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Title: Reliability of ultrasonographic measurement of muscle and tendon morphology using the Overlapping Images Method

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Brief running title (70 characters or less): Overlapping Images Method for triceps surae

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Abstract

Problem addressed: Previous attempts have been made to superimpose ultrasound images (Overlapping Images Method). The merit of this method is that it enables capturing the entire morphology of the muscle and tendon in the longitudinal direction beyond the width of the ultrasound probe. However, the division points between tissues were identified visually, and the reliability of the measurement was not clarified.
Experimental approach: This study identified the division points for the overlapping images method using the following two procedures: (1) visual-only identification and (2) a combination of visual and custom-built software-based identification. Finally, the reliability of the measurements for each procedure was examined by estimating the intra- and inter-rater correlation coefficients (ICC 1.1, ICC 2.1). The length of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle was measured using the overlapping images method for 19 volunteers. **Main results and findings:** The ICC 1.1 and ICC 2.1 scores for visual identification alone ranged between 0.61–0.91 and 0.70–0.93, respectively. The scores for the combination of visual and software-based identification ranged 0.90–0.99 and 0.58–0.96, respectively. **Conclusions:** The high intra- and inter-rater reliability in the overlapping images method was demonstrated in almost all measurements of the three tissue lengths and the applicability of overlapping

images method was demonstrated. In particular, the intra-rater reliability was better when a combination of visual and custom-built software-based identification was used.

Keywords: Achilles tendon, gastrocnemius muscle, ultrasound image, reliability

Information in Japanese

タイトル：「オーバーラッピング画像法」による超音波を用いた筋及び腱の形態評価の信頼性

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Problem addressed: 先行研究において、「オーバーラッピング画像法」と呼ばれる個別の超音波画像を重ね合わせる手法によって、筋や腱の形態を測定する試みがされている（Urlando, A., & Hawkins, D. (2007). Med Sci Sports Exerc., 39(7): 1147-1152）。この手法のメリットは、プローブ幅に依存せず、長軸方向で組織の全長を計測できる点である。しかしながら、先行研究では重ね合わせた

超音波画像に描出される組織の分岐点について目視で識別されており、この手法の信頼性について明確に示されていない。**Experimental approach:** そこで本研究では、(1) 目視により識別、(2) 目視とカスタマイズしたソフトウェアの両方による識別、以上 2 つの手順により超音波画像上の組織の分岐点を定義し、それぞれの手順によるオーバーラッピング画像法の検者内及び検者間信頼性（ICC1.1, ICC2.1）を検討することとした。対象は健常成人 19 名とし、アキレス腱、筋腱部複合部、腓腹筋の全長をオーバーラッピング画像法を使用して計測した。**Main results and findings:** 目視による識別に基づく測定において ICC1.1 と ICC2.1 のスコアは、それぞれ 0.61-0.91 と 0.70-0.93 の範囲内であった。一方、目視とソフトウェアの両方による識別に基づく測定において ICC1.1 と ICC2.1 のスコアは、それぞれ 0.90-0.99 と 0.58-0.96 の範囲内であった。
Conclusions: オーバーラッピング画像法における検者内及び検者間信頼性はおおよそ高いものと評価され、応用可能性が示された。特に、目視とソフトウェアの両方による識別に基づく測定では、目視のみの識別の場合と比べて検者内信頼性が高かった。

Introduction

Most previous studies have used ultrasound to evaluate muscle morphology, as it is non-invasive and measurements are performed in real time. It is well-known that ultrasound has been widely used to capture morphological characteristics in sports sciences. For example, we previously measured muscle morphology and extensibility using b-mode ultrasound to investigate the effect of massage interventions before and after the intervention [1,2]. In addition, based on the morphological characteristics reflected on ultrasound images, attempts have been made to detect age-related changes in muscle mass in growing children and to explain the effects of long-term strength training on muscle mass [3,4]. However, a limitation of morphological measurements by b-mode ultrasound is that the measurable area depends on the width of the ultrasound probe. Therefore, morphological characteristics generally cannot be measured over an area wider than the width of the ultrasound probe. Although the panoramic view function has been used in recent years to overcome this limitation and seems to be reliable and valid [5], devices equipped with this function are limited.

