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**Validity of measuring distal vastus medialis muscle using rehabilitative ultrasound
imaging versus magnetic resonance imaging**

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Abstract

Objective quantification of muscle size can aid clinical assessment when treating musculoskeletal conditions. To date the gold standard of measuring muscle morphology is magnetic resonance imaging (MRI). However, there's a growing body of evidence validating rehabilitative ultrasound imaging (RUSI) against MRI.

Objective: This study aimed to validate RUSI against MRI for the linear measurements of the distal fibres of vastus medialis muscle in the thigh.

Twelve healthy male participants were recruited from a local university population. The distal portion of their right vastus medialis was imaged with the participant in long-sitting, using MRI and RUSI whilst the leg was in extension and neutral hip rotation. Cross sectional area (CSA) and three linear measures were taken from the MRI and compared with the same linear measures from RUSI. Statistical analysis included comparison of MRI and RUSI measures using the paired t-test and correlation using intra-class correlation coefficients (ICC 3,1).

Mean differences between the linear measures taken from the MRI and RUSI were -0.5mm to 2.9mm (95% confidence intervals -0.6 to 8.3mm), which were not statistically different ($p > 0.05$) and were highly correlated (ICCs_{3,1} 0.84-0.94). Correlations between the three linear measurements and muscle CSA ranged from $r = 0.23$ to 0.87, the greatest being muscle thickness. Multiplying the linear measures did not improve the correlation of 0.87 found for muscle thickness.

Linear measures of vastus medialis depth made using RUSI were shown to be as valid as using MRI. Muscle thickness measures using RUSI could be used within an objective assessment of this muscle.

1. Introduction

The current gold-standard for measuring the size of soft-tissues in humans is magnetic resonance imaging (MRI) (Reeves et al. , 2004). However, MRI scans can be difficult to access, is expensive and requires skilled clinicians to interpret the images. Alternative valid, accurate and reliable techniques are needed to measure muscle size for research and clinical purposes. Rehabilitative ultrasound imaging (RUSI) can offer a safe, objective and relatively inexpensive means of evaluating muscle and related soft tissue morphology, and can provide visual feedback to aid interventions in research and clinical practice (Whittaker et al. , 2007, Whittaker and Stokes, 2011).

Several studies have reported RUSI as a valid method for measuring the size of muscles when compared to MRI, including; the lumbar multifidus (Hides et al. , 1995), cervical multifidus (Lee et al. , 2007), abdominals (Hides et al. , 2006), trapezius (O'Sullivan et al. , 2009), quadriceps (Walton et al. , 1997, Reeves, Maganaris, 2004, Thomaes et al. , 2012) and anterior hip muscles (Mendis et al. , 2010). A systematic review of 13 studies on the validity of using RUSI compared with MRI or computed tomography (CT) concluded that RUSI can provide valid measurements of skeletal muscles (Pretorius and Keating, 2008). They hypothesised that, due to the varied nature of the populations in the reviewed studies, it was possible to generalise this statement, rather than restrict it to a particular muscle.

Several muscles have yet to be evaluated for validity using RUSI. An example is vastus medialis, where substantial alterations in fibre alignment are seen between proximal and distal muscle portions (Smith et al. , 2009). The distal oblique fibres are thought to play a pivotal role in the kinematics and alignment at the patellofemoral joint (Berry et al. , 2008, Fagan and Delahunt, 2008, Lin et al. , 2008) and physiological or biomechanical dysfunction of this muscle are reported to contribute to patellofemoral pain (PFP) (Lankhorst et al. , 2012). Recent research has used RUSI to assess the pennation angle of these oblique muscle fibres in cadaveric specimens (Engelina et al. , 2012) and assess muscle volume in

individuals with PFP (Lin, Lin, 2008, Jan et al. , 2009). There is, however, a need to assess the validity of RUSI measurements of vastus medialis (VM). Therefore, the present study aimed to assess the distal portion of vastus medialis using both MRI and RUSI.

2. Methods

2.1 Participants

Twelve healthy, recreationally active adult males, aged 18 - 30 years, were recruited from the local community. Participant had no history of neurological or musculoskeletal disease, lower limb pathology or injury requiring treatment and were recreationally active.

2.2 Magnetic Resonance Imaging

The MRI system used was an extremity open Esaote MR scanner (MRE) with a 0.2 Tesla magnet. The right knee was scanned at 7mm intervals, between the upper third of the tibia, through to the middle third of the thigh, capturing the distal end of the relaxed VM muscle (Figure 1). Briefly, each participant was positioned in long-sitting with their right knee extended and a soft support on the outside of the knee to prevent lateral hip rotation. The images were examined by the researcher looking first at the distal images through to the proximal ones until the patella was in view. Slices were then added in the cephalad direction until the patella was not in the field of view and this image was selected for the measurements.

