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Upper and Lower Limb Muscle Thickness Measurements in Older Women: Analysis of Variability and Development of Prediction Equations

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SUMMARY

Purpose. The present study aimed to describe the variability of muscle thickness measures (MT) of arm and thigh muscles, obtained by ultrasound (USD) from community-dwelling healthy older women; and to obtain a predictive equation model for arm, thigh and leg MT based on upper and lower limb circumference measures using a frequentist and Bayesian approach.

Methods. Sixty-two older women (74.5 ± 8.5 years) volunteered and were submitted to MT measures. Assessments of the Brachial (Br), Biceps Brachii (BB), Vastus Lateralis (VL), Rectus Femoris (RF) were obtained and grouped to infer elbow flexors (EF), and knee extensors (KE) muscles. Thigh (TC) and arm (AC) circumference were also measured. Variability of MT and circumference measures were inferred by the coefficient of variation (CV) and Huber's M- estimator. Age-adjusted linear regression models were generated to predict MT. The magnitude of evidence was obtained using Bayesian inference, determining posterior probabilities based on our data.

Results. The MT measure of the EF presented lower CV (15.9%). The CV of limb circumference measures was 10.8% (TC) and 12.5% (AC) and Huber's M-estimator varied 0.18 cm (AC). Circumference measurements were significantly associated with MT with age-adjusted coefficients of determination (R^2) of 0.268 and 0.173 ($p < 0.05$), and standard error of the estimate of 0.36 cm and 0.56 cm for EF and KE, respectively. Bayesian inference confirmed the good posterior probability of the model.

Conclusions. Measure of MT of the EF presented lower between-subject variability, and a better predictive equation model for MT based on AC.

KEY WORDS

Anthropometry; muscle mass; lean mass; body composition; aging.

INTRODUCTION

The muscle mass assessment is a valuable tool in the process of screening older adults at risk for many clinical outcomes. Thus, the early detection of muscle mass reduction helps to detect early older adults at risk of functional impairments and co-morbidities development. Ultrasound (USD) has been successfully used to obtain muscle thickness (MT) measurements (1-6), including investigations involving older adults (7, 8).

It is known that the pattern of muscle mass decrease along the aging process is not homogeneous among muscle groups (8), but the between-subject variability of muscle thickness leads to difficulty the establishing reference values for comparisons. Additionally, ethnic differences, a recognized factor determining factor of body composition, also limits the use of reference values and predictive equations from studies previously developed studies (9), since mostly predictive equations of muscle mass using USD were developed with Asian populations (7, 10).

However, despite the extensive knowledge about MT, data from the variability of muscle thickness measurements from arm and thigh muscles, obtained from the USD, are scarce, and predictive equations for estimating MT using limb circumference measurements are very desirable. Despite being less accurate in estimating segmental muscle mass, the limb circumference measurement is an easily accessible tool, with an even lower cost than the USD and less demand for technological knowledge (11).

Menopause accelerates muscle mass reduction in older women, which could lead to greater between-subject variability in MT, than in older men. Therefore, in this study we aimed to 1) describe the variability of MT from arm and thigh muscles among older women; 2) obtain a predictive equation model to estimate arm and thigh MT by limb circumference measurements using a frequentist and Bayesian approach.

MATERIALS AND METHODS

Sample

Seventy-two community-dwelling older women (74.5 ± 8.5, 60 to 100 years old) with no acute diseases or cognitive impairment (12, 13) volunteered for this study. All participants presented independent ambulation and the absence of limb amputations or skin lesions. Additionally, volunteers were instructed about all procedures and signed an informed consent form. **Table I** presents the sample characteristics. The local Research Ethics Committee approved (protocol n. 2.783.516 – Date of approval: July 24, 2018) all procedures according to the Declaration of Helsinki.

Table I. Sample characteristics.

