Original Article

Verification of the accuracy of measuring the muscle cross-sectional area and muscle intensity of the rectus femoris using ultrasonography

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ABSTRACT

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Objective: This study aimed to establish ultrasonography as a method of measuring the muscle cross-sectional area and muscle intensity of the rectus femoris and to assess its measurement accuracy.

Methods: Three testers measured the muscle cross-sectional area and muscle intensity of the rectus femoris using ultrasonography in 16 healthy male volunteers. Intra-rater and inter-rater reliabilities were evaluated using the intraclass correlation coefficient (ICC), and Bland-Altman (B-A) analysis was used to confirm the systematic error and measure the minimum detectable change.

Results: Intra-tester and inter-tester ICCs of the muscle cross-sectional area and muscle intensity were ≥0.9 and ≥0.7, respectively. On B-A analysis, no systematic error was observed in each measurement. The minimum testable changes were 0.39 cm^2 for the intra-tester cross-sectional area, 0.15 cm^2 for the inter-tester cross-sectional area, 6.77 for the intra-tester intensity, and 4.47 for the inter-tester intensity.

Conclusion: The muscle cross-sectional area and muscle intensity of the rectus femoris can be measured with high accuracy using this measurement method. In addition, changes larger than the minimum detectable change can be used as a true change in clinical efficacy assessments.

Key words: ultrasonography, rectus femoris, muscle intensity, muscle cross-sectional area, measurement accuracy

Introduction

Aging leads to a decrease in the number and size of muscle fibers [1], an increase in connective tissue and infiltration of fat into skeletal muscle [2], and disease, resulting in decreased quality and quantitative function of skeletal muscle, such as inflammatory fasciitis and myonecrosis [3]. Such skeletal muscle dysfunction has attracted attention as an important outcome, since it is associated with physical function and secondary complications such as mortality [4] and falling fractures, rehospitalization rates [5, 6], and quality of life (QOL) [7]. In recent years, techniques such as ultrasonography (US), bioelectrical impedance, dual-energy X-ray absorptiometry, computed tomography, and magnetic resonance imaging have been used to evaluate the quality and quantity of skeletal muscle. Among them, skeletal muscle evaluation using US can provide information such as muscle cross-sectional area (CSA), echo intensity (EI), muscle thickness, and pennation angle for each muscle. US is a useful evaluation method for frail patients or those who are critically ill in the medical setting because it can easily be conducted noninvasively at the bedside [8].

Previous studies have shown that measurement of

CSA and EI using US is as accurate as that using a conventional device [9, 10]. US is used in a variety of fields because it can simultaneously assess skeletal muscle mass and quality in a quick and noninvasive manner. Recently, EI has been used as a biomarker for aging and disease changes, such as skeletal muscle fat infiltration and inflammatory response, and it has been reported to be associated with physical frailty and a reduced QOL [3, 11]. EI can be expressed as an objective numerical value in the range of 0 (black) to 255 (white) on a grayscale using a histogram function. The larger the numerical value of EI, the greater the percentage of connective tissue and adipose tissue in the muscle. Since Heckmatt et al. [12] reported on EI in 1982, it has been used in longitudinal studies, such as cross-sectional studies of age-related changes and assessment of the effectiveness of training. However, few reports have specified the 95% minimal detectable change (MDC_{95}), which is a clinically significant difference. Radaelli et al. [13] reported improved EI with strength-specific training for the elderly, but did not consider MDC_{95}. Owing to the characteristics of the device, which visualizes reflected waves (echoes) obtained by applying ultrasonic waves emitted by an ultrasonic diagnostic imaging device to an object, measurement errors may be caused by the method of operating the probe or by the type of instrument. Therefore, it is important to examine the reliability of the measurement sites and methods and to confirm the error range.

This study aimed to verify the accuracy of CSA (RF_{CSA}) and EI (RF_{EI}) of the rectus femoris (RF) using US as an evaluation method, as it can be used at the bedside without requiring extra equipment.