Some scientists have attempted to superimpose ultrasound images obtained separately by moving the probe longitudinally over the skin of the Achilles tendon and gastrocnemius muscle. This method is called the “Overlapping Images Method (OIM)”

[6,7,8]. However, a serious problem is the reliability of this method for visually identifying the division points reflected in ultrasound images, such as the proximal and/or distal tendon and muscle ends, has not been fully clarified.

Against this backdrop, the present study identified the division point of OIM using the following two procedures: (1) conventional visual-only identification and (2) a combination of visual and custom-built software-program-based identification, which was conducted out of concern regarding the low reliability of visual-only identification. We then examined the intra- and inter-rater reliabilities of the OIM measurements for each procedure.

Based on the only report showing that the high intra-rater reliability was achieved for the OIM measurement of Achilles tendon length [7], we hypothesized that the OIM measurement would be a reliable method for both procedures. We also predicted that the reliability would improve by using a combination of visual and software identification.

Materials and Methods

Study population

This study was approved by the Research Ethical Review Committee with

Human Subjects of Waseda University, and all procedures were performed in accordance with the Declaration of Helsinki (approval number: 2016-101). Data obtained from nineteen volunteers (12 men and 7 women) was analyzed. All participants were fully informed of the procedure and purpose of the study, and provided written informed consent.

Procedure

Participants were instructed to lie on their backs on a Biodex chair (Biodex System 3; SAKAI Medical Co., Ltd., Tokyo, Japan), with the seat placed approximately parallel to the floor. The right ankle joint was fixed to a plate attached to Biodex, and the knees and hips were maintained in neutral positions. When the fixed ankle was moved passively with the plate to prevent excessive varus and valgus joint motions, the maximum dorsiflexion and plantar flexion ranges of motion of the ankle displayed on the Biodex screen were recorded. Simultaneously, an electromagnetic 2-dimensional goniometer (DTS EM-852; SAKAI Medical Co., Ltd., Tokyo, Japan) was attached to the skin along the right femur and fibula lines to monitor the knee joint range of motion during the ankle's passive movement so that the compensation movement could be regulated to within ± 5°.

Ultrasound measurement

Ultrasound images of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle were obtained based on the processes of the OIM [6,7,8] with the ankle joint in three different positions: neutral, maximum dorsiflexion, and maximum plantar flexion. These processes include the following steps:

[Step-1] Pieces of cut thread were arranged and stuck with a tape on the skin on the back of the lower leg in a straight line from the center of the calcaneal tuber to the most proximal end of the gastrocnemius muscle, serving as landmarks at intervals smaller than the width of the ultrasound probe (i.e., approximately 3 cm) (SNiBLE; Konica Minolta, Inc., Tokyo, Japan). (Figure 1).

[Step-2] The ultrasound probe was placed on the skin over the calcaneal tuber, and the first ultrasound image was acquired by visualizing the Achilles tendon insertion site and landmarks at both ends of the image.

[Step-3] The probe was applied over the skin from the calcaneal tuber to the most proximal end of the gastrocnemius muscle and the ultrasound images were acquired by visualizing landmarks at both ends of each image.

[Step-4] Each image acquired in Step-3 was overlapped based on marker lines, and an image showing the overall Achilles tendon, muscle-tendon unit, and gastrocnemius muscle was created by superimposing each image (Figure 2) in the neutral, maximum dorsiflexion, and maximum plantar flexion positions, respectively.

