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Original article

Body composition using bioelectrical impedance: Development and validation of a predictive equation for fat-free mass in a middle-income country

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SUMMARY

Objective: We developed and described a new equation to estimate fat-free mass derived from BIA using a sample population from Brazil and having Dual-energy X-ray absorptiometry (DXA) as the reference method. We also compared this new equation with two published and widely used equations developed in high-income countries.
Methods: Cross-sectional study with 294 healthy adults from Pelotas, Brazil. DXA was used to assess total fat mass and fat-free mass aiming to obtain reference measures for the development of the new BIA equation. Multivariable linear regression models including fractional polynomials were used to find the best predictive model for FFM, using resistance, reactance, age, weight and height as the independent variables. Models were developed separately for men and women. The bootstrapping method was used to test the validity of the new equation. Finally, the Bland-Altman approach was used to assess the agreement of our equations and the two others widely used equations with the FFM measured by DXA.
Results: The new equations explained more than 80% of the variation in fat-free mass percentage from DXA. In the bootstrapping analysis, the new equations presented good validity, as the corrected RMSE was similar to those found in regression analysis. Finally, the new equations presented a better concordance when compared to two validated equations from US and Switzerland.
Conclusion: The new developed equations appear to be the best options to predict fat-free mass percentage in Brazilian adults by bioelectrical impedance and appear to fit well in all Brazilian population due to the good validity presented.

1. Background

Obesity is a major public health problem and very high levels of body fat play a significant role in the development of non-communicable diseases and mortality [1,2]. Obesity traditionally has been assessed using body mass index (BMI) but the accuracy of this approach has been questioned because it does not distinguish fat mass (FM) from fat-free mass (FFM) [3].

Despite the good accuracy of some body composition methods, such as the four-compartment model, their complexity along with prohibitive cost make them unsuitable to be frequently used in epidemiological studies. The applicability of these methods is limited to few research centers mostly located in high-income countries [4].

Bioelectrical impedance analysis (BIA) has been proposed as an alternative method for body composition assessment in the 1980's [5,6]. Compared to other body composition methods, such as dual X-ray absorptiometry (DXA) and four-compartment model, BIA is cheaper and easier to apply in epidemiological studies. These characteristics make BIA the most used tool to assess body composition worldwide [7,8].

On the other hand, this technique has some limitations. For example, it is a doubly indirect method which uses predictive equations to estimate body composition derived from comparisons

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with reference methods. Most studies using BIA to predict body composition in the last years developed equations based on samples from Europe or US [9–11]. These equations could not fit well in populations with other ethnic characteristics, as Brazil, due to possible differences in distribution of body composition measures as well as in body frame.

The objective of this study was to develop and describe a new predictive equation to estimate fat-free mass percentage derived from BIA for Brazilian subjects, using DXA as the reference method, and compare this new equation with two published and widely used equations developed in high income countries.

2. Methods

2.1. Subjects

Cross-sectional study carried out between August 2007 and December 2008 with 302 healthy individuals aged 20–79 years from Pelotas, Brazil. Pelotas is a city located in Southern Brazil with 330,000 inhabitants, according to the last Brazilian census. For this study, a sample with 302 individuals was considered enough to allow the development of a predictive equation to assess adult body composition using BIA parameters. To guarantee that different age groups were equally represented, 50 participants were selected in six groups based on sex and age (20–39 years, 40–59 years and 60–79 years).

Individuals aged 20–79 years, with BMI $\geq$16 kg/m^2, without any medical condition such as heart and kidney failure, chronic hepatitis, and without medical treatment with diuretics or corticoids were considered eligible for the study. Pregnant women, people with any physical disability, and those who had been hospitalized or who had received any medical care using contrast in the 90 days before the study were excluded.

This study was not registered as a clinical trial since all individuals included were not assigned to any intervention and did not receive any dose of investigational drug. This study was approved by Federal University of Pelotas' Research Ethics Committee and all participants gave their written consent to participate.

2.2. Anthropometric and body composition measures

A trained technician measured weight using a digital Filizola scale (0.01 kg precision), and height using a metal stadiometer (1 mm precision) coupled to the scale. For these measurements, individuals were wearing light clothes suitable for the exam.

