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Original Contribution

Reliability of Muscle Quantity and Quality Measured With Extended-Field-of-View Ultrasound at Nine Body Sites

Jona Van den Broeck [a,*], Savanah Héréus [a], Erik Cattrysse [a], Hubert Raeymaekers [b], Michel De Maeseneer [b], Aldo Scafoglieri [a]

[a] Experimental Anatomy Research Group, Department of Physiotherapy and Human Anatomy, Faculty of Physical Education and Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium
[b] Department of Radiology, University Hospital Brussels, Brussels, Belgium

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ABSTRACT

Objective: Measuring muscle quantity and quality is very important because the loss of muscle quantity and quality is associated with several adverse effects specifically in older people. Ultrasound is a method widely used to measure muscle quantity and quality. One problem with ultrasound is its limited field of view, which makes it impossible to measure the muscle quantity and quality of certain muscles. In this study, we aimed to evaluate the intra- and inter-rater reliability of extended-field-of-view (EFOV) ultrasound for the measurement of muscle quantity and quality in nine muscles of the limbs and trunk.
Methods: Two examiners took two ultrasound EFOV images with a linear probe from each of the muscle sites. The intraclass correlation coefficient (ICC) was used, and the standard error of measurement and coefficient of variation were calculated.
Results: Intra-rater reliability was good to excellent (ICC = 0.2–1.00) for all muscle measurements. The inter-rater reliability for most of the muscle measurements was good to excellent (ICC = 0.82–0.98). Inter-rater reliability was moderate (0.58–0.72) for some muscle quantity measurements of the tibialis anterior, gastrocnemius, rectus femoris, biceps femoris and triceps brachii muscles.
Conclusion: Muscle quantity and quality can be measured reliably using EFOV US.

Introduction

Measurement of muscle quantity and quality is very important in the context of sarcopenia, cachexia and muscle atrophy [1]. Loss of muscle quantity and quality is associated with loss of function, disability, decreased physical performance, falls and mortality [2–4]. Low muscle quantity and quality are still difficult to diagnose because they are difficult to measure accurately in a clinical setting [5]. To diagnose loss of muscle quantity and quality, computed tomography (CT) and magnetic resonance imaging (MRI) can be used [3]. CT and MRI are the gold standards for measuring muscle quantity and quality, but are expensive and not portable, and CT exposes patients to ionizing radiation [6,7].

Ultrasound (US) is widely used to measure muscle quantity and quality without any of these disadvantages. Muscle quantity can be measured as cross-sectional area (CSA) or muscle thickness (MT), and muscle quality can be measured as echo intensity (EI) [3,5,8,9]. EI reflects muscle quality because it reflects non-contractile tissue associated with myosteatosis [10,11]. As it is possible to measure both muscle quantity and quality, ultrasound can be expanded in clinical practice to support the diagnosis of low muscle mass and quality [12,13]. Several researchers have tried to create an equation for determining whole-body muscle mass using selected ultrasound measurements [14]. MT was measured with ultrasound at nine sites of the body: lower leg anterior and posterior, upper leg anterior and posterior, trunk anterior and posterior, upper arm anterior and posterior and forearm lateral [14]. For ultrasound-derived equations to be applicable, the inter-rater reliability for measuring MT must be high, which has been reported previously [15–17]. However, there is some disagreement over how MT should be measured [14]. It would be better to measure the MT of a specific muscle rather than a site on the body, where more than one muscle is measured at the same time [18]. Measurement of a single muscle is more difficult because it is essential to measure the maximal muscle bulk of a single muscle, and the point of maximal muscle bulk differs between muscles [19]. In addition, muscle quality has not been taken into account in the equations, which, according to the European Working Group on Sarcopenia in Older People (EWGSOP), is an important part of the diagnosis

* Corresponding author. Experimental Anatomy Research Group, Department Physiotherapy and Human Anatomy, Faculty of Physical Education and Physiotherapy, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090, Brussels, Belgium.
E-mail address: jona.van.den.broeck@vub.be (J. Van den Broeck).

