J. Phys. Ther. Sci. 34: 515–521, 2022

The Journal of Physical Therapy Science

Original Article

Reliability of lower leg muscle thickness measurement along the long axis of the muscle using ultrasound imaging, in a sitting position

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Abstract. [Purpose] To verify the reliability and validity of lower leg muscle thickness (MT) assessment along the muscle's long axis using ultrasound imaging (USI) in a sitting position. [Participants and Methods] Twenty healthy adult female participants (aged, 20.3 ± 0.9 years) were included in the study. Intra- and inter-examiner reliability of the proximal, middle, and distal MT of the tibialis anterior (TA) and medial head of the gastrocnemius (GM) were verified using USI in a sitting position. Additionally, the relationship between MT measurement using USI and muscle cross-sectional area (MCSA) measurement using magnetic resonance imaging (MRI), as well as the ankle joint's maximum muscle strength, were examined. [Results] The reliability of TA and GM MT measurement using USI was high for all regions. The relationship between MCSA measurement using MRI and MT measurement using USI showed a significant correlation in all the regions for both muscles. The relationship between ankle muscle strength and USI of MT was not significantly correlated in any region for both muscles. [Conclusion] Measurement of MT using USI is reliable and valid for MCSA, but must be combined with assessments of other factors for muscle strength.

Key words: Lower leg muscle, Ultrasound imaging, Reliability

(This article was submitted Mar. 29, 2022, and was accepted Apr. 27, 2022)

INTRODUCTION

Evaluation of lower leg muscles is often performed to assess muscle damage due to trauma, muscle atrophy due to disuse, such as unloading, and recovery from these injuries. The assessment of leg muscles is used not only to identify the local condition of the lower leg but also as an index of total body muscle mass[1)]. However, assessments from the body surface, such as measurements of the lower leg circumference, are limited in accuracy due to the influence of factors, such as subcutaneous fat, bone, and edema. Therefore, imaging devices, such as computed tomography, magnetic resonance imaging (MRI), and dual-energy X-ray absorptiometry are often used to assess muscle conditions[2, 3)].

Ultrasound imaging (USI) has been used in various situations because it can easily confirm the condition of individual muscles[4, 5)]. The USI has an advantage of being able to capture images in any posture, without the disadvantages of radiation exposure or restrictions on patients with pacemakers or metal implants, unlike measurements with the above-mentioned devices[2)]. The relationship between muscle thickness (MT) measurement by USI and muscle mass measurement by MRI has been reported[2, 6)], and its usefulness in the assessment of muscle morphology has been clarified. In recent years, low-cost

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portable devices linked to applications, such as smartphones, have been developed, and their popularity is increasing as a quick method for obtaining information concerning the morphology of the body from any location.

However, there are still several issues regarding the reliability and validity of USI measurements. It is commonly believed that evaluation by USI depends on the skill of the examiner, and that the value varies, depending on the compressive and shear stress and angle of probe inclination[7]. Therefore, the reliability of USI measurements has been investigated for various muscles[8–10]. Additionally, muscles do not have a uniform morphology in the long axis direction, and the MT and muscle cross-sectional area (MCSA) vary across the long axis of muscle regions[11]. It has been reported that not only resting morphology but also changes due to muscle atrophy and hypertrophy vary, depending on the long axis region of the muscle[12, 13]. Therefore, the evaluation of muscle morphology, considering the long axis region, is expected to detect muscle changes sensitively. Furthermore, differences are reported in muscle morphology in the lying and sitting positions due to the effects of gravity. The extent of these differences varies across the muscle long-axis regions[11, 14]. Changes in muscle morphology due to postural changes may be influenced by the muscle mass of the area[11], and the "maximal muscle belly", which is frequently used as an index of the measurement region, may not be at a constant position due to postural changes. The reliability of measurement values in the sitting position must be ensured because measurement by USI in the sitting position is often necessary for reasons, including ease of measurement and limitations of the measurement position. However, most reports on the reliability of lower leg muscle assessment using USI have been conducted in the lying position[15], and most lower leg muscle measurements using USI do not take into account differences due to the measurement region and position.

