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Original article

Reliability and validity of ultrasound to estimate muscles: A comparison between different transducers and parameters

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SUMMARY

Background and aims: We aimed to investigate the test-retest reliability and validity of ultrasound for two commonly used types of transducer, using different methods for the estimation of muscle size and echo intensity (EI).

Methods: Fourteen healthy adults were included in this study. Ultrasound images of the rectus femoris size (thickness in cm and cross-sectional area [CSA] in cm²), obtained at the mid-thigh, were validated against MRI. Both a linear and a curved array transducer were used to assess rectus femoris size and EI (values 0–255, higher scores indicating increased intramuscular fat and interstitial fibrous tissue). To assess test-retest reliability of ultrasound, participants were tested twice, with a one-week interval. Validity and reliability were evaluated using paired sample t-tests, intraclass correlation coefficient (ICC), and Bland–Altman plots.

Results: No significant differences between the repeated evaluations of rectus femoris thickness, CSA and EI were found. Reliability for thickness and CSA evaluations was excellent for both transducers (ICC = 0.87–0.97) and moderate for EI (ICC = 0.42–0.44). Mean difference between MRI and ultrasound for CSA (curved = 0.59 cm², p = 0.086; linear = 2.1 cm², p = 0.002) and thickness (curved = 0.31 cm, p = 0.01; linear = 0.21 cm, p = 0.043) were small but significant, except for CSA using a curved transducer. Agreement between ultrasound and MRI ranged from moderate for thickness (ICC = 0.45) to excellent for CSA (ICC = 0.92).

Conclusions: Our study demonstrates that the test-retest reliability and validity of muscle size estimation by ultrasound for both curved and linear array transducers seems to be adequate. Future studies should focus on the longitudinal evaluation of muscle size and EI by ultrasound.

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1. Introduction

The number of older adults in the world's population is increasing [1]. Ageing brings challenges that are often related to loss of muscle mass. The decrease in muscle mass, accompanied with a decrease in muscle function, is referred to as sarcopenia [2]. As sarcopenia is associated with an increased risk of adverse outcomes, sarcopenia is an important nutrition (-related) disorder [3].

Muscle mass is one of the diagnostic measures for sarcopenia [4]. A commonly used method to assess fat-free mass, of which

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muscle mass is a large component, is bioelectrical impedance analysis. However, the validity of this method on the individual level is limited [5]. Alternatively, ultrasound is used to estimate peripheral muscle size [6–9], and based on multiple peripheral muscle size measurements, to estimate total muscle mass [6,10,11].

From our recent systematic review, we concluded that ultrasound is valid and reliable to quantify muscles in older adults [6]. However, we observed that most of the reliability studies focused on the interpretation of ultrasound images, instead of assessing the complete ultrasound procedure. Although the interpretation of ultrasound images are the final steps in the complete ultrasound procedure, the whole ultrasound procedure consists of more steps, including identifying the scanning position and performing the scan. Another part in the ultrasound procedure is the type of parameters used. In previous studies, both thickness [9,12–14] and cross-sectional area (CSA) [15–17] were measured. Both parameters reflect the size of a peripheral muscle, but it has been suggested that thickness measurements are more prone to measurement errors as compared to CSA [18]. Yet, it remains unclear which parameter is preferable for use in daily practice, since it is not clear whether these methods differ in reliability and validity. Beyond the size of a peripheral muscle, the echo intensity (EI) of the muscle is also of great interest, since increased EI, which results from intramuscular fat and interstitial fibrous tissue [19,20], is associated with impaired physical functioning [21,22]. However, limited information on the test-retest reliability of ultrasound for the estimation of EI is available.

Another important part in the complete ultrasound procedure is the selection of the transducer. For the estimation of muscle size, linear array transducers are mainly used because this type of transducer is more adapted to muscle anatomy. Linear transducers often use higher frequencies, which leads to higher resolution. However, higher frequencies result in a limited penetrating ability, and therefore the high frequency linear array transducer is not feasible for use in deeper structures [23]. In contrast, curved array transducers often use lower frequencies, resulting in a greater penetrating ability, and has a large field of view. Therefore, the curved array transducer is also being used for the estimation of muscle size [16,22,24]. A previous study compared the curved array transducer with the linear array transducer, and concluded that the curved array transducer is valid and reliable for assessing CSA of the rectus femoris [16]. However, this study did not include thickness measurements and more importantly, did not compare with a gold standard.

