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Influence of Probe Position in the Measurement of Muscle Thickness and its Association with Lean Mass: an Ultrasound Study

Influencia de la Posición de la Sonda en la Medición del Grosor Muscular y su Asociación con la Masa Magra: Un Estudio Ecográfico

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SUMMARY: Skeletal muscles play a fundamental role in people's lives and their evaluation provides significant information on health. Different tools have been used to evaluate muscle mass, and the evaluation of muscle thickness (MT) using ultrasound has been included as an alternative, which can be performed with the probe in different positions; however, these could present differences. The objectives of this study were to determine whether there are differences in the measurement of MT in the vastus lateralis (VL) muscle using the probe in the longitudinal or transverse position, and to determine its association with the lean mass of the lower limbs. The results indicated no significant differences between MT measurements with the probe in the longitudinal and transverse positions ($p = 0.084$). However, when associating these measurements with lower limb lean mass, it was found that transverse measurements had a strong association ($r = 0.547$; $p < 0.001$), while longitudinal measurements had a moderate association ($r = 0.351$; $p = 0.007$). This suggests that measurements with the probe positioned transversely to measure the MT would be the best option. Therefore, it could be useful as an indicator of lower limb lean mass in the absence of tools, such as bioelectrical bioimpedance or magnetic nuclear resonance.

KEYWORDS: Lean mass; Sarcopenia; Muscle quality; Muscle thickness; Muscle architecture.

INTRODUCTION

Skeletal muscle plays a fundamental role in people's lives, and its ability to produce mechanical energy makes it possible to move, maintain posture, perform daily activities, and function in society (Frontera and Ochala, 2015). Muscle tissue accounts for approximately 40% of the body mass, maintains body temperature (Guderley, 2004), fulfills endocrine functions, and contributes to the maintenance of glucose levels (Pedersen and Febbraio, 2012). Therefore, studies on muscle mass have been of interest to the scientific community over the years, as it has been used as a powerful indicator of health (Heymsfield *et al*., 2015).

It is now known that skeletal muscles undergo morphological changes in addition to mass loss, such as ectopic infiltration of fat tissue (De Carvalho *et al*., 2019), loss of innervation, alterations in neuromotor junctions (Jerez-Mayorga *et al*., 2020), and adaptations in muscle architecture (Narici *et al*., 2021). This leads to a loss of muscle functionality in terms of strength, speed, and power (Suchomel *et al*., 2018), which leads to an increase in comorbidities and mortality, and therefore, an increase in public health expenditure (Cruz-Jentoft and Sayer, 2019; Tieland *et al*., 2018).

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Different tools have been used to estimate the cross-sectional area (CSA). A method that has gained space in the last time is the use of B-mode ultrasound, as it is considered to have a very low radiation risk and allows live assessments (Tagliafico *et al*., 2022). Similarly, the equipment is becoming increasingly portable, allowing for the estimation of muscle mass at the bedside (Tosato *et al*., 2017). This allows the performance of different types of assessments in the muscle, which vary depending on the position of the probe, such as muscle architecture, when the probe is placed parallel to the longitudinal axis of the muscle (Narici *et al*., 2021), or echogenicity if the probe is placed perpendicular to the longitudinal axis of the muscle (Lopez *et al*., 2017). In particular, muscle thickness (MT) could be a potential health indicator because of its association with muscle function (Ikezoe *et al*., 2012; Abe *et al*., 2015a) and has been proposed as a valid method to obtain the CSA of muscles in the lower limbs (Sponbeck *et al*., 2021) and, in this way, early screening for decreased muscle mass or monitoring of therapeutic interventions (Strasser *et al*., 2013). Different muscle groups of both the upper and lower limbs have been used to determine the MT (Miyatani *et al*., 2004). One of the commonly used protocols is the vastus lateralis (VL) muscle, which has been studied at different lengths using a measurement with the probe in the longitudinal position (Lima *et al*., 2014; Marzilger *et al*., 2020).