Methods

1. Subjects

The study participants included 16 healthy male volunteers (age 21.5 ± 0.82 years) without a history of severe orthopedic problems of the lower extremities or cerebrovascular disease. Before the start of the study, the subjects were fully informed and their consent to participate in the study was obtained.

2. Testers

Three testers (testers A, B, and C) who had no experience with ultrasound imaging performed US. Testers A, B, and C were physical therapists with clinical experience of 4, 6, and 10 years, respectively. To standardize the proficiency of the US imaging techniques, none of the selected testers had experience with US. The tester received an explanation of the evaluation method and practiced measurement for about 1 hour, then the testing was started.

3. Imaging method of US

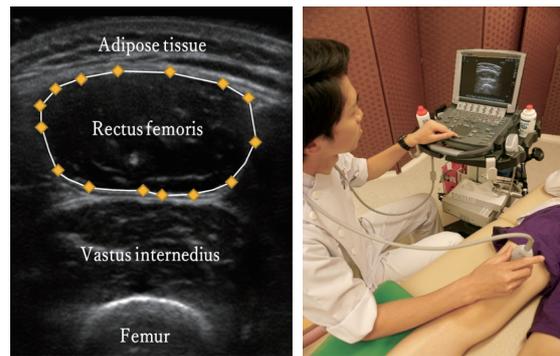The posture during the US was modified in

Figure 1. Cross-sectional imaging and measurement of the femoral region using US.
Superficial views of the adipose tissue, rectus femoris, vastus intermedius, and femur can be obtained by placing an ultrasound probe perpendicular to the femur at the distal third of the subject's femur. Image analysis of the rectus femoris was performed using a convex hull to analyze polygonal-enclosed areas, not including the fascia superior, right and the tendon of the insertion of the rectus femoris inferior.

accordance with the method of Fukumoto et al. [14]. The subjects were positioned in the supine position. After the lower extremities had become sufficiently weak, the posture was adjusted to the neutral position of the hip joint with a cushion (Figure 1). US equipment (SonoSite M-turbo, FujiFilm) and a linear probe (56 mm, 6–15 MHz) were used for measurement. The setting was standardized to the Brightness mode, which can show the intensity of the echo. The echo-jelly used was a hard-type jelly (Conductor™ Transmission Gel, Chattanooga) that prevented the probe from coming in contact with the skin.

Image evaluation of RF was based on three criteria: (1) the probe did not come in contact with the skin; (2) the RF was placed in the image as a left-to-right indicator; and (3) the probe angle was adjusted so that the femur became high intensity as a vertical indicator (Figure 1). In this study, the RF needed to be placed in the image, so the measurement site was located in the distal third of the line connecting the superior anterior superior iliac spine to the superior border of the patella. At the time of measurement, the probe was manipulated, and the image was taken while the monitor screen was viewed. The three testers evaluated 16 subjects for three consecutive evaluations. Because there was a possibility of the measurement results of each tester causing bias (measurement bias) because of the cross-sectional area and intensity information, the measurement images and measurements of each tester were managed so that they could not be seen by the other testers.

4. Image analysis

All image analyses were performed by a single analyst (author) who was experienced in such analysis. Data processing and US were performed on the same

day. Image J software (15.0i, NIH) was used for image analysis. Berger et al. [15] performed image analysis in a partially modified fashion. First, the magnification rate was adjusted using the marks for every 1 cm displayed on the side of the image. The entire RF was then surrounded by a polygon using a convex hull (Figure 1). With respect to the boundary between the soft tissue around the RF, the fascia was circumferentially surrounded with the upper and right and left sides, and the cessation tendon of the RF was not included in the lower part. The enclosed area was designated RF_{CSA}, and the 8-bit gray scale using histogram analysis was quantified in the range from 0 to 255. The mean value of the area was designated as RF_{EI}.