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of sarcopenia [3]. However, it is necessary to measure CSA first to derive the EI within this perimeter. In some muscles, the field of view is not large enough, and the CSA of the muscles is therefore not fully visible, so the EI cannot be measured for the entire surface [20–22].

The development of the extended-field-of-view (EFOV) US technique has made it possible to construct a 2-D image of muscles with a large CSA [23]. In EFOV US, the probe is moved over the region of interest and combines new image frames with prior frames to form a panoramic image [22]. With this panoramic image, it becomes possible to measure MT, CSA and EI on one scan. For some muscles, the intra-rater reliability of the EFOV ultrasound technique in measuring CSA, MT and EI was good (ICC ranged between 0.88–0.99) [1,24]. However, to assess muscle quantity and quality, more research is needed regarding the inter-rater reliability and reproducibility of the nine muscle sites used in the equations, as well as the CSA and EI of the different muscles.

In this study, our aim was to provide an overview of the reliability of CSA, MT and EI measurements at nine different sites: lower leg anterior (tibialis anterior muscle) and posterior (gastrocnemius muscle), upper leg anterior (rectus femoris muscle) and posterior (biceps femoris muscle), trunk anterior (rectus abdominis muscle) and posterior (erector spinae), upper arm anterior (biceps brachii muscle) and posterior (triceps brachii muscle) and forearm lateral (extensor muscles).

Methods

Participants

Healthy participants 18 y of age and older were included in this study. Non-community-dwelling individuals, those having undergone surgery <16 wk before enrollment and those with partial or artificial limbs or currently or ongoing neuromuscular diseases or musculoskeletal injuries were excluded.

General procedure

All measurements were performed at the University of Brussels (Vrije Universiteit Brussel). Participants had to sign an informed consent document to participate in this study. The informed consent and the study were approved by the medical ethics committee (B.U.N. 1432020000265). General characteristics of the participants were collected including sex, age, height, weight, body mass index, dominant side, comorbidities and prostheses. The protocol described below is based on studies previously performed by Lopez et al. [25], Ahtiainen et al. [26] and Jenkins et al. [27]. The participants were asked not to exercise for 30 min before the examination [28]. For the anterior muscles, the participants had to lie supine 5 min before the first measurement. Participants were asked to relax with the legs 15 cm apart and a firm sculptured support (10 cm) under the knees. The arms were abducted 45° and supported, and the elbows were extended. For the posterior muscles, participants had to lie prone. The participants were asked to relax the with legs 15 cm apart, with arms abducted 90° and supported and elbows extended. The right side of every participant was measured. The first researcher (trained specialist in anatomy) drew a mediolateral line on the skin to mark the location of the measurement point. The second researcher measured at the same location marked by the first researcher. Ultrasound measurements were performed using a Mindray M7 (Mindray Bio-medical Electronics Co., Shenzhen, Guangdong, China) with a linear probe (7L4s, 4.5 mm). All scans were obtained perpendicular to the direction of the muscle fibers, at a depth of 6.5 cm, frequency of 8 MHz, gain of 0, IP of 1, frame rate of 47 and dynamic range of 65. The measurements were performed with the transducer in a transverse plane, by moving the transducer manually with slow and continuous movement from the medial to the lateral side along the marked line on the skin. The transducer was held perpendicular to the skin with minimal pressure. A generous amount of transmission gel (VUE ultrasound gel) was used to enhance the acoustic coupling and reduce near-field artefacts. In all participants, each muscle was measured four times, twice by researcher one and twice by researcher two. The two researchers were blinded for each other's image analysis. At the lower leg, the muscles were measured anterior and posterior at the proximal 30% point between the lateral condyle of the tibia and the lateral malleolus of the fibula [29]. In the upper leg, the muscles were measured anterior and posterior at the 50% point between the greater trochanter femoris and the lateral condyle of the femur [7]. At the trunk anterior, the rectus abdominis muscle was measured 2 cm above and lateral of the umbilicus [30]. At the trunk posterior, the erector spinae was measured lateral of the spinal process of the third lumbar vertebra [31]. In the upper arm, the muscles were measured anterior and posterior at the proximal 40% point between the acromion of the scapula and the lateral epicondyle of the humerus [32]. The forearm lateral was measured at the proximal 30% point between the head of the radius and the styloid process of the radius [30].