Accordingly, the purpose of this study was to verify the reliability and validity of the morphological assessment of the lower leg muscles by USI in a sitting position in the long axis of the muscle.

PARTICIPANTS AND METHODS

Twenty healthy adult female individuals (age, 20.3 ± 0.9 years; height, 159.6 ± 5.2 cm; weight, 51.3 ± 5.2 kg) were included in the study. The inclusion criteria were as follows: (1) no pain that interferes with daily life; (2) no typical physical disability due to diseases, such as cerebrovascular disease or rheumatoid arthritis; (3) no history of fracture or surgery of the spine or lower limbs; and (4) no contraindications to MRI, such as hearing aids, tattoos, or insertion of metal objects. As the size of the coil used during the MRI measurement was 300 mm, female participants were selected to fit the measurement area of the lower leg muscles into one coil.

Participants were informed about the purpose and content of the study in advance, and that the data obtained would not be used for any purpose other than the study, with care to prevent leakage of personal information. Written informed consent was obtained from all the participants. The study was approved by the Medical Ethics Committee of Kanazawa University (Approval No.: 1028-1).

Measurements included MT by USI, MT and MCSA by MRI, and maximal muscle strength. The MT measurement by USI was used to examine the intra- and inter-examiner reliability. The MT measurement by USI, MT and MCSA measurement by MRI, and maximal muscle strength were used to examine the validity.

In a study of intra-examiner reliability of MT measurement by USI, the same examiner (examiner A) performed a second measurement a few days after performing the first. For inter-examiner reliability, there were two examiners (examiners A and B), and examiner B performed the measurement for a certain period of time after examiner A recorded the measurement.

The measurement position was 90° knee joint flexion, 0° ankle joint dorsiflexion, and in a sitting position with the lower leg perpendicular to the floor. The USI was performed using a portable ultrasonography system (Pocket Echo Miruco, Nippon Sigmax Co., Ltd., Tokyo, Japan) with a 10 MHz linear probe in B-mode. The target muscles were the tibialis anterior (TA) and medial head of the gastrocnemius (GM) on the right side. To measure the position of the muscle belly, the distance from the fibular head to the lateral malleolus was divided into eight parts, and the TA muscle was imaged at 2/8 (proximal region: 25%), 4/8 (middle region: 50%), and 6/8 (distal region: 75%) from the proximal region, and the GM was imaged at 1/8 (proximal region: 25%), 2/8 (middle region: 50%), and 3/8 (distal region: 75%) from the proximal region. The short-axis image was obtained in such a way that the maximum MT region on the horizontal plane was at the center of the image[11]. To avoid pressure on the muscle by the probe, imaging was performed within the range where the probe did not directly contact the lower leg by using a hard-type gel. The point in the long-axis direction (distance from the fibular head) and short-axis direction (distance from the tibial margin) were measured at the time of the first measurement in order to determine the position of the measurement. The image was captured at that point in the second and subsequent measurements. The MTs of the TA and GM were measured from the acquired images using the image analysis program, ImageJ, with reference to the fascia (Fig. 1).

The MRI was performed using a vertical open MRI (Gravity MRI with 0.4-T permanent magnets, Hitachi, Ltd., Tokyo, Japan), and 30 T1-weighted images were captured from the fibular head to 290 mm distal to the right lower leg. The measurement position was the same as that for USI. Other measurement parameters were as follows: slice plane, axial; pulse sequence, Rf-Spoiled Steady-state Gradient echo; field of view, 280 mm; repetition time, 110.0 ms; echo time, 8.6 ms; flip angle, 35°; slice thickness, 10.0 mm; slice spacing, 10.0 mm; matrix size, 256 × 256; number of averaged signals, 2; receiver bandwidth, 20.6 kHz; and scan time, 4 min 32 s. Additionally, an oral refreshing agent (Breath Care, Kobayashi Pharmaceutical Co., Ltd., Osaka, Japan) was attached to identify the USI measurement point during MRI MT measurement.

Image analysis was performed using the image analysis program, ImageJ. The MTs of TA and GM were measured based on the border of the muscle (Fig. 2a). The area of the region inside the muscle boundary was also measured and used as the MCSA of each muscle (Fig. 2b).

