Shear elastic modulus is a reproducible index reflecting the passive mechanical properties of medial gastrocnemius muscle belly

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Abstract

Background: Passive mechanical properties are important in muscle function because they are related to the muscle extensibility. Recently, the assessment of muscle shear elastic modulus using shear-wave elastographic (SWE) imaging was developed. However, reliability and validity of shear elastic modulus measurements during passive stretching remain undefined.

Purpose: To investigate the reproducibility and validity of the shear elastic modulus measured by SWE imaging during passive stretching.

Material and Methods: Ten healthy men volunteered for this study. The shear elastic modulus of medial gastrocnemius (MG) muscle belly was measured using ultrasonic SWE imaging during passive dorsiflexion. To assess the intra-session and inter-day reliabilities, the protocol was performed twice by the same investigator with a 5-min rest period between measurement sessions and twice on two different days by the same investigator with a 1–2-week interval between the two sessions. To assess the inter-investigator reliability, the protocol was performed on the same day by two investigators with a 5-min rest between measurement sessions. In addition, B-mode ultrasonography was used to determine the displacement of myotendinous junction (MTJ) of MG during passive ankle dorsiflexion.

Results: The intra-session, inter-day, and inter-investigator reliabilities of the method was confirmed on the basis of acceptably low coefficient of variations and substantially high intraclass correlation coefficients. In addition, a significant correlation was found between MTJ displacement and shear elastic modulus.

Conclusion: These results suggested that shear elastic modulus measured using SWE imaging is a reproducible index reflecting the passive mechanical properties.

Introduction

Passive mechanical properties are important in muscle function because they are related to muscle extensibility (1,2). Generally, they are measured as per the range of motion (ROM) (3,4). However, previous studies (5,6) challenged that measurements of ROM as an indicator of passive mechanical properties have many limitations. For example, ROM measurements are influenced by many factors, such as pain, stretch tolerance, and reflex activation of the agonist muscle. Therefore, in human experiments, many studies (5–7) recommend measuring passive torque and passive torque–angle curves using a dynamometer. However, passive torque

measurements are influenced by several factors such as synergistic muscles, aponeurosis, tendon, joint capsules,

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and ligaments (2,8). Therefore, non-invasive methods of measuring the passive properties of muscles, such as myotendinous junction (MTJ) displacement, have been developed using ultrasonography during passive movement (7,9–12). However, the measurement of MTJ displacement is not possible in all muscles. Therefore, the assessment of passive mechanical properties in individual muscles *in vivo* remains challenging.

Recently, the assessment of muscle shear elastic modulus using a shear-wave elastographic (SWE) imaging technique was developed (13,14). This technique is based on calculation of the shear elastic modulus by measuring the local shear-wave velocity propagation from a remote mechanical vibration, which may be a valuable tool for measuring the passive mechanical properties of individual muscles during passive stretching. Maisetti et al. (2) reported that the assessment of the shear elastic modulus of medial gastrocnemius (MG) using SWE imaging showed good intra-session reliability during passive stretching, and that the relationship between the shear elastic modulus and length of the gastrocnemius muscle tendon unit (GMTU) calculated by a mathematical model was very well fitted ($R^2 = 0.944$–1). Therefore, the authors concluded that the shear elastic modulus determined with SWE imaging can be used to accurately estimate the stretching level. In addition, Hug et al. (15) have reported that the assessment of slack length in MG and Achilles tendon during passive stretching with SWE imaging showed good intra-session reliabilities. However, inter-day and inter-investigator reliabilities of shear elastic modulus measurements made using SWE imaging during passive stretching remain undefined. In addition, the relationship between the shear elastic modulus and the actual muscle elongation, such as MTJ displacement during passive stretching, is also unclear, because the previous study estimated the length of GMTU using a mathematical model.