The MT was defined as the distance between the deep and superficial aponeuroses. When the probe is positioned in the longitudinal axis, the aponeuroses are described as two parallel lines appreciated in the ultrasound image (Blazevich and Sharp, 2005), whereas in a transverse measurement, the aponeuroses are presented with a curvilinear disposition, even with an irregular shape, showing different MT lengths in the same image (Zhou & Zheng, 2021). This situation could generate variation in the MT measurement, even if the probe was positioned at the same specific point. In this context, the MT determined on the longitudinal axis could be altered or incongruent to estimate the real muscle thickness at that specific point. Despite the possible incongruities in the measurement, we did not find studies that determined the variation between the measurements with the probe positioned on the longitudinal or transverse axis for the LV. Therefore, the aim of this study was to determine whether there are differences between LV MT measurements determined using the probe in the longitudinal or transverse position, and to determine its association with lower limb lean mass.

Table I. Participant characteristics.

	n = 60
Female / Male	22/38
Age (years)	39.5 ± 12.5
Height (cm)	163.1 ± 9.3
Body mass (kg)	77.5 ± 14.5
Body mass index (kg x m^2)	29.1 ± 5.0
Lower limb length (m)	0.8 ± 0.05
Mean lean mass of lower limbs (kg)	9.3 ± 1.9

Values are shown as mean ± Standard deviation.

MATERIAL AND METHOD

This study was conducted at the Laboratory of the Basic Sciences Department of the Universidad San Sebastián, Valdivia, Chile. Its protocol evaluation was approved by the scientific ethics committee of the University of Granada, Spain (2380/CEIH/2021) and the Universidad San Sebastián, Chile (55-2021-20).

Participants. Participants of both sexes and over 18 years of age were included. Each participant was provided with an informed consent form detailing the study information and adhering to the principles of the Declaration of Helsinki. The participant characteristics are presented in Table I.

Sample size estimation . A study with similar characteristics was conducted (Vega *et al*., 2018). The number of participants was required to obtain a statistical power of 80%, a statistical significance level of 5%, and an effect size of 0.4. The estimated sample size for this study corresponded to 44 participants. Sixty participants were recruited for the study.

Ultrasonography. Ultrasound imaging was used to assess muscle thickness (MT) in the dominant limb of each participant. A linear probe programmed in B-mode with a frequency range of 7.5 10 MHz and a depth of 8 cm (Sonus, DUO LCP) was positioned on the longitudinal and transverse axes of the vastus lateralis (VL) muscle. The probe was covered with a large amount of conductive gel to prevent skin pressure. The participants were positioned on the edge of a stretcher with 90º knee flexion and instructed to relax their musculature at the time of the measurement, and they were asked not to perform any physical exercise 48 h before the evaluation. Measurements were taken at 50% of the distance between the most proximal point of the greater trochanter and most distal point of the lateral femoral condyle. Three images were captured at the evaluation site during the session, and the femur was placed at the center of the image to standardize each evaluation for transverse measurement. software (ImageJ 1.42; National Institutes of Health, Bethesda, Maryland, USA) was used to process and analyze the images. MT was determined as the distance between the deep and superficial aponeurosis. To calculate the total MT value along the longitudinal axis, three measurements were taken in each image, from the lower limit of the superficial aponeurosis to the upper limit of the deep aponeurosis. The first measurement was taken at the left end of the image, the second at the center, and the third at the right end, and the results were averaged. To determine the total MT value in the transverse axis, two measurements were taken for each image: the first from the point of the deep aponeurosis closest to the superficial aponeurosis and the lower limit of the superficial aponeurosis and the second from the farthest point of the deep aponeurosis to the superficial aponeurosis and the lower limit of the superficial aponeurosis. All measurements were averaged to calculate total MT value (Fig. 1).

