Measurement consistency of dynamic stretching muscle stiffness evaluated using shear wave elastography: comparison among different stretched levels and ROI sizes

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Abstract

Aim: To investigate the reliability of quantitative analysis of dynamic stretching muscle stiffness using shear wave elastography (SWE), and to evaluate the influence of stretched levels and region of interest (ROI) sizes on the repeatability of SWE measurements. Materials and methods: SWE videos of the gastrocnemius medius were collected during ankle movement from plantar flexion (PF) 40° to dorsiflexion (DF) 30°. Shear wave images were collected of ankle angles at PF 25°, 0°, DF 15°, and DF 30°, representing the slack status, mildly stretched level, moderately stretched level, and maximal stretched level of the gastrocnemius medius, respectively. ROI circles with diameters of 2 mm, 5 mm, and 8 mm were applied to measure the shear modulus. Intra-observer, and inter-observer repeatability of the measurements were compared among different stretched levels and ROI sizes. Results: Twenty-one healthy volunteers were enrolled. Muscle stiffness increased as the ankle DF increased. Intraclass correlation coefficients (ICCs) of intra-observer and inter-observer repeatability obtained for ROI sizes of 2 mm, 5 mm and 8 mm indicated good to excellent repeatability at all stretched levels. Conclusions: Shear wave elastography appeared to be a reliable tool to evaluate the dynamic stretching muscle stiffness with satisfactory repeatability at various stretched levels of gastrocnemius medius. Good to excellent repeatability was found using different ROI sizes.

Keywords: repeatability; skeletal muscle; passive stretching; elastography; ultrasonography

Introduction

Since shear wave elastography (SWE) was first introduced in clinical practice in 2009 [1], it has become an important imaging technique providing quantitative measurement of tissue stiffness. SWE was initially applied for evaluation of the breast, thyroid and liver [2-4]. Currently, there is no standard protocol for SWE measurement [5]. There has been continuing discussion to explore the optimal ROI settings [1,5,6]. Moon et al [6] compared the impact of two types of region of interest (ROI) (2 mm round ROI and ROI drawn along the margin of the lesion) in differentiating solid breast lesions and recommended a 2 mm circle for the stiffest region. Skel et al [1] investigated the diagnostic efficiencies of ROIs of 1, 2, and 3 mm diameters in solid breast lesions and found that the benign/malignant threshold was lower with increasing ROI size. Yauk et al [5] compared three circular ROIs and four free-hand ROIs and confirmed that SWE values and their diagnostic performance varied based on ROI settings. Although studies have evaluated ROIs at an increasingly rapid rate, no consensus has been reached yet.
Recently, the application of SWE on skeletal muscle has been gradually explored. Previous studies have suggested that increased muscle stiffness is correlated with the stage of muscular diseases, such as Duchenne muscular dystrophy [7] and cerebral palsy [8]. Increased stiffness contributes to muscle adaption and decreased passive joint range of motion, further disrupting motor function over time and adversely impacting quality of life [8,9]. Longitudinal assessment of passive muscle stiffness could provide information of the muscle function in monitoring disease progression and treatment response. However, the quantification of passive muscle stiffness involves a series of muscle stretched levels, which would be more challenging. Moreover, the inherent anisotropy of skeletal muscle, various muscle volumes, and routinely diffuse lesions in muscle disorders are unique when compared to other tissues. In previous studies, various sizes of ROI were applied in SWE for muscle evaluation. For example, ROI sizes of 1.5, 5, 6, and 10 mm, etc., were applied in SWE for evaluation of gastrocnemius [8-15]. However, the influence of stretched levels and ROI sizes on the SWE measurement in skeletal muscles has not been clarified.

Based on the different opinions in ROI size selection in the evaluation of muscle stiffness, we aimed to investigate the ROI size-associated measurement repeatability in dynamic stretched muscle stiffness using SWE. We chose to analyze the passive muscle stiffness as a valuable indicator for evaluating the mechanical properties of skeletal muscles, which could provide information for diagnosis and monitoring of disease progression and treatment response. In addition, the muscle stiffness range during the dynamic stretching process would provide more information than relaxed muscles would.

