

## ARTICLE



Health issues and nutrition in the elderly

# Predictive equations for fat mass in older Hispanic adults with excess adiposity using the 4-compartment model as a reference method

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**BACKGROUND:** Predictive equations are the best option for assessing fat mass in clinical practice due to their low cost and practicality. However, several factors, such as age, excess adiposity, and ethnicity can compromise the accuracy of the equations reported to date in the literature.

**OBJECTIVE:** To develop and validate two predictive equations for estimating fat mass: one based exclusively on anthropometric variables, the other combining anthropometric and bioelectrical impedance variables using the 4C model as the reference method.

**SUBJECTS/METHODS:** This is a cross-sectional study that included 386 Hispanic subjects aged  $\geq 60$  with excess adiposity. Fat mass and fat-free mass were measured by the 4C model as predictive variables. Age, sex, and certain anthropometric and bioelectrical impedance data were considered as potential predictor variables. To develop and to validate the equations, the multiple linear regression analysis, and cross-validation protocol were applied.

**RESULTS:** Equation 1 included weight, sex, and BMI as predictor variables, while equation 2 considered sex, weight, height squared/resistance, and resistance as predictor variables.  $R^2$  and RMSE values were  $\geq 0.79$  and  $\leq 3.45$ , respectively, in both equations. The differences in estimates of fat mass by equations 1 and 2 were 0.34 kg and  $-0.25$  kg, respectively, compared to the 4C model. This bias was not significant ( $p < 0.05$ ).

**CONCLUSIONS:** The new predictive equations are reliable for estimating body composition and are interchangeable with the 4C model. Thus, they can be used in epidemiological and clinical studies, as well as in clinical practice, to estimate body composition in older Hispanic adults with excess adiposity.

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## INTRODUCTION

Obesity is one of the most common alterations of nutritional status in older Hispanic people [1] and a disease now considered a global pandemic [2]. In older adult populations, it is associated with physical disability, morbidity, and mortality [3–7]. The pathophysiology of obesity is reflected in body composition [8], which in these cases is defined as an abnormal or excessive accumulation of fat, diagnosed by a body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup> [9]. The BMI, however, is not a direct marker of adiposity [10] and has only low sensitivity for identifying subjects with obesity [11]. As a result, the prevalence of obesity and its associations with health risks could be underestimated and/or misclassified [11–13]. For this reason, fat mass index (FMI) cut-off points have been proposed

as an alternative approach to diagnosing and classifying obesity [14]. Implementing the classification based on FMI cut-off points requires measuring fat mass (FM) with tools that are reliable for assessing body composition, such as dual-energy X-ray absorptiometry (DXA). One practical, low-cost option for assessing FM when laboratory methods are unavailable consists in using predictive equations.

Several factors can affect the predictive power of predictive equations, including age, health conditions such as obesity, ethnicity, and even the reference method applied. Deurenberg et al. [15], for example, demonstrated that equations based on general adult populations overestimated fat-free mass (FFM) by 6–7 kg in certain samples of older people. This error can be attributed to differences in body composition between younger

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and older adults, especially in bone mineral content (BMC) and total body water (TBW) [16–18]. Regarding the presence of obesity, incorporating measures of skinfolds and resistance index or height squared/resistance ( $Ht^2/R$ ) variables into equations designed to predict body composition is of only limited usefulness due to factors like skin compressibility and alterations in the hydration factor, respectively [19]. Studies have also demonstrated that body composition differs among ethnic groups [20–22]. For example, FFM and FM are lower and higher, respectively, in Mexican compared to Caucasian and African American older adults, despite similar BMI [20]. Therefore, differences in body composition by ethnicity could influence the accuracy of the equations applied.

Regarding the reference method, studies show that estimates of body composition using equations based on the 2-compartment (2C) model differ statistically from those based on the 4-compartment (4C) model, suggesting that the former has an associated bias [19, 23, 24]. In the case of older Hispanic-American adults with obesity, densitometry has been shown to overestimate FM by 0.57 kg, while hydrometry and DXA may underestimate this compartment by 0.89 kg and 0.79 kg, respectively, compared to the 4C model [25]. These findings suggest that predictive equations based on the 2C model and DXA may not be accurate for estimating FM in older non-Caucasian adults with obesity. It is important to note that the 4C model is considered the best option as a reference method for designing and validating predictive equations [23, 26].