A handheld dynamometer (μTas F-1, Anima Co., Ltd., Tokyo, Japan) was used to measure muscle torque as an index of maximum muscle strength.

Ankle plantar flexion was measured in the supine position with the lower limb extended, and ankle dorsiflexion was measured in the sitting position with the lower leg perpendicular to the floor and the knee joint flexed at 90°. The ankle joint position was set at 0° of plantar dorsiflexion and 0° of rotation in and out for both plantar flexion and dorsiflexion motions. The sensor pad of the hand-held dynamometer was placed on the second metatarsal head, and the lower limb was fixed with a fixation belt to prevent it from moving from that position. In this position, maximal contraction of the right ankle joint during plantar flexion and dorsiflexion was performed, and the muscle torque was measured. For the measurement, we instructed the patient to move the ankle joint without flexing or extending the toes. The measurements were performed twice, and the average value was used as the representative value.

Statistical analysis was performed using SPSS version 27 (IBM SPSS Statistics, Japan IBM, Tokyo, Japan), and the rejection region was set at $p<0.05$. The concordance of MT measurement by USI was analyzed using the intraclass correlation coefficient (ICC) after confirming the normality of the data using the Shapiro–Wilk test. The intra-examiner reliability ICC (1, 1) using the first and second measurements of examinee A, inter-examiner reliability ICC (2, 1) using the first measurement of examinee A, measurement of examinee B, and 95% confidence interval were calculated. The Bland-Altman analysis was used to investigate systematic bias (fixed and proportional biases). To determine the presence of fixed bias, the 95% confidence interval of the difference between the two measurements was calculated, and the presence of fixed bias was determined when the interval did not contain 0. Proportional bias was determined to be present if the regression equation of the difference between the two measurements and mean of the two measurements was significant[16, 17)]. When no systematic bias was observed, the 95% confidence interval of the minimal detectable change (MDC95: standard deviation of the difference between the two measurements × 1.96) was calculated to investigate the range of measurement error[17, 18)]. When only fixed bias was observed, limits of agreement (LOA) in the population were calculated to investigate the tolerance of bias between the two measurements[16)]. When a proportional bias was observed, the LOA was calculated by converting to a

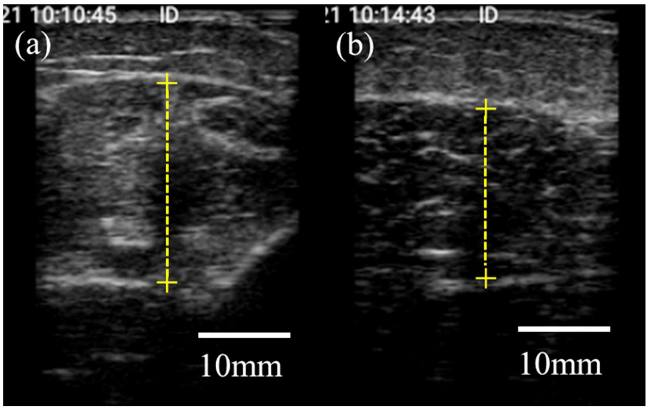

Fig. 1. Muscle thickness measurement by ultrasound imaging. (a) Muscle thickness of the tibialis anterior; (b) muscle thickness of the medial head of the gastrocnemius.

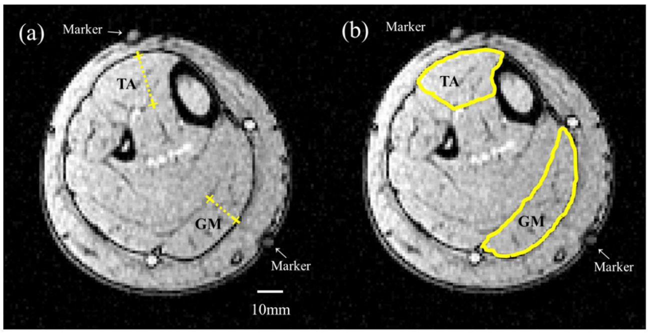

Fig. 2. (a) muscle thickness (broken line) and (b) muscle cross-sectional area measurement (encircled) by magnetic resonance imaging.

percent difference plot[16]. The ICC (3, 1) and Bland-Altman analysis were performed on MT measurement by USI and MT measurement using MRI for evaluating the validity of USI measurement using MRI[9]. The MT of the USI was used as the first measurement of examinee A. Pearson's correlation analysis was performed for the relationship between MT measurement by USI, MCSA measurement by MRI, and maximal ankle muscle strength.