**Materials and methods**

**Participants**

The study protocol was approved by the institutional Ethics Board and written informed consent was obtained from each subject. The inclusion criterion was normal motor function of both legs. Participants were excluded if they reported any history of neuromuscular or musculoskeletal disease, lower extremity injury, or surgery. The descriptive properties of the participants, including age, gender, height, weight and circumference of the left calf in the area of the greatest bulk were recorded. Body mass index (BMI) was calculated using the formula: BMI = Weight (kg)/Height (m)^2.

**Shear wave elastography image acquisition**

SWE was conducted using a Resona version 7.0 ultrasound scanner (Mindray Imagine, Shenzhen, China) with a linear transducer (L11-3U), by two sonographers (L.X. and P.M.) with more than 10 years of musculoskeletal ultrasound experience and 4 years of SWE imaging experience.

Any vigorous exercise was refrained in one week prior to the testing, as it could affect the measurements. Each participant lay supine on a flat bed with their left leg relaxed and left foot firmly secured to the footplate by two straps. The knee was bent at 30° flexion to avoid overstretching of the gastrocnemius medius (GM) during stretched cycles [16]. SWE videos that focused on the center of the greatest GM muscle bulk in the upper calf were collected during ankle movement from plantar flexion (PF) 40° to dorsiflexion (DF) 30° at a constant velocity of 2°/s using an ankle exercise machine, with a rectangular-shape elastography window nearly cover the overall thickness of GM. The transducer was oriented perpendicularly to the skin and parallel to the GM fascicles consistently during the stretching process. An adequate amount of gel was used to minimize the probe load to the GM muscle. Participants were instructed to stay as relaxed as possible through the passive stretching process. All B-mode and elastographic system-setting parameters, such as gain, depth (4 cm), transducer frequency (7 MHz), and focus were kept constant throughout the study.

The stretching cycle was repeated three times with the 1st cycle regarded as preconditioning; two sets of SWE videos were collected from the latter two stretched cycles. The shear modulus was measured in the two sets of SWE movies and the average value of the measurements adopted for analyses. Two sonographers (L.X. and P.M.) performed the above process and the inter-observer repeatability was assessed accordingly. After one week, SWE was performed by L.X. on the same group of participants to assess the intra-observer repeatability.

**Data processing**

SWE images were collected for ankle angles at PF 25°, 0°, DF 15°, and DF 30° (fig 1), which represented the slack status [17], mildly stretched level, moderately stretched level, and maximal stretched level, respectively.

ROIs with diameters of 2, 5 and 8 mm were applied to measure the shear modulus (fig 2A-C). The mean shear modulus and the standard deviation (SD) over the whole elastography window was also measured (fig 2D), representing the overall shear modulus of the GM muscle.

**Statistical analysis**

Statistical analyses were performed using SPSS (Version 21.0, SPSS Institute, Chicago IL, USA) and MedCalc (Version 18.2.1, MedCalc Software, Mariakerke, Belgium). Intra-observer and inter-observer agreement
of the measurements were statistically analyzed among different stretched levels and different ROI sizes by intraclass correlation coefficient (ICC). The 95% confidence interval and ICC were used to assess the repeatability of the shear modulus test results. Generally, ICC values in the ranges 0–0.40, 0.41–0.60, 0.61–0.79, and 0.80–1.00 indicate poor, moderate, good, and excellent reliability, respectively [13,18]. All quantitative parameters were expressed as mean ± standard deviation (SD). The one-way ANOVA test was used to determine if there was a statistically significant difference in shear modulus among different stretched levels, with post-hoc Tukey-corrected pairwise multiple comparisons to highlight differences between each group and another. The Normal distribution was tested by Kolmogorov-Smirnov (K-S). The Homogeneity test was performed by Levene’s Test. Pearson correlation analysis was used to analyze the correlation between overall mean shear modulus and SD as well as shear moduli measured with different ROI sizes. A confidence level of 0.05 was chosen for all statistical tests.