5. Evaluation of measurement accuracy

Measurement accuracy was evaluated for relative reliability and absolute reliability, and the efficacy of the measurement was investigated in clinical applications and in the reproducibility of the measurement. Relative reliability was assessed in terms of intra-class correlation coefficients (ICCs) to assess inter- and intra-rater reliabilities. Absolute reliability was assessed using the Bland-Altman analysis (B-A analysis) to assess the presence or absence of systematic error (additivity error, proportional error) and MDC_{95}. B-A analysis is the recommended method for clinical application of a new evaluation because it can determine the error caused by the measurement method and the performance of the analyst, and can obtain MDC_{95} [16].

6. Statistical analysis

Data normality was confirmed using the Shapiro-Wilk test. The stability of the measurements of RF_{CSA} and RF_{EI} was evaluated by the intra-tester coefficient of variation. The coefficient of variation was calculated by dividing the mean value by the standard deviation. The mean values were measured for each tester after the first and second, first and third, and second and third examinations.

ICC (1, 1) and ICC (2, 1) of RF_{CSA} and RF_{EI} were calculated from the results of each tester. ICC (2, 1) was analyzed using the first measurements of each tester in three patterns: A and B, A and C, and C and B. Subsequently, the Spearman-Brown formula was used to calculate the number of repeated measurements that assured a reliability of ICC (1, 1) and ICC (2, 1) of 0.9 or higher.

The B-A analysis used the first and second measurements for each tester and generated a scatter plot (B-A plot) with the difference between the two measurements on the y-axis and the mean of the two measurements on the x-axis. The additivity error was determined by calculating the 95% confidence interval (CI) for the mean difference between the two measurements and determining the presence or absence of the additivity error when the interval did

not include zero [17, 18]. The proportional error was then tested for significance of the correlation, and when the t-value obtained in Eq. 1 was greater than $n-2$ degrees of freedom and the t-value of the 5% level of significance, proportional error was considered to have existed [19]. MDC_{95} was calculated using Eq. 2. Statistical analysis was performed using the Shapiro-Wilk test, the ICC using SPSS statistics version 24 (IBM Corp., Armonk, NY, USA), and the B-A analysis using R2.8.1. The significance level was set at <5%.

$$t = r\sqrt{\frac{n-2}{1-r^2}} \qquad \text{(Eq. 1)}$$

$$MDC_{95} = 1.96 \times SD_d \qquad \text{(Eq. 2)}$$

r: Correlation coefficient between the difference between the two measurements and the mean of the two measurements in the data group
n: sample size
SD: standard deviation of the difference between the two measurements

Results

US images of all subjects were able to be accurately imaged, meeting the three measurement criteria described above. Table 1 shows the measured values and coefficients of variation for each analyst.

ICCs (1, 1) were >0.9 for RF_{CSA} and 0.765–0.882 for RF_{EI}. ICCs (2, 1) were >0.9 for RF_{CSA} and 0.772–0.875 for RF_{EI}. Using the lower limit of the RF_{EI}, three replicate measurements were used to assure a reliability of ≥0.9 for ICC (1, 1), (2, 1) from Spearman-Brown's formula (Tables 2 and 3).

As a result of the B-A analysis, there were no additional and proportional errors intra- and inter-tester; MDC_{95} had an RF_{CSA} of A, 0.33 cm²; B, 0.39 cm²; C, 0.37 cm²; RF_{EI} of A, 5.69; B, 6.77; C, 6.57; inter-tester RF_{CSA} of A, 0.27 cm²; B, 0.33 cm²; C, 0.37 cm²; and RF_{EI} of A, 5.17; B, 4.64; C, 6.66 (Table 4, 5, Figure 2).

Discussion

This study investigated the precision of measuring RF_{CSA} and RF_{EI} using ICC and B-A analysis as a basic study for clinical application of US. To the best of our

Table 1. Measurement value and Coefficient of variation of RF_{CSA} and RF_{EI}.

Tester	RF_{CSA} (cm²)	CV (%)	RF_{EI}	CV (%)
A	5.49±1.2	2.4	26.7±5.1	5.9
B	5.46±1.1	2.5	27 ±5.7	7.0
C	5.51±1.2	2.5	26.5±4.5	7.9

Mean ± standard deviation, %
RF, rectus femoris; CSA, cross-sectional area; EI, echo intensity; CV, coefficient of variation.