Body resistance (R) and reactance (Xc) were measured by BIA Quantum (RJL Systems), following manufacturer's instructions by a trained technician. All individuals stayed in supine position in the 15 min previous to the examination. We collected three measures of R and Xc for everyone included in the study using a single 50 kHz frequency and 800 μA.

A DXA (LUNAR DPX; GE HealthCare®) with the software enCORE (Lunar DPX DXA Systems) was used to assess fat mass and fat-free mass percentage of our sample and these measures were used as the reference. Fat mass percentage was calculated by dividing total fat mass from DXA (Kg) by total body mass (Kg) multiplied by 100. Fat-free mass percentage was calculated by subtracting fat mass percentage from 100. All DXA examinations were carried out by a trained technician following manufacturer's instructions.

2.3. Statistical analyses

Stepwise regression with a significance level of 5% was used to select the best independent predictors of fat-free mass percentage from DXA to be included in the equation. Akaike's information criterion, coefficient of determination (R^2) and root mean squared error (RMSE) were checked to include the independent predictors in the model. The best predictors of fat-free mass percentage were weight, height, resistance and reactance.

Least square multi variable models were used to construct predictive equations for this Brazilian sample. Firstly, multivariable fractional polynomial models were performed to derive the best-fitting powers of covariables included in the model [12]. After selecting covariables' best-fitting powers, a linear regression model was performed to derive the new equation.

In the linear regression model, FFM percentage from DXA, used as the reference method, was included as the dependent variable, and weight, height, resistance, and reactance as the independent variables. All variables were introduced with estimated powers of 1, except weight which was introduced with an estimated power of −0.5.

To test the external validity of this new equation, the individuals included in the study were randomly resampled with replacement to hypothetically apply this new equation in N different samples using the bootstrapping method through the bootstrap command in Stata [13]. To assess if the equation presented good validity, the optimistic mean and the corrected RMSE of the new FFM equation was assessed based on 10,000 replications with the same size of the dataset. The corrected RMSE was calculated by the difference between original RMSE and the optimistic mean.

Finally, to assess how better our novel equation is when compared to other published equations to estimate FFM, we assessed the concordance coefficients of the FFM estimated by our novel equation and by two other previously published equations from US and Switzerland [9,10]. Bland-Altman graphics were used to assess these concordance coefficients using the FFM measured by DXA as parameter. All analyses were stratified by sex and performed using Stata 13.1.

3. Results

We included 294 individuals (47.3% men) who average aged 46.3 years. Eight individuals were excluded due to inability to perform DXA exams (presence of prosthesis or BMI $\geq$40 kg/m^2). Regarding anthropometric characteristics, the mean weight was 72 ± 13.4 kg while the mean height was 164 ± 9.3 cm. When the results were stratified by age and sex, we observed that both men and women $\geq$60 years of age presented higher BMI and waist circumference and lower height. There was no difference in mean weight according to age-groups in men and women (Table 1).

When the body composition parameters measured by DXA were assessed, we observed that $\geq$60-year-old women presented higher total fat mass than the younger ones. In addition, those older age-group ($\geq$60) men and women presented lower fat-free mass percentage. Resistance and reactance measured by BIA were also lower in the older age-groups, independent of sex. These BIA parameters were higher in women than in men (Table 2).

Results for the predictive equation based on this Brazilian sample are presented in Table 3. In the regression analyses, we observed that weight, height, resistance and reactance explained more than 80% of the variation in fat-free mass percentage measured by DXA (R^2_{adj} = 80.3% in men and R^2_{adj} = 85.5% in women), and the RMSE was 3.65 in men and 2.92 in women. From these results, we obtained the following equations to predict fat-free mass percentage in the Brazilian population:

Table 1
Anthropometric measures of healthy Brazilian subjects according to sex and age group (N = 294).

