



APPENDICULAR LEAN SOFT TISSUE: DEVELOPMENT AND CROSS-VALIDATION OF PREDICTIVE MODELS FOR OLDER MEN AND WOMEN

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Abstract: *Background:* Sarcopenia may explain, in a large proportion, physical disability, falls and fractures, especially in aged elderly. However, a diagnosis in an operationally systematic, simple and low cost way is extremely important, particularly for home-based, epidemiological studies. *Objective:* The purpose of this study was to develop and validate predictive equations of appendicular lean soft tissue (ALST) in elderly older than 80 years. *Design and settings:* A validation study was performed in 106 elderly (men and women) aged 80 years and older. *Measurements:* Body weight, height, circumference (arm, midcalf, hip and waist) and triceps skinfold were measured in the elderly. ALST were measured using as the reference method dual-energy X-ray absorptiometry (DXA). *Results:* Two models were predicted. The first model (ALST, in kg = $0.074 \times \text{height} + 0.277 \times \text{weight} - 0.144 \times \text{triceps skinfold} - 0.103 \times \text{waist circumference} + 1.831 \times \text{gender} - 0.966$), which considered all possible variables in stepwise multiple regression, presented better statistical performance ($r^2 = 0.82$; SEE = 1.67 kg), compared to the second model (ALST, in kg = $0.138 \times \text{height} + 0.103 \times \text{weight} + 3.061 \times \text{gender} - 12.489$), a more practical equation, due to a lesser quantity of predictive variables ($r^2 = 0.75$; SEE = 1.94 kg). Both models were validated, however, it was verified trend ($p < 0.05$) for overestimation of predicted ALST. *Conclusion:* In summary, two models for predicting ALST in men and women with age ≥ 80 years were developed and cross-validated. Model 1, with a greater number of predictive variables, presented a better accuracy than did the model with only three variables (height, weight, and gender). Validation studies are needed to test the usefulness of both models in other populations.

Key words: Skeletal muscle mass, appendicular lean soft tissue, elderly, cross-validation.

Introduction

Sarcopenia, initially defined as an age-related decline in skeletal muscle mass (SMM) among elderly (1), has become an issue of public health since it has shown to (in a large proportion) explain physical disability, falls and fractures (2, 3). Currently, the most practical way to diagnose sarcopenia is through the skeletal muscle mass assessment performed using the dual energy x-ray absorptiometry (DXA) (2, 4-7). The measurement of appendicular lean soft tissue (ALST) by DXA is highly correlated to SMM by magnetic resonance imaging (8). Although DXA is cheaper compared to other imaging techniques, it still presents several limitations, for example when the SMM assessment is required in mobility impaired patients, in large epidemiological studies, or in developing countries (where the equipment may still not so widely available).

The proposal of equations predictive of ALST in older persons is particularly important for clinical and research professionals in order to facilitate the assessment of SMM and the diagnosis of sarcopenia. Thus, the purpose of the present study is double: 1) to develop predictive equations of ALST in persons aged 80 years and older, and 2) to validate the

developed equations.

Methods

Study Design

Persons aged 80 years and older were invited to participate in a pilot study of the SABE Study (Health, Well-being and Aging) conducted in the city of Presidente Prudente, São Paulo State, Brazil. The SABE Study, a multicenter, epidemiological and household based study coordinated by the Pan American Health Organization (PAHO), was performed in seven centers of Latin America, in 2000. In Brazil, the study was conducted in São Paulo City, and it was coordinated by the School of Public Health (FSP) at the University of São Paulo (USP), supported by PAHO and funded by the Foundation for Research Support of São Paulo (FAPESP) and the Ministry of Health (MS). All procedures of the sampling process, collecting, and designing database have been previously published (9).

Subjects

Individuals invited to participate in this study were informed about the objectives and methodology of data collection, and





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signed the "Statement of Informed Consent". All protocols were reviewed and approved by the Ethics Committee in Research of the São Paulo State University (Process no. 26/2009). From a total of 2,100 persons aged 80 years and older living in the city of Presidente Prudente, 106 community-dwelling individuals with no limb amputation or mobility impairment were included in the study sample.