[please insert Figure 1 here]

The CSA and linear measures from the MRI images were made using Image J software (<http://rsbweb.nih.gov/ij/>), with all measures estimated by the same investigator (FK). The CSA of the distal VM muscle was obtained by manually tracing around the inside edge of the muscle border. Three linear measures were also taken to examine their correlation with CSA. These included: Line A – the longest line drawn from where the VM meets the medial

border of the femur, to the outer edge of the muscle; Line B – the line 90 degrees clockwise from Line A; Line C – the line mid-way between lines A and B. The cursor was placed on the inside edge of the muscle border to exclude fascia (Figure 2).

[please insert figure2 here]

2.3 Ultrasound Imaging

Ultrasound images were obtained on the same day as the MRI scan, with participants positioned in long-sitting to replicate the MRI posture. Two ultrasound images were taken of the relaxed right VM muscle, at the proximal border of the patella, using an ultrasound scanner (Aquila, Pie Medical, UK), with an 8MHz linear transducer (width 60mm; Figure 3). The same investigator took all ultrasound images, ensuring consistency of imaging location and minimal pressure was applied onto the muscle belly. Once the ultrasound images were obtained (Figure 4), linear measures A, B and C (Figure 2) were taken offline using Image J, with all measures being taken by the same investigator (FK) using the first RUSI image. Measures of MRI scans were undertaken first and then the process was repeated with the RUSI scans for all participants. The gap between the two sets of measures and their analysis was at least two weeks. Participant codes were used to de-identify all images.

[please insert figures 3 & 4 here]

2.4 Reliability of measurements on MRI & RUSI scans

The reliability of the investigator's ability to make repeated measurements on the same scans on different days was high for CSA and linear measures on MRI scans (intra-class correlation coefficient ICCs 0.95-0.99, confidence intervals 0.87-0.99) and linear measurements on RUSI scans (ICCs 0.90-0.98, confidence intervals 0.45-0.98).

2.5 Statistical Analysis

Data were tested for normality of distribution using the Shapiro Wilks test. Subsequently parametric descriptors for measurements were calculated (mean and standard deviations) and compared between MRI and RUSI using ICCs (3,1) and Bland and Altman analysis. A threshold ICC of 0.9 or above was used as this is an appropriate level of validity for measures used for decision-making or diagnosis (Portney and Watkins, 2000). Paired t-test was used to examine significant differences between measures using the two imaging techniques. The relationship between linear and CSA measures of MRI data were examined using Pearson's correlation coefficients (r).

3. Results

3.1 RUSI versus MRI

There were no significant differences in the linear measures using the two imaging techniques ($p > 0.05$), with mean differences ranging from 0.5-2.9mm (95% confidence intervals -0.6 to 8.3mm, Table 1). Comparison of measures from RUSI and MRI using ICC analysis showed good agreement for Line A, exceeding the threshold of ICC=0.9. Lower agreement between modalities was found for Line B and C, which showed moderate agreement of ICC 0.84 (Table 2). The Bland and Altman results support the ICC findings, showing small mean differences (<1mm) and limits of agreement for linear measures A & B, with greater differences (mean 2.9mm) between the measures for Line C (Table 2, Figure 5).

[please insert Tables 1 & 2, & Figure 5 here]

3.2 Correlation between linear measures and CSA

Pearson's correlation coefficients ranged between 0.23 and 0.87 when comparing single and combined linear measurements against CSA from the MRI scans. Line C had the highest

level of correlation of $r=0.87$ (Figure 6), with line B the poorest correlation of $r=0.23$ (Table 3). The linear measures were then multiplied and examined for correlation with CSA, which gave correlations ranging from $r =0.60$ to 0.86 .

[please insert Table 3 & Figure 6 here]

4. Discussion

Linear measures taken from RUSI had good to excellent levels of agreement when compared to MRI images of the same muscle, making it a viable option for clinical evaluation of vastus medialis muscle size. The CSA of the muscle belly assessed using MRI was highly correlated with the single linear measurement (line C) and a combination of linear measures.

