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Original article

Muscle fascicle length obtained by panoramic ultrasound and estimated by prediction equations

Comprimento do fascículo muscular obtido pela ultrassonografia panorâmica e estimado por equações de predição

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ABSTRACT

Introduction: B-mode ultrasound is limited to the size of the transducer. However, the panoramic technique allows measuring the fascicle length without using prediction equations. **Objective:** To compare the fascicle length of the vastus lateralis obtained by panoramic ultrasound and estimated by trigonometric equations. **Methods:** Fifteen men aged 24 ± 6 participated in the study. The research is characterized as a single-visit comparative observational study. A panoramic ultrasound image of the vastus lateralis was performed using a 4 cm linear transducer, with a frequency of 10 MHz and 6 cm of image depth, through a 13 cm scan. After collecting the images, the panoramic fascicle length was compared and estimated by two different prediction equations. **Results:** One-way ANOVA detected no significant difference ($P = 0.093$). The analysis of percentage difference, coefficient of determination, standard error of the estimate, and Pearson correlation coefficient between the difference and the average of the panoramic measure compared to equation 1 ($\Delta = 24.1\%$; $R^2 = 0.68$; SEE = 0.9 cm; $r = 0.796$; $p = 0.000$) and equation 2 ($\Delta = 17.4\%$; $R^2 = 0.48$; SEE = 1.1 cm; $r = 0.695$; $p = 0.004$) indicated proportion bias. **Conclusion:** Although no significant difference was observed between the prediction equations and the panoramic measure, the trigonometric equations showed an overestimated fascicle length and a low agreement with the reference measure.

Keywords: exercise; hypertrophy; quadriceps muscle; reproducibility; resistance training.

RESUMO

Introdução: A ultrassonografia no modo B é limitada ao tamanho do transdutor, entretanto a técnica panorâmica permite mensurar o comprimento do fascículo sem a necessidade de utilizar equações de predição. **Objetivo:** Comparar o comprimento do fascículo do vasto lateral obtido pela ultrassonografia panorâmica e estimado por equações trigonométricas. **Métodos:** Participaram do estudo 15 homens com idades de 24 ± 6 anos. A pesquisa caracteriza-se como um estudo observacional comparativo de visita única. Foi realizada uma imagem de ultrassonografia panorâmica do vasto lateral através de um transdutor linear de 4 cm, com frequência de 10 MHz e 6 cm de profundidade de imagem, através de uma varredura de 13 cm. Após a coleta das imagens, foi comparado o comprimento do fascículo panorâmico e estimado por duas equações de predição diferentes. **Resultados:** A ANOVA de uma via não detectou diferença significativa ($P = 0,093$). As análises de diferença percentual, coeficiente de determinação, erro padrão da estimativa e coeficiente de correlação de Pearson entre a diferença e a média da medida panorâmica em comparação a equação 1 ($\Delta = 24,1\%$; $R^2 = 0,68$; EPE = 0,9 cm; $r = 0,796$; $p = 0,000$) e equação 2 ($\Delta = 17,4\%$; $R^2 = 0,48$; EPE = 1,1 cm; $r = 0,695$; $p = 0,004$) indicaram viés de proporção. **Conclusão:** Apesar de não ter sido observada diferença significativa entre as equações de predição e a medida panorâmica, as equações trigonométricas apresentaram uma superestimativa do comprimento do fascículo e uma baixa concordância com a medida de referência.

Palavras-chave: exercício físico; hipertrofia; músculo quadríceps; reprodutibilidade dos testes; treinamento de força.

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Introduction

B-mode ultrasound (US) is commonly used to monitor muscle adaptations resulting from injury rehabilitation [1], resistance training [2], stretching [3], or muscle atrophy [4]. US is a simple and reliable technique to determine muscle architecture variables, such as muscle thickness (MT), fascicle length (FL), and fascicle angle (FA) [5,6].