Anthropometric measures

Body weight was measured using an electronic scale (Plenna, Brazil) to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm. Subjects were measured barefoot, with light clothes. Body mass index (BMI) was calculated as the ratio of body weight (in kg) by squared height (in m), and the cut-off value of 30 kg/m² was used to classify individuals as obese.

Circumferences (in cm) of mid-arm, calf, hip and waist were performed three times to the nearest 0.1 cm in the plane orthogonal according to standardized procedures (10), and the median value used for analysis. Triceps skinfold (SKF) was measured (in mm) three times to the nearest 0.1 mm according to the standardized anatomic location and methods (10), and the median value used for analysis. SKF measurements were made on the right site of the body using a Lange caliper (Cambridge Scientific, Cambridge, MD).

DXA

Whole-body and regional body composition were estimated using DXA (Lunar DPX-NT, Lunar /GE Corp, Madison, Wisconsin, USA). After completion of scans, the system provided the total and the regional body composition results of lean soft tissue, body fat, and bone mineral content. Appendicular lean soft tissue (ALST) was considered as the sum of lean soft tissue (in kg) of arms and legs. The same laboratory technician positioned the subjects, performed the scans, and executed the analyses using standard protocol.

Statistical Analysis

Descriptive statistics were performed to present mean values (\pm standard deviations, SD) of anthropometric variables, and ALST (both measured by DXA and predicted by each model). After confirming the normality of the data using both the Shapiro–Wilk and Levene test, independent Student t-test was performed to compare means between genders.

Forward stepwise regression analysis was performed to identify the model which best predicted ALST assessed using the reference method. During the model development, homogeneity of variance and normality of residuals were tested. The criterion for inclusion (addition and retention) of a

predictor was statistical significance set at the $p=0.05$ level. If more than one variable was retained by the model, a variance inflation factor (VIF) for each independent variable was also calculated to evaluate multi-collinearity.

Two anthropometric prediction models were proposed: 1) with all the variables included in the stepwise regression; and 2) only anthropometric variables (height, weight), plus gender (male or female), ethnicity (Caucasian and African descent), and age were included in the stepwise regression.

Cross-validation was performed in the same sample, using PRESS statistics, which is an internal cross-validation procedure alternative to data splitting, convenient when insufficient independent data are available, and also providing a useful case diagnostic (11). The PRESS statistic is obtained by 1) fitting a regression equation with one observation excluded, 2) determining the predicted value of the excluded observation, 3) calculating the residual for that predicted value (observed – predicted), 4) repeating steps 1–3 for all observations, and 5) taking the sum of squares (SS) of all residuals. Thus, the PRESS statistic is a function of these residuals:

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$$\text{PRESS} = \text{SS} (\text{PRESS residuals}) \quad (1)$$

The PRESS statistic is never smaller than SS (error) from the ANOVA table. Hence, an alternative measure of model adequacy, as suggested by Myers (12), is defined as:

$$r^2 = 1 - [\text{PRESS}/\text{SS}(\text{total})] \quad (2)$$

Similarly, an alternative measure to the ordinary standard error of estimation (SEE), termed the SEE PRESS can be defined as:

$$\text{SEE PRESS} = \sqrt{\left(\frac{\text{PRESS}}{n}\right)} \quad (3)$$



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where n , is number of observations. Validation using the PRESS procedure is similar to applying the equation to an independent sample because the PRESS residual is obtained for the observations that are not included in the data when the equation is derived (13).

As part of the cross-validation process, dependent Student t -test was performed to compare means between ALST measured by DXA and predicted by each equation. The relationships between ALST predicted by the new equations and ALST measured by DXA were verified by regression analysis, where slopes and intercepts were examined as well as the r^2 and the SEE. Agreement between the reference method and the ALST predictive equations was also explored by analyzing the mean differences between methods, limits of agreement, and trends according to the Bland and Altman's method[14], and the Lin's concordance coefficient of correlation (CCC) (15). Analysis of trends was verified to observe under- or overestimation statistically significant.

Bland and Altman's method and Lin's CCC analysis were performed using Med-Calc Statistical Software (MedCalc Software, Mariakerke, Belgium). PRESS statistic was performed using SigmaPlot for Windows version 11.0 (Systat Software, Inc., Germany). Data were analyzed using PASW Statistics 18 (IBM Corp., Somers, NY, USA) with type I error set at $P < 0.05$.