The purposes of this study were to investigate the reproducibility of the shear elastic modulus measured by SWE imaging during passive stretching, to examine the relationship between MTJ displacement and shear elastic modulus and to identify the indicator that reflect the passive mechanical properties during passive stretching.

Material and Methods

Participants

Ten healthy men volunteered for this study (age, 23.3 ± 1.1 years; height, 171.6 ± 2.7 cm; body weight, 63.5 ± 9.2 kg). Participants with a history of neuromuscular disease or musculoskeletal injury involving the lower limbs were excluded. All participants were fully informed of the procedures and purpose of the study, and written informed consent was obtained. This study was approved by the ethics committee of Kyoto University Graduate School and the Faculty of Medicine (E-1162).

Experimental protocol

Participants were familiarized with the procedure and instructed to remain relaxed during measurement. They lay in the prone position on a dynamometer table, with their hips secured by adjustable lap belts (MYORET RZ-450, Kawasaki Heavy Industries, Kobe, Japan). The knee of the dominant leg was in full extension and the foot of the same leg was attached securely to the footplate of the dynamometer. The ankle was passively dorsiflexed at a constant velocity of $5°/s$, starting from $10°$ plantarflexion to $30°$ dorsiflexion. Each ankle joint position was maintained for 10 s using the dynamometer for the measurements of the shear elastic modulus (shear wave speed) and MTJ displacement.

Assessment of muscle shear elastic modulus using ultrasound SWE imaging

The shear elastic modulus of the MG muscle belly was measured during passive dorsiflexion using ultrasonic SWE imaging (Aixplorer, MSK mode, SuperSonic Imagine, Aix-en-Provence, France). According to the methods of previous studies (16–18), the shear elastic modulus was measured at 30% of the proximal lower leg length from the popliteal crease to the lateral malleolus, almost where the maximal cross-sectional area in the lower leg is observed (19). Ultrasound images were identified as described in Fig. 1 and visualized as continuous sagittal plane ultrasound images using an ultrasound transducer (50 mm long, 4–15 MHz, Liner ultrasound transducer, SuperSonic Imagine). As described by Koo et al. (20), this apparatus uses an acoustic radiation force created by ultrasound beams to perturb muscle tissues by inducing shear waves that propagate within the muscle. As the shear waves propagate, they are captured by the ultrasound transducer at an ultrafast frame rate. The shear wave propagation speed is estimated at each pixel using a cross-correlation algorithm. The ultrasonic SWE images show a color-coded box presentation of shear wave speed superimposed on the sagittal B-mode ultrasound image of MG. As shown in Fig. 1, the ultrasonic SWE images represented a scale from blue (soft) to red (stiff) depending on the magnitude of the shear wave speed. In every measurement, the region of interest (ROI) was set near the center of the SWE image where the muscle was thickest. In addition, a 5-mm diameter circle was set near the center of ROI (17). The circle for the
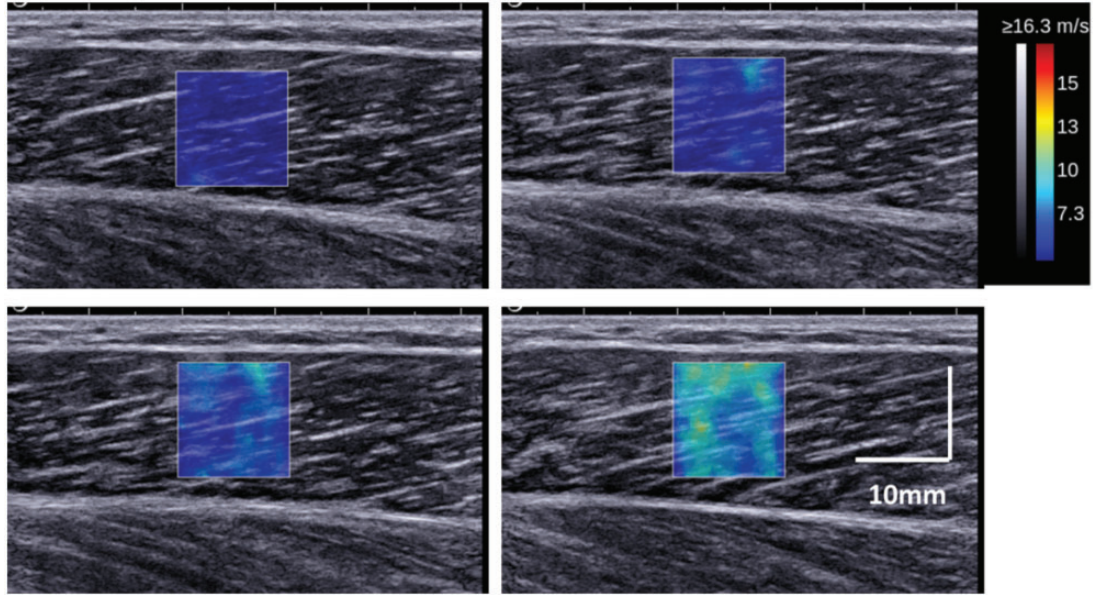
Fig. 1. Typical examples of muscle shear elastic modulus measured by ultrasonic shear-wave elastography (SWE) during passive stretching.