FL adaptations are associated with the type [7] and speed of contraction [8], as well as the range of motion [9]. Therefore, motor gestures such as jumps [10], sprints [11], and muscle power actions, in general, are benefited from the addition of sarcomeres in series [12]. The determination of the FL is justified in studies with treatment that seek to improve the force-velocity relationship [13].

More recently, the panoramic US technique has demonstrated the possibility of consistently providing an extended field of view in a single scan [14]. The panoramic US allows the evaluator to observe an extensive part of the muscle of interest without the limitation of the transducer size through the rendering of superimposed static images [15]. Thus, it is possible to observe the MT in different portions of the same muscle [16] and to be able to quantify the entire FL without the need to use prediction equations.

The FL prediction equations assume that the superficial and deep aponeuroses of the muscle are parallel. The trigonometric equations, such as those by Finni *et al.* [17] and Kawakami *et al.* [18] assume that FL, MT, and deep aponeurosis form a right triangle. The assumption is not necessarily supported, as the aponeuroses and fascicles are not linear [19]. The FL prediction equations assume that the superficial and deep aponeuroses of the muscle are parallel. The prediction equations were developed when the size of the transducer and software were limited by the technology available. However, with the improvement of software and hardware, it is currently possible to render panoramic images in an US device, being able to perform comparisons between the measurements of panoramic and estimated FL by equations.

Therefore, the present study aimed to determine the difference and agreement in the FL of the vastus lateralis obtained by panoramic US and estimated by trigonometric equations.

Methods

Sample

Fifteen university men aged 24 ± 6 years, with a stature of 1.77 ± 0.6 m and a body mass of 74.6 ± 8.1 kg, participated in the study. The sample was for convenience and composed of Physical Education undergraduate students. The eligibility criteria were: being between 18 and 40 years old, having a BMI < 30 kg/m^2, not having a musculoskeletal injury or existing health restrictions according to the PAR-Q questionnaire, and not using nutritional supplementation or pharmacological aids.

Before entering the study, all participants read and signed an informed consent form. The research was approved by the research ethics committee of *Hospital Universitário Pedro Ernesto of the Universidade do Estado do Rio de Janeiro*, under protocol number 2.531.389 (CAAE: 76693917.2.0000.5259).

Study design

The research is characterized as a comparative observational study. The participants made a single visit to the laboratory and were previously instructed not to perform any moderate or intense physical activity for at least 48 hours before the visit. Before starting the procedures, the subjects were left to rest on a stretcher for 20 minutes to stabilize the intramuscular fluid.

Procedures

The participants remained supine to perform a panoramic US image of the vastus lateralis muscle. A guide rail made of polyethylene foam was positioned at 40% of the upper lateral border of the greater trochanter of the femur and the upper limit of the lateral epicondyle of the tibia. The guide rail was placed at a 15º inclination angle between the greater trochanter of the femur, and the superior region of the patella, as shown in figure 1 [20]. Panoramic US images were recorded by a researcher using a GE LOGIQe ultrasound scanner (*GE Healthcare, Chicago, IL, USA*) with a 4 cm 12L-RS linear transducer, with a frequency of 10 MHz and 6 cm of image depth. Subsequently, a 13 cm scan was performed on the skin with a conductive gel (*Mercur, Santa Cruz do Sul, RS, Brazil*), applying minimal pressure to avoid deforming the tissues.