Image analysis

The primary variables were MT, CSA, and EI. MT can be defined in two ways. The first MT (MT site) is defined as the lengths of the adipose–muscle and muscle–bone interfaces, as depicted in Figure 1 [30]. The second muscle thickness measure (MT muscle) is defined as the distance between the deep and superficial aponeuroses of one single muscle, as illustrated in Figure 1 [33]. MT site was measured at the lower leg posterior, upper leg anterior and posterior and forearm lateral. MT muscle was measured in the tibialis anterior, gastrocnemius (caput mediale), rectus femoris, biceps femoris, rectus abdominis, erector spinae, biceps brachii and triceps brachii muscles. For CSA, the circumference of the muscle was manually drawn with a cursor in the ImageJ (ImageJ bundled with 64-bit Java 1.8.0_172) program, as illustrated in Figure 2. CSA was measured in the tibialis anterior, gastrocnemius (caput mediale), rectus femoris, biceps femoris, rectus abdominis, erector spinae, biceps brachii and triceps brachii muscles. EI was defined as the brightness of the image acquired through ultrasound [34]. It is expressed in a gray-scale (0–255) within a defined area (*i.e.,* CSA) of the ultrasound image [34] and is expressed in arbitrary units (AU) [35,36]. EI was measured in the tibialis anterior, gastrocnemius (caput mediale), rectus femoris, biceps femoris, rectus abdominis, erector spinae, biceps brachii and triceps brachii muscle.

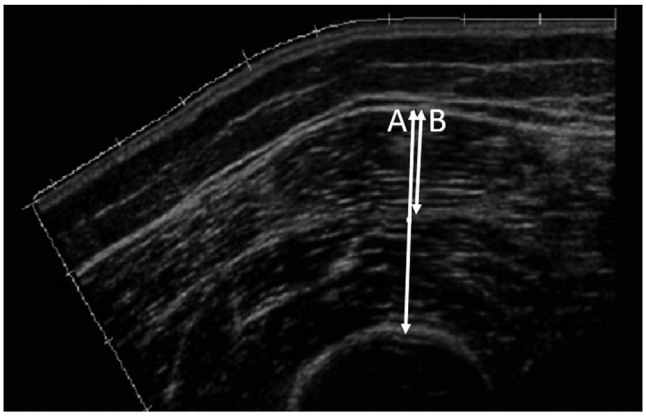

Figure 1. Example of upper leg anterior and rectus femoris muscle thickness

(A) Thigh anterior muscle thickness was measured from the middle of the superior aponeurosis of the rectus femoris muscle, through the middle of the inferior aponeurosis of the rectus femoris muscle, to the femur. (B) Muscle thickness of the rectus femoris muscle is measured from the middle of the superior aponeurosis to the inferior aponeurosis of the rectus femoris muscle.

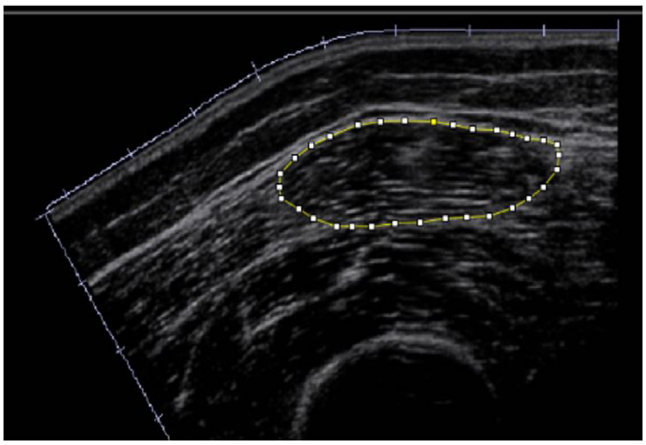

Figure 2. Extended-field-of-view cross-sectional area of the rectus femoris muscle.