Results

Characteristics of the study sample are presented in Table 1. According to the BMI classification, 12 participants were classified as obese ($BMI \geq 30 \text{ kg/m}^2$), with a mean BMI of 34.6 kg/m^2 , while 94 were classified as normal or overweighted (mean BMI 24.2 kg/m^2). Compared to women, men had significantly higher weight, height, waist circumference, and ALST, but lower triceps skinfold ($p < 0.05$).

Two anthropometric prediction models were proposed. In the first model, the entire group of possible independent predictors was included in the stepwise regression analysis. The order of entrance in the final model was height, gender, weight, triceps skinfold, and waist circumference ($F \text{ value} = 90.37$; $p < 0.001$; Table 2). VIF's values ranged from 2.29 to 6.44.

Due to its simplicity and easiness to be performed in epidemiological studies, weight, height, age, gender, ethnicity (and interactions between each of these predictors with ethnicity and gender) were only considered in the second model. In this model, height, gender, and weight (in order of entrance) were retained as significant predictors of ALST ($F \text{ value} = 105.14$; $p < 0.001$; Table 2). VIF's values ranged from 1.37 to 1.82.

Table 1
Descriptive statistics of the sample

Variables (units)	Total (n=106) Mean (SD)	Women (n=71) Mean (SD)	Men (n=35) Mean (SD)
Age (yrs)	83.6 (3.0)	83.5 (3.1)	83.9 (2.6)
Weight (kg)*	59.9 (11.6)	56.4 (10.1)	66.9 (11.2)
Height (cm)*	153.8 (10.3)	149.3 (8.6)	162.9 (7.2)
BMI (kg/m^2)	25.4 (5.3)	25.5 (5.9)	25.2 (3.9)
ALST (kg)*	15.9 (3.9)	13.9 (2.0)	20.0 (3.5)
Circumferences (cm)			
- Mid-arm	27.4 (3.7)	27.5 (3.8)	27.4 (3.4)
- Calf	33.2 (3.6)	32.9 (3.9)	33.7 (2.8)
- Waist*	88.1 (11.3)	85.9 (10.9)	92.6 (11.0)
- Hip	95.5 (8.1)	95.9 (8.4)	94.9 (7.7)
Skinfold thickness (mm)			
- Triceps*	18.0 (7.9)	20.9 (7.7)	12.2 (4.4)

Abbreviations: BMI, body mass index; ALST, appendicular lean soft tissue, measured by DXA. * Statistically different across gender ($p < 0.05$)

The PRESS statistic method was used for the main cross-validation analysis (Table 2). Both models presented similar r^2 and SEE PRESS values compared to the performances obtained in the development process, although values from Model 2 were slightly worse ($< 0.7\%$).

The comparison of the values predicted by both models with values measured by DXA showed lower differences and bias for the second model (Table 3). For both models, differences were not significant ($p > 0.05$) and bias were close to zero, with low limits of agreement, and high Lin's CCC.

Predicted and measured values were highly correlated (coefficients of correlation > 0.8) with the slope and the intercept of the regression line of identity not different from 1 and 0, respectively (Figure 1, left column). In the Bland and Altman's analysis, both models showed a trend ($p < 0.05$) towards overestimation of predicted ALST in the subjects with fewer quantity of appendicular muscle mass (Figure 1, right column). Normal distribution of the residuals was verified for both models.

Discussion

The loss of skeletal muscle mass begins around forty years (16, 17), and that by the eighth decade of life, both men and women have about 50% less muscle mass than young adults (18-20). Therefore, the study of sarcopenia in the most advanced age groups (e.g., persons with 80 years and older) is of great interest, especially due to the risk of disability, morbidity, and mortality associated with it (21-24). In this context, the development of ALST models may be particularly useful in order to provide an alternative solution to the use of DXA (and other imaging techniques) for those individuals and settings presenting specific limitations.



