



## Inter-rater and intra-rater reliability of ultrasound imaging for measuring quadriceps muscle and non-contractile tissue thickness of the anterior thigh

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20 **Inter-rater and intra-rater reliability of ultrasound imaging for measuring**  
21 **quadriceps muscle and non-contractile tissue thickness of the anterior thigh**  
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50 subcutaneous fat thickness, vastus intermedius

1 **Title**

2 Inter-rater and intra-rater reliability of ultrasound imaging for measuring quadriceps  
3 muscle and non-contractile tissue thickness of the anterior thigh

4  
5 **Abstract**

6 *Objective:* To determine intra-rater and inter-rater reliability of ultrasound imaging for  
7 measuring muscle and non-contractile (subcutaneous fat and perimuscular fascia)  
8 tissue thickness of the anterior thigh.

9 *Approach:* Ultrasound imaging has been used for clinical research to assess the  
10 morphology and cross-sectional area of muscles and other musculoskeletal  
11 structures. Repeatability of measurements with the ultrasound imaging technique  
12 between operators and test re-test reliability are important and need to be  
13 established for specific muscles. Twenty-four healthy moderately active adults (aged  
14 36-64 years), underwent B-mode ultrasound imaging by two investigators. The  
15 anterior thighs were scanned at a site two-thirds of the distance between the antero-  
16 superior iliac spine and the superior pole of the patella. Intraclass Correlation  
17 Coefficients (ICCs, model 3,1 for inter-rater and 3,2 for intra-rater reliability between-  
18 days) with 95% confidence intervals (CI) was used to assess reliability.

19 *Main results:* Inter-rater reliability of ultrasound imaging measurements were ICC<sub>3,1</sub>  
20 of 0.98 (95% CI: 0.95–0.99) for muscle thickness, 0.81 (95% CI: 0.60–0.91) for  
21 subcutaneous fat, 0.78 (95% CI: 0.56–0.90) for non-contractile tissue (subcutaneous  
22 fat combined with perimuscular fascia), and 0.70 (95% CI: 0.42–0.86) for  
23 perimuscular fascia. Intra-rater reliability values were ICC<sub>3,2</sub> 0.96 (95% CI: 0.90–  
24 0.98) for muscle thickness, 0.99 (95% CI: 0.97–0.99) for subcutaneous fat, 0.98  
25 (95% CI: 0.96–0.99) for non-contractile tissue, and -0.02 (95% CI: -0.41–0.38) for  
26 perimuscular fascia.

27 *Significance:* The present findings indicate very high inter-rater and intra-rater  
28 reliability of ultrasound imaging thickness measurements of the quadriceps muscles  
29 and non-contractile tissue of the anterior thigh, while perimuscular fascia  
30 measurements alone were not reliable between days.

## 1 **Introduction**

2 Ultrasound (US) imaging has been successfully applied in clinical practice and  
3 clinical research to evaluate the architecture, thickness, and cross-sectional area of  
4 muscles, and in studying morphology and/or pathology of other musculoskeletal  
5 structures, such as tendons and ligaments (Whittaker *et al* 2007). Quadriceps  
6 muscle weakness and atrophy are commonly reported in patients with knee  
7 osteoarthritis (Pettersen *et al* 2008) or any painful condition that affect the knee  
8 (Henriksen *et al* 2011, Rice *et al* 2014), and in critically ill patients in intensive care  
9 units, in whom muscle weakness occurs rapidly (Hadda *et al* 2017). The US imaging  
10 technique is non-invasive, sufficiently accurate, relatively low cost, widely available  
11 and a safe modality to study musculoskeletal tissues, but the accuracy of the  
12 procedure is operator-dependent (Wakefield *et al* 2005). Accurate, reliable  
13 measurement of muscle thickness is a powerful tool for use in research and in the  
14 clinical setting if the technique is shown to be reliable for a specific muscle, in this  
15 case, the quadriceps and its associated non-contractile tissues (i.e. subcutaneous fat  
16 and perimuscular fascia). The relative proportions of muscle and subcutaneous fat  
17 would be useful to know, for example, when losing weight to ensure fat and not  
18 muscle was being lost, and when gaining weight to ensure muscle and not fat was  
19 increasing, such as in critically ill patients receiving nutritional support. Obtaining a  
20 clear image and taking accurate measurements both require the ability to interpret  
21 the image, so this could vary between operators and also between images of  
22 different complexity, so reliability needs to be examined between different operators  
23 for specific tissues (Whittaker *et al* 2007). Therefore, ensuring the repeatability of  
24 measurements between investigators and measurements made on different  
25 occasions need to be established when imaging a particular muscle.