Anthropometric measurements. Body composition, along with lower limb lean mass, was assessed using tetrapolar bioelectrical bioimpedance analysis (Rice Lake Body Composition D1000-3; Full Body, USA). The stature was assessed using a portable stadiometer (SECA, Model 213, Hamburg; Germany to 0.1 cm). The dominant lower limb length was assessed manually using a tape measure based on an anthropometric measurement protocol (Norton 2019), and the lower limb length was determined as the distance (in meters) between the most proximal prominent point of the femur and the most prominent distal point of the lateral malleolus of the fibula (Marzilger *et al*., 2020).

Statistical analysis. Descriptive data are presented as mean ± standard deviation (SD), and normality was verified using the Shapiro-Wilk normality test. Pearson's correlation coefficient was used to determine the correlation between the MT measurements and lower limb lean mass. The paired samples Student's t-test was used to determine if there was a difference between transverse and longitudinal MT measurements, and Cohen's d effect was used to measure the effect size. The following scale was used to interpret the magnitude of effect size: < 0.20 = trivial, 0.20 - 0.59 = small, 0.60 - 1.19 = moderate, 1.20 - 2.00 = large, and > 2.00 = very large (Hopkins *et al*., 2009). All analyses were performed using the JASP software (version 0.17), and statistical significance was set at P ≤ 0.05.

RESULTS

All variables showed a normal distribution. When comparing the types of measurements there was only a "small" difference, which is not considered significant between those obtained with the probe positioned in the longitudinal and transverse axis (p value:0.084) (Table II), However, when determining its correlation with the lean mass of the lower limb, the measurements obtained with the probe positioned in the transverse axis presented a "strong" positive association (r:0. 547; p value: < 0.001), on the other hand, measurements taken with the probe on the longitudinal axis only presented a "moderate" positive association with lean mass (r:0.351; p value:0.007) (Fig. 2).

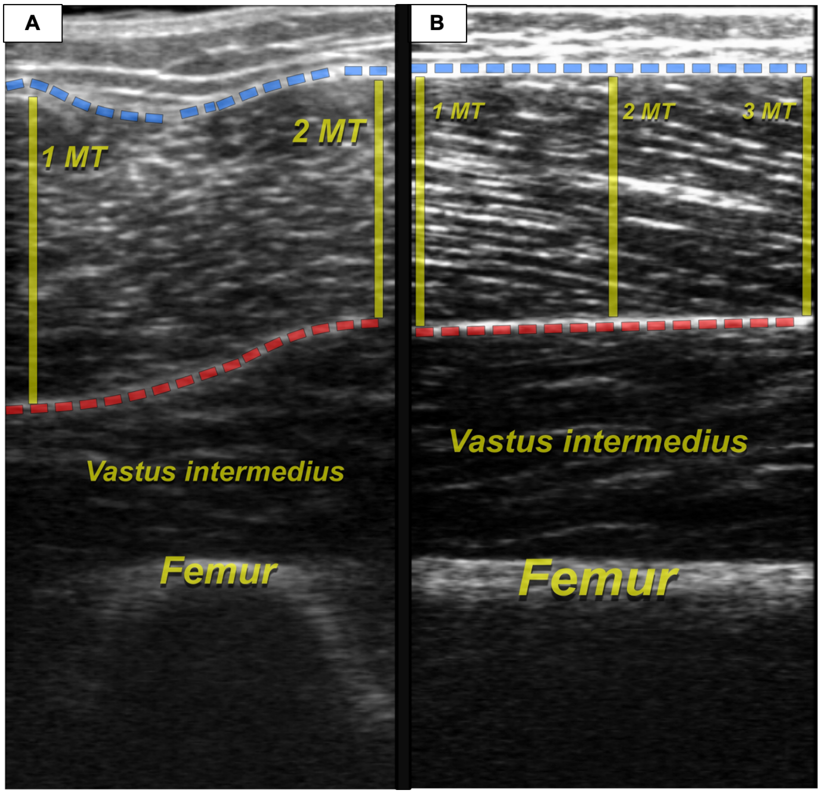

Fig. 1. The measurements were performed for each image. A: Image extracted with the probe in the transverse position; B: Image extracted with the probe in the longitudinal position; blue dashed lines: superficial aponeurosis; red dashed lines: deep aponeurosis; MT1, 2, and 3: Number of muscle thickness measurements.