Table 2

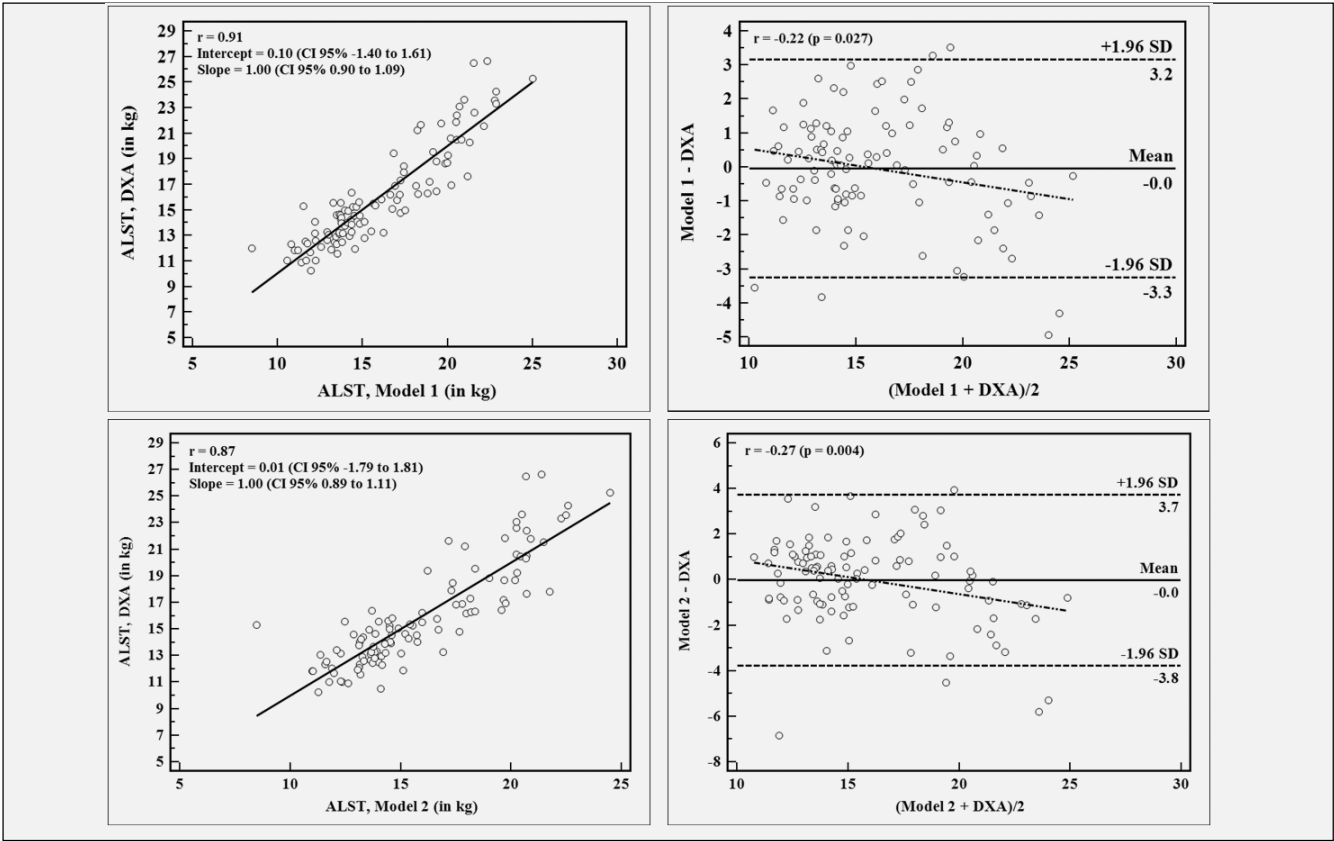
Models predicting appendicular lean soft tissue (using DXA as reference method) and PRESS statistics internal cross-validation method

Model	Predictor Variables	β	SE	Adj r^2	SEE (kg)	Cross validation PRESS	
						r^2	SEE (kg)
1 (n=102)	Intercept	-0.966	3.741	0.817	1.668	0.814	1.668
	Height	0.074	0.024				
	Gender ^a	1.831	0.560				
	Weight	0.277	0.037				
	Triceps SKF	-0.144	0.034				
	Waist Circ.	-0.103	0.031				
2 (n=106)	Intercept	-12.489	3.496	0.748	1.938	0.743	1.950
	Height	0.138	0.025				
	Gender ^a	3.061	0.519				
	Weight	0.103	0.019				

Notes: a Gender, men = 1, women = 0; SKF = skinfold.