Age group	Anthropometric measures[a]			
	Weight (kg)	Height (cm)	BMI (kg/m^2)	Abdominal circumference (cm)
Men (N = 139)				
p-value[b]	*0.245*	*0.009*	*<0.001*	*<0.001*
20–39 years	77.6 (10.6)	174.1 (6.7)	25.6 (3.3)	86.5 (8.6)
40–59 years	81.5 (16.9)	168.5 (14.3)	27.5 (3.5)	95.3 (9.9)
60 or more	81.3 (10.4)	169.0 (5.8)	28.4 (2.7)	100.5 (7.3)
Women (N = 155)				
p-value[b]	*<0.001*	*<0.001*	*<0.001*	*<0.001*
20–39 years	60.1 (9.8)	161.4 (5.6)	23.1 (3.8)	73.9 (9.4)
40–59 years	64.8 (10.5)	156.7 (4.4)	26.3 (4.4)	84.0 (9.9)
60 or more	69.2 (9.2)	154.8 (4.9)	28.9 (3.4)	89.9 (7.9)

[a] Means (standard deviations) are displayed for all measures.
[b] p-values are displayed for Analysis of Variance.

Table 2
DXA and BIA measures of healthy Brazilian subjects according to sex and age group (N = 294).

Age group (years)	DXA measurements[a]			BIA characteristics[b]	
	Total mass by DXA (kg)	Fat-free mass (kg)	% of fat-free mass	Resistance (Ω)	Reactance (Ω)
Men (N = 139)					
p-value[b]	*0.320*	*0.055*	*<0.001*	*0.027*	*<0.001*
20–39	76.4 (10.4)	58.9 (6.4)	77.8 (9.0)	466.7 (58.0)	64.3 (6.9)
40–59	78.5 (11.9)	57.0 (6.8)	73.2 (6.9)	443.0 (43.4)	57.8 (6.3)
60 or more	79.9 (10.1)	55.7 (4.2)	70.4 (6.4)	441.6 (50.7)	51.7 (6.3)
Women (N = 155)					
p-value[b]	*<0.001*	*0.758*	*<0.001*	*<0.001*	*<0.001*
20–39 years	58.3 (9.5)	37.4 (3.2)	65.1 (8.1)	630.5 (60.3)	65.5 (6.9)
40–59 years	62.9 (10.2)	37.9 (3.9)	61.0 (6.8)	585.2 (67.9)	61.1 (7.3)
60 or more	67.3 (9.0)	37.8 (3.9)	56.5 (5.4)	549.9 (56.4)	56.9 (5.7)

[a] Means (standard deviations) are displayed for all measures.
[b] p-values are displayed for Analysis of Variance.

FFM (%)

Men: $\% \text{ of FFM} = -145.7735 - [0.0947 \times \text{resistance } (\Omega)] + [0.2014 \times \text{reactance } (\Omega)] + [0.6995 \times \text{height (cm)}] + \left(\frac{1159.3860}{\sqrt{weight\ (kg)}}\right)$

Women: $\% \text{ of FFM} = -118.1866 - [0.0556 \times \text{resistance } (\Omega)] + [0.1378 \times \text{reactance } (\Omega)] + \left(\frac{847.0996}{\sqrt{weight\ (kg)}}\right) + [0.6153 \times \text{height (cm)}]$

Bootstrapping analysis based on 10,000 replications showed that Brazilian equations showed a good validity, since the bootstrap corrected RMSE was very similar to those found in linear regression models: the bootstrap corrected RMSE was 3.70 (vs. 3.65 in regression analysis) and 2.97 (vs. 2.92 in regression analysis) for men and women, respectively.

When compared with different validated equations developed for US and Swiss populations, the Brazilian equation presented higher concordance with the FFM estimated by DXA in both sexes.

Table 3
Brazilian equations based on anthropometric and BIA characteristics for prediction of fat-free mass percentage estimated by DXA.

Variable	Coefficient	SE	[95% CI]	
Men (N = 139)[a]				
Resistance (Ω)	−0.0947	0.0079	−0.1102	−0.0792
Reactance (Ω)	0.2014	0.0460	0.1113	0.2916
Weight$^{-0.5}$ (kg)	1159.3860	52.4992	1056.4876	1262.2844
Height (cm)	0.6995	0.0561	0.5895	0.8094
Intercept	−145.7735	12.3672	−170.0133	−121.5337
Women (N = 155)[b]				
Resistance (Ω)	−0.0556	0.0058	−0.1102	−0.0792
Reactance (Ω)	0.1378	0.0422	0.1113	0.2916
Weight$^{-0.5}$ (kg)	847.0996	32.9572	1056.4876	1262.2844
Height (cm)	0.6153	0.0455	0.5895	0.8094
Intercept	−118.1866	7.9716	−170.0133	−121.5337

Bootstrap corrected RMSE: Men = 3.70; Women = 2.97.
Brazilian equations:

Men: $\% \text{ of FFM} = -145.7735 - [0.0947 \times \text{resistance } (\Omega)] + [0.2014 \times \text{reactance } (\Omega)] + [0.6995 \times \text{height (cm)}] + \left(\frac{1159.3860}{\sqrt{weight\ (kg)}}\right)$.