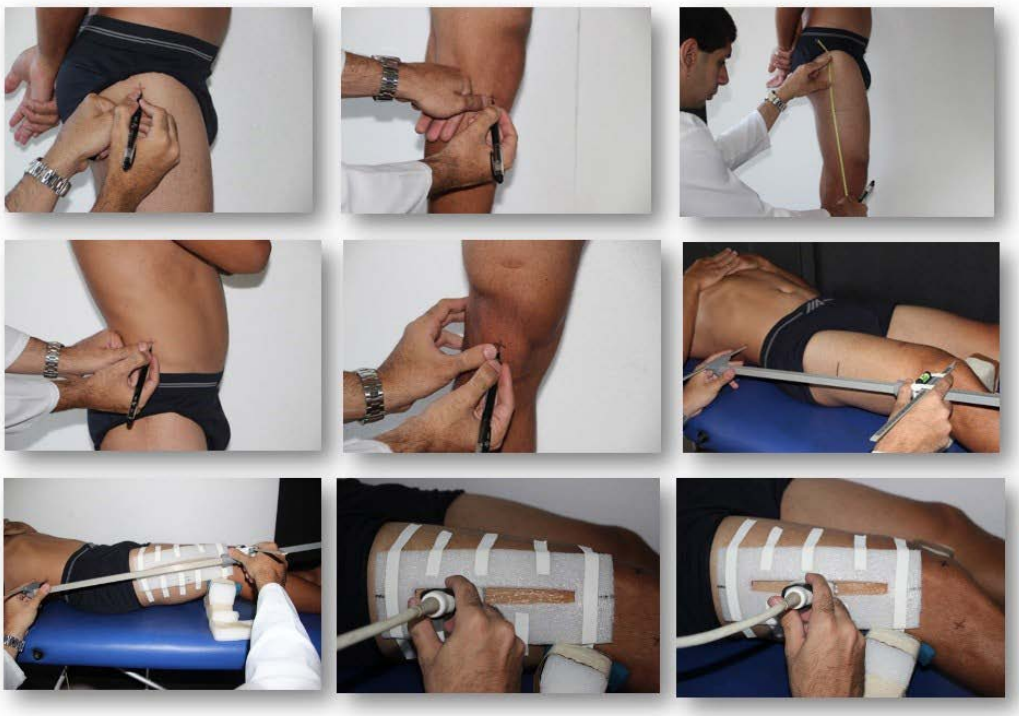
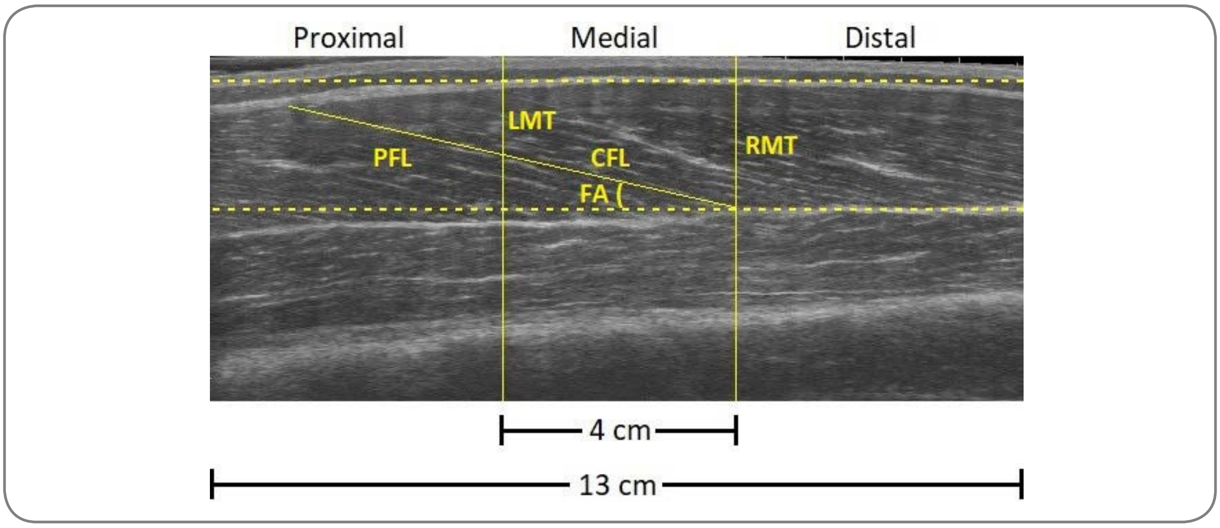


Figure 1 - Identification of anatomical sites for placement of the guide rail and manipulation of the transducer over the vastus lateralis muscle