[Step-5] Four division points (i.e., the most distal end of the Achilles tendon, the muscle-tendon junction of the soleus muscle, the muscle-tendon junction of the gastrocnemius muscle, and the most proximal end of the gastrocnemius muscle) in the image made in Step-4 were identified in two ways; the first was by the raters' visual identification, and the second was using custom-built MATLAB-based program (MathWorks) in addition to visual identification. In the software identification approach, the division points were decided by scanning within regions of interest (ROI) set into MATLAB after importing the ultrasound image into the software program. First, the ROI-reference (red square in Figure 3) was placed at the most proximal position at each division point, and an ROI-scan range (orange square in Figure 3) was placed at the whole division point covering the ROI-reference (step-5-1 in Figure 3). Next, the area within the ROI-scan (green square in Figure 3) was scanned while moving from the distal to the proximal side (step-5-1 in Figure 3). Finally, texture features in ROI-reference and scan were calculated by gray-scale histogram analysis and squared euclid distance (SED), which is an indicator for traditional similarity evaluation [9], between ROI-reference and each ROI-scan (step-5-2 in Figure 3). The position where SED values were maximum (i.e., the lowest degree of similarity) was defined as the division point between tissues.

[Step-6] The lengths of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle were measured using ImageJ (National Institution of Health) based on the definition in Step-5.

All steps were performed by rater A, whereas only step 5 was performed by all three examiners (raters A, B, and C) to examine the inter-rater reliability.

Statistical analysis

In order to examine the intra- and inter-rater reliabilities of OIM measurement by visual-only identification and a combination of visual and software identification, the ICC 1.1 and ICC 2.1 scores were estimated. ICC 1.1 was calculated using the data measured by rater A, whereas ICC 2.1 was conducted using the data measured by raters A, B, and C. In addition, based on the definition that the value obtained by visual-only identification is the “true value,” the value differences between visual-only and combination identification were examined using the paired *t*-test or Wilcoxon signed-rank test following the Shapiro–Wilk normality test to investigate the validity of the combination identification. The level of significance was set as $p < .05$. IBM SPSS Statistics for Windows, Version 25.0. (IBM Corp., Armonk, NY, IBM Corp.) was used for all the statistical analyses.

Results

The age, height, and weight of the 19 participants were 23.36±2.54 years, 164.36 ± 8.05 cm, 60.76 ± 10.57 kg (mean ± standard), respectively.

Tables 1 and 2 show the evaluated ICC scores, and Table 3 shows each tissue length and the differences in absolute values between visual-only and combination identification. The ICC 1.1 and ICC 2.1 scores by the visual-only identification were 0.61–0.91 and 0.70–0.93, respectively. These scores by the combination identification ranged 0.90–0.99 and 0.58–0.96, respectively. There were significant differences between visual-only and combination identification in the values of the Achilles tendon at the neutral and maximum plantar flexion positions.

Discussion

We investigated the intra- and inter-rater reliabilities of visual-only and combination identification in OIM by comparing the lengths of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle measured based on ultrasound image divisions. Based on previously reported criteria: poor, < 0.40; fair, 0.40–0.59; good, 0.60–0.74; and excellent ≥ 0.75 [10,11)], the intra- and inter-rater reliability was "good" or "excellent" for both visual-only and combination identification in the present study. In particular, the OIM determined by combination identification showed a high intra-rater reliability of the length measurements of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle, which supported our hypothesis. To investigate the validity of the combination identification, we also examined the differences between visual-only and combination identification based on the definition that the value determined by the visual-only identification is the "true value." There were no significant differences between visual-only and combination identification, except in the length of the Achilles tendon at the neutral and maximum-plantar flexion positions. The findings of the present study suggest that OIM is applicable in the field of sports science as a method for investigating the influence of massage intervention, training, or growth in children on the overall length of muscles and tendons.

Notably, the ICC score on the gastrocnemius muscle measurement at neutral position was lower than that of other measurements. We identified four division points (the distal end of the Achilles tendon, soleus muscle-tendon junction, gastrocnemius muscle-tendon junction, and gastrocnemius proximal end). Among them, the black-and-white ultrasound images were less clear at the proximal end of the gastrocnemius relative to the gastrocnemius length measurement than at the other division points. Because we determined the division point using the MATLAB program grayscale histogram in the combination identification, black-and-white ultrasound image ambiguity may make grayscale histogram identification difficult, reducing the measurement reliability. This is a limitation of the combination identification.

