REPRODUCIBILITY AND REPEATABILITY OF MUSCLE THICKNESS MEASUREMENTS WITH ULTRASOUND: STTUDY OF VARIATIONS ACROSS DAYS AND EVALUATORS

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SUMMARY

Muscle hypertrophy refers to the increase in total muscle mass, characterized by a rise in the number of actin and myosin filaments in each muscle fiber. Skeletal muscle is dynamic, maintaining a balance between protein synthesis and breakdown. Physical training stimulates adaptations like strength and hypertrophy, while inactivity leads to muscle atrophy. Magnetic resonance imaging (MRI) has historically been used to assess muscle tissue with high accuracy, but its application is limited to laboratory settings due to high costs and impracticality in clinical environments. To address this, ultrasound has emerged as a viable tool for evaluating muscle areas with high validity. This study aims to determine the validity of intra- and inter-rater ultrasound measurements of rectus femoris muscle thickness. The reproducibility sample size was determined using interclass correlation with a power of 80% and a significance level of 5%, requiring 52 participants. For repeatability, an ANOVA for repeated measures with a power of 95% was used, requiring 86 participants. Ultrasound (Mindray model M6) with a linear probe (6-14 MHz) will measure muscle thickness at 40% of the femur's length from the proximal region. Skin markings and standardized probe positioning will ensure consistency. Participants will be in a relaxed, dorsal decubitus position during imaging, with water-soluble gel applied to minimize distortion. All visits will take place in an air-conditioned room, supervised by a professional to ensure procedural accuracy.

Keywords: Muscle hypertrophy; ultrasound; reproducibility; muscle cross section.

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Introduction

In humans, muscle mass remains relatively stable during early life, but after the age of 30, a natural process of muscle mass reduction begins at a rate of 0.5 to 1.0% per year. With aging, the impaired balance between protein synthesis and proteolysis in skeletal muscle results in a progressive decline in muscle mass, strength, and function, a condition known as sarcopenia. To date, physical activity, particularly resistance training, is endorsed as a first-line therapy to manage sarcopenia. Randomized controlled clinical trials have shown positive effects of resistance training on muscle mass, strength, and physical performance.

There are various ways to assess muscle mass gain. One common method is to measure muscle circumference before and after strength training through perimeter assessment. Bone densitometry can also be used to evaluate muscle mass gain, as muscle mass is directly related to bone density. Additionally, bioelectrical impedance is a non-invasive technique that can measure body composition, including muscle mass. Another method is to measure muscle thickness using ultrasound. The muscle cross-sectional area is a measure of the muscle surface area cut by a plane perpendicular to the muscle's longitudinal axis, often used to assess muscle strength and hypertrophy.

Since its first reports in the literature, anatomical section images have been used to evaluate muscle tissue with a high degree of precision and reliability through procedures such as magnetic resonance imaging (MRI), which allows an accurate assessment of muscle tissue from cross-sectional area analysis or to diagnose muscle injuries. However, its use is limited to laboratory environments, making it impractical for clinical settings due to its high cost. Therefore, new techniques and tools were needed to identify muscle area images that are highly valid and accessible. The aim of this study was to determine the reliability of rectus femoris muscle thickness (MT) measurements, both intra- and inter-rater, as well as inter-day reliability, and to present the absolute and relative typical error of measurement (TEM) for MT in these conditions.

Material and methods

The study was approved by the Human Research Ethics Committee of the Evangelical University of Goiás (UniEvangélica), as per the CEP report No. 6.210.982, with the Certificate of Presentation for Ethical Consideration (CAAE) No. 69796523.7.0000.5076. The individuals agreed to participate and signed an Informed Consent Form (ICF), in accordance with Resolution 196/96 of the National Health Council (CNS).

The Mindray M6 ultrasound was used to determine the thickness of the rectus femoris (RF) muscle using a linear transducer with a frequency of 6-14 megahertz (MHz), model L14-6Ns. Anatomical points from the greater trochanter and lateral epicondyle of the femur of the right thigh were adopted, followed by the positioning at 40% of the femur's length towards the proximal region for the analysis of the rectus femoris (RF) muscle thickness. The study included one hundred and eight (108) participants of both sexes, aged between 18 and 67 years. Recruitment and selection began in August 2023, immediately after the protocol was approved by the Ethics Committee of the Evangelical University of Goiás.