Three previous studies have compared measures taken from US and MRI images of the quadriceps muscle group (Walton, Roberts, 1997, Reeves, Maganaris, 2004, Thomaes, Thomis, 2012). Direct comparison to the present study is limited for two of these studies (Walton, Roberts, 1997, Reeves, Maganaris, 2004), as they compared CSA rather than linear measurements using a compound scanning technique (Cavalieri technique). Walton et al (1996) compared the agreement of CSA and volume measures taken from MRI and US images of the quadriceps femoris muscle group in 10 healthy participants. They found no significant difference between data sets, with a mean difference of 0.49cm^2 between CSA measures with each imaging modality. Reeves et al (2004) compared the agreement of CSA measures, taken from MRI and RUSI images of the vastus lateralis muscle in six healthy participants. Their ICC's ranged between 0.997-0.999 with a mean error of 2.6%. They also showed very high correlation coefficients between MRI and RUSI CSA measures ($r=0.99$). This level of agreement was higher than that of our VM data, although the landmark

identification for vastus lateralis may have offered a better opportunity for more accurate and repeatable measures. Finally, Thomaes et al (2012) assessed rectus femoris CSA using RUSI and CT in a cohort of patients with coronary artery disease. Reliability of measures was achieved (ICCs of 0.97) and the ICC computed between US and CT was 0.92 (95%CL: 0.81 - 0.97). The absolute difference between both techniques was 0.01 ± 0.12 cm ($p = 0.66$) resulting in a typical percentage error of 4.4%.

Other studies comparing MRI and RUSI measures of different muscles have shown good results but differing analytical techniques limits the comparison to the present study. The CSA of lumbar multifidus was compared using MRI and RUSI at vertebral levels from L2 to S1 and no significant differences were found, despite differences in position for imaging (Hides, Richardson, 1995). Measures of cervical multifidus muscle from C4 to C6 was also shown to be valid for thickness ($R^2 = 0.42$ to 0.64) but not for CSA ($R^2 = 0.11$ to 0.39) or width ($R^2 = 0.16$ - 0.69) (Lee, Tseng, 2007). The small CSA of the muscle (approximately 1 cm^2) may have amplified errors, thus influence the accuracy of measurements. Finally, the abdominal muscles were examined at rest and contraction with good agreement found between RUSI and MRI for transversus abdominis and internal oblique muscles (ICC=0.84-0.95) (Hides, Wilson, 2006).

The present study showed Line C was most highly correlated with VM CSA. However, this was the measurement that showed the lowest agreement between MRI and RUSI (ICC 0.84). Correlation values for lines A ($r=0.66$) and B ($r=0.23$) were too low to be of use clinically (Kline, 1986) and their combinations with line C did not increase the predictive value. The opposite was shown in lumbar multifidus, where the greatest correlations with CSA were for combined linear measures ($r<0.9$) rather than any single measure ($r=0.7$ to 0.8) (Hides et al. , 1992). This difference between studies may be explained by the contrasting shape between the muscles. Linear measures of muscle size could be a clinically useful dimension when CSA is unobtainable. Although CSA of vastus medialis can be estimated from a single linear measure, Line C, it requires line A to be drawn (but not measured) in order to locate it. It

would be worth exploring other ways to measure VM muscle thickness, so that only one line needs to be drawn, thus saving time.

4.1 Limitations

Only a small sample size was used for this validation study, which limited the statistical power of the results and ability for conclusion to be generalised across differing populations. However, it included more participants than most previous studies. A formal reliability study was not conducted to test the investigator's RUSI technique and obtaining measurements on different days. The ability to measure the same scans on different days was, however, shown to be highly reliable for both MRI and RUSI (all ICCs above 0.90). Further examination of between-day and between observer (inter-rater) reliability is needed for VM, as has been demonstrated for several other muscles (Whittaker et al 2007). Finally, since the largest difference between modalities was found for line C, which was also found to have the best correlation with CSA, a more clinically meaningful measurement of vastus medialis size could be sought in future studies.

4.2 Recommendations

Linear measures of distal vastus medialis muscle can be measured using both MRI and RUSI techniques. A single linear measurement of VM thickness, line C, was highly correlated with CSA in the MRI images.

5. Conclusions

Linear measures made on RUSI scans were comparable to those on MRI scans, with small mean differences, indicating that RUSI is a valid technique for measuring vastus medialis muscle depth. The strong correlations found between a muscle thickness measure and CSA using MRI images, indicated that the linear measure have potential to estimate CSA. The

validity of measuring muscle thickness might be improved by further research to find an even more robust linear measurement of vastus medialis in cross section. (Walton, Roberts, 1997)

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Figure Legends

Figure 1. MRI scan of a typical participant. Muscle tissue is darker than bone or other soft tissues, and the vastus medialis oblique (VMO) is the main muscle (kidney-shaped) seen on the right of the image, the central pale area is the femur bone.