Table 2. Intra-rater reliabilities of RF_{CSA} and RF_{EI}.

Tester	ICC (1, 1)			
	RF_{CSA}	95%CI	RF_{EI}	95%CI
A	0.99	0.96–0.99	0.85	0.64–0.95
B	0.98	0.95–0.99	0.83	0.60–0.94
C	0.99	0.96–0.99	0.75	0.42–0.90

ICC, intraclass correlation coefficient; RF, rectus femoris; CSA, cross-sectional area; EI, echo intensity; 95% CI, 95% confidence interval.

Table 3. Inter-rater reliabilities of RF_{CSA} and RF_{EI}.

Tester	ICC (2, 1)			
	RF_{CSA}	95%CI	RF_{EI}	95%CI
A-B	0.99	0.98–0.99	0.88	0.69–0.96
A-C	0.98	0.90–0.99	0.81	0.53–0.93
B-C	0.97	0.84–0.99	0.77	0.46–0.91

ICC, intraclass correlation coefficient; RF, rectus femoris; CSA, cross-sectional area; EI, echo intensity; 95% CI, 95% confidence interval.

knowledge, this is the first report both in Japan and abroad that specifies MDC_{95} by verifying the precision of intra- and inter-tester measurements at the same measurement sites of RF_{CSA} and RF_{EI} using US.

With regard to ICC, Kuwahara et al. [20] concluded that ≥0.9 indicates excellent performance; ≥0.8, good performance; ≥0.7, usual availability; ≤0.6, reconsider; and ≥0.8, recommended reconsideration for Landis et al. [21]. In this study, the mean ICC values were ≥0.9 for RF_{CSA} and ≥0.8 for RF_{EI} for both intra- and inter-tester reliability. This indicates that the measurement is reliable regardless of the tester. In addition, the results of the B-A analysis showed that both intra- and inter-tester reliability 95% CIs were zero, and no additional error was found. No significant intra- and inter-tester correlations were found in the significance of the correlations, indicating that there was no proportional error. This suggests that an increase in the number of measurements can improve the precision of measurement and that three mean values must be used when both RF_{CSA} and RF_{EI} are secured with an ICC ≥0.9.

The reliability of US is related to measurement posture, relaxation of the target muscles, and manipulation of the probe (e.g., angle, skin compression), but standardized methods to minimize these effects have not been

Table 4. Systematic error of intra-tester and MDC_{95} by Bland-Altman analysis.

	Tester	Additivity error		Proportional error		MDC_{95}
		95%CI	existence	*p*-value	existence	
RF_{CSA}	A	−0.24〜0.11	No	0.89	No	0.33 (cm^2)
	B	−0.29〜0.11	No	0.52	No	0.39 (cm^2)
	C	−0.10〜0.29	No	0.25	No	0.37 (cm^2)
RF_{EI}	A	−3.47〜2.55	No	0.95	No	5.69
	B	−3.69〜3.47	No	0.57	No	6.77
	C	−3.62〜3.33	No	0.76	No	6.57

MDC_{95}, 95% minimal detectable change; RF, rectus femoris; CSA, cross-sectional area; EI, echo intensity; 95% CI, 95% confidence interval.

Table 5. Systematic error of inter-tester and MDC_{95} by Bland-Altman analysis.

	Tester	Additivity error		Proportional error		MDC_{95}
		95%CI	existence	*p*-value	existence	
RF_{CSA}	A-B	−0.04〜0.11	No	0.28	No	0.27 (cm^2)
	A-C	−0.23〜0.05	No	0.56	No	0.33 (cm^2)
	B-C	−0.27〜0.07	No	0.79	No	0.37 (cm^2)
RF_{EI}	A-B	−2.15〜0.66	No	0.67	No	5.17
	A-C	−0.98〜1.54	No	0.25	No	4.64
	B-C	−0.78〜2.84	No	0.24	No	6.66