Women: $\% \text{ of FFM} = -118.1866 - [0.0556 \times \text{resistance } (\Omega)] + [0.1378 \times \text{reactance } (\Omega)] + \left(\frac{847.0996}{\sqrt{weight\ (kg)}}\right) + [0.6153 \times \text{height (cm)}]$.

[a] Men: R^2adj = 80.3%; RMSE = 3.65;
[b] Women: R^2adj = 85.5%; RMSE = 2.92.

Table 4
Concordance coefficients between different equations for estimating percentage of fat-free mass and DXA estimates.

Equation	Concordance correlation coefficient	Difference (average)	95% Limits of agreement	
Men				
FFM measured by DXA	0.894	0	−7.053	7.053
Kotler et al. [9]	0.519	−5.252	−15.237	4.732
Kyle et al. [10]	0.800	−2.152	−10.202	5.898
Women				
FFM measured by DXA	0.924	0	−5.654	5.654
Kotler et al. [9]	0.710	−6.027	−12.165	0.112
Kyle et al. [10]	0.769	−3.081	−10.069	3.907

The Lin's concordance coefficient found in men was 0.894 using the Brazilian equation, being higher when compared to the equations proposed by Kotler et al. [9] (Lin's concordance coefficient = 0.519) and by Kyle et al. [10] (Lin's concordance coefficient = 0.800). Similar results were observed in women: Brazilian equation showed higher concordance when compared to those equations proposed by Kotler et al. and Kyle et al. (Lin's concordance coefficient = 0.924 vs. 0.710 and 0.769, respectively) (Table 4 & Fig. 1).

4. Discussion

BIA is an alternative method to estimate body composition and has been used in several investigations [14–18], since it is less invasive and less expensive than other techniques like DXA, computerized tomography, and magnetic resonance imaging. Bioelectrical impedance analysis is an easy-to-use and low-cost method for the estimation of fat-free mass (FFM) in healthy individuals and in physiological and pathological conditions [19–21]. However, the BIA equation's choice should be made very carefully, because BIA prediction models differ according to the sample's characteristics in which they have been derived.

The Swiss equation was one of the first developed, using 343 healthy volunteers and DXA as the reference method [10]. The second equation was developed for American population using a multicompartmental method as reference, with a sample of 1474 white and 355 African Americans [22]. Despite the excellent accuracy and precision obtained by the American equation, Trippo et al. [23] were not able to replicate its results in a Germanic sample, endorsing that predictive equations to estimate overall body composition should be population-specific. Since then, several equations have been developed using different populations [7,8,24–26].

Our study created and validated a predictive equation in Brazilian population using adequate statistical approaches. The new equations were developed to predict FFM percentage using body composition measures from DXA as reference, and their results confirmed the potential of BIA to predict body composition in this Brazilian population. The best model used to predict fat free mass from DXA considered weight, height, resistance and reactance and explained more than 80% of variation in FFM percentage measured by DXA. The parameters used in these new predictive equations were the same as those used by the Geneva (weight, height, R and Xc) [10] and US equations (weight, height and impedance) [22].

When these new equations were compared to those validated by Kyle et al. [10] and Kotler et al. [9] , we found that these new Brazilian predictive equations presented higher concordance