After collecting the images, an independent researcher coded the files and divided the 13 cm panoramic images into three portions: proximal, medial, and distal. The medial portion, in the center of the image, was limited by the typical size of a 4 cm transducer. Images were analyzed using open-access imaging software (ImageJ, ver. 1.50f, National Institutes of Health, Bethesda, MD, USA). The following variables were quantified: panoramic fascicle length (PFL), conventional fascicle length (CFL), right muscle thickness (RMT), left muscle thickness (LMT), and FA. MT was measured in straight line mode, FL in segmented line mode, and FA in angle tool mode. PFL measurements were compared to estimated FL using equations 1 and 2 by Finni *et al.* [17] and Kawakami *et al.* [18], respectively. Figure 2 shows a typical image of the vastus lateralis muscle observed by panoramic US and typical demarcations of those generally used with the conventional measure for predicting FL through trigonometric equations.



CFL = conventional fascicle length; PFL = panoramic fascicle length; FA = fascicle angle; LMT = left muscle thickness; RMT = right muscle thickness
Figure 2 - Panoramic ultrasound image of the vastus lateralis muscle and typical demarcations of conventional ultrasound

Equation 1, Finni *et al.* [17]: $EFL = EFL + \frac{\text{LMT}}{\sin(\text{FA})}$

Equation 2, Kawakami *et al.* [18]: $EFL = \frac{\text{RMT}}{\sin(\text{FA})}$

Reliability and measurement error

In a recent study carried out by our laboratory, it was observed that measurements performed on different days have high consistency (ICC_{MT} = 0.964; ICC_{FL} = 0.947; ICC_{FA} = 0.942), low absolute error (TEM_{MT} = 0.07 cm; TEM_{FL} = 0.31 cm; TEM_{FA} = = 0.92º), and low variability between measurements (CV_{MT} = 2.9%; CV_{FL} = 2.9%; CV_{FA} = 4.1%) [21]. Thus, it is possible to attribute a greater power of certainty to the results of the comparison between the FL obtained by the panoramic US and estimated by equations 1 and 2 that used the variables MT, FA, and the FL itself for the prediction calculation.

Statistical analysis

Shapiro-Wilk test was used to test the normality of the distribution of the FL measurements and for the prediction variables (CFL, EFL, FA, LMT, and RMT). To determine the difference between the three measurement techniques used to determine the FL, a one-way analysis of variance (ANOVA) was performed, as they are different measures, therefore, independent. The mean of individual percentage differences (Δ = [PFL - EFL] x 100 / PFL) between the FL measurement techniques was also calculated. Linear regression analysis, followed by the coefficient of determination (R^2) and the standard error of the estimate (SEE), were used to verify how the FL variation predicted by equations 1 and 2 explains the variation of the panoramic measure. The agreement between techniques was determined using the Bland-Altman graphical analysis [22], followed by the Pearson correlation coefficient (r) between the difference and the average of the FL measurements for each of the two equations used. The $P < 0.05$ level of significance level was adopted for all analyses performed using the Statistical Package for the Social Sciences software (*IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.*).

Results

Shapiro-Wilk test did not identify deviations from normality for all FL measures and the prediction variables (CFL, EFL, FA, LMT, and RMT) ($P > 0.05$). One-way ANOVA detected no significant difference between the panoramic FL and those predicted by equations 1 and 2 ($F = 2.519$; $P = 0.093$). However, comparing the percentage difference between the panoramic measurement and equation 1 (Δ = 24.1%) and equation 2 (Δ = 17.4%) suggests an overestimation of the FL measurement. Figure 3 graphically shows the mean and standard deviation of the FL measurement techniques. Figure 4 presents the linear regression analysis, R^2, SEE, Bland-Altman, and Pearson's r between the difference and the average of the FL measurements.