Table II. Comparison between measurements made with the probe in a longitudinal and transverse position.

	MT Longitudinal	*MT Transversal*	*p value*	*Effect Size (Cohen's d)*	
Mean MT (cm)	2.65 ± 0.539	2.56± 0.421	0.051	0.257	small

MT Longitudinal: Muscle thickness determined with the probe in a longitudinal position. MT Transversal: Muscle thickness determined with the probe in a transversal position. Values are shown as mean ± SD. Statistical significance was established at p < 0.05.

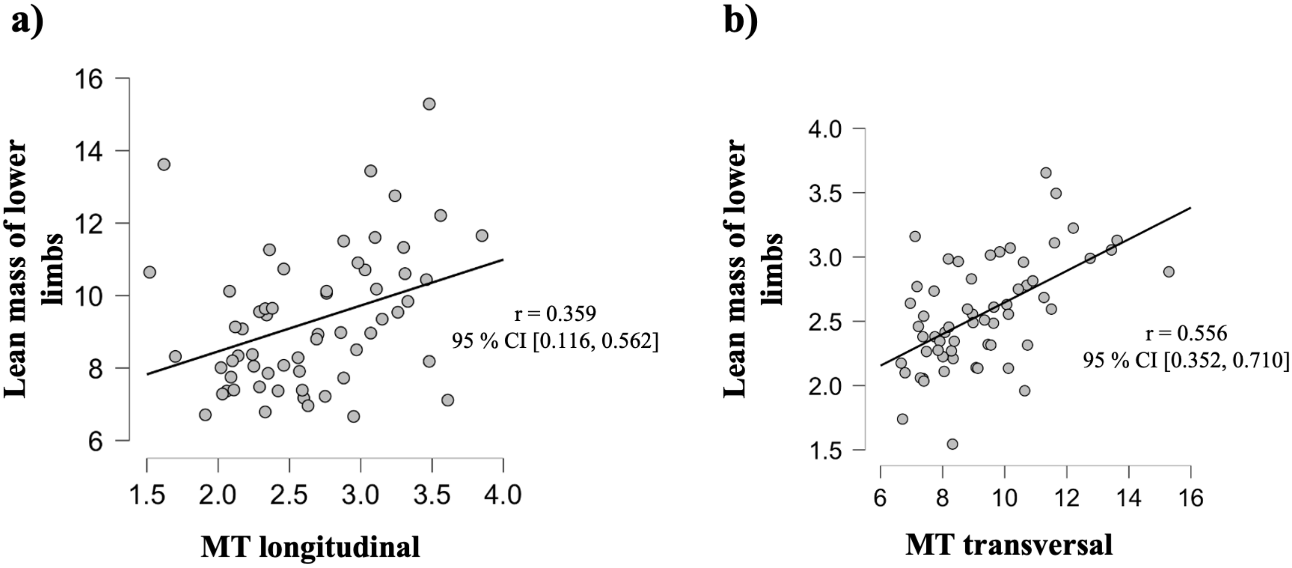

Fig. 2. Association between MT measurements and lower limb lean mass. a: MT measurements performed with the probe in the longitudinal position; b: MT measurements performed with the probe in the transverse position.

DISCUSSION

The objectives of this study were I) to determine whether there are differences in the measurement of MT in the LV using the probe in the longitudinal or transverse position and II) to determine its association with lower limb lean mass. The results indicated no significant differences between MT measurements with the probe in the longitudinal and transverse positions, suggesting that both positions can be used interchangeably in the measurement protocol. However, when associating these measurements with lower-limb lean mass, it was found that transverse measurements possessed a strong association, whereas longitudinal measurements presented a moderate association, suggesting that measurements with the probe positioned transversely present a higher predictive value for lower-limb lean mass. Therefore, it could be useful as an indicator of lower limb lean mass in the absence of bioelectrical bioimpedance or magnetic resonance imaging.