MDC_{95}, 95% minimal detectable change; RF, rectus femoris; CSA, cross-sectional area; EI, echo intensity; 95% CI, 95% confidence interval.
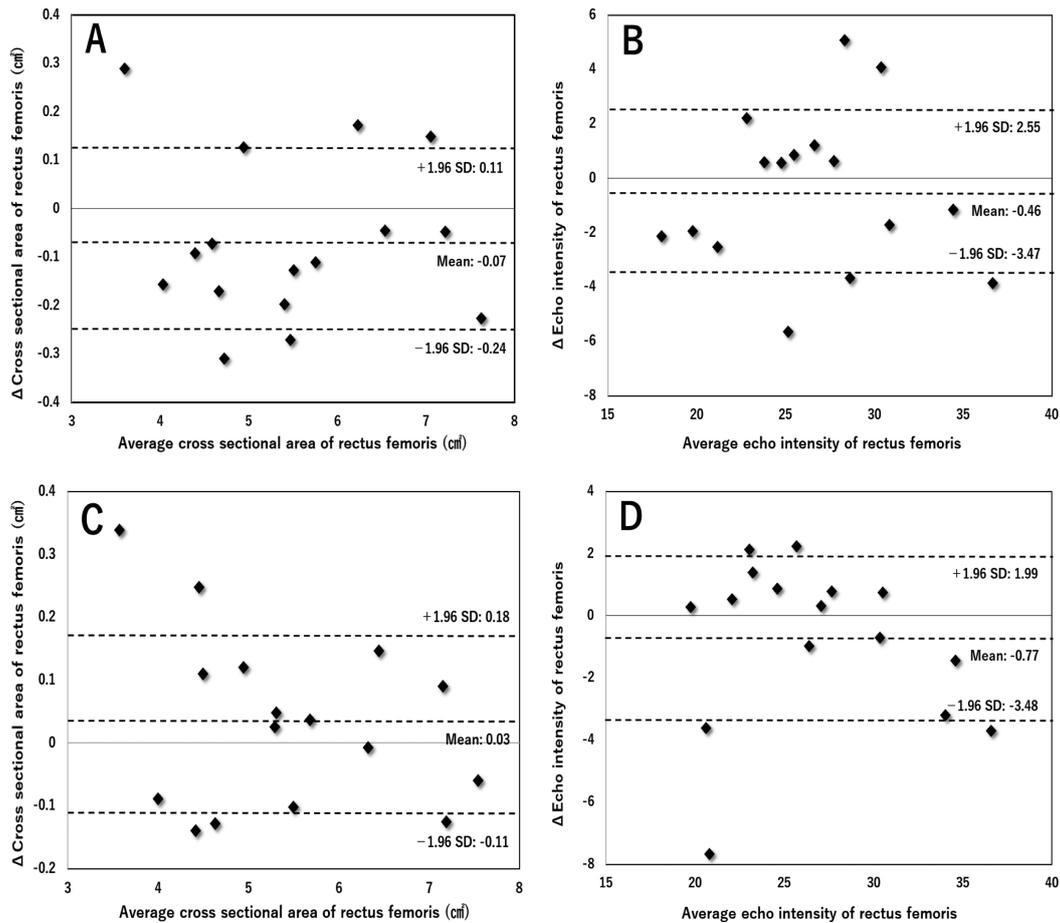
Figure 2. B-A plot of intra- and inter-tester.
(A) Intra-tester of the muscle cross-sectional area; (B) Inter-tester of the muscle cross-sectional area; (C) Intra-tester of muscle intensity; (D) Inter-tester of muscle intensity.
The mean and error tolerances for the differences in muscle cross-sectional area and muscle intensity measurements are shown as dashed lines. The mean difference between the measured values approximates zero and is distributed almost evenly with respect to both positive and negative values, indicating that there is no fixing error. Also, there is no tendency for the difference in the measurement value to increase or decrease with an increase in the muscle cross-sectional area and the muscle intensity.