26 It was necessary to conduct the present study of healthy participants to establish the  
27 variability when measuring healthy tissues before assessing reliability in pathological  
28 cases, as pathology could contribute to variability in measurements due to poorer  
29 tissue quality.

30 Test-retest (intra-rater) reliability of US imaging has already been determined for  
31 various muscles: e.g. O' Sullivan *et al* (2007) reported excellent reliability for lower  
32 trapezius thickness measurements, with intraclass correlation coefficient (ICC)  
33 values greater than 0.90; Herbert *et al* (2009) performed a systematic review on the

1 reliability of the US imaging technique for measurements of abdominal and lumbar  
2 trunk muscle thickness, and reported moderate to excellent reliability found from  
3 high quality studies, with ICC values ranging from 0.62 to 0.97. Similar reliability (ICC  
4 values) was reported by Costa *et al* (2009) in a systematic review for studies that  
5 measured abdominal muscle thickness. Excellent reliability of lumbar multifidus  
6 muscle thickness was established by Wallwork *et al* (2007), for both experienced  
7 raters (ICC<sub>3,1</sub> of 0.94) and for a novice rater (ICC<sub>3,1</sub> of 0.89). Very high inter-rater and  
8 intra-rater reliability, with ICC values above 0.90, were reported for supraspinatus  
9 (Temes *et al* 2011), gluteus medius, gluteus minimus, and vastus medialis muscles  
10 (Whittaker and Emery 2014), and intra-rater reliability of anterior thigh tissues,  
11 including muscle and non-contractile tissues (Agyapong-Badu *et al* 2014).

12 The contribution of fascia thickness relative to measurements of muscle thickness  
13 has received little attention but a study of the abdominal muscles that measured  
14 fascia separately found that thickness of fascia was greater in participants with back  
15 pain than those without, whereas muscle was thinner in those with back pain,  
16 suggesting that measuring these tissues separately may help us understand  
17 mechanisms of muscle contractile abnormalities (Whittaker *et al* 2013). This was an  
18 important finding, as fascia is often included in muscle thickness measurements, so  
19 the implication is that assessment of muscle atrophy may be underestimated if fascia  
20 is included in US measurements of muscle thickness. Regarding quadriceps, a  
21 novelty of the present study is that inter-rater reliability of the non-contractile tissue  
22 measurements has not been studied previously, i.e. subcutaneous fat and fascia  
23 (superficial perimuscular fascia of the rectus femoris muscle and deeper  
24 intermuscular fascia between the rectus femoris and vastus intermedius muscles.  
25 Another novel aspect is that the relative contributions of muscle and non-contractile  
26 tissues have only been studied in young and older age groups (Agyapong-Badu *et al*  
27 2014), and not the present middle-aged group that would typically be the age of  
28 osteoarthritis onset. The purpose of the present study was to determine inter-rater  
29 and intra-rater between-day reliability of US imaging in measuring rectus femoris and  
30 vastus intermedius muscles of the quadriceps, and non-contractile tissue  
31 (subcutaneous fat and perimuscular fascia) thickness of the anterior thigh, in healthy  
32 middle-aged individuals.

## 1 **Methods**

### 2 **Participants**

3 A group of 24 (12 females, 12 males) healthy moderately active adults (Physical  
4 Activity Guidelines Advisory Committee, 2018), aged (years)  $48.91 \pm 9.78$  (36-64),  
5 height (m)  $1.71 \pm 0.06$  (1.59–1.82), body mass (kg)  $72.87 \pm 12.66$  (47.8–100.2)  
6 participated in the study. Exclusion criteria included diseases and conditions that  
7 affect muscle function, lower limb musculoskeletal injuries and pathologies including  
8 fracture, surgery, neoplasm, and neurological conditions. Participants were asked  
9 not to undertake vigorous exercise within the 24 hours prior to testing. The study was  
10 approved by the local Ethics Committee on human experimentation (CESU 1/2015).  
11 Written informed consent was obtained from all participants after full explanation of  
12 the aims and procedures, and the study was conducted in accordance with the  
13 Helsinki Declaration of 1975, as revised in 2008. Participants' rights were protected.