Variable	Mean ± SD
Age (years old)	74.5 ± 8.5
Weight (Kg)	65.1 ± 11.1
Height (cm)	152.4 ± 6.3
BMI (Kg/m^2)	28.0 ± 4.5

Muscle thickness measurements

Transverse ultrasound images of the right Brachial (Br), Biceps Brachial (BB), Vastus Lateralis (VL), and Rectus Femoris (RF) muscles were obtained with a B-mode 2-dimensional ultrasound (USD) imaging device (Figlabs® FP 102). Volunteers remained upright with their upper and lower limbs relaxed and with palms facing forward, as described by Takai *et al.* (7). A single trained and qualified evaluator performed all USD measurements.

The linear array transducer (Figlabs®, L471), and the image was obtained in B-mode because it is a widely used technique in muscle tissue imaging (7). The transducer with a sampling frequency of 7.0 MHz was positioned perpendicularly to muscular tissue and underlying bone. During the image recording, the pressure was kept to a minimum to avoid excess compression and distortion, and a generous amount of contact water-soluble gel was applied (1).

The assessment sites were marked precisely, with the reference points marked using a dermatologic pen. The MT of Br and BB muscles were recorded as proposed by Abe *et al.* (9), at 60% of the distance between the lateral epicondyle and the acromial process. For the MT of VL and RF muscles, the reference point used corresponded to 2/3 of the distance between the greater trochanter and the lateral femoral epicondyle, 3 cm from the midline of the anterior thigh, as proposed by Chilibeck *et al.* (6). The chosen muscles are frequently requested during daily activities and commonly analyzed in similar studies, besides being easy to identify through palpation techniques and also presenting lower chances of picking up signals from neighboring muscles during recordings (3, 4).

Subcutaneous adipose tissue at the tissue-muscle and muscle-bone interfaces were identified in the USD image and used to identify the muscle limits, guiding the MT measurements for each of the elbow flexors (EF) and knee extensors (KE) muscle groups (10).

Limb circumference measurements

An inelastic anthropometric tape measure (ABNTM, Brazil) was used to assess thigh (TC) and arm (AC) circumference as proposed by Callaway *et al.* (14), on the medial portion of the right thigh (5). The upper and lower body limb was relaxed during the measurements and the medial portion of the thigh and arm was used as reference. All measurements were performed in a standardized way, with three measurements, and the mean value was used for the study analysis.

Statistical analysis

Descriptive analysis was carried out with mean, standard deviation, coefficient of variation (CV = [(Standard Deviation/mean) × 100]), minimum, maximum, and 95% confidence interval (upper and lower limits), as well as median and 25th and 75% percentiles of MT, TC and AC. As the mean is a central tendency measure highly influenced by extreme values (outliers), Huber's M-estimator was also reported, since it represents a measure of robust estimate, as suggested by Maumet *et al*. (15) and Proisy *et al*. (16). Additionally, all variables of interest were submitted to the Shapiro-Wilk normality test.

Age-adjusted linear regression models were generated to predict muscle thickness from limb circumference measurements. Adequacy of each regression model (simple and multivariate) was verified by 1) analyzing residues using Shapiro-Wilk test; 2) visual inspection was also conducted to identify extreme observations in histograms of residues; 3) Durbin-Watson index, adopting a value closer to 2, the more adequate (17). These statistical procedures were carried out using SPSS (SPSS Inc., Chicago, IL) and the critical alpha was set at 0.05.

The Bayes factor hypothesis testing was used to check qualitative outcomes and the probability of replicating results (*i.e*., magnitude of the evidence) as done by Souza-Júnior *et al*. (18). Briefly, Bayes factor (BF10) represents the change from prior to posterior odds of the model given a set of data. In regression models, it indicates the difference between prior odds and posterior odds (19, 20), indicating the relative predictive performance of the model for the analyzed data (*i.e*., ratio of marginal likelihoods) (21). Interpretation of Bayes factor is intuitive: BF10 = 5 indicates that data are 5-fold more likely under alternative hypothesis than null hypothesis, whereas BF10 = 0.2 indicates that the observed data are 5-fold more likely under null hypothesis than under alternative hypothesis (21, 22). It is worth emphasizing that, in linear regression models, the null hypothesis states that the true slope of the line (*i.e*., beta) is zero (23).