Statistical analysis

Sample size was estimated based on the study of Walter et al. [37], with $\alpha = 0.05$, $\beta = 0.20$, $\rho_0 = 0.5$ and $\rho_1 = 0.8$. When using two examiners in a reliability study, 22 participants had to be included [37].

SPSS Statistics software, Version 29 (IBM, Armonk, NY, USA) was used to perform a Shapiro–Wilk test to examine the normal distribution of the data. For the reliability analysis, the intraclass correlation coefficients ($ICC_{2,1}$) (two-way mixed, single measures) for intra-rater and inter-rater reliability were used. In the ICC range 0–0.20, reliability was considered slight; in the range 0.21–0.50; poor; in the range 0.51–0.75, moderate; in the range 0.76–0.90, good; and >0.91, excellent [38]. In the case of skewed data, bootstrapping was used to check whether the ICC value could be interpreted correctly. When bootstrap values were <0.80, bootstrap was assumed not to confirm the interpretation of the ICC value. The standard error of measurement (SEM) was calculated as the standard deviation (SD) of the sample scores multiplied by the square root of one minus the reliability of the scores ($SEM = SD\sqrt{(1 - ICC)}$). The coefficient of variation (CV%) was determined using the formula CV% = (SEM/great average) * 100. Values ≤10% were judged to be sufficiently reliable. For inter-rater reliability, the absolute difference was also calculated. For this purpose, the absolute difference between the values of raters 1 and 2 was determined. The means ± standard deviation of these differences were calculated. The significance level for all analyses was set at $p < 0.05$.

Results

In this study, 31 participants were included. The participants' characteristics are summarized in Table 1. Some of the participants had comorbidities: diabetes, thrombopenia and allergy. One participant was pregnant, and one took anticoagulants.

Table 1
Participant characteristics

Sex (n)	
Male	13
Female	18
Age (y)	30 (24–61)
Height (m)	1.71 ± 0.10
Weight (kg)	67.0 (59.8–71.3)
Body mass index	22.2 (20.8–24.6)
Right-handedness (n)	28

All variables, except sex and right-handedness, are followed by the interquartile range in parentheses.

Intra-rater reliability

The results of the Shapiro–Wilk test indicated that some data were not normally distributed. Bootstrapping was used for these data when calculating the ICC. The ICC results are outlined in Table 2. Intra-rater reliability for most muscle measurements was good to excellent (ICC = 0.82–1.00). Only the ICC of the measurement of rectus femoris muscle EI could not be assessed because of the bootstrap value for one rater.

For MT (sites and muscles), the SEM ranged between 0.05 and 0.21 cm. The CV% for most of muscles was <10% (2.3%–7.7%). Only for the triceps brachii muscle was the CV% slightly higher than the preset 10% (14.0% for examiner 1, 12.5% for examiner 2). For CSA, the SEM ranged between 0.10 and 1.63 cm². For all CSA measurements, the CV% was lower than 10% (1.7%–9.6%).

For the EI, the SEM ranged between 0.00 and 5.74 AU. Most CV% values were lower than 10% (0.0%–8.8%), except for the biceps femoris, rectus abdominis, erector spinae and biceps brachii muscles (11.4% –14.5%). For the rectus abdominis muscle, there are no SEM and CV% values because the bootstrap was not confirmed.

Inter-rater reliability

Inter-rater reliability is outlined in Table 3. For the rectus femoris, erector spinae, biceps brachii and forearm extensor muscles, all ICC values for MT, CSA and EI were good to excellent (0.78–0.92). In the tibialis anterior, gastrocnemius, rectus femoris, biceps femoris and triceps brachii muscles, some ICC values were only moderate (0.58–0.72) or not confirmed by the bootstrap in the case of the triceps brachii muscle. The ICC for EI was in all muscles good to excellent (0.88–0.98).

The SEM values for MT, CSA and EI were between 0.08 and 0.33 cm, 0.40 and 1.40 cm² and 3.57 and 8.05 AU.