Therefore, in this study we aimed to determine the:

- Test-retest reliability, i.e., the capacity to consistently reproduce the complete ultrasound procedure over a period of time, and validity, i.e., the extent to which ultrasound measures what it is intended to measure, of ultrasound measurements of CSA and thickness, using a linear and a curved array transducer against measurements obtained with MRI;
- The test-retest reliability of ultrasound for the estimation of EI.

2. Materials and methods

2.1. Population

Healthy individuals aged between 18 and 65 years were included in the study. Participants were recruited through advertisements within the Hanze University of Applied Sciences and by using the individual networks of the researchers. Respondents were excluded if they had a pacemaker or implants, claustrophobia,

severe neuromuscular diseases, or were pregnant. All participants were informed about the study procedures and provided informed consent prior to the start of this study. This study was approved by the Medical Ethical Committee of the University Medical Center Groningen (reference 2014/432).

2.2. Design and setting

Magnetic Resonance Imaging (MRI) was used as reference for the measurement of muscle size. The ultrasound and MRI scans were performed on the same day. For evaluating the test-retest reliability of ultrasound, the scans were conducted by one examiner with 3 years of experience in muscle ultrasound and repeated one week later. The participants were instructed to maintain their physical activity level throughout the study. Within this timeframe, no differences in muscle status were expected in this sample of healthy adults.

2.3. Measurements

2.3.1. Ultrasound

Brightness Mode (B-mode) (Honda HS-2100 MSK) ultrasound was used to measure rectus femoris size and EI, using a 10 MHz linear array transducer with a length of 5 cm, and a 5 MHz curved array transducer with a length of 6 cm. Gain (85 dB) and depth (75 mm) were kept constant and time gain compensation, i.e., a setting used to account for tissue attenuation, was in a neutral position throughout the study for each subject. For the estimation of rectus femoris size and EI, the participant was in supine position with the leg extended and relaxed on the examination table. The measurements were performed on the right leg. Measurements were taken after 20–30 min of rest to avoid fluid shifts, and at 50% distance between the greater trochanter and the lateral condyle [25]. This point was marked with indelible ink. While obtaining images, minimal contact pressure was applied to avoid compression of the muscle. The transducer was placed perpendicular to the muscle surface.

Rectus femoris size was estimated afterwards in two ways: (1) the thickness and (2) cross-sectional area (CSA). All measurements were conducted three times, and the mean of the three measurements was used for analyses. Rectus femoris size was determined with the free hand tool using the built-in software. Rectus femoris thickness (in cm) was defined as the greatest distance between the superficial and deep aponeuroses. The CSA was measured by tracing the inner echogenic line of the rectus femoris and is expressed in cm [2]. The EI of the rectus femoris was determined afterwards using a standard gray-scale histogram in ImageJ 1.51j8 (National Institute of Health, USA) [26]. A region of interest was manually drawn to include as much of the rectus femoris as possible, without any surrounding fascia. EI is expressed as a value between 0 (black) and 255 (white), with higher scores indicating increased intramuscular fat and interstitial fibrous tissue. The ultrasound data were analyzed blindly, i.e., without patient characteristics and thickness and EI results visible.

2.3.2. MRI

MRI scans (3.0 T, Philips Achieva, Philips Healthcare, Best, The Netherlands) were performed using a body coil with the participant placed in supine position. Axial-plane images of the right leg were acquired using a T1-weighted spin-echo sequence with the following scanning parameters: echo time (TE), 20 ms; repetition time (TR), 674 ms; 1 acquisition field; field of view 100 × 180 mm; matrix, 256 × 192; 3 mm slice thickness and 0 mm interslice gap. Fish-oil tablets were used as external marker and

used to identify the corresponding sections measured with ultrasound. The size of the rectus femoris was measured following the same procedure as the ultrasound measurements, using ImageJ 1.51j8.

2.4. Statistical analyses

Normality was tested using a histogram. Descriptive statistics were used to characterize the study population. Categorical variables were expressed as relative frequencies and continuous variables were presented as mean $\pm$ standard deviation (SD) or median and interquartile range (IQR) for not-normally distributed variables.