According to our search, few predictive equations for aged people have been designed and validated based on the 4C model. Researchers like Gallagher et al. [27] and Sun et al. [28] developed equations based on African American and Caucasian populations with wide age ranges (12–97 years). Specific equations for older adults have been developed by Baumgartner et al. [23] and Dey et al. [29] based on Caucasians aged 65–94, while Huerta et al. [30] studied a Mexican population aged 60–89. Their equations, however, were based on subjects with wide BMI ranges (15.6–34.6 kg/m<sup>2</sup>), most of whom were Caucasians. Our search did not identify any specific equations to estimate body composition in older people with obesity based on the 4C model. If equations derived from sample groups with a wide age range, wide BMI range, or a distinct ethnicity are applied to assess the body composition of older Hispanic adults with excess adiposity, the accuracy of the resulting estimates may be poor. Therefore, the objective of the present study was to develop and validate two predictive equations for estimating fat mass in older Hispanic adults with excess adiposity: one based exclusively on anthropometric variables, the other combining anthropometric and bioelectrical impedance variables using the 4C model as the reference method.

## METHODS

### Study design

This is a cross-sectional, multi-site study conducted in 2016–2019, based on convenience sampling in three cities in northern Mexico: Hermosillo, Sonora; Ciudad Juárez, Chihuahua; and Monterrey, Nuevo León. To apply the inclusion, exclusion, and elimination criteria, the study protocol was divided into two stages. In the first stage, potential volunteers underwent clinical and laboratory assessments. In the second stage, all the volunteers who met the inclusion criteria underwent anthropometric and body composition measurements (details are described below). The same protocol was conducted in all three Body Composition Laboratories by trained personnel. All procedures were approved by the Ethics Committee of the Centro de Investigación en Alimentación y Desarrollo, (CIAD) A.C. (CE/008/2014), the Universidad Autónoma de Ciudad Juárez (UACJ) (CBE-ICB/023.10-14), and the Universidad Autónoma de Nuevo León (UANL) (15-FaSPyN-SA-19). The protocol was fully explained to all subjects and their informed, written consent was obtained.

## Subjects

This study included older adults (aged ≥60 years) with excess adiposity, stable weight (±3 kg) over the previous 3 months, hypertension and hypothyroidism under treatment, and the absence of other diseases or clinical conditions that could influence body composition (e.g., edema, diabetes, history of heart disease or stroke, kidney or liver failure, cancer). Significantly, in the present study, the excess fat (men >6–9 kg/m<sup>2</sup>, women >9–13 kg/m<sup>2</sup>) and obesity (men >9 kg/m<sup>2</sup>, women >13 kg/m<sup>2</sup>) categories by FMI [31] were considered as excess adiposity. Exclusion criteria were age <60 years, unstable body weight over the previous 3 months, one or more of the aforementioned medical conditions that affect body composition, and physical disability, all by self-report. Elimination criteria during the first stage were subjects with diabetes according to fasting plasma glucose ≥126 mg/dL or 2-h plasma glucose ≥200 mg/dL during oral tolerance glucose testing [32], cognitive dysfunction by Pfeiffer's test [33], physical dependence by Lawton and Brody's scale [34], inadequate hydration status by specific gravity urine and hematocrit values [35]. The elimination criteria applied after the second stage were subjects without excess adiposity (men ≤6 kg/m<sup>2</sup>, women ≤9 kg/m<sup>2</sup>) according to the FMI and subjects who did not complete all stages of the study.

### Dependent variables: fat mass and fat-free mass by the 4C model

Percentages of FM were estimated by the 4C model using Baumgartner equation (23),

$$\%FM = 205 * \left( \frac{1.34}{Bd} - 0.35 * A + 0.56 * M - 1 \right)$$

where Bd is body density, and A and M represent the aqueous and mineral fractions of the weight, respectively. FM in kg was obtained from the percentage of FM multiplied by weight and divided by 100. The 4C model equation requires three specific, independent measurements, as follows:

- Body density (Bd, kg/L), assessed by air displacement plethysmography (BodPod® Body Composition System, Life Measurement Instruments; Concord, CA, USA) following the protocol outlined in Aleman-Mateo and collaborators [36]. For this test, total body volume (TBV) was corrected by thoracic gas volume (TGV) according to the manufacturer's recommended protocol. In some volunteers, TGV could not be measured by the BodPod® protocol, so the predicted TGV value was used. To ensure that the use of the predicted TGV value did not affect the estimates of FM by the 4C model, the determination coefficient ( $R^2$ ) between the FM estimated by considering both TGV values (predicted and measured) was calculated. The  $R^2$  found in the present study was 0.96. The BodPod® system was calibrated daily following the manufacturer's guidelines.