The CV% values for all measurements of MT, except those of the rectus femoris and triceps brachii muscles, were <10% (6.2%–9.0%). For the CSA, the CV% was <10% for the gastrocnemius, rectus abdominis and biceps brachii (8.0%–8.5%). The CV% values for the EI were all >10%, except for the EI of the gastrocnemius muscle (9.3%).

The absolute difference for MT measurements ranged between 0.06 and 0.40 cm. For the CSA, the absolute difference ranged between 0.42 and 1.96 cm². For the EI, the absolute difference ranged between 2.63 and 5.38 AU.

Discussion

Intra-rater reliability

The results of this study indicate that intra-rater reliability, when measuring the MT, CSA and EI of muscles with EFOV ultrasound, was good to excellent for all muscles, except for the measurement of the EI of the rectus femoris muscle. Other studies report similar values for reliability (ICC) for the gastrocnemius (CSA), rectus femoris (CSA), biceps femoris (MT, CSA, EI), rectus abdominis (CSA, EI), erector spinae (MT, CSA) and biceps brachii (MT, CSA, EI) muscles [23,27,35,36,39-41].

For the gastrocnemius muscle, it seems that measuring the EI at two different time points influences intra-rater reliability. The ICC values are lower when the measurements are not done on the same day [23]. In our study, participants were measured at the same time point. Despite being asked not to exercise for the 24 h preceding the examinations in other studies, the activity on those days may still affect the difference in EI. It has already been reported that physical activity increases EI because the expansion of muscle size after exercise can at least partly be explained by the relative expansion of extracellular water [28]. We therefore conclude that a longitudinal study decreases the reliability of EFOV ultrasound.

The angle of incidence of the probe and the contour of the measured part of the body may also affect the EI [22,23,34]. We also suspect that