Results

Basic characteristics

Twenty-one healthy volunteers participated in the study, including 10 men and 11 women. The mean±SD (min-max) age was 31.48±7.17 (23-44) years, with a mean BMI of 21.86±1.28 (19.1-24.5). The mean weight was 61.67±6.89 (52-75) kg, and mean height was 167.57±6.64 (159-178) cm. The calf circumstance was 37.62±2.22 (33-41) cm, and the GM muscle thickness was 1.95±0.38 (1.39-2.52) cm.

Intra-observer and inter-observer repeatability

The ICCs of intra-observer and inter-observer of different ankle angles with different ROI sizes are shown in table I and figure 3. The ICCs of intra-observer and inter-observer with ROI size of 2 mm were all above 0.6, which indicated good to excellent repeatability. The ICCs of 8 mm ROI were higher than that of 5 mm ROI.
Overall shear modulus at different stretched levels

There was a positive correlation between the overall mean shear modulus and SD ($r=0.873$, $p=0.000$) (fig 4A). The overall shear modulus and the SD increased significantly as the stretched level increased ($F=145.60$, $p=0.000$; and $F=74.67$, $p=0.000$, respectively) (fig 4B,C, table II).

Relevance of shear modulus measured by different ROIs to the overall shear modulus

The shear moduli measured for different ROI sizes had excellent correlations with the overall shear moduli. The correlation coefficient $r$-values were 0.990 (ROI = 2 mm), 0.994 (ROI = 5 mm) and 0.997 (ROI = 8 mm), respectively (all $p=0.000$).

Discussion

The current study investigated the intra-observer and inter-observer reliability in the SWE measurement of dynamic stretching muscle stiffness of GM. By adopting different ROI sizes to measure passive muscle stiffness at four stretched levels, including slack status, mildly stretched level, moderately stretched level and maximal stretched level, an overall view of the reliability of SWE would be presented.

Our results demonstrated that intra-observer and inter-observer repeatability of the SWE technique were good to excellent in the relaxed condition, as well as in mildly, moderately and maximal stretched muscles. A similar investigation was performed in a prior study of SWE on the lateral gastrocnemius for four ankle angles (PF20°, PF10°, 0°, and DF10°), and the reliability of the measurements was good to excellent; the ICC range of 0.67-0.80 was lower, which may be due to the inconsistent ROI sizes from 4.6 to 6.2 mm, and the measurement area exceeding the epimysium of the gastrocnemius may also have affected the measurement consistency [19]. Similarly, good to excellent reliability of muscle stiffness was observed in another study conducted on the GM in relaxing and submaximal contracted conditions, with an ICC range of 0.891-0.979 [20].

The shear moduli measured by different ROI sizes in our study had excellent correlation with the overall shear modulus, which confirmed the reliability of SWE application in evaluating dynamic stretched muscle stiffness. Ultrasound elastography technique was initially applied to superficial tissues such as the breast [2]. The most valuable application of SWE in the breast is the differentiation of benign and malignant tumors [1,6,21] and the local stiffest area has always been a concern, which
is different from the application of SWE in the musculoskeletal system. In most muscular diseases, skeletal muscles are commonly impaired in their entirety [8,12,22-26]. Diffuse pathology exists and makes the overall stiffness more valuable in evaluating muscle stiffness. For muscle stiffness assessment, the mean shear modulus in the middle of the muscle belly is a more reasonable indicator, which has been widely adopted in previous studies [8,15,27,28]. Based on the inherent pathologic differences between muscle diseases and breast tumors, the ROI selection for muscular application cannot be directly inferred from the experience of breast SWE.