Figure 2. Diagram of a typical cross-section of the vastus medialis (VM) muscle on a magnetic resonance image taken at the base of the patella, showing the three linear measures: Line A – the longest line drawn from the corner where the VM meets the medial border of the femur, to the outer edge of the muscle, remaining inside the muscle border; Line B – the line 90 degrees clockwise from Line A, remaining inside the muscle border; Line C – the line mid-way between lines A and B, remaining inside the muscle border.

Figure 3. Participant having an ultrasound scan taken of the right vastus medialis muscle in long-sitting

Figure 4. Ultrasound scan of right vastus medialis taking at the same level as MRI images, just above and medial to the proximal border of the patella.

Figure 5. Bland and Altman Plot showing difference between measurements of linear measurement C taken from MRI and ultrasound scans. The dashed line represents the mean difference, solid lines are 95% upper and lower limits of agreement, representing two standard deviations.

Figure 6. Regression plot of cross-sectional area (CSA) against linear measurement C from MRI images of the vastus medialis muscle ($r=0.87$)

Figure 1

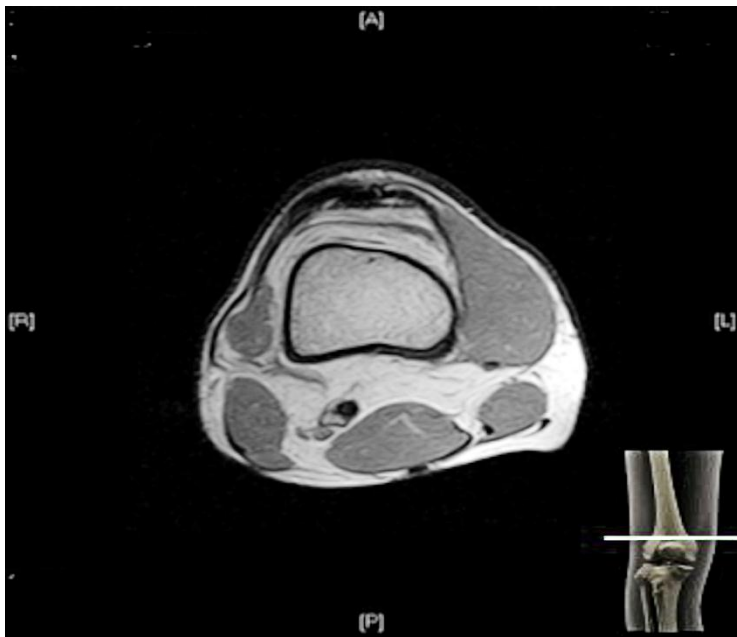


Figure 2

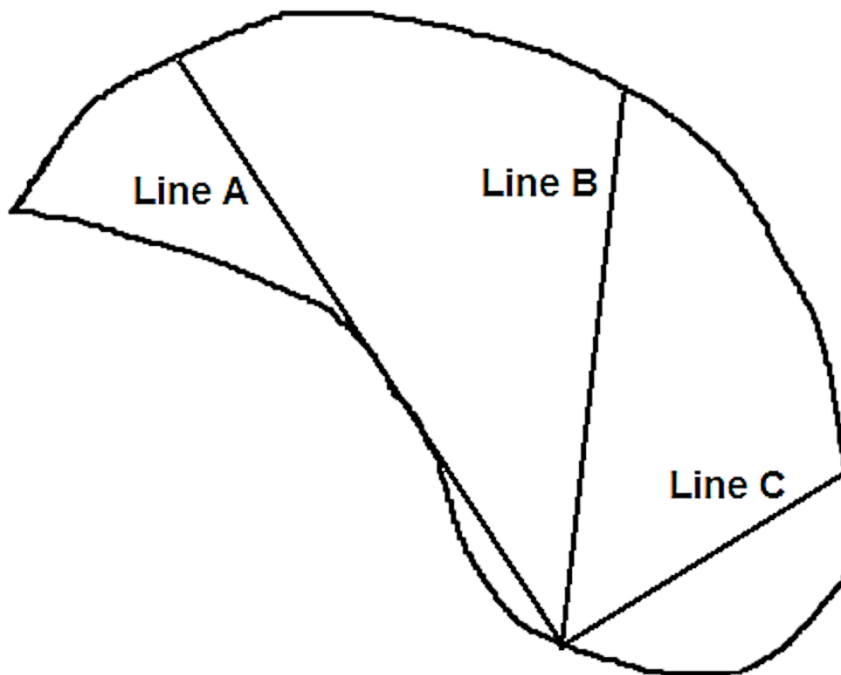


Figure 3



Figure 4



Figure 5

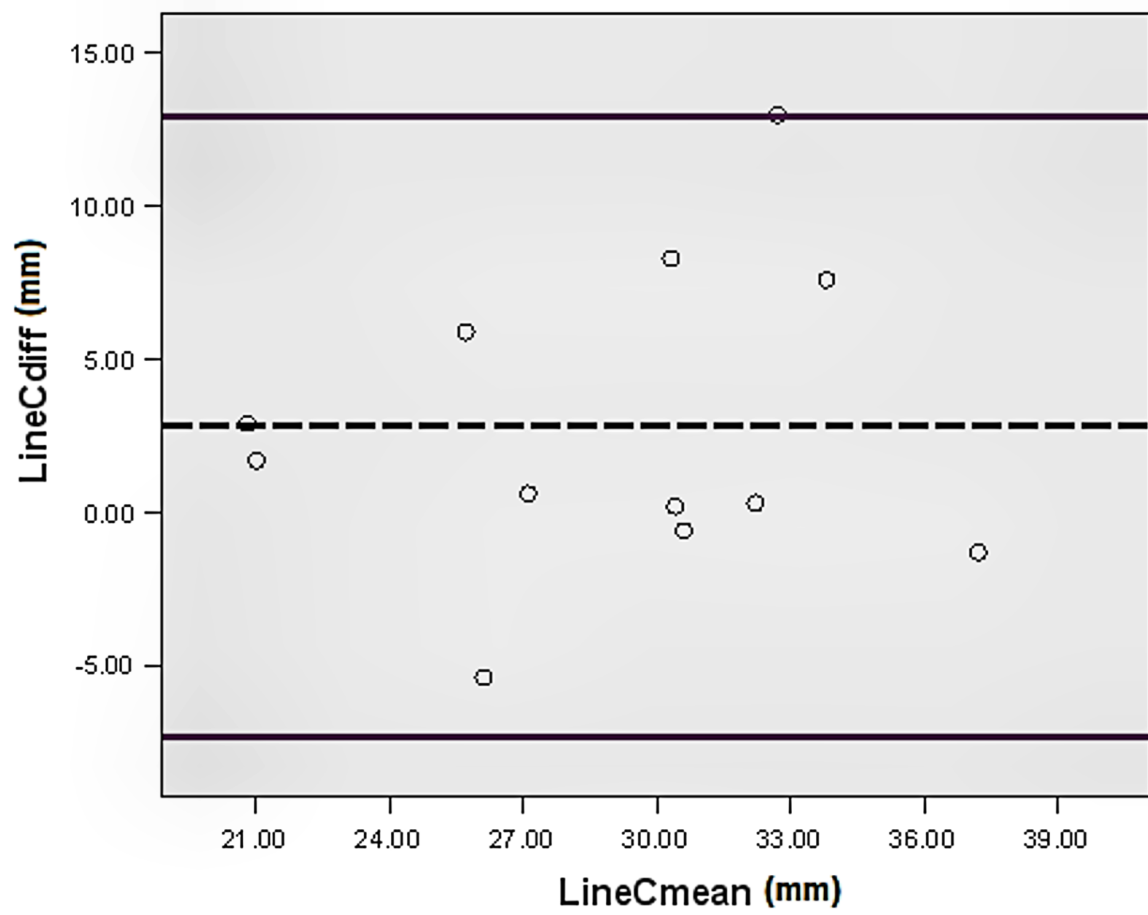


Figure 6

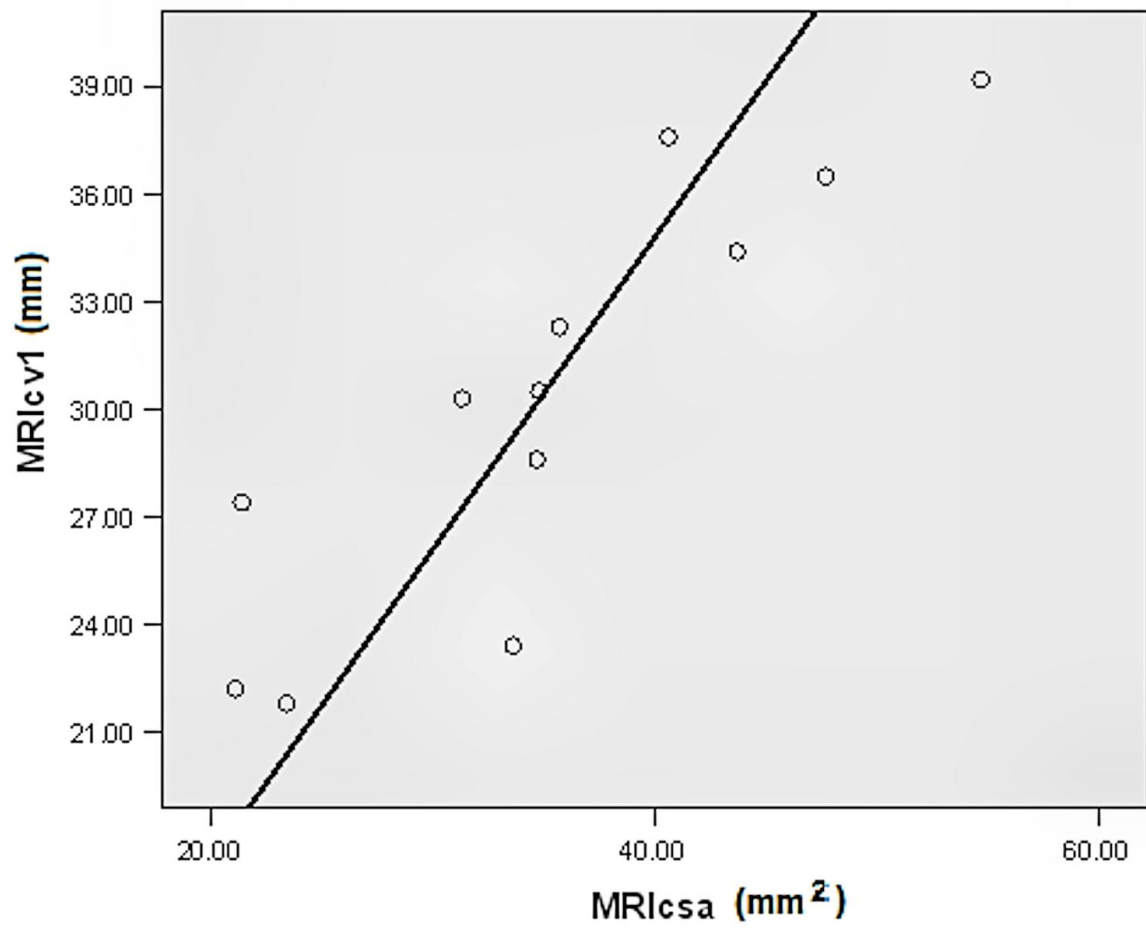


Table 1. Measurements of vastus medialis muscle size made using magnetic resonance imaging (MRI) and rehabilitative ultrasound imaging (RUSI)

	Imaging Technique					
	MRI			RUSI		
	Mean	SD	Range	Mean	SD	Range
CSA (mm²)	35.2	10.4	21.1 - 54.7	-	-	-
Line A (mm)	42.0	7.3	29.8 - 57.8	41.3	7.4	25.1 - 55.2
Line B (mm)	23.0	4.6	15.2 - 33.4	23.5	4.5	17.5 – 30.3
Line C (mm)	31.0	5.5	21.8 – 39.2	28.1	5.5	19.3 – 36.2

SD standard deviation

There were no significant differences between MRI and RUSI measurements

Table 2. Comparison of linear measurements of vastus medialis from MRI and RUSI scans by intra-class correlation coefficients (ICC) and Bland and Altman analysis.

	ICC		Bland and Altman		
	ICC Coefficient	95% Confidence Limits	Mean difference (mm)	SD of differences (mm)	95% Limits of Agreement (mm)
Line A	0.94	0.79 - 0.98	0.7	2.6	-4.9 to 4.7
Line B	0.84	0.53 – 0.95	-0.5	2.6	-6.2 to 3.1
Line C	0.84	0.54 – 0.95	2.9	3.1	-0.6 to 8.3

Table 3. Pearson correlation coefficients for each line measure and multiplication of line measures with the CSA. Measures were taken from the MRI images of the VMO muscle.

Linear measure	Correlation with CSA measure (r)	P-value
A	0.66	0.019
B	0.23	0.477
C	0.87	0.000
AxB	0.60	0.041
AxC	0.86	0.000
BxC	0.66	0.019