Table 2
Muscle measurements and intra-rater reliability

Biomarker	Rater 1				Rater 2			
	Average	ICC (bootstrap 95% interval)	SEM	CV (%)	Average	ICC (bootstrap 95% interval)	SEM	CV (%)
Tibialis anterior muscle								
MT muscle (cm)	2.52 ± 0.34[a]	0.92	0.10	3.8	2.39 ± 0.32	0.93	0.08	3.5
CSA (cm²)	7.11 ± 1.95	0.95	0.44	6.1	7.28 ± 2.75	0.96	0.55	7.4
EI (AU)	37.85 (29.35–53.32)	0.99 (0.99–1.00)	2.32	5.0	30.85 (24.83–50.60)	0.99 (0.98–1.00)	2.63	5.9
Gastrocnemius muscle								
MT muscle (cm)	1.62 ± 0.31	0.94	0.08	4.7	1.59 ± 0.28	0.93	0.07	4.7
MT site (cm)	3.41 ± 0.52	0.86	0.19	5.7	3.64 ± 0.51	0.84	0.20	5.6
CSA (cm²)	9.17 ± 2.03	0.94	0.50	5.4	9.71 ± 2.42	0.91	0.73	7.4
EI (AU)	28.49 (19.35–54.17)	0.99 (0.97–1.00)	2.57	6.6	26.17 (21.40–58.52)	1.00 (0.99–1.00)	0.00	0.0
Rectus femoris muscle								
MT muscle (cm)	1.18 ± 0.25	0.90	0.08	6.6	1.16 ± 0.25	0.92	0.07	6.0
MT site (cm)	2.79 ± 0.44	0.90	0.14	4.9	2.65 ± 0.44	0.98	0.06	2.3
CSA (cm²)	3.67 ± 1.08	0.91	0.32	8.7	3.74 ± 1.35	0.96	0.27	7.2
EI (AU)	34.36 (22.52–57.78)	0.98 (0.95–0.99)	3.80	8.5	34.36 (25.56–46.39)	X[b] (0.39–0.99)	X	X
Biceps femoris muscle								
MT muscle (cm)	2.96 ± 0.41	0.87	0.15	4.9	2.82 ± 0.50	0.85	0.19	6.8
MT site (cm)	3.90 ± 0.45	0.87	0.16	4.1	3.77 ± 0.45	0.82	0.19	5.0
CSA (cm²)	10.06 ± 2.43	0.90	0.77	7.6	10.46 ± 2.78	0.89	0.92	8.7
EI (AU)	29.02 (21.30–37.79)	0.94 (0.86–0.98)	3.84	11.4	27.73 (19.10–35.39)	0.98 (0.95–0.99)	2.52	7.3
Rectus abdominis muscle								
MT muscle (cm)	0.88 ± 0.23	0.95	0.05	5.8	0.93 ± 0.23	0.91	0.07	7.4
CSA (cm²)	4.71 ± 1.28	0.96	0.26	5.4	4.82 ± 1.26	0.95	0.28	5.8
EI (AU)	30.70 (16.52–61.94)	0.99 (0.99–1.00)	2.87	7.0	30.28 (16.16–68.00)	0.96 (0.91–0.99)	5.74	14.1
Erector spinae muscle								
MT muscle (cm)	2.82 ± 0.48	0.90	0.15	5.3	2.70 ± 0.55	0.85	0.21	7.7
CSA (cm²)	5.76 ± 1.63	0.94	0.10	1.7	5.49 ± 1.61	0.89	0.53	9.3
EI (AU)	15.79 (6.99–25.17)	0.96 (0.92–0.99)	2.18	12.6	17.24 (10.21–25.43)	0.95 (0.87–0.99)	3.20	14.5
Biceps brachii muscle								
MT muscle (cm)	1.98 ± 0.53	0.92	0.15	7.5	1.94 ± 0.43	0.93	0.11	5.9
CSA (cm²)	5.78 ± 1.81	0.95	0.40	5.2	6.31 ± 1.75	0.93	0.46	7.3
EI (AU)	23.74 (14.87–35.81)	0.97 (0.94–0.99)	3.22	10.9	22.38 (16.96–35.20)	0.99 (0.98–1.00)	2.46	7.3
Triceps brachii muscle								
MT muscle (cm)	1.10 (0.83–1.44)	0.90 (0.82–0.97)	0.17	14.0	1.10 (0.88–1.55)	0.95 (0.92–0.99)	0.17	12.5
CSA (cm²)	15.92 ± 6.90	0.97	1.20	7.5	14.34 (11.97–20.87)	0.95 (0.88–0.99)	1.63	9.6
EI (AU)	23.82 (16.59–43.73)	0.98 (0.96–0.99)	2.76	8.8	23.06 (15.58–36.12)	0.98 (0.95–0.99)	2.56	8.4
Forearm extensor muscles								
MT site (cm)	1.64 ± 0.36	0.94	0.09	5.3	1.62 ± 0.35	0.91	0.11	6.4

CSA, cross-sectional area; CV, coefficient of variation; EI, echo intensity; ICC, intraclass correlation coefficient; MT, muscle thickness; SEM, standard error of measurement.

[a] Mean ± standard deviation or median (interquartile range).

[b] X indicates that the bootstrap does not confirm the correct interpretation of the ICC value.

gain, depth, frame rate and dynamic range could influence EI, but further investigation is required.

The SEM values in the literature for measurements of gastrocnemius (CSA), biceps femoris (MT, CSA, EI), rectus abdominis (CSA, EI), erector spinae (EI) and biceps brachii (CSA) muscles are similar to ours [23,35,36,40]. In all studies in which the SEM values for the gastrocnemius (SEM EI: 2.63 AU), rectus femoris muscles (SEM CSA: 1.0 cm²) and erector spinae (SEM CSA: 1.4 cm²) were higher, the participants were not measured on the same day [23,36,40].