Test-retest reliability and validity were determined by three statistical tests: (1) a paired samples t-test or Wilcoxon Signed Rank in case of not-normally distributed data to assess systematic differences between the repeated measurements and between ultrasound and MRI, (2) intraclass correlation coefficient (ICC) (two-way mixed, absolute agreement, single measures) with a 95% confidence interval (95% CI) was used to assess the agreement between the repeated measurements and between ultrasound and MRI, and (3) Bland–Altman plots were constructed to assess the level of agreement and systematic bias or outliers [27]. Furthermore, typical percentage errors were calculated to assess the typical error of ultrasound by dividing the difference between the repeated evaluations by the mean of the first evaluation, multiplied by 100 [16,28]. The level of statistical significance was set at $p < 0.05$. Common cut-off points for the interpretation of ICC scores were used: 0.81–1.00 (excellent), 0.61–0.80 (good), 0.41–0.60 (moderate), 0.21–0.40 (fair) and <0.21 (poor) [29].

3. Results

Fourteen participants with a median age of 32.5 years (IQR:28–43), of which nine females (64%), were included in this study (Table 1). In six participants, of whom five males, the linear array transducer did not capture the complete CSA of the rectus femoris.

3.1. Test-retest reliability

Table 2 shows no significant differences between the 1st and the 2nd evaluation of rectus femoris thickness, CSA, and EI. Compared to the curved array transducer, mean rectus femoris thickness was greater with the linear array transducer, both on the 1st and 2nd evaluation (evaluation 1: $p = 0.014$; evaluation 2: $p = 0.001$). In contrast, lower mean values of the CSA were observed when the linear array transducer was used, compared to the curved array transducer (evaluation 1: $p = 0.003$; evaluation 2: $p = 0.001$). Reliability of ultrasound for the estimation of rectus thickness and CSA was excellent for both the linear and curved array transducer (ICC = 0.87–0.97). Typical percentages errors ranged from 1.1% to 2.6% (Table 3). Figs. 1 and 2 demonstrate the agreement between the repeated evaluations of muscle size. Fig. 1 shows close to zero bias for the thickness evaluations (-0.04 cm for both linear and curved array transducer). For the CSA evaluations, the bias

Table 2

Reliability of the estimation of rectus femoris thickness and cross-sectional area.

	1st evaluation	2nd evaluation	p-value
<i>Thickness in cm</i>			
Linear array transducer	1.91 (0.38)	1.97 (0.32)	0.399
Curved array transducer	1.79 (0.28)	1.85 (0.28)	0.150
<i>CSA in cm²</i>			
Linear array transducer	8.48 (1.56)	8.67 (1.74)	0.443
Curved array transducer	10.02 (2.75)	10.22 (2.77)	0.208
<i>Echo intensity^a</i>			
Linear array transducer, [IQR]	70.77 (68.79–79.76)	71.38 (50.93–75.45)	0.133
Curved array transducer, [IQR]	60.26 (54.60–68.32)	56.82 (47.86–68.84)	0.311

Data expressed as mean (SD), unless stated otherwise; IQR, interquartile range; CSA, cross-sectional area.

^a values ranging from 0 to 255.

Table 3

Intra-rater reliability for estimating rectus femoris thickness, CSA and echo intensity using different transducers.

	Linear array transducer		Curved array transducer	
	ICC (95%CI)	TPE	ICC (95%CI)	TPE
Thickness	0.87 (0.64–0.96)	2.3	0.95 (0.83–0.98)	2.1
CSA	0.97 (0.91–0.99)	1.1	0.97 (0.89–0.98)	2.6
Echo intensity	0.44 (–0.06–0.78)	10.6	0.42 (–0.11–0.77)	11.2

ICC, intraclass correlation coefficient; 95%CI, 95% confidence interval; TPE, typical percentage error; CSA, cross-sectional area.

was -0.09 cm for the linear array and -0.27 cm for the curved array transducer (Fig. 2). For the estimation of EI, reliability was moderate (curved array transducer ICC = 0.42, linear array transducer ICC = 0.44) (Table 3).