Bayes factor analysis was carried out using JAMOVI®, and the parameter Jeffreys-Zellner-Siow prior (JZS prior) was set as r scale = 0.354. Bayes factor was reported for the multivariate model since it quantifies the probability favoring to alternative hypothesis (*i.e*., the ability of observed data under models to predict the outcome).

RESULTS

Descriptive characteristics of the MT from the studied population are presented in **table II**. Based on the descriptive analysis it is possible to infer that the EF thickness measurement exhibited less variability (CV = 15.9%), whereas the Br measurement the greater variability (CV = 24.3%). The Huber's M-estimator of MT varied by 0.01 cm (Br and BB) from the mean MT, indicating that the mean measures suffered little influence from extreme values. Only the MT measurement from the RF did not meet the assumption of normality of the data distribution ($p > 0.05$).

The descriptive parameters from limb circumferences are shown in **table III**. The variability (*i.e*., CV) of limb circumference measurements was between 10.8%, for TC, and 12.5%, for AC. The Huber's M-estimator of MT varied by 0.18 cm (AC) from the mean limb circumference, indicat-

Table II. Descriptive statistical parameters of muscle thickness measurements.

Statistical Parameter	Br	BB	VL	RF	EF	KE
Mean	0.95	1.70	1.62	1.70	2.65	3.32
Huber's M-estimator	0.94	1.69	1.59	1.66	2.63	3.29
SD	0.23	0.33	0.34	0.39	0.42	0.62
95%CI - Lower	0.88	1.60	1.53	1.59	2.52	3.15
95%CI - Upper	1.02	1.81	1.71	1.80	2.78	3.48
CV	24.3	19.1	21.1	22.7	15.9	18.7
Minimum	0.51	1.16	1.00	1.08	1.91	2.25
Maximum	1.57	2.63	2.60	2.84	3.56	4.76
Median	0.78	1.42	1.42	1.37	2.30	2.75
Percentage 25	0.95	1.70	1.57	1.67	2.70	3.35
Percentage 75	1.09	1.88	1.83	2.01	2.92	3.68

Br: Brachial; BB: Biceps brachial; VL: Vastus lateralis; RF: Rectus femoris; EF: Elbow flexors; KE: Knee extensors.

Table III. Descriptive statistical parameters of limb circumference measurements.

Statistical Parameter	AC	TC
Mean	30.9	50.2
Huber's M-estimator	30.7	50.5
SD	3.9	5.4
95%CI - Lower	30.0	48.9
95%CI - Upper	31.9	51.6
CV	12.5	10.8
Minimum	22.2	36.3
Maximum	39.5	62.5
Median	27.9	45.7
Percentage 25	31.2	50.5
Percentage 75	33.1	53.58

AC: arm circumference; TC: thigh circumference.

ing that the mean measures suffered little influence from extreme values. The TC and AC measures met the assumption of normality of the data distribution ($p > 0.05$).

Table IV presents parameters from linear regression models to predict muscle thickness. Limb circumferences were significantly associated with MT measurements of the respective body segments ($p < 0.05$), and the age-adjusted coefficients of determination (R^2) were 0.268 and 0.173 in predicting EF and KE thickness, respectively. The standard error measures of the estimate were small, 14 and 17% for the EF and KE thickness estimation models, respectively. Additionally, the analysis of the residuals analyzing residues and the Durbin-Watson index indicated the good fit of the regression models obtained in this study.

Results from Bayesian analysis (**table IV**) indicated high BF10 for proposed linear regression models, suggesting 8.98 and 13.19-fold probability favoring the alternative hypothesis for models obtained to predict MT of KE and EF, respectively.

DISCUSSION

The present study investigated the variability of MT from arm and thigh muscles among older women and generated a predictive equation model to estimate arm and thigh MT by limb circumference measurements using a frequentist and Bayesian approach. Our results indicate that the MT of EF exhibits somewhat less between-subject variability, indicating a more homogeneous pattern of presentation, which may explain the better equation model fit for predicting MT of EF from arm circumference.