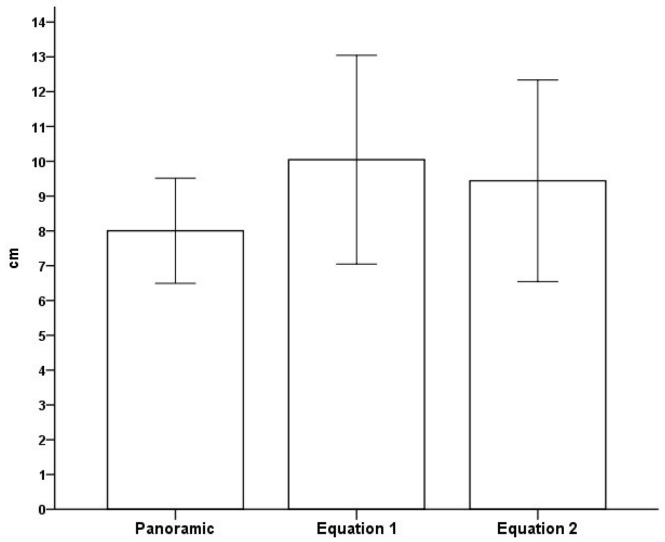


Figure 3 - Descriptive analysis (mean ± standard deviation) of the fascicle length in centimeters (cm) obtained by panoramic ultrasound and prediction equations

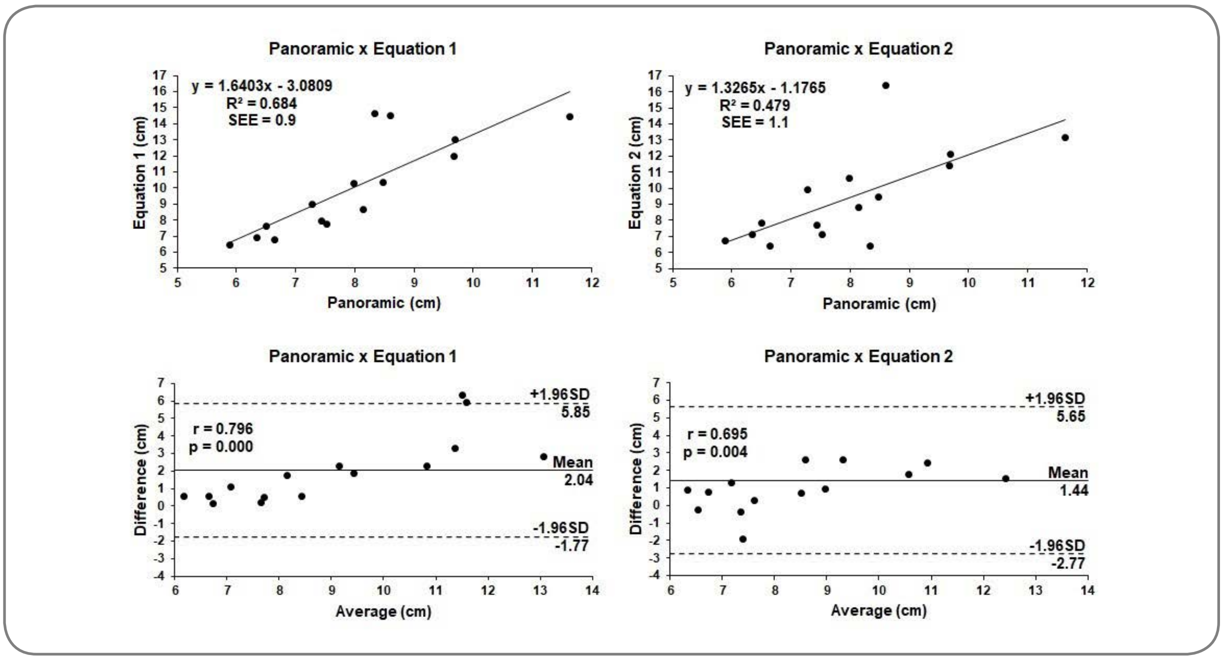


Figure 4 - Linear regression analysis followed by the coefficient of determination (R^2), standard error of the estimate (SEE), and Bland-Altman followed by the Pearson correlation coefficient (r) between the difference and the mean of the measures of the panoramic fascicle length and predicted by equations 1 and 2

Discussion

FL obtained by the panoramic US served as a reference to compare with the FL predicted by the equations of Finni *et al.* [17] and Kawakami *et al.* [18]. However, no significant difference was observed between the vastus lateralis FL obtained by the panoramic US and predicted by trigonometric equations ($P = 0.093$).