Other studies have assessed the reliability of MT measurements using ultrasound and their association with functional parameters in different populations (Endo *et al*., 2021.; De Souza Silva *et al*. 2018; Strasser *et al*., 2013), showing strong or moderate associations with physical activity level (Ichinose *et al*., 1998), muscle strength, and stiffness (Ikezoe *et al*., 2012). Similarly, a few years ago, Ticinesi *et al*. (2018) proposed an assessment of sarcopenia using MT evaluated using ultrasound in the LV (Ticinesi *et al*., 2018). A similar situation was considered by Abe *et al*., in 2015 who proposed predictive models to determine appendicular lean mass using ultrasound measurements of MT (Abe *et al*., 2015c).

The association between MT and the cross-sectional area (CSA) of the lower limb muscles has been reported in previous studies (Abe *et al*., 2015b), which have established strong relationships between MT of different lower limb muscles and their CSA, such as the tibialis anterior (r =0.9) (Martinson and Stokes 1991), hip adductors (r =0.95) (Ogawa *et al*., 2012), and quadriceps muscles (r =0.82) (Franchi *et al*., 2018). The findings of the present study add to those reported by these authors, where a moderate to strong association was found between LV MT and lower limb lean mass.

To date, the differences between longitudinal and transverse MT measurements of the LV have not been considered. Although the results indicate that there were no significant differences in this measurement protocol, it is possible that there are other affecting factors, such as the position of the knee at the time of the evaluation, which could be determinant because changes in muscle architecture have been demonstrated for different degrees of articulation (Kellis and Blazevich 2022). The individual anatomy of each subject and the specific point of measurement in the muscle did not have a uniform size (Franchi *et al*., 2018). In this context, it is possible that a larger sample or measurement at another point in the muscle with another knee position may reveal different results.

It is important to note that, although the study showed an association between lean mass and muscle thickness measured using ultrasound, a causal relationship could not be established. Furthermore, the correlation found does not necessarily mean that greater muscle thickness always translates into greater lean mass, or vice versa. Other factors such as hydration (Cheng *et al*., 2023) and adiposity (De Carvalho *et al*., 2019) may also influence muscle thickness, as measured using ultrasound.

CONCLUSION

In conclusion, these results suggest that there are no significant differences in muscle thickenss measurements with the probe in the transverse or longitudinal position. However, the transverse probe position had a stronger association with lower limb lean mass; therefore, this measurement may be the most suitable for this purpose. However, further studies are needed to confirm these findings and to determine the best probe position for estimating lean mass in different parts of the body.

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RESUMEN: El músculo esquelético cumple un rol fundamental en la vida de las personas, y su evaluación entrega mucha información de la salud. Se han utilizado diferentes herramientas para evaluar la masa muscular, y el último tiempo se ha incluido la evaluación del grosor muscular (MT) a través de la ecografía como una alternativa para estimarla, las cuales se pueden realizar con la sonda en distintas posiciones, sin embargo, estas podrían presentar diferencias. Los objetivos del estudio fueron determinar si existen diferencias en la medición de MT en el músculo vasto lateral (VL) utilizando la sonda en posición longitudinal o transversal y determinar su asociación con la masa magra de los miembros inferiores. Los resultados indican que no existen diferencias significativas entre las mediciones de MT con la sonda en posición longitudinal y transversal (valor p: 0.084). Sin embargo, al asociar estas mediciones con la masa magra de los miembros inferiores, se encontró que las mediciones transversales poseen una asociación fuerte (r: 0.547; valor p < 0.001), mientras que las mediciones longitudinales presentan una asociación moderada (r: 0.351; valor p: 0.007). Esto sugiere que las mediciones con la sonda posicionada transversal para medir MT serían la mejor opción. Por lo tanto, podría ser de utilidad como un indicador de masa magra de los miembros inferiores en caso de no contar con herramientas como la bioimpedancia bioeléctrica o resonancia nuclear magnética.

KEYWORDS: Masa magra; Sarcopenia; Calidad muscular; Grosor muscular; Arquitectura muscular.

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