RESULTS

Table 1 shows MT measurement by USI and MRI, and Table 2 shows the results of ICC and systematic bias. The ICC (1, 1) of MT measurement by USI was more than 0.8 in all the regions (25%, 50%, and 75%) for both TA and GM. In the ICC (2, 1), both TA and GM were above 0.8 in all the regions.

The 95% confidence intervals for fixed bias included 0 for all the regions of the TA and GM, for both intra- and inter-examiners. For proportional bias, there was no significant regression between the difference and the mean of the two measurements at all regions of the TA and GM for both intra- and inter-examiners. The MDC95 ranged between 1.3–2.1 mm overall.

Table 1. Muscle thickness by ultrasound imaging and magnetic resonance imaging

Muscle	Region	USI Examiner A 1st (mm) (n=20)	USI Examiner A 2nd (mm) (n=20)	USI Examiner B 1st (mm) (n=20)	MRI (mm) (n=20)
TA	25%	23.6 ± 3.3	23.9 ± 3.2	23.8 ± 3.2	27.6 ± 3.1
	50%	21.8 ± 3.0	22.0 ± 3.1	22.1 ± 3.1	24.5 ± 3.9
	75%	14.8 ± 2.9	14.9 ± 2.5	14.9 ± 2.6	10.2 ± 2.8
GM	25%	17.6 ± 2.7	17.4 ± 2.5	17.2 ± 2.6	20.9 ± 3.0
	50%	17.1 ± 2.6	17.3 ± 2.7	17.3 ± 2.6	20.1 ± 3.1
	75%	15.9 ± 3.0	15.8 ± 2.9	15.8 ± 2.9	19.8 ± 2.9

Data are presented as the mean ± standard deviation.
USI: ultrasound imaging; MRI: magnetic resonance imaging; TA: tibialis anterior; GM: medial head of the gastrocnemius.

Table 2. Intraclass correlation coefficients and systematic bias of muscle thickness measurements by ultrasound imaging and magnetic resonance imaging

Condition	Muscle	Region	ICC (95% CI)	Fixed bias 95% CI of difference	Proportional bias Correlation coefficient	MDC_{95} (mm)	LOA
Intra- examiner reliability (n=20)	TA	25%	0.95 (0.88 to 0.98)	−0.53 to 0.44	0.17	2.0	-
		50%	0.96 (0.91 to 0.99)	−0.48 to 0.31	0.04	1.6	-
		75%	0.95 (0.87 to 0.98)	−0.62 to 0.22	0.28	1.8	-
	GM	25%	0.93 (0.83 to 0.97)	−0.41 to 0.59	0.06	1.8	-
		50%	0.94 (0.86 to 0.98)	−0.57 to 0.30	0.20	2.1	-
		75%	0.92 (0.82 to 0.97)	−0.87 to 0.13	0.27	2.1	-
Inter- examiner reliability (n=20)	TA	25%	0.96 (0.90 to 0.98)	−0.65 to 0.23	0.08	1.8	-
		50%	0.95 (0.88 to 0.98)	−0.72 to 0.17	0.12	1.9	-
		75%	0.96 (0.90 to 0.98)	−0.44 to 0.30	0.32	1.5	-
	GM	25%	0.93 (0.82 to 0.97)	−0.15 to 0.78	0.11	1.3	-
		50%	0.97 (0.93 to 0.99)	−0.35 to 0.26	0.05	2.0	-
		75%	0.94 (0.86 to 0.98)	−0.38 to 0.57	0.13	2.0	-
USI and MRI (n=20)	TA	25%	0.79 (0.55 to 0.91)	3.00 to 4.95*	0.09	-	1.6 to 6.4 (mm)
		50%	0.87 (0.69 to 0.94)	1.81 to 3.52*	0.52*	-	0.0 to 0.2 (%)
		75%	0.45 (0.02 to 0.74)	−5.96 to −3.21*	0.05	-	−8.0 to −1.2 (mm)
	GM	25%	0.83 (0.61 to 0.93)	2.50 to 4.05*	0.17	-	1.4 to 5.2 (mm)
		50%	0.91 (0.79 to 0.96)	2.35 to 3.48*	0.47*	-	0.1 to 0.2 (%)
		75%	0.89 (0.74 to 0.95)	3.24 to 4.56*	0.05	-	2.3 to 5.5 (mm)