14

### 15 **Procedure**

16 Transverse B-mode images of the anterior thigh were acquired by two physical  
17 therapists, using an ultrasound scanner (MicrUs EXT-1H; Telemed, Vilnius,  
18 Lithuania) with a 5 MHz linear transducer (39 mm length). Ultrasound scans were  
19 obtained with the participant resting in supine lying (Figure 1), with the hip in neutral  
20 and the knee fully extended. Sandbags were placed at the ankle to avoid lateral  
21 rotation of the hip, with the ankle relaxed in slight plantar flexion. The distance  
22 between the antero-superior iliac spine and the superior border of the patella was  
23 measured, then all ultrasound measurements were performed at two thirds of the  
24 measured distance from the antero-superior iliac spine (Delaney *et al* 2010). For  
25 inter-rater reliability, measurement of this distance was performed independently by  
26 each investigator. Ultrasound scans were taken by two investigators on the same  
27 session but they did not observe one another scanning, which was performed  
28 independently. For image acquisition, a thick layer of ultrasound gel was applied  
29 between the transducer and the skin, and minimal contact pressure was applied  
30 when placing the transducer on the skin to obtain the image, to avoid compression of  
31 the underlying tissues, which would influence the measurement of tissue thickness.  
32 For intra-rater reliability, participants returned one week later for scans to be  
33 repeated by only one investigator, to examine test-retest reliability.

## 1 **Ultrasound Imaging Data Processing**

2 Images were analyzed off-line using ImageJ software (available from  
3 <https://imagej.nih.gov/ij/>). Subcutaneous fat thickness was measured from the skin to  
4 the outside edge of the superficial fascial layer (Figure 2), muscle thickness of rectus  
5 femoris (RF) and vastus intermedius (VI) were measured between the inside edges  
6 of muscles borders to exclude perimuscular fascia. The superficial perimuscular  
7 fascial layer was considered between the outside edges of the connective tissue  
8 layers superior to RF, while deep fascial layer between RF and VI. Each anonymized  
9 ultrasound image was measured twice and the mean used in the analysis. The same  
10 investigator (FM) performed the measurements on all images.

11

## 12 **Data Analysis**

13 Data analysis was conducted using SPSS 22 (SPSS Inc, Chicago, IL). The data  
14 were examined for normality using the Shapiro-Wilk test and found to be normally  
15 distributed and therefore parametric. Descriptive statistics were used to summarize  
16 the data as means and standard deviations. Intraclass coefficients (ICC) provide a  
17 measure of how consistent measurements from multiple observations are, so ICCs  
18 were used to assess agreement between measurements made on the two occasions  
19 (intra-rater, test-retest).

20 For intra-rater reliability, the association between measurements made by the same  
21 investigator from the two sessions was analyzed using a two-way mixed repeated  
22 measure ANOVA (ICC<sub>3,2</sub>).

23 Agreement between the two raters was analyzed using the ICC calculated by a two-  
24 way mixed single measure ANOVA (ICC<sub>3,1</sub>) to test inter-rater reliability. Precision of  
25 measurements was assessed using the Standard Error of Measurement (SEM),  
26 which provides values in meaningful units for measuring thickness (mm) and was  
27 used to calculate Minimum Detectable Change (MDC), which is the minimal change  
28 that falls outside the measurement error. The SEM and MDC were calculated from  
29 the ICC as follows:  $SEM = SD \sqrt{1 - ICC}$ ;  $MDC = 1.96 \times \sqrt{2} \times SEM$

30 Interpretation of ICCs was based on criteria described by Munro (2005), in which  
31  $ICC > 0.90$  indicate very high reliability, 0.70-0.89 high reliability, 0.50-0.69 moderate  
32 reliability, and 0.26-0.49 low reliability.

33

## 1 **Results**

### 2 *Inter-rater reliability*

3 The measurements obtained by rater 1 and rater 2 demonstrated very high reliability  
4 for muscle thickness (ICC<sub>3,1</sub> of 0.98; 95% CI: 0.95–0.99), and high reliability for  
5 subcutaneous fat (ICC<sub>3,1</sub> of 0.81; 95% CI: 0.60–0.91) and perimuscular fascia  
6 (ICC<sub>3,1</sub> of 0.78; 95% CI: 0.56–0.90), as seen in (Table 1). Inter-rater reliability of  
7 perimuscular fascia thickness was high, ICC<sub>3,1</sub> of 0.70 (95% CI: 0.42–0.86).  
8 Standard error of measurement (SEM) was 0.99mm for muscle thickness, 1.44mm  
9 for subcutaneous fat, 1.55mm for non-contractile tissue and 0.16mm for fascia  
10 thickness. Minimal detectable change (MDC) was 2.74mm for muscle thickness,  
11 3.99mm for subcutaneous fat, 4.29mm for non-contractile tissue and 0.44mm for  
12 perimuscular fascia.