- The aqueous weight fraction (A), calculated from the ratio of total body water in kg (TBW) to body weight in kg. TBW was measured using the stable isotope of deuterium oxide (D<sub>2</sub>O; 99.8 atom percent, Lot. No. 14G-316, Cambridge Isotope Laboratories, Inc., USA). D<sub>2</sub>O quantification in saliva samples was performed following two protocols; one reported by Aleman-Mateo and collaborators [36] using Isotope Ratio Mass Spectrometry (IRMS; DELTA PLUS, Thermo Finnigan, Bremen, Germany), the other published by the International Atomic Energy Agency [37] using Fourier Transform Infrared Spectrophotometry (FTIR; 8400S, Cat No. 206-72400-92, Shimadzu Corporation, USA).

- The mineral weight fraction (M), calculated from the ratio of total body mineral mass in kg (TBMM) to body weight in kg. TBMM was calculated by multiplying BMC in kg × 1.279 (the sum of bone plus cell mineral content) [23]. BMC was measured by DXA using a General Electric Lunar DPX-MD+ at CIAD, by Lunar iDXA at UANL, and by Lunar Prodigy Advance at UACJ. All DXA-body composition measurements and calibration procedures strictly followed the manufacturers' guidelines. To estimate BMC in subjects whose body size exceeded the dimensions of the DXA bed, the unmeasured part was added by a programming function included in the DXA software. All DXA scans were analyzed according to the protocol published previously by Aleman-Mateo et al. [36]. In addition, the regions of interest (ROI) were marked manually by a qualified technician, following the procedures described by Ramos et al. [38], using Encore software (LU43616ES©2015, GE Healthcare Lunar).

At that point, it was possible to calculate FFM in kg based on the difference between body weight and the 4C-derived FM model, both in kg.

Note that the body weight used in all procedures was measured by the BodPod® scale.

### Potential predictor variables: anthropometric and bioelectrical impedance variables

In addition to age and sex, certain anthropometric and BIA variables were considered as potential predictor variables of FM and FFM due to their biological association with body composition. Body weight, BMI, mid-arm circumference, hip circumference, calf circumference, and skinfolds are all associated with FM [19, 39]. The BIA variables associated with FFM include resistance ( $R$ ), reactance ( $X_c$ ), and the  $Ht^2/R$  [15, 28]. Since all these variables are easily measured, they can be used in clinical practice and epidemiological studies. All measurements were obtained with subjects barefoot, under fasting conditions, and with minimal clothing. Anthropometric measurements were repeated twice with each subject by trained personnel following the protocol of the International Standards for Anthropometric Assessment published by the International Society for the Advancement of Kinanthropometry [40].

-Body weight in kg was measured by the BodPod® digital scale. Standing height in meters (m) was measured to the nearest 0.1 centimeter using a stadiometer (SECA 264, Germany). The scale and stadiometer were calibrated daily using 20 kg dumbbells and a 1-meter-long metal bar, respectively. BMI ( $\text{kg}/\text{m}^2$ ) was calculated from the ratio of body weight to height squared.

-Mid-arm circumference was measured at the point equidistant from the acromial and radial points. Gluteal or hip circumference was taken at the level of the greatest posterior protuberance of the buttocks. Calf circumference was taken at the maximum girth of the calf. All circumferences were measured using a flexible steel tape measure ( $0\text{--}200 \pm 0.01$  cm, Rosscraft, Canada).

-The thickness of four skinfolds (triceps, biceps, subscapular, suprailiac) was measured in mm with a Harpenden skinfold caliper ( $0\text{--}80 \pm 0.2$  mm, model HSB-BI, Burgess Hill, England). The sum of these four skinfolds (sum4) in mm was then calculated. The caliper was calibrated as per the manufacturer's guidelines.

-Resistance and reactance were measured using single-frequency (50 kHz) BIA (Model Quantum X BIA Analyzer system; RJL Systems, Detroit, MI). Measurements were taken under fasting conditions after subjects had lain in a supine position for at least 5 min with arms slightly abducted from the trunk and legs slightly separated. Care was taken to ensure that neither the subjects nor their clothes contained any metal. The skin of the right hand and foot were swabbed with 70% alcohol before surface electrodes were placed on the dorsal surface of the wrist, hand, ankle, and foot, with neighboring sets separated by 5 cm.  $Ht^2/R$  ( $\text{cm}^2/\Omega$ ) was calculated from the ratio of height squared to  $R$ . The instrument was calibrated daily as per the manufacturer's guidelines.