3.2. Validity

Figs. 3 and 4 depict the agreement between ultrasound and MRI in measuring rectus femoris thickness (Fig. 3) and CSA (Fig. 4). Compared to MRI, lower values of both thickness and CSA were found with ultrasound. Except for estimating CSA with the curved array transducer, significant differences were found between MRI and ultrasound (Table 4). The mean difference in thickness between MRI and ultrasound was 0.21 cm for the linear array and 0.31 cm for the curved array transducer. The mean difference in CSA between MRI and ultrasound was 2.1 cm² for the linear array transducer and 0.59 cm² for the curved array transducer. The validity of ultrasound compared to MRI ranged from moderate for thickness (ICC = 0.45; curved transducer) to excellent for CSA (ICC = 0.92; curved array transducer) (Table 4).

A subanalysis in the participants in which the complete CSA could be estimated with the linear transducer ($N = 8$) showed no significant difference between MRI and ultrasound (CSA_{MRI} = 9.83 cm²; CSA_{ultrasound} = 8.14 cm², $p = 0.127$). The validity of ultrasound using the linear transducer compared to MRI for the estimation of CSA was good (ICC = 0.62; 95%CI:–0.031–0.919).

4. Discussion

Our study demonstrates that the test-retest reliability and the validity of ultrasound for the estimation of rectus femoris size for both the curved- and the linear array transducer is good. The test-retest reliability for the estimation of EI is moderate. Interestingly, for thickness estimation, measurements performed with the

Table 1

General characteristics of the study population ($N = 14$).

Age in years, [IQR]	32.50 (28–43)
Female, N (%)	9 (64)
Weight in kg	70.60 (10.22)
BMI in kg/m ²	22.90 (2.59)

Data expressed as mean (SD), unless stated otherwise; IQR, interquartile range; BMI, body mass index.

Fig. 1. Bland–Altman plots illustrating the agreement between two evaluations for the estimation of rectus femoris thickness for the linear (a) and curved array transducer (b). The dotted lines represent the limits of agreement.

curved array transducer have shown a higher intra-rater reliability than those obtained with the linear transducer.

The validity estimates for the estimation of muscle thickness are comparable with other studies [28,30,31]. One previous study found that the correlation between computed tomography (CT) and ultrasound measured quadriceps size was higher for the CSA estimates than for the thickness estimates [32]. Furthermore, the results of this study suggest that the CSA is a slightly better parameter for the estimation of rectus femoris size than thickness, since the ICC scores for the CSA estimations were higher, although just slightly, than those for thickness. Nevertheless, we found a moderate to excellent agreement between MRI and ultrasound, implicating that ultrasound can be used for both parameters.

Our results also indicate that ultrasound slightly underestimates the size of the rectus femoris compared to MRI in healthy adults.

This underestimation was observed for both the CSA and the thickness evaluations, except for the CSA evaluation with the curved array transducer. Our results are in line with a previous study that also observed that ultrasound slightly underestimates the size of the quadriceps compared to CT [32]. This slight underestimation might be a result of the scanning position. Although we used a common method, i.e., fish-oil tablets as external marker in order to match the MRI images with the ultrasound images [14,17], this method introduces a small error because of the positioning of the fish-oil tablets. We furthermore observed a significant difference between the linear array transducer and MRI for estimating CSA. This could possibly be explained by the fact that in six of our healthy participants, the size of the linear array transducer was insufficient to capture the complete CSA of the rectus femoris at 50% of distance between the greater trochanter and the lateral

Fig. 2. Bland–Altman plots illustrating the agreement between two evaluations for the estimation of rectus femoris CSA for the linear (a) and curved array transducer (b). The dotted lines represent the limits of agreement.

Thickness

Fig. 3. Bland–Altman plots illustrating the agreement between ultrasound and MRI for the estimation of rectus femoris thickness for the linear (a) and curved array transducer (b). The dotted lines represent the limits of agreement.