The error rate (CV%) in our study for measuring muscle quantity (MT and CSA) was in most muscles <10%. Only in the triceps brachii muscle MT were the values higher. This result is probably owing to the difficulty encountered when measuring the triceps brachii muscle. To measure the total muscle, the examiner has to move the probe around the upper arm because the triceps muscle inserts medially, posteriorly and laterally at the humerus. This very difficult technique increases the risk of errors. The triceps brachii muscle also has a difficult shape, which makes it difficult to decide where to measure the MT. Those two reasons could have resulted in the higher CV% value. In the literature, CV% values could be found only for the rectus femoris (CSA) and biceps brachii (CSA) muscles [40]. These CV% values were similar to our results. For the EI, the values for CV% were <10% only in the tibialis anterior, gastrocnemius and triceps brachii muscles, confirming that EI is highly sensitive to several factors during measurement (*e.g.,* curvature of the body, angle at which the probe is held and pressure placed on the probe) [22,23,34].

To the best of our knowledge, there are no studies regarding the intra-rater reliability of the measurements of the tibialis anterior, triceps brachii and forearm extensor muscles. Only one study on fascicle length

Table 3
Inter-rater reliability of the muscle measurements

	Absolute difference[a]	ICC (bootstrap 95% interval)	SEM	CV (%)
Tibialis anterior muscle				
MT muscle (cm)	0.06 (0.03–0.18)	0.65	0.20	8.0
CSA (cm²)	0.78 ± 0.87	0.82	1.01	13.8
EI (AU)	4.55 (2.26–7.34)	0.96 (0.94–0.99)	4.84	10.7
Gastrocnemius muscle				
MT muscle (cm)	0.12 ± 0.08	0.88	0.10	6.2
MT site (cm)	0.20 (0.12–0.31)	0.60	0.33	9.0
CSA (cm²)	0.77 (0.22–1.36)	0.88	0.77	8.0
EI (AU)	3.99 ± 2.66	0.98 (0.94–0.99)	3.57	9.3
Rectus femoris muscle				
MT muscle (cm)	0.10 ± 0.07	0.87	0.09	7.3
MT site (cm)	0.24 (0.12–0.31)	0.58	0.32	11.6
CSA (cm²)	0.47 ± 0.43	0.86	0.45	12.1
EI (AU)	5.08 (1.4–9.8)	0.93 (0.85–0.98)	6.86	15.5
Biceps femoris muscle				
MT muscle (cm)	0.40 ± 0.65	0.72	0.24	8.2
MT site (cm)	0.40 ± 0.64	0.69	0.25	6.4
CSA (cm²)	1.96 ± 2.82	0.71	1.40	13.4
EI (AU)	3.76 (0.97–8.19)	0.94 (0.87–0.97)	4.08	12.4
Rectus abdominis muscle				
MT muscle (cm)	0.08 (0.03–0.16)	0.88	0.08	8.7
CSA (cm²)	0.55 ± 0.69	0.90	0.40	8.3
EI (AU)	5.83 (2.66–10.46)	0.92 (0.82–0.97)	8.05	20.0
Erector spinae muscle				
MT muscle (cm)	0.16 (0.10–0.35)	0.78	0.24	8.6
CSA (cm²)	0.42 (0.22–0.94)	0.87	0.59	10.2
EI (AU)	5.16 ± 4.09	0.88 (0.80–0.96)	4.44	21.0
Biceps brachii muscle				
MT muscle (cm)	0.16 ± 0.14	0.90	0.15	7.7
CSA (cm²)	0.60 (0.32–0.99)	0.91	0.53	8.5
EI (AU)	3.05 (1.51–6.79)	0.90 (0.88–0.98)	6.87	20.5
Triceps brachii muscle				
MT muscle (cm)	0.14 (0.07–0.30)	X[b] (0.68–0.93)	X	X
CSA (cm²)	1.31 (0.50–3.77)	X (0.63–0.96)	X	X
EI (AU)	2.63 (1.02–6.20)	0.97 (0.94–0.99)	3.23	10.5
Forearm extensor muscles				
MT site (cm)	0.18 ± 0.24	0.83	0.14	8.8

CSA, cross-sectional area; CV, coefficient of variation; EI, echo intensity; ICC, intraclass correlation coefficient; MT, muscle thickness; SEM, standard error of measurement.