*Bias was observed.
USI: ultrasound imaging; MRI: magnetic resonance imaging; ICC: intraclass correlation coefficient; CI: confidence interval; MDC: minimal detectable change; LOA: limits of agreement; TA: tibialis anterior; GM: medial head of the gastrocnemius.

The ICC (3, 1) of MT measurement by USI and MRI was more than 0.8 in all regions except for 25% (0.79) and 75% (0.45) of the TA. For fixed bias, the 95% confidence interval of the difference did not include 0 for any TA and GM regions. For proportional bias, only 50% of both the TA and GM showed a significant regression between the difference in the two measurements and mean value.

The MRI measurements of MCSA and correlation coefficients between the MCSA and MT measurement by USI are shown in Table 3. Both the TA and GM showed significant positive correlations in all the regions.

For maximum ankle muscle strength, plantar flexion strength was 10.2 ± 3.0 (kgf), and dorsiflexion strength was 16.5 ± 4.7 (kgf). The correlation coefficients between maximum ankle muscle strength and MT measurements by USI and MCSA measurements on MRI are shown in Table 4. There was no significant correlation between the ankle dorsiflexor strength and MT of the TA and between the ankle plantar flexor strength and MT of the GM in any region. Ankle dorsiflexor strength and MCSA of TA were significantly correlated only in the 50% region, and ankle plantar flexor strength and MCSA were significantly correlated only in the 25% region.

DISCUSSION

This study investigated the reliability and validity of the measurement of lower leg muscle morphology using portable USI in a sitting position at the muscle long axis region.

The results of this study showed that the intra- and inter-examiner ICC values were greater than 0.9 for all the regions in the TA and GM, and that the lower limits of the 95% confidence intervals for ICC were all greater than 0.8. Intraclass correlation coefficient is considered to be “almost perfect” above 0.8[19]. For systematic bias, there was no fixed or proportional bias for the intra- and inter-examiners in all regions of the TA and GM. In a previous study, MT measurement by USI of the lower leg muscles showed high measurement reliability regardless of the presence or absence of markings or other measurement point specifications[20, 21], which was consistent with the results of this study. Therefore, it can be interpreted that the reliability of lower leg MT measurement by USI in the sitting position was high for all regions of the TA and GM. Other than

Table 3. Correlation coefficient between muscle cross-sectional area measured by magnetic resonance imaging and muscle thickness measured by ultrasound imaging

Muscle	Region	Muscle cross-sectional area (mm^2)	Correlation coefficient (n=20)
TA	25%	793.3 ± 143.2	0.77*
	50%	457.0 ± 119.9	0.78*
	75%	70.3 ± 38.8	0.57*
GM	25%	1,229.1 ± 316.6	0.74*
	50%	1,360.4 ± 331.6	0.91*
	75%	1,275.1 ± 348.6	0.91*

*Significant correlation (p<0.05).
TA: tibialis anterior; GM: medial head of the gastrocnemius.