quantitative analysis automatically calculated the maximum, minimum, standard deviation, and mean value of shear wave speed. The mean shear wave speed was used for the analysis (21). Shear wave speed of MG muscle belly was measured every 10° from 0° to 30° of dorsiflexion. Subsequently, the shear elastic modulus (μ) in the main direction of the probe was calculated as follows:

$$\mu(\text{kPa}) = \rho \text{Vs}^2$$

where Vs represents the shear wave speed and ρ is the muscle mass density ($1000 \, \text{kg/m}^3$).

To assess the intra-session reliability for each ankle joint position, the protocol was performed twice by the same investigator with a 5-min rest period between measurement sessions. Similarly, to assess the inter-day reliability, the protocol was performed twice on two different days by the same investigator with a 1–2-week interval between the two sessions. To assess the inter-investigator reliability, the protocol was performed on the same day by two investigators with a 5-min rest between measurement sessions.

Assessment of MTJ displacement

B-mode ultrasonography (Famio Cube SSA-520A; Toshiba Medical Systems Corporation, Tochigi, Japan) was used to determine the displacement of MTJ of MG during passive ankle dorsiflexion. MTJ was identified and visualized as a continuous sagittal plane ultrasound image using an 8-MHz linear-array probe. An acoustically reflective marker was placed on the skin under the ultrasound probe to confirm that the probe did not move during measurement (10,11). We defined MTJ displacement as the distance between MTJ and the acoustically reflective marker. A custom-made fixation device was used to secure the probe to the skin. Ultrasound images of MTJ were quantified using open-source digital measurement software (ImageJ, National Institutes of Health, Bethesda, MD, USA). To accurately measure MTJ, MTJ was identified at the inner-most edges of the fascia surrounding the muscle where it fuses with the tendon. MTJ displacement was measured every 10° from 0° to 30° dorsiflexion with reference to 10° plantar flexion. The measurement order for shear elastic modulus and MTJ displacement was done randomly to avoid any order effect.

EMG

EMG (TeleMyo2400; Noraxon USA, Inc., Scottsdale, AZ, USA) was used to confirm that the participants were relaxed and to ensure that muscles were inactive during passive dorsiflexion. Surface electrodes (Blue Sensor M, Ambu, Olstykke, Denmark) with a 2.0-cm interelectrode distance were placed on certain portions of MG muscle bellies. EMG sampling rate was 1500 Hz.