condyle. The subanalysis in the sample in which the complete CSA could be estimated with the linear array transducer supports this, and yielded higher validity scores for the linear transducer. These results suggest that, in particular for smaller muscles, the linear array transducer is valid for the estimation of the size of the rectus femoris. Nevertheless, the mean differences between MRI and ultrasound were rather small (thickness: 0.21–0.31 cm, CSA: 2.1 cm²). These differences, and the observed typical percentage errors (ranging from 1.1% to 2.6% for muscle size) for the reliability of ultrasound for the estimation of muscle size, are smaller than the observed changes in muscle size in longitudinal studies [33–38]. Previous studies reported an age-related decrease in CSA of the quadriceps, ranging between 12.5% and 24% [34,35]. Also, muscle

thickness tends to decline with ageing, with estimates ranging from 3.4% to 12.6% [33,39]. On the other hand, muscle size tends to increase after a period of progressive resistance training [36–38,40,41]. During a training period ranging from 10 to 13 weeks, an increase of around 9% in quadriceps CSA [37,41], and ranging from 4.1% to 14.9% in quadriceps thickness [36,38,40,41], was found. Given the small errors in our study as compared to the effect of ageing and training on muscle size, our study suggest that ultrasound has the potential to evaluate changes in muscle status in clinical practice.

In this study, we found a moderate agreement between the repeated evaluations of EI. However, the reliability estimates of the EI analyses had large confidence intervals, which could

CSA

Fig. 4. Bland–Altman plots illustrating the agreement between ultrasound and MRI for the estimation of rectus femoris CSA for the linear (a) and curved array transducer (b). The dotted lines represent the limits of agreement.

Table 4
Validity of ultrasound versus MRI for estimating rectus femoris thickness and CSA.

	MRI	Linear array transducer			Curved array transducer		
	Mean (SD)	Mean (SD)	p-value*	ICC (95%CI)	Mean (SD)	p-value*	ICC (95%CI)
Thickness in cm	2.15 (0.44)	1.94 (0.37)	0.043	0.60 (0.11–0.86)	1.85 (0.25)	0.010	0.45 (–0.10–0.80)
CSA in cm ²	10.76 (3.20)	8.61 (1.55)	0.002	0.51 (–0.08–0.83)	10.17 (2.80)	0.086	0.92 (0.73–0.97)

SD, standard deviation; ICC, intraclass correlation coefficient; CSA, cross-sectional area. * difference between ultrasound and MRI.

possibly be explained by the small sample size. Despite the small sample size, our findings are in line with a previous study on the test-retest reliability of ultrasound for the estimation of EI [22,42]. Compared to these studies, we observed higher greyscale values which could be explained by the small sample size, equipment used [43], or the measurement procedure (e.g. free hand tool or rectangular region of interest and its size and position) [44,45].

The current study shows that a curved array transducer is as valid as a linear array transducer in measuring muscle size compared to MRI. Although our findings are based on a small sample size, these are in line with a previous study in 17 patients with COPD that concluded that a curved array transducer is valid for the estimation of rectus femoris CSA compared to a linear array transducer [16]. Our study confirms that finding and suggests that the curved array transducer is also valid and reliable for thickness measurements.

This study provides valuable information for nutrition support professionals on the usefulness and limitations of ultrasound for the quantification of muscles. Ultrasound might be of great importance for evaluating peripheral muscle size and EI and may be helpful in monitoring therapeutic effects. This study should be considered as a first step. Future studies should focus on the use of ultrasound in the estimation of muscles in older adults and on its role in diagnosing sarcopenia.

A limitation of our study is the small sample size. In addition, caution must be taken in generalizing our results to other populations. Since EI might be increased in older adults [20,46], caution must be taken in generalizing the results of this study to the older population. Moreover, since only healthy adults participated within a normal BMI range, and without peripheral edema, the current findings cannot be generalized to obese persons and persons with peripheral edema. Furthermore, in six out of 14 participants we were not able to capture the complete CSA with the linear array transducer. We consider these results as an important finding, rather than a limitation of the study design. Nevertheless, it might have led to bias, and therefore we performed additional analyses in the group in which the complete CSA could be evaluated. Major strengths of the current study are the strict scanning protocol, including both the linear and curved array transducer and use of the MRI, which is considered a gold standard for muscle assessment [20,47].

In conclusion, this study suggests that both the curved array transducer and a linear array transducer are valid and reliable for the estimation of rectus femoris thickness and cross-sectional area. Future studies should focus on the longitudinal evaluation of muscle size and EI by ultrasound.

Statement of authorship

WN: conceptualization, methodology, resources, data analyses, visualization, writing original draft, review and editing, data curation; HJW: conceptualization, methodology, supervision, review and editing; ISR: methodology, data analyses, review and editing;

CPVDS: conceptualization, methodology, resources, supervision, review and editing; HH: conceptualization, methodology, supervision, review and editing.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

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