established. Even in actual evaluations, there are often errors because of compression of the skin that are not experienced when searching for the target muscles and adjusting the probe angle while observing the monitor images of the ultrasonic device. Recently, it has been reported that the rectus femoris is relaxed by lifting the leg in the edge-sitting position [15], that the probe is vertically depicted by holding the cable and suspending it [22], and that the probe contact pressure can be unified by using a fixator [23]. However, when evaluating frail patients at the bedside, adjustment of the fixing device or postural adjustment may affect the duration of the assessment or restrict the measurement environment. In this study, to stabilize the angle of the probe, a hard-type echo-jelly was used to prevent soft tissue compression, and a cushion was used to secure the femur in the neutral position. As a result, even inexperienced individuals can easily adjust the vertical index, and it is believed

that this leads to high measurement accuracy. Because this method does not require special equipment or environment, it is considered that it can be used for measurement at the bedside for patients who are under bed rest.

It is important to evaluate the effectiveness of this measurement method in relation to the outcomes of the study and the clinical application, taking into account random errors generated by measurements in the pre- and post-intervention comparisons and assessment of the effects of the intervention. In this study, MDC_{95} was used for the measurement of chance error. MDC_{95} represents the threshold value after which the difference in two measurements is due to measurement error, and if the difference is within the MDC_{95} value, it is possible to judge the "true change" [18]. Previous studies have reported muscle thickness MDC_{95} in various muscles [13, 15]. However, reports on CSA and EI are lacking.

Vieira et al. [22] reported an EI MDC_{95} of 10.23 for the biceps brachii muscle, and Rosenberg et al. [24] reported an MDC_{95} of CSA for the medial gastrocnemius muscle to be 1.995 cm². O'Brien et al. [25] reported that the EI of the biceps brachii, deltoid, flexor digitorum superficialis, tibialis anterior, gastrocnemius medialis, and peroneus tertius varies according to the muscle, with coefficients of variation of 12.7%, 8.4%, 8.4%, 10.0%, 7.4%, and 9.6% even for proficient assessors. Since the measurement accuracy varies according to the measured muscle, it is necessary to evaluate the measurement accuracy for each measurement muscle and measurement method. This study also showed that the mean intra-test coefficients of variation were 2.5% for RF_{CSA} and 6.9% for RF_{EI}, and MDC_{95} was better than that in previous studies [14, 16, 24, 25] in which RF_{CSA} testers were 0.36 cm², inter-tester were 0.32 cm², inter-tester were 6.34, and inter-tester were 5.49 for RF_{EI}. There are two reasons why the measurement accuracy of RF_{EI} was slightly inferior to that of RF_{CSA}. First, the intensity of the femur was used as an index. As the intensity of the femur is measured only visually with a subjective judgment of the image on the monitor, it is not possible to confirm the previous image once the probe is released. Therefore, it is considered that errors occurred in the continuous measurement. Second, the deeper the tissue is, the greater the attenuation of the echo and the more obscure the image due to the characteristics of US. The femur lies deepest, and the distance to the femur varies between subjects due to differences in subcutaneous fat and muscle thickness. Because the echo settings were uniform, differences in the distance to the femur may have altered the degree of difficulty in modulating intensity. However, this measurement method did not cause systematic errors within and between the testers. In addition, it has the potential to be clinically applicable as one of the methods for measuring RF_{CSA} and RF_{EI}, since it has the same accuracy as reports using special equipment and reports by experienced testers.

The limitation of this study is the lack of evaluation accuracy among proficient testers. Although there are no objective indicators of US proficiency, obviously the trainees tend to identify the target muscles and probe angles quickly and accurately. Because testers' blinds require many testers with randomize controlled trial, it is necessary to minimize errors due to proficiency. It is also necessary to compare measurement accuracy in terms of proficiency in the future. In addition, in terms of image analysis, only one experienced image analyst performed the analysis, so it is necessary to examine the errors that arise in the process of image analysis.

Abbreviations

ICC, intraclass correlation coefficient
B-A analysis, Bland-Altman analysis
QOL, quality of life
US, skeletal muscle ultrasonography
CSA, muscle cross-sectional area
EI, muscle echo intensity
MDC_{95}, 95% minimal detectable change
RF, rectus femoris

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