EMG activity within 3 s was recorded from MG while the participants performed an isometric maximum voluntary contraction (MVC). MVC was

achieved during maximal isometric plantar flexion with the ankle at 0° using the dynamometer. Strong verbal encouragement was provided during the contraction to promote maximal effort. EMG activity was calculated using the root mean square (RMS), and full wave rectification was performed using an RMS smoothing algorithm with a window interval of 50 ms. EMG activity recorded during the measurements was expressed as a percentage of MVC.

Statistical analysis

SPSS version 21.0 (SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses. The reliability of measurements of shear elastic modulus for each ankle joint position were assessed using the intraclass correlation coefficient (ICC) and coefficient of variation (CV). Intra-session, inter-day, and inter-investigator reliabilities were assessed using ICC (1, 1), ICC (1, 1), and ICC (2, 1), respectively.

Significant differences in shear elastic modulus and MTJ displacement measurements taken for each ankle joint position were assessed using a paired t-test with Holm correction. In addition, MTJ displacement and shear elastic modulus was averaged by all subjects for each ankle joint position, and the relationships between MTJ displacement and shear elastic modulus was determined using Pearson's product-moment correlation coefficients. Differences were considered statistically significant at an alpha level of $P < 0.05$. Descriptive data are shown as mean ± standard errors of the mean (SEM).

Results

Reproducibility of shear elastic modulus measurement

The reproducibility of shear elastic modulus measurements is demonstrated in Table 1. There were no shear elastic modulus values that reached saturation. For the intra-session reliability, ICC (1, 1) was 0.936–0.985 and CV was 4.6–6.1%. For the inter-day reliability, ICC (1, 1) was 0.830–0.897 and CV was 5.9–10.0%. For inter-investigator reliability, ICC (2, 1) was 0.836–0.960 and CV was 5.8–8.4%.

Relationships of shear elastic modulus and MTJ displacement

The measured values of shear elastic modulus and MTJ displacement for each ankle joint position are listed in Table 2. Shear elastic modulus and MTJ displacement significantly increased with dorsiflexion ($P < 0.05$). A significant correlation was found between MTJ

Table 1. Reproducibility of shear elastic modulus measurements.

	0°	10°	20°	30°
Intra-session reliability				
ICC (1, 1)	0.936	0.94	0.955	0.985
95% CI	0.776–0.983	0.790–0.984	0.838–0.988	0.945–0.996
CV	4.7 ± 3.6%	5.0 ± 3.4%	6.1 ± 3.2%	4.6 ± 2.0%
Inter-day reliability				
ICC (1, 1)	0.897	0.851	0.848	0.830
95% CI	0.660–0.973	0.533–0.960	0.525–0.959	0.479–0.954
CV	5.9 ± 3.1%	8.1 ± 8.7%	10.0 ± 6.7	9.6 ± 14.6
Inter-investigator reliability				
ICC (2, 1)	0.908	0.836	0.911	0.960
95% CI	0.682–0.976	0.460–0.957	0.700–0.977	0.851–0.990
CV	5.8 ± 3.5%	6.4 ± 4.2%	6.2 ± 4.9%	8.4 ± 3.1%

95% CI, 95% confidence intervals; CV, coefficient of variation; ICC, intraclass correlation coefficient.

displacement and shear elastic modulus (r = 0.964, $P = 0.036$).

EMG

For each participant, the EMG activity of GM was < 2.0% MVC during all tests, which confirmed the lack of a contractile component contribution to the shear elastic modulus and MTJ displacement.

Discussion

For each participant, the EMG activity of GM was very low (<2.0% MVC) during measurement. Therefore, we assumed that no voluntary or reflex contraction occurred during the measurement and that values obtained for the shear elastic modulus and MTJ displacement reflected the passive mechanical properties of MG (22,23).