Significant differences in the Achilles tendon length measurements were found between visual-only and combination identification at neutral and maximum dorsiflexion positions using the paired t-test. This result suggests that it may be somewhat more difficult to identify the division points of the tissues involved in measuring the Achilles tendon length (i.e., the distal end of the Achilles tendon and soleus muscle-tendon junction). The clarity of the distal end of the Achilles tendon is likely to vary depending on the ultrasound incidence angle, and some of the images acquired in the present study were unclear. Therefore, it is important to clearly reflect the distal end of the Achilles tendon in ultrasound image acquisition to guarantee the high reliability and validity of the OIM measurement.

Conclusion

This study examined the reliability of OIM by incorporating both visual-only and combination identification procedures. This demonstrates that the high intra- and inter-rater reliability can be obtained in almost all length measurements of the AT, muscle-tendon unit, and gastrocnemius muscle at three different positions (neutral, maximum dorsiflexion, and maximum plantar flexion positions). In particular, OIM using combination identification showed a higher intra-rater reliability. The applicability of the overlapping imaging method is demonstrated by the findings of the present study.

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Conflicts of interest: None in particular.

Author contributions

A.Y.: Conceptualization, Data curation, Writing-Original Draft preparation, Investigation. **M.H.:** Methodology, Software, Writing-Reviewing and Editing. **S.M.:** Data curation, Investigation, Writing-Reviewing and Editing. **K.M.:** Data curation, Investigation. **N.H.:** Conceptualization, Writing-Reviewing and Editing.

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Figure legends

Figure 1. Cut thread arranged on the skin from the calcaneal tuber to the proximal end of the gastrocnemius muscle.

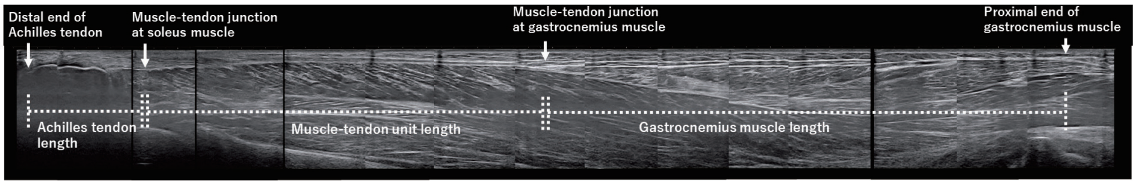

Figure 2. Images superimposed for length measurement of the Achilles tendon, muscle-tendon unit, and gastrocnemius muscle.