[a] Mean ± standard deviation or median (interquartile range).

[b] X indicates that the bootstrap does not confirm the correct interpretation of the ICC value.

of the triceps brachii reported good results for intra-rater reliability [42]. Based on results of our study and the literature, we can conclude that intra-rater reliability is good to excellent for all muscle measurements described in this study. However, more research is needed on the factors that affect EI and on an easier and clearer way to measure the triceps brachii muscle.

Inter-rater reliability

Inter-rater reliability was good to excellent for all muscle measurements of the rectus abdominis, erector spinae, biceps brachii and forearm extensor muscles. Measuring the CSA shows good results for the gastrocnemius and rectus femoris muscles, as in earlier research [1].

The SEM values from our study could not be compared with SEM values from the literature because these have not been described previously.

The CV% for the tibialis anterior (MT), gastrocnemius (MT, CSA), rectus femoris (MT), biceps femoris (MT, CSA), rectus abdominis (MT, CSA), erector spinae (MT), biceps brachii (MT, CSA) and forearm extensor (MT) muscles were all <10%. For the muscles with higher CV% values, where exactly these muscles should be measured must be clarified. The CV% values for the EI were >10% in all muscles except the gastrocnemius. In another study with healthy participants, where the muscles were measured in the same manner as in our study, the same results were obtained for the tibialis anterior (EI) and biceps brachii (EI) muscles [24]. In a study in which two examiners measured the gastrocnemius (CSA) and rectus femoris (CSA) muscles each on the same scan, the CV% was <10% [1]. However, the ultrasound scan was taken by one researcher [1], whereas in our study, two scans were taken by two different researchers. The reliability of two measurements taken on the same scan cannot be influenced by the pressure of the transducer, the proportion of gel used and the movement of the examiner while taking the EFOV. The inter-rater reliability of the other muscles has not been investigated before. In addition, to our knowledge, no studies have previously investigated the absolute difference between two examiners. Therefore, little can be compared with the literature.

The limitations of this study are (i) the settings that could have influenced the results, (ii) the use of only one ultrasound device and (iii) the small sample.

The visibility of the muscles depends on depth [43]. Superficial muscles are best visualized at a higher frequency, while muscles that lie deeper are more visible at a low frequency [43]. The absorption of sound through the skin and subcutaneous fat plays an important role in this visibility [43]. The consequence of this difficulty is that the frequency should be captured per muscle. The frequency should be adjusted depending on the thickness of the skin and fat and the depth of each muscle. This may make the practical applicability more difficult, and thus, more research on reliability is necessary.

Inter-machine variability is a known problem in ultrasound. Research has already revealed that reliability do not differ much between machines when the researcher is the same [44,45]. However, more research is needed as it has already been reported that it is mainly muscle quality is influenced more than muscle quantity in muscle measurements with ultrasound [45]. Preferably, muscle quality should be measured over the entire surface of the muscle rather than just a small area, as this has been shown to improve reliability [46].

As this study had a small sample, how BMI, age and sex influence the reliability of muscle measurements with EFOV ultrasound should be examined further.

Our research reveals that several muscle measurements exhibit good reliability. Measuring muscle quantity with EFOV ultrasound is certainly a reliable measurement to use for body composition purposes. With these muscle measurements, it is possible to proceed and establish reference values against which to compare muscle measurements in daily practice in the future. To measure muscle quality, it is important to choose muscles that have been found to have good reliability in measurements in further research or practice.

Conclusion

In this study, for measurements of muscles with EFOV ultrasound were found to have good to excellent intra-rater reliability, probably because the muscles were measured at the same time point.

Inter-rater reliability was good for measurements of muscle quantity and quality in the muscles studied. More research is needed to assess why some muscle measurements more reliable than others and how we can improve the reliability.

Conflict of interest

The authors declare no competing interests.

Data availability statement

Because the data are confidential and disclosure was not approved for this purpose, we are unable to share the data. We are, however, willing to share the data in the context of research collaboration. For this purpose, an e-mail can be sent to the corresponding author.

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