ICC > 0.75 is generally considered good (18,24). In this study, ICC for measuring the shear elastic modulus were in the range of 0.936–0.985 for intra-session reliability, 0.830–0.897 for inter-day reliability, and 0.836–0.960 for inter-investigator reliability. CV < 12% is regarded as acceptably low for a biological measurement (18). In this study, CVs for measuring the shear elastic modus for intra-session, inter-day, and inter-investigator reliabilities were in the range of 4.6–6.1%, 5.9–10.0%, and 5.8–8.4%, respectively. On the basis of these values, the reproducibility of measuring shear elastic modulus using the SWE imaging technique during passive stretching was confirmed.

In this study, the shear elastic modulus significantly increased with ankle dorsiflexion; this finding is consistent with that of previous studies (2,16,18). Similar to

Table 2. Measured values of shear elastic modulus and MTJ displacement.

	0°	10°	20°	30°
Shear elastic modulus (kPa)	8.1 ± 0.6	14.4 ± 1.0*	26.6 ± 2.6*†	49.6 ± 4.8*†‡
MTJ displacement (cm)	0.30 ± 0.01	0.60 ± 0.02*	0.90 ± 0.03*†	1.20 ± 0.04*†‡

Data are means ± standard error of the mean (SEM).

*$P < 0.01$; significant difference from value at 0°.

†$P < 0.01$; significant difference from value at 10°.

‡$P < 0.01$; significant difference from value at 20°.

the change in shear elastic modulus, MTJ displacement also increased with ankle dorsiflexion, a finding consistent with that of previous studies examining the relationship between muscle elongation and ankle dorsiflexion (7,9,12). These results suggest that the shear elastic modulus increases with passive stretching of GM. Although the reason of this change is not clear, it could be assumed that collagen fibers are aligned in the direction of the stress when a muscle is stretched (25), which partially increases passive muscle tension during stretching (1,26). In fact, previous studies reported a strong linear relationship between the shear elastic modulus and passive muscle tension (27,28). Therefore, the change in collagen fiber alignment with muscle stretching could partly explain the increase in the shear elastic modulus.

To the best of our knowledge, this is the first study to demonstrate the relationship between MTJ displacements and shear elastic modus during passive stretching. Our results showed that all measurements were significantly increased with ankle dorsiflexion and that MTJ displacement was correlated with shear elastic modulus ($r = 0.964$, $P = 0.036$). These results suggest that shear elastic modulus is correlated with MTJ displacement as an index of muscle elongation, and that the shear elastic modulus measured via the SWE imaging technique could be related to muscle elongation. However, Maisetti et al. (2) reported that changes in muscle shear elastic modulus were due to changes in muscle passive tension. Therefore, when taken together, shear elastic modulus measurement could provide an accurate estimation of the change in muscle elongation, when the muscle is not in a slack statement (i.e. under muscle passive tension).

Other advantages of shear elastic modulus measurement by the SWE imaging technique are that this technique permits quick and easy *in vivo* evaluation of the passive properties of individual muscles compared with other devices, such as dynamometer and magnetic resonance elastography (29), and evaluation of the passive properties of both the surface and deep muscles by easily changing the ROI position (14). Previous studies (17) reported that shear elastic modulus measured via the SWE imaging technique decreased after static stretching, which suggests that this imaging technique is a useful tool for quick and easy evaluation of the effects of stretching on individual muscles. However, we investigated the reproducibility and validity of the SWE imaging technique only for MG, which is a superficial muscle. In addition, the number of subjects ($n = 10$) was too small. Therefore, further studies with larger samples are required to clarify the reproducibility and validity of the SWE imaging technique for superficial and deep muscles during passive stretching.

In conclusion, our results showed that the intra-session, inter-day, and inter-investigator reliabilities of shear elastic modulus measurements obtained by the SWE imaging technique during passive stretching are very high (e.g. $ICC > 0.830$ and $CV < 10.0\%$). In addition, the results of this study revealed a high correlation of MTJ displacement with shear elastic modulus. These results suggested that shear elastic modulus measured using the SWE imaging technique is a reproducible index reflecting the passive mechanical properties of MG muscle belly.

Declaration of conflicting interests

Funding

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