Figure 1

Regression analysis of the models developed, using DXA as reference method (left column) and Bland and Altman analysis of the models (right column). The solid line represents the mean differences between predictive models and DXA and the dashed lines represent (± 1.96 SD) confidence intervals





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Table 3
Comparison of means of ALST from predictive models and DXA

Model	Mean (SD)		t	Bias	Limits of agreement	CCC (95% CI)
	DXA	Model				
1 (n=102)	16.01 (3.88)	15.97 (3.53)	0.272*	-0.04	-3.25 ; 3.17	0.91 (0.88–0.94)
2 (n=106)	15.93 (3.86)	15.91 (3.36)	0.077*	-0.01	-3.76 ; 3.73	0.86 (0.80–0.90)

CCC: concordance coefficient of correlation. * $p > 0.05$.

In this study, two models to predict ALST in elderly with age ≥ 80 years were developed. The first model considered in the stepwise regression analysis all variables proposed. It reported a higher adjusted r^2 and lower SEE compared to the second one. Nevertheless, the second equation (proposed as a simplification of the former) still presented acceptable accuracy and agreement.

Most of anthropometric models used to estimate total skeletal muscle mass were proposed in populations with a wide range of age (18 to 86 years old) (25, 26). These models were developed considering magnetic resonance imaging as reference method, which allowed the prediction of total skeletal muscle mass. Differently, the use of DXA as reference allows to specifically predict ALST. In literature, only one study developed an ALST-based anthropometric model specific for older persons. Baumgartner et al. (2), in a subsample of the Aging Process Study and the Rosetta Study, developed a model for men and women with age ≥ 65 years, with better accuracy than the present study ($r^2 = 0.91$; SEE = 1.58 kg). However, that equation also included handgrip strength as a predictor variable, which would imply the routine implementation of this measure (with the acquisition of a hand-held dynamometers) in the clinical setting.

More recently, an Australian study (27) proposed three predictive equations of ALST, also taking DXA as the reference method, in subjects aged between 50 and 83 years. A predictive model with variables weight, BMI, age and gender presented the highest statistical performance (adjusted $r^2 = 0.91$; SEE = 1.87). Although this study used a sample of elderly, the average age of the three cohorts involved in the development and validation of the models ranged between 50 and 64 years. The ALST predictive equations proposed in our study is applied to older persons aged 80 to 95 years old, that is subjects with ages not considered in the models proposed by Janssen et al. (25), Lee et al. (26), and Visvanathan et al. (27).

Despite the lower accuracy compared to the more complex model, model 2 could be more appropriate for studies with very large populations, because fewer variables are measured. It is noteworthy, however, that models which use predictors positively correlated to the dependent variable, as in our model

2, will hardly identify subjects considered as sarcopenic obese because the higher the weight, the higher will also be the muscle mass. For this specific individuals, it is to privilege models including measures of the adipose tissue (as in our model 1 adopting waist circumference).

The sample size used in this study can be indicated as a major limitation. Compared to previous studies, the present analyses are performed with the smallest sample. However, as mentioned before, the age group we studied (i.e., community-dwelling older persons aged 80 years and older) is very characteristic, making our both models very specific. The models should be applied in subjects with similar characteristics in terms of anthropometric variables and ethnicity. Another limitation of the study resides in the relatively few anthropometric variables considered in the analyses. Unfortunately, data are from a larger study in which only these parameters were collected. Other anthropometric variables (e.g., thigh circumference, thigh and calf skinfolds) might have led to different models. Finally, the Bland and Altman's analysis showed a trend towards the overestimation of ALST in subjects with low amount of ALST, especially in model 2. These findings highlight the need for a careful individual interpretation in these individuals.

In conclusion, two models for predicting ALST in men and women with aged 80 years and older were developed and cross-validated in a subsample of the SABE Study. The model 1 (including height, weight, waist circumference, triceps skinfold, and gender) reported higher accuracy than the second model with fewer variables (i.e., height, gender, weight). Validation studies are needed to test the usefulness of both models in different populations, as well as longitudinal studies to track changes in ALST.

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