inter-rater reliability. We followed the protocol of previous studies regarding the probe placement procedure [16,23,24]. Upon identified the targeted muscle, one step forward has been taken by marking the edges of the muscle and mid-point between the edges. In this way, we made sure the probe was in the central area and positioned along the muscle contraction direction. In a previous study, the intra-rater reliability of GM at MRL condition was reported to be moderate to good (ICC = 0.68) [24]. Another study reported moderate to good inter-rater reliability for the muscle measurement (ICC: TA = 0.616, RF = 0.679, GM = 0.728) [27]. Our probe placement procedure achieved better intra-rater (ICC: GM_RST = 0.696, GM_MRL = 0.862) and inter-rater reliability (ICC: TA_RST = 0.871, TA_MRL = 0.851, RF_RST = 0.732, RF_MRL = 0.711, GM_RST = 0.707, GM_MRL = 0.895) with the increase ranging from 4% to 26%. The reliability of GM was found to be the lowest among the three target muscles. This might be because the curved shape of the GM muscle makes the ultrasound probe slides easily on the GM muscle during the muscle contraction. This made it difficult to maintain the same probe location, resulting in the lowest ICC values of the GM muscle. According to our experience throughout the experimental procedure, we understand that the standardization of the probe placement procedure prior to any measurement of shear modulus is important, in

order to achieve accuracy and reliability. It is clear that shear modulus gets easily affected by changing intramuscular properties and dynamics, such as fiber orientation [8] and increasing intramuscular pressure during either reflex or voluntary muscle contraction [14]. In addition to this sensitivity caused by changing internal muscle dynamics, body position is another important factor which needs to be considered during the measurement. One of the body positions that can affect the measurement is the external and internal rotation of the lower extremity in supine position that can be observed anteriorly. This may cause a shift of the muscle beneath the ultrasound probe. This particular artifact needs to be considered especially when a probe is placed over TA muscle. Previous works took consideration of the ultrasound probe location along muscle contraction direction of the lower extremity; however, the probe location between the muscle boarders was usually undermined during experimental procedure [12,16]. We believe that our defined probe placement procedure is an improvement on standardizing the measurement technique using SWE. The reliability of this method was investigated and showed a moderate to excellent intra-rater and inter-rater reliability. This novel probe placement procedure provides more detailed guidance to the SWE operator, which can help to eliminate bias during the SWE measurement.

4.2. Effect of RST vs. MRL Position on Muscle Hardness

In our study, the shear modulus for all tested muscles was found to be significantly higher under MRL than RST position. This finding is in line with previous works [28,29]; however, our study provides more information on the intrinsic qualities of skeletal muscles. Regardless of the anatomical location of the tested muscle, when skeletal muscle is elongated, there is a measurable resistance even when motor neurons are quiescent [7]. The MRL position caused a lengthening of the muscle fibers within its physiological limits hence showed a measurable resistance. However, a total change produced by lengthening of the muscle-tendon unit under the MRL condition was much smaller for the RF muscle when the knee is set off the table in comparison to lengthening beyond 90° knee flexion and for the GM muscle when the outsole was in touch with the wall in comparison to lengthening beyond 0° neutral ankle position. Therefore, the detected increase in the stiffness or local hardness, by shear-wave elastography, can mainly be attributed to the mechanical property representing transverse muscle stiffness, which is different from muscular-tendon complex stiffness along the longitudinal axis of the muscle [30].

It is also assumed that the increase in local muscle hardness by lengthened muscle fibers was due to collagen fibers aligned in the direction of the stress applied by muscle lengthening [31]. Consequently, the transverse muscle stiffness increases along increasing an intramuscular pressure. Another factor attributable to this is several attached cross-bridges changing linearly with the force developed by the lengthening fibers [32]. The composition of the muscle fibers is another known factor contributing to the muscle stiffness where fast contracting fibers are known to have lower stiffness [33]. All the contributors need to interact with each other to produce an active stiffness required to maintain a quiet standing position [34]. According to our results, when muscle hardness was compared between RST and MRL positions for the TA and GM muscles, as expected, the lengthened muscle up to its mid-range or when joint torque was supposedly minimal, the shear modulus was found to be significantly higher in the lengthened than the shortened condition. The RF muscle has also displayed increased local hardness under lengthened condition. If a change in the muscle hardness, revealed as an increase under MRL position without being stretched beyond this level is evident, it would be of interest to find out whether there is any change induced by pathology or related to age, yet detectable at this particular muscle position while the subject remains relaxed with the fibers not overstretched.

5. Limitations of the Study

There were some limitations to the study. Although our novel probe placement procedure managed to eliminate the influence of the measurement errors caused by the probe

location, attention should be paid to the fact that we did not eliminate the orientation between muscle fiber direction and the probe. The shear modulus does not necessary assume isotropy. In a transverse isotropic medium, there are two shear moduli: μ_perpendicular and μ_parallel, which are measured depending on the orientation of the muscle fibers in relation to the shear wave propagation and the shear wave deflection. For in vivo materials such as muscle, the mechanical properties are not isotropic, resulting in the hardness varying with different directions of muscle fibers and probe. The influence of muscle fiber direction has been reported previously [14], and future studies should take this issue into consideration. In this study, we reported the muscle hardness in terms of shear modulus within the region of interest for TA, RF and GM under both RST and MRL conditions. Due to the limitation of the probe size and the nature of the in vivo materials, it should be noted that the localized hardness cannot represent the whole muscle. Mapping the muscle hardness along the whole muscle area could be considered as a potential future research direction.

6. Conclusions

In this study, we demonstrated that the muscle hardness detected by shear wave elastography (SWE) increased when TA, GM and RF muscles were positioned at their mid-range lengthened position. Our probe positioning method revealed a moderate to excellent reliability, emphasizing the importance of recognizing the muscle fibers' cross-sectional view first and then positioning a probe along the muscle fibers longitudinally between the marked points on the skin from the cross-sectional view. Further investigation is needed with a larger sample size to compare the changes in the hardness between resting and upright standing positions. It is also necessary to determine the influence of muscle fiber direction instead of muscle contraction direction to muscle stiffness measurement. We believe that the muscle positioned at its mid-range lengthened condition could be sensitive to reflecting the intramuscular changes caused by specific pathological conditions. This novel probe placement procedure could help to eliminate bias during the SWE measurement.

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