Table 4. The correlation coefficient between muscle thickness measurement by ultrasound imaging, muscle cross-sectional area measurement by magnetic resonance imaging, and maximum ankle muscle strength

Direction of motion	Muscle	Region	Correlation coefficient (n=20)
Ankle dorsiflexion	TA muscle thickness	25%	0.27
		50%	0.42
		75%	0.14
	TA MCSA	25%	0.41
		50%	0.46*
		75%	0.33
Ankle plantarflexion	GM muscle thickness	25%	0.13
		50%	0.16
		75%	0.13
	GM MCSA	25%	0.49*
		50%	0.30
		75%	0.35

*Significant correlation (p<0.05).
TA: tibialis anterior; GM: medial head of the gastrocnemius; MCSA: muscle cross-sectional area.

systematic bias, there is a random error, which can be divided into biological variation and measurement error[16]. The MDC is the degree of measurement error in the change of two values obtained by repeated measurements, and changes within the MDC are considered to be due to measurement error[17]. In this study, the MDC95 ranged between 1.3–2.1 mm, which means a difference of at least 1.3 mm in the TA and GM can be regarded as an error when remeasuring; a difference greater than 2.1 mm can be interpreted as more than an error.

Regarding the degree of agreement with MT measurement by MRI, 25% of the TA was “substantial” (0.6–0.8), 75% was “moderate” (0.4–0.6), and others were “almost perfect”[19]. Systematic bias was observed at all sites for the TA and GM. This is because USI measures the distance inside the fascia as MT, but MRI cannot identify the boundary between the muscle and fascia; therefore, MRI may include the fascia while measuring the distance. It is possible that this effect caused the overall value of MT measurement by MRI to be greater than that of USI. In contrast, there was a positive correlation between the MCSA measurement by MRI and MT measurement by USI in all the regions. In our previous study[11], the only region with a significant positive correlation between MT measurement by USI and MCSA measurement on MRI was the 50% region of the GM, which was different from the present study. The reason for this is that in this study, the measurement point was precisely defined in the MRI measurement. These results suggest that MT measurement by USI is closely related to MCSA measurement on MRI, and it is a valid morphological index for the muscle.

There was no significant correlation between muscle strength and MT measurement by USI in any region for both the TA and GM. The anatomical cross-sectional area perpendicular to the muscle is not a sufficient indicator of muscle strength, and the importance of the physiological cross-sectional area perpendicular to the muscle fibers has been highlighted[22]. The MT in this study was measured perpendicular to the muscle, as well as the anatomical cross-sectional area, which was not considered to reflect the physiological cross-sectional area. In addition to the above, it is possible that the activity of muscles other than the TA and GM occurred, although the participants were instructed not to perform toe movements during the measurement of maximum muscle strength in this study. Ankle plantar flexion and dorsiflexion are not performed by a single muscle, but by the activity of several muscles. Particularly, for ankle joint plantar flexion, there are other muscles besides GM, such as the lateral head of the gastrocnemius and soleus muscle, and the soleus muscle, in particular, is known to have a physiological cross-sectional area several times larger than that of the GM[23]. In this study, the fact that there were many regions where MCSA measurement by MRI was not related to ankle muscle strength supports that MT measurement by USI is not related to ankle muscle strength for the two reasons mentioned above. These results suggest that although MT measurement of the TA and GM by USI in the sitting position is useful as a morphological index, such as the MCSA, it is not sufficient as an estimator of muscle strength and should be evaluated in combination with other factors.

One of the limitations of this study is that the measurement point was defined. In a study that investigated the reliability of MT measurement using USI, those that used marking to define the measurement point, as in this study, showed higher reliability than those that did not define the point[15]. However, in actual measurements, it is often necessary to determine the measurement point of MT each time; therefore, the reliability while including this point must be considered in the next step. Magnetic resonance imaging is also more likely to be used as a criterion for criterion-related validity in USI because of its ability to produce accurate images[2, 6, 24]. However, MT measurement by MRI had unclear fascial boundaries, which may have led to errors. Therefore, it is necessary to investigate whether MT measurement by MRI is appropriate as a standard when considering the validity of MT measurement by USI.

In conclusions, this study investigated the reliability and validity of the morphological assessment of the lower leg muscles in the sitting position by USI in the long axis of the muscle. As a result, the reliability of the USI measurement of MT in the sitting position was satisfactory. The MT measurement by USI was positively correlated with MCSA on MRI, but not with maximal muscle strength. Therefore, we believe that the measurement of MT by USI in the sitting position is useful for the evaluation of muscle morphology, but that it needs to be combined with the evaluation of other factors for muscle strength.

Funding

This research was funded by JSPS KAKENHI grant number JP20K11204.

Conflict of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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