Although equation 1 showed a lower variability when compared to the panoramic measure ($R^2 = 0.684$; SEE = 0.9 cm), there was a higher percentage overestimation ($\Delta = 24.1\%$) when compared to equation 2 ($\Delta = 17.4\%$), which showed a higher variability ($R^2 = 0.479$; SEE = 1.1 cm). In addition, the Bland-Altman graphical analysis, followed by Pearson's r between the difference and the average, showed proportion bias in comparing the panoramic FL against equation 1 ($r = 0.796$; $p = 0.000$) and equation 2 ($r = 0.695$; $p = 0.004$). The observed errors and the FL values overestimated by equations 1 and 2 reinforce the need for caution when using FL prediction equations in the vastus lateralis muscle.

Reeves *et al.* [23] observed a 10.7% increase in FL using the equation by Finni *et al.* [17]. However, the adaptation was lower than the percentage difference of 24.1% found by equation 1 compared to the reference measure. Alegre *et al.* [24] detected a 10.5% increase in FL using Kawakami *et al.* [18] equation. However, the percentage difference between equation 2 and the panoramic measure was 17.4% in the present study.

Although there was no statistical difference between the techniques, it cannot be assumed that when interventions, such as resistance training, are performed, predicted FL measures of the vastus lateralis will be reproducible over time.

Equation 1 [17] was developed from the measurement of FL limited to a conventional US field of view size. FL prediction value was based on the distance between the end of the FL in the proximal part of the image to the superficial aponeurosis and the sine of the FA. The measure of FL, estimated by equation 2 [18], used a trigonometric relationship where measurements of the MT of the distal part of the image and the sine of the FA are necessary. On the other hand, the image produced by the panoramic US allows the total visualization of the target fascicle.

Prediction equations are not limited to studies with the vastus lateralis muscle but any pennate muscle. A similar study compared different FL prediction equations with the panoramic measurement of the FL of the biceps femoris obtained by panoramic US [25]. However, it is worth mentioning that previous studies that aimed to determine the difference between the measurement of the reference FL and the prediction equations used different statistical methods. Some studies considered the different techniques as repeated measures [19,25,26], while others did not [27,28]. The procedure used can be a limitation when comparing the results of different studies. The present study considered the different FL prediction techniques as independent measures.

As previously shown, the prediction equations assume that the superficial and deep aponeuroses of the muscle are parallel and that the fascicle is always a straight line, making clear the limitations of conventional US to measure the FL in its entirety [28]. On the other hand, to perform the panoramic measurement, it is necessary to have an experienced rater who maintains constant practice to obtain rendered images without tissue deformation [29]. Therefore, our laboratory suggests that before collecting and quantifying the panoramic or estimated FL measurements, the rater should be trained in the entire process (marking anatomical sites, image acquisition by the US, and FL measurement via software) and have its reliability and measurement error previously tested.

Conclusion

Although no significant difference was observed between the prediction equations and the reference measure, the trigonometric equations overestimated the FL. Therefore, in the absence of the panoramic technique, equations 1 and 2 can be used to predict the FL of the vastus lateralis muscle obtained by the conventional US. However, one should be cautious when interpreting the results, as there was no high agreement with the reference measure, which can be an error factor in cases of interventions that perform repeated measures.

Academic affiliation
This article represents the final product of a graduate discipline carried out by André Luiz Conveniente Soares, M.Sc., and supervised by Paulo Sergio Chagas Gomes, Ph.D.

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Conflict of interest
The authors declare no conflict of interest.

Authors' contribution
Research conception and design: Soares ALC, Gomes PSC; **Data collection:** Soares ALC; **Data analysis and interpretation:** Soares ALC, Gomes PSC; **Statistical analysis:** Soares ALC, Gomes PSC; **Writing of the manuscript:** Soares ALC; **Critical review of the manuscript:** Gomes PSC

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