From our results, the SD of shear modulus increased with the stretched level, which suggested higher heterogeneity in stretched muscles with higher shear modulus [19]. This could explain why the repeatability decreased with the increased stretched level when adopting an ROI of 2 mm, since a smaller ROI size may increase the measurement bias when the overall tissue is more heterogeneous. As the ROI size increased, the sampling error decreased and values obtained using a larger ROI size were more capable of representing the overall shear modulus. This could explain why a larger ROI had higher repeatability as compared to a smaller ROI in assessing muscle stiffness using SWE. Also, as mentioned above, the stiffest value of breast masses has the highest value in clinical evaluation, which is unsuitable for SWE measurements in muscle disorders with diffuse pathologic characteristics. In our study, the measurement repeatability maintained good to excellent at the maximal stretched level of GM despite the highest heterogeneity. The shear moduli measured with different ROI sizes also had excellent correlations with the overall shear moduli, which suggested that shear modulus measured by these ROI sizes were capable of representing the overall shear modulus.

Moreover, the high repeatability obtained in our study may also be attributed to the consistency of other potential variables, such as the depth of the ROI, and probe load. Ewertsen et al [29] demonstrated that the shear wave velocity decreased with increasing scanning depth. On the other hand, Alfuraih et al [11] pointed out readings acquired from depths less than 4 cm yielded excellent reliability, and variance in SWE readings only increased significantly at depths greater than 6 cm. In our study, the ROI depths in all participants were in the range of 4 cm to minimize the effect of depth. The influence of the probe load in our study was controlled by minimizing the required pressure of the probe on the skin using adequate gel, while preserving SWE images by experienced sonographers.

The findings of this study were subject to several limitations. Firstly, the number of enrolled participants was small. Secondly, the overall stiffness of the GM muscle was represented by a portion in a cross-section of the muscle and may differ from that of the realistic overall muscle. Thirdly, only one muscle was selected in

### Table I. Intra-observer, and inter-observer repeatability at different ankle angles with different ROI sizes.

| ROI sizes (mm) | Stretched levels | Intra-observer | Inter-observer |
|----------------|------------------|----------------|----------------|
|                |                  | ICC            | 95% Confidence interval | ICC          | 95% Confidence interval |
| 2              | PF25° 0° DF15° DF30° | 0.8511 (0.6690-0.9368) | 0.7597 (0.4963-0.8950) |
|                |                  | 0.8274 (0.6224-0.9262) | 0.7445 (0.4696-0.8878) |
|                |                  | 0.7737 (0.5215-0.9015) | 0.6910 (0.3790-0.8619) |
| 5              | PF25° 0° DF15° DF30° | 0.9123 (0.7970-0.9635) | 0.9090 (0.7723-0.9586) |
|                |                  | 0.9111 (0.7943-0.9630) | 0.8997 (0.7697-0.9581) |
|                |                  | 0.9065 (0.7843-0.9610) | 0.8922 (0.7537-0.9548) |
| 8              | PF25° 0° DF15° DF30° | 0.9209 (0.8157-0.9671) | 0.9064 (0.7841-0.9609) |
|                |                  | 0.9302 (0.8364-0.9711) | 0.9169 (0.8069-0.9654) |
|                |                  | 0.9372 (0.8519-0.9740) | 0.9215 (0.8169-0.9674) |
|                |                  | 0.9287 (0.8330-0.9704) | 0.9077 (0.7870-0.9615) |

ROI – region of interest; ICC – intraclass correlation coefficient; PF – plantar flexion, DF – dorsiflexion

### Table II. Overall shear modulus at different stretched levels.

|               | PF 25° | 0°       | DF15°    | DF30°    | F       | P       |
|---------------|--------|----------|----------|----------|---------|---------|
| Mean shear modulus (kPa) | 4.86±1.20 | 10.16±2.49 | 20.05±5.49 | 43.20±11.32 | 145.60   | 0.000   |
| Standard deviation (kPa)  | 1.60±1.04 | 1.99±1.23 | 3.77±1.75 | 10.43±3.64 | 74.67    | 0.000   |

PF – plantar flexion; DF – dorsiflexion
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Conflict of interest: none

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