Miyatani *et al*. (2) estimated muscle volume from MT measurements of the upper and lower limbs, and their results indicated that the EF measurements lead to a better adjustment than KE measurements, in the prediction models of the cited study. Previous studies have reported that the pattern of muscle mass decline during the aging process differs among muscle groups, which has a direct influence on the pattern of demand/overload/activation of the different muscles (24-27). The muscles in the lower limbs tend to be more demanded throughout life because they are directly involved in standing orthostatic position and walking, while the muscles in the upper limbs are less demanded throughout life. Thus, the reduction in mobility that occurs with aging may have a greater impact on the muscles of the lower limbs (24, 27, 28).

Thus, it is plausible to hypothesize that the decline in muscle thickness of the lower limbs has a more variable behavior during aging since the level of mobility seems to directly influence this variable. In contrast, despite a smaller proportional decline associated with aging, the MT of EF muscles may show a more homogeneous decline pattern, which would justify a better fit in prediction models, as observed in the present study.

The results of the Bayesian analysis allow us to infer a higher probability of reproducibility of the regression model obtained for MT of the EF, based on arm circumference, and adjusted for age, with a probability of about 93%

Table IV. Statistical parameters obtained from regression models and Bayes factors.

Variable	Obtained equation	R2 Adjusted	P-value	Standard error of the estimate	Index Durbin-Watson	Bayes Factor (BF10)
Muscle thickness of EF (cm)	$2.00 + 0.048 \times (AC) + (-0.011) \times (age)$	0.268	0.001	0.361	1.65	13.19
Muscle thickness of KE (cm)	$2.50 + 0.039 \times (TC) + (-0.015) \times (age)$	0.173	0.003	0.563	1.63	8.98

EF: Elbow flexors; KE: Knee extensors; AC: arm circumference; TC: thigh circumference. Measurements of circumference and muscle thickness were reported in centimeters; Age was reported as years old.

[BF10/(BF10-1] ×100), while the models generated for MT of the KE, based on thigh circumference, and adjusted for age, was 90%. Thus, the Bayes Factor obtained for the models generated for predicting the MT of KE and EF were considered moderate and strong, respectively.

Based on the presented results, it is possible to predict the MT of the KE and EF from specific anthropometric measurements of the thigh and arm circumference, respectively, adjusted for age in older women. In this sense, the use of anthropometric measurements has been relevant, because they represent non-invasive, low-cost methods, with simple equipment, easy handling, and low complexity.

Importantly, unlike previously reported studies, we did not aim to compare the rate of MT decline in different muscle groups in different aged groups, but rather to identify the pattern of MT variability between different muscle groups within a sample of older women, and then to propose a model for predicting MT of upper and lower limb muscle groups from limb circumference, identifying which models have the best fit.

Although we included only women because menopause accelerates muscle mass reduction in older women, it represents a limitation, since the predictive equation should not be applied to older men. Additionally, the error of estimate is a limitation intrinsic to all predictive equations, however, our results expose all parameters to allow clinicians to decide to use the generated prediction equation, aware of the standard error of the estimate.

USD represents a tool with great potential for MT assessment when compared to limb circumference, but it requires greater financial investment and technical knowledge, which is not accessible to all healthcare professionals. Thus, our study presents results that enable the prediction of older women's MT based on limb circumference with an excellent fit. Future studies should investigate the applicability and the reproducibility of this equation to similar populations.

CONCLUSIONS

The results of this study showed that, in older women, MT measures of EF showed lower between-subject variability, which can explain better equation model adjust for prediction of EF MT by arm circumference, while the adjustment of the predictive model to estimate KE MT by thigh circumference, was moderate.

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

CMA, RP, MHF: conceptualization, project administration, investigation, resources, supervision, reading, and approving the final manuscript. CMA, JAOC, LS, RSC: methodology, formal analysis, supervision, reading, and approving the final manuscript. CMA, RP, LS: visualization, writing – original draft.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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