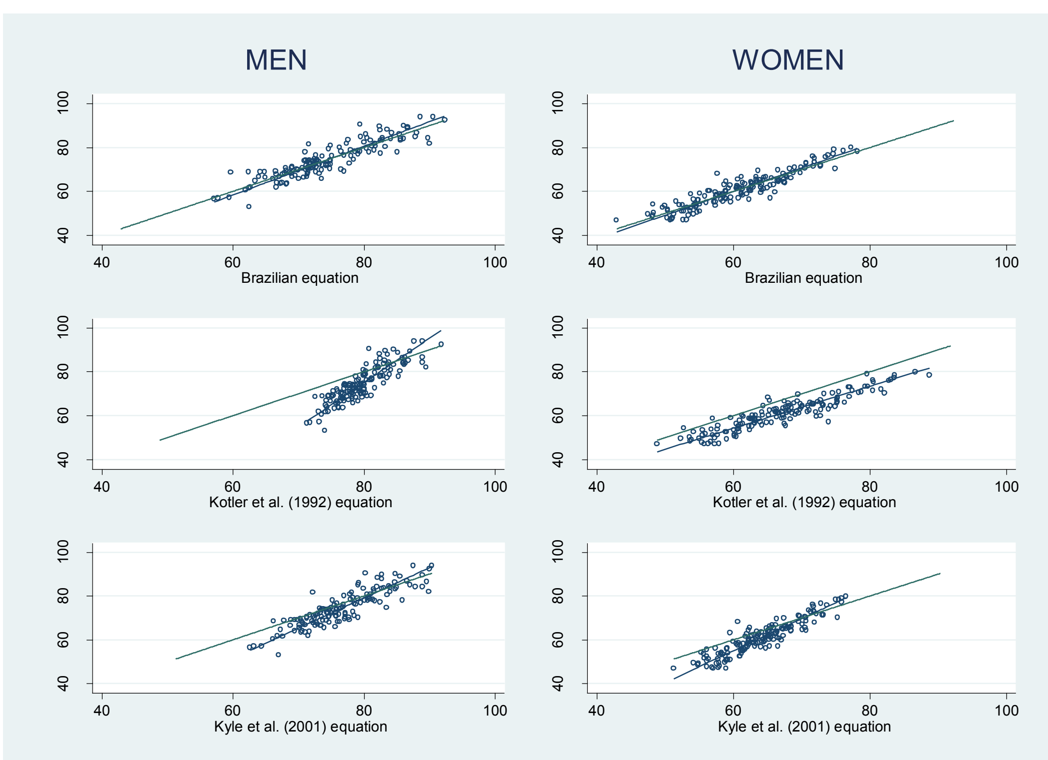

Fig. 1. Concordance between different equations for estimating percentage of fat-free mass and DXA estimates stratified by sex.

coefficient correlation in both men and women. Although Kotler et al. [9] used the same BIA device used in our study (RJL Systems), their predictive equation presented poor concordance correlation to estimate FFM percentage in Brazilian sample, mainly in men. In addition, for the three equations analyzed in this study, the concordance coefficients were always higher in women than in men. It is an interesting result, since another study had already compared these two equations (Kyle and Kotler) and found that they estimated body composition better in men, with some discrepancies in women [27].

The coefficients of determination obtained using the Brazilian predictive equations were all above 80%, being higher in women. Moreover, this study also showed that the Brazilian equation presented a good validity, as the bootstrap parameters were very similar to regression results after 10,000 replications. This is not the first time bootstrapping is used to assess external validity of a predictive equation derived from BIA. Dung et al. [21] , assessing BIA in children and adolescents with Crohn disease, also used bootstrapping method to test external validity of their equation and found an acceptable validation of the derived equation to estimate FFM in that young population [21].

The selection of sample based on sex and different age-groups, including young adults as well as elderly people, may be considered a strength of this study, since this allowed us to evaluate body composition differences according to age and gender variation. In addition, the use of DXA as the reference method can also be considered a strength as DXA is considered a good method to estimate body composition in adults [28,29]. On the other hand, the non-randomly selected and not representative sample may be considered a limitation of this study.

In conclusion, the new developed equations appear to be the best options to predict fat-free mass percentage in Brazilian adults by using Bioelectrical Impedance Analysis, as it showed better results when compared to already published and widely used predictive equations from US and Switzerland. In addition, these equations also appear to fit well in all the Brazilian population due to the good validity presented in bootstrapping analysis. Nonetheless, further researches using Brazilian samples from different regions are required to confirm this hypothesis.

Conflict of interest

The authors declare no conflict of interest related to this study.

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This study was conducted by MCG, SPO, LPS and AJDB. MCG designed and conducted the research, helped in analyzing data and in drafting the manuscript. SPO helped in data collection, in analyzing data and drafted the manuscript. LPS performed the statistical analyses and drafted the manuscript. AJDB designed and conducted the research, helped in analyzing data and reviewed the final version of the manuscript.

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