The sample for the reproducibility study was established considering the tests to be conducted (interclass correlation), with a power of 80%, an average effect size of 0.5, and a significance level of 5%, requiring 52 participants. For repeatability analysis, the G*Power software (version 3.0) was used, considering the collection from two groups and three measurements from the same participant (ANOVA for repeated measures) for each group, with a power of 95%, an effect size (f) of 0.25, a significance level of 5%, and a 20% loss, requiring 86 participants.

Statistics

The analysis was constructed based on the concepts of technique reproducibility (inter- and intra-observer or evaluator) and measurement repeatability, which can also be inter- and intra-observer. If two evaluators are used, and each makes at least three measurements, the results were described as mean and standard deviation for variables with a normal (Gaussian) distribution, and as median and 95% confidence interval (CI 95%) for non-normal distributions. The test used to verify the distribution was the Shapiro-Wilk test for fewer than 50 observations or the Kolmogorov-Smirnov test for more than 50 observations. The analysis was conducted using one measurement and/or the average of the three measurements.

Reproducibility refers to whether the technique or instrument used follows the same standard without generating errors. It indicates if the technique or instrument can be replicated with a high degree of reliability. The agreement between the measurements (whether good or poor) was assessed by two experienced evaluators. The test used to determine this agreement will be the kappa coefficient.

Results

After analyzing the statistical assumptions, the data were described using the mean, standard deviation, and 95% confidence interval. The characteristics of the sample used are described in Table 1. The Shapiro-Wilk normality test demonstrated a normal distribution for the repeated measurements of rectus femoris muscle thickness ($p = 0.815$; $p = 0.175$), for both men and women.

Tabela 1. Sample Characteristics (N = 108)

	Male (N = 50)				Female (N = 58)			
	Age (years)	IC 95% (inf-sup)	MT (cm)	IC 95% (inf-sup)	Age (years)	IC 95% (inf-sup)	MT (cm)	IC 95% (inf-sup)
Average	36,0	31,9 - 40,0	2,41	2,30 - 2,52	29,9	26,2 - 33,5	1,86	1,77 - 1,93
STDV	14,1		0,38		14,0		0,31	

Legenda: STDV = Standard Deviation; MT = Muscle Thickness

The intra-rater intraclass correlation coefficient (ICC) analysis (internal consistency of the measurement) showed an excellent classification for evaluator 1 (Visit 1; ICC = 0.998; p = 0.0001 – Visit 2; ICC = 0.998; p = 0.0001) and for evaluator 2 (Visit 1; ICC = 0.997; p = 0.0001 – Visit 2; ICC = 0.998; p = 0.0001).

When we analyzed the inter-rater reliability within the same session (session 1), we observed an ICC = 0.994 (p = 0.0001). The same result was found for the measurements between evaluators in session 2 (ICC = 0.994; p = 0.0001).

Finally, when evaluating the reliability between measurement sessions of rectus femoris muscle thickness (stability), an ICC of 0.963 (p = 0.0001) was observed for evaluator 1, and an ICC of 0.962 (p = 0.0001) for evaluator 2.

Table 2 presents the relative and absolute typical error of measurement (TEM) for all reliability analyses conducted.

	Internal Consistency				Inter Raters		Inter Days	
	Session 1		Session 2		Session 1	Session 2	Rater 1	Rater 2
	Rater 1	Rater 2	Rater 1	Rater 2				
TEM Abs (cm)	0,03	0,03	0,03	0,04	0,10	0,09	0,11	0,11
TEM Rel (%)	1,3	1,5	1,3	1,5	4,8	4,7	5,7	5,5

Legend: Typical Error of Measurement (TEM);

Table 2. Presentation of relative and absolute TEM for the muscle thickness (MT) measurements observed across evaluation sessions and different evaluators. This

table will summarize the TEM values, providing a clear comparison of the consistency and accuracy of the measurements for each session and evaluator.

Conclusion

The results demonstrated intra- and inter-rater reliability with an ICC of 0.9 and $p < 0.001$. Therefore, B-mode ultrasound is a reliable tool for measuring muscle thickness. This analysis helped establish the validity and consistency of the measurement methods used, allowing researchers and healthcare professionals to make accurate inferences and informed decisions based on these results. The intra-rater consistency suggests its applicability by the same professional at different times. The inter-rater variability highlights the need for standardized approaches. The temporal stability of the measurements reinforces its usefulness in follow-up studies. Ultimately, this study contributes to advancing knowledge in the field, providing solid evidence on the reliability of B-mode ultrasound for muscle evaluation, paving the way for more robust and reliable clinical and scientific applications.

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