### 13 *Intra-rater reliability*

14 Intra-rater reliability of the measurements obtained by rater 1 on day 1 compared  
15 with the measurements taken by the same rater one week apart, are shown in Table  
16 2. The US imaging measurements showed a very high test-retest reliability between  
17 days with ICC<sub>3,2</sub> of 0.96 (95% CI: 0.90–0.98) for muscle thickness measurements,  
18 ICC<sub>3,2</sub> of 0.99 (95% CI: 0.97–0.99) for subcutaneous fat, ICC<sub>3,2</sub> of 0.98 (95% CI:  
19 0.96–0.99) for non-contractile tissue. Inter-rater reliability of perimuscular fascia  
20 thickness was negative with ICC<sub>3,2</sub> of -0.02 (95% CI: -0.41–0.38). Standard error of  
21 measurement (SEM) was 1.3mm for muscle thickness, 0.47mm for subcutaneous  
22 fat, 0.66mm for non-contractile tissue and 0.3mm for fascia. Minimal detectable  
23 change (MDC) was 3.6mm for muscle thickness, 1.3mm for subcutaneous fat,  
24 1.83mm for non-contractile tissue and 0.83mm for perimuscular fascia.

25

## 26 **Discussion**

27 US imaging is an operator-dependent technique (Wakefield *et al* 2005), so  
28 demonstrating the level of repeatability of measurements between investigators and  
29 test re-test reliability is of high importance and needs to be established for individual  
30 muscles. Several factors can influence variability of tissue thickness measurement,  
31 many of which can be minimized by standardizing the data collection protocol  
32 (Whittaker *et al* 2007). For example, participant positioning in lying was  
33 standardized, in terms of posture, position of the limb affecting muscle length and

1 joint angles. The scanner settings were kept constant for each individual, such as  
2 type and frequency of the transducer, as well as minimal contact pressure, so as not  
3 to distort the image through compression of the tissues. The status of the tissues can  
4 also affect their thickness, hence participants were asked not to undertake vigorous  
5 exercise within the 24 hours prior to testing and were studied at the same time of day  
6 when they returned for repeated testing, to keep conditions as constant as possible.  
7 The present study demonstrated that US imaging is a highly reliable methodology for  
8 measuring anterior thigh muscle thickness, though reliability was poor for measuring  
9 perimuscular fascia on its own.

10 Results of the present study are in keeping with the very high intra-rater reliability  
11 reported by Agyapong-Badu *et al* (2014) for rectus femoris and vastus intermedius,  
12 as well subcutaneous fat thickness of anterior thigh in young and older adults. The  
13 present study provides further evidence producing data for both intra- and inter-rater  
14 reliability of anterior thigh measurements in middle aged adults.

15 The MDC values recorded in the present study for intra-rater reliability were relatively  
16 lower (muscle thickness=3.6mm, subcutaneous fat=1.3mm) compared to those  
17 reported (young: muscle thickness=5.51mm, subcutaneous fat=2.28mm; older:  
18 muscle thickness=5.86mm, subcutaneous fat=2.49mm) by Agyapong-Badu *et al*  
19 (2014) in healthy people. The authors reported differences with sex and ageing,  
20 highlighting the sensitivity to differences between groups but not changes in  
21 individuals over time or minimal differences with the technique. Comparisons with  
22 data in patient groups may be more informative to identify clinically meaningful  
23 differences for specific patient groups (Sabatino *et al* 2017).