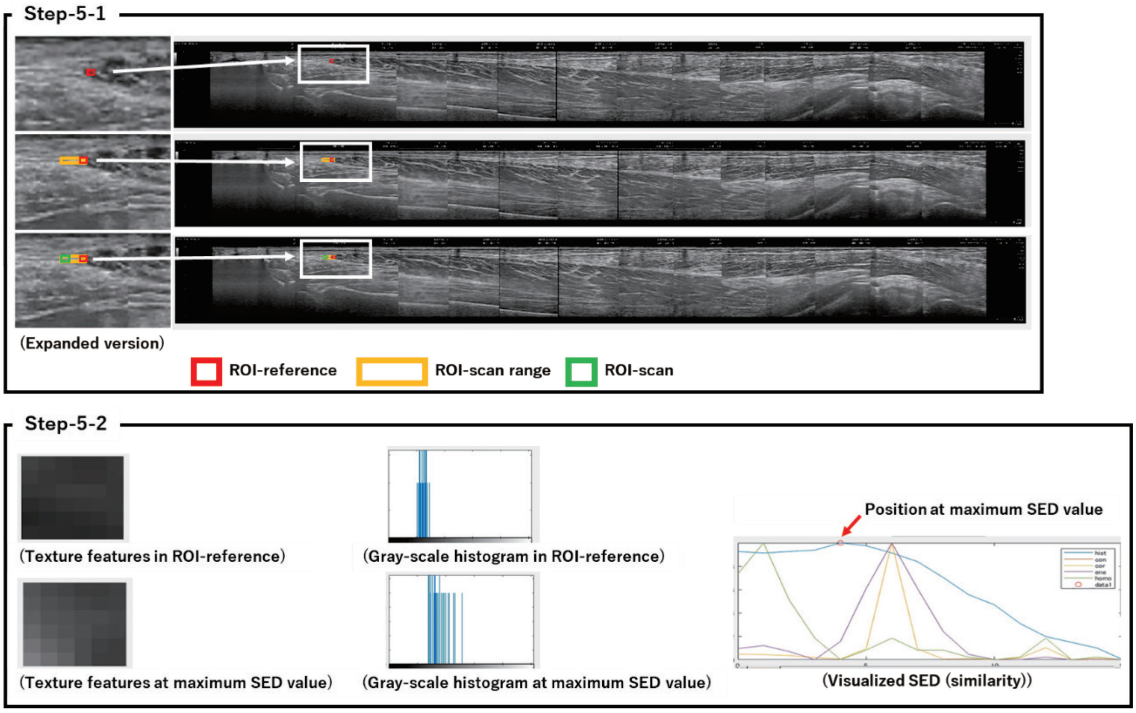

Figure 3. Process in [Step-5] using custom-built MATLAB-based program.

ROI = regions of interest, SED = squared euclid distance

Tables

Table 1. Intraclass correlation coefficient scores for intra-rater reliability in visual-only and combination of visual and software identification (ICC 1.1).

		Visual-only identification	Combination identification
Neutral position	Achilles tendon	0.91	0.99
	Muscle-tendon unit	0.84	0.98
	Gastrocnemius muscle	0.69	0.99
Max-dorsiflexion	Achilles tendon	0.76	0.95
	Muscle-tendon unit	0.89	0.98
	Gastrocnemius muscle	0.81	0.90
Max-plantar flexion	Achilles tendon	0.70	0.95
	Muscle-tendon unit	0.61	0.96
	Gastrocnemius muscle	0.90	0.94

Max-dorsiflexion = maximum dorsiflexion position; max plantar flexion = maximum plantar flexion position.

Table 2. Intraclass correlation coefficient scores for inter-rater reliability in visual-only identification and combination of visual and software identification (ICC 2.1).

		Visual-only identification	Combination identification
Neutral position	Achilles tendon	0.85	0.91
	Muscle-tendon unit	0.84	0.96
	Gastrocnemius muscle	0.79	0.58
Max-dorsiflexion	Achilles tendon	0.77	0.92
	Muscle-tendon unit	0.89	0.89
	Gastrocnemius muscle	0.93	0.82
Max-plantar flexion	Achilles tendon	0.70	0.91
	Muscle-tendon unit	0.72	0.83
	Gastrocnemius muscle	0.88	0.89

Max dorsiflexion = maximum dorsiflexion position; max plantar flexion = maximum plantar flexion position.

Table 3. Length of each tissue and differences in the absolute values between in visual-only identification and combination of visual and software identification (cm).

		Visual-only identification	Combination identification	Value differences	p
Neutral position	Achilles tendon	7.11±1.93	7.47±1.91	0.60±0.73	<.01
	Muscle-tendon unit	13.50±2.28	13.35±2.48	0.71±0.94	.95
	Gastrocnemius muscle	19.34±2.24	19.37±2.63	1.20±1.76	.91
Max-dorsi flexion	Achilles tendon	7.67±1.75	7.80±1.55	0.56±0.82	.30
	Muscle-tendon unit	13.07±2.30	13.31±2.39	0.68±0.78	.13
	Gastrocnemius muscle	20.11±2.39	20.17±2.53	0.78±0.87	.67
Max-plantar flexion	Achilles tendon	6.94±1.74	7.21±1.60	0.68±1.01	<.01
	Muscle-tendon unit	13.48±2.08	13.74±1.98	0.79±1.26	.19
	Gastrocnemius muscle	17.37±2.48	17.71±2.95	0.92±1.06	.06

Values are presented as the mean ± standard deviation. Max dorsiflexion = maximum dorsiflexion position; max plantar flexion = maximum plantar flexion position.