24 The present results for perimuscular fascia thickness measurements alone were not  
25 reliable between-days, with ICC values of -0.2, which is below the level of 0.9  
26 recommended for clinical measurements by Portney and Watkins (2000). Similarly,  
27 low intra-rater reliability has been reported for abdominal wall perimuscular  
28 connective tissue by Whittaker *et al* (2014). Inter-rater reliability for fascia was more  
29 acceptable (ICC 0.7), possibly because the two raters performed imaging on the  
30 participants within the same session, when participants remained in the same  
31 position and the same scanning site marked on the skin. It may be that relocating the  
32 scanning site for test-retest reliability was not adequately robust in the present study  
33 protocol. From a clinical point of view, it may be more valuable to evaluate the  
34 integrity and the continuity of the fascia, as it transmits mechanical tension

1 generated by muscular activity (Maas and Sandercock 2010, Wilke *et al* 2018,  
2 Yucesoy 2010), rather than measuring its thickness. The contrary applies to muscle,  
3 where reliable measurement of thickness is of high clinical importance. For example,  
4 in situations where quadriceps muscle wasting is known to occur, such as critically ill  
5 patients in intensive care units (Hadda et al 2017), in patients with knee osteoarthritis  
6 (Pettersen et al 2008) or any painful condition affecting the knee (Henriksen et al  
7 2011, Rice et al 2014). Monitoring of recovery in these patients could be aided using  
8 US imaging to ensure that gain in weight with nutritional loading is due to increases  
9 in muscle size and not subcutaneous fat. Other clinical areas where reliable  
10 measurement of quadriceps muscle thickness is potentially valuable include  
11 assessing quadriceps atrophy due to disuse, sarcopenia, knee pain, and to evaluate  
12 quadriceps hypertrophy during specific training programs.

13

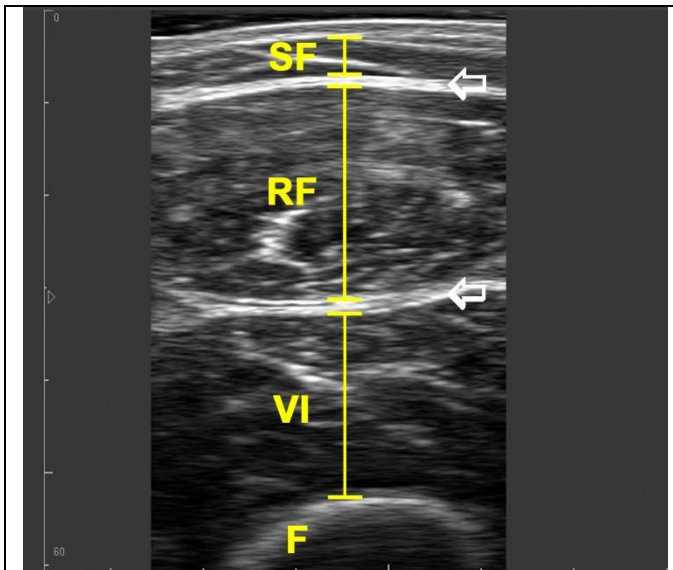
## 14 **Conclusion**

15 The present findings provide evidence for excellent inter-rater and intra-rater  
16 reliability of US imaging for measuring quadriceps muscle and non-contractile tissue  
17 (subcutaneous fat combined with perimuscular fascia) thickness of the anterior thigh  
18 in healthy middle-aged adults. These results add to the body of knowledge about  
19 musculoskeletal soft tissues that can be measured reliably using ultrasound imaging,  
20 supporting its use as an objective research and clinical tool.



**FIGURE 1.** The experimental set-up, with a subject positioned in supine lying, with the ultrasound transducer placed to obtain a transverse section of the anterior mid-thigh, and sandbags around the legs to keep the hips in a neutral position.

1



**FIGURE 2.** Subcutaneous fat (SF); rectus femoris (RF); vastus intermedius (VI); femur (F); arrows indicate superficial and deep fascial layers.

2

1 **TABLE 1. Inter-rater reliability between two raters**

Participants n=24	Subcutaneous fat	Fascia	Non-contractile tissue	Muscle thickness
ICC <sub>3,1</sub>	0.81	0.70	0.78	0.98
95% CI	0.6-0.91	0.42-0.86	0.56-0.9	0.95-0.99
SEM (mm)	1.44	0.16	1.55	0.99
MDC (mm)	3.99	0.44	4.29	2.74

2 CI=Confidence interval; SEM=standard error of measurement; MDC=minimum detectable change

3

4 **TABLE 2. Intra-rater reliability on two days**

Participants n=24	Subcutaneous fat	Fascia	Non-contractile tissue	Muscle thickness
ICC <sub>3,2</sub>	0.99	-0.02	0.98	0.96
95% CI	0.97-0.99	-0.41-0.38	0.96-0.99	0.90-0.98
SEM (mm)	0.47	0.3	0.66	1.3
MDC (mm)	1.3	0.83	1.83	3.6

5 CI=Confidence interval; SEM=standard error of measurement; MDC=minimum detectable change

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