### STATISTICAL ANALYSES

Normal distribution was tested by the skewness and kurtosis tests for normality, and by normality plot (histogram). Sex differences were probed by a two-sample independent  $t$ -test. The cross-validation protocol was used to design and validate two predictive equations; one based on anthropometric variables only (Eq.#1), the other on anthropometric and BIA variables combined (Eq.#2). To perform the cross-validation protocol, the total sample was divided into two equal groups (Group 1 to develop, Group 2 to validate) using a random procedure stratified by sex. To confirm the randomization process, the equality of all general characteristics—anthropometric, body composition, and BIA variables—between the two groups was verified by a two-sample independent  $t$  test with a  $p$  value  $>0.05$ . The following consecutive statistical procedures were then performed to design and generate each equation in Group 1:

- (1) Selection of the potential predictor variables: as mentioned above, the variables considered are associated biologically with body composition. In the case of FM, the following variables were tested as potential predictors: age in years,

sex (women = 0, men = 1), region or city (Juárez = 0, Monterrey = 1, Hermosillo = 2), body weight in kg, height in meters, BMI in  $\text{kg}/\text{m}^2$ , mid-arm circumference in cm, hip circumference in cm, calf circumference in cm, four skinfolds in mm (triceps, biceps, subscapular, suprailiac), and the sum4 in mm. Regarding FFM, the following were tested as potential predictor variables: age in years, sex (women = 0 and men = 1), region or city (Juárez = 0, Monterrey = 1, and Hermosillo = 2), body weight in kg, height in meters, BMI in ( $\text{kg}/\text{m}^2$ ), mid-arm circumference in cm, waist circumference in cm, hip circumference in cm, calf circumference in cm,  $R$  in  $\Omega$ , reactance in  $\Omega$ , and  $Ht^2/R$  in  $\text{cm}^2/\Omega$ . The potential predictor variables that showed a statistical association with the variable to be predicted by simple linear regression ( $p \leq 0.2$ ) were chosen based on univariate analysis.

- (2) Model generation and selection: model generation was conducted using "all-possible-subsets" regression procedures. An optimum model was considered as one with the highest  $R^2$  value ( $\geq 0.70$ ), lowest root-mean-square error (RMSE) value ( $\leq 5.0$ ), and the Mallows's  $C_p$  value closest to the number of regressors. In addition, all variables had to contribute statistically to the model ( $p \leq 0.05$ ), according to multiple linear regression analysis.
- (3) Model evaluation: the assumptions of linear regression—linearity, normality, homoscedasticity—were evaluated. The linearity of each quantitative (independent) variable with respect to the variable to be predicted (dependent) was assessed graphically using a scatter plot. The normality of the residuals was evaluated by a histogram or normality plot. Homoscedasticity was assessed by graphing the residual (dependent variable) and predicted (independent variable) values using a scatter plot. Finally, the absence of collinearity was evaluated by the variance inflation factor ( $VIF < 10$ ).

Upon completing these procedures, the following statistical procedures were conducted with Group 2 to validate the equations:

1. Estimates of body composition: the equations generated (Eq.#1 and Eq.#2) were applied in Group 2 to estimate FM and FFM, respectively. Note that estimating FM by Eq.#2 required subtracting FFM in kg from body weight. For this reason, the cross-validation protocol was applied using only the FM variable. The FM in kg derived from the 4C model was used as the reference method.
2. Accuracy at the group level was tested by a two-way analysis of variance (ANOVA) with sex and method as factors. If the mean FM values between each new equation and the 4C model were not significantly different ( $p \geq 0.05$ ;  $p$  value corresponding to the method factor), the equation was considered accurate at the group level for estimating FM.
3. Accuracy at the individual level was probed by a simple regression procedure using FM from the 4C model and FM calculated by each equation as the dependent and independent variables, respectively. If the intercept did not differ significantly from zero ( $p \geq 0.05$ ), but the slope did differ significantly from zero ( $p < 0.05$ ; assuming it is not significantly different from 1.0), the equation was considered accurate at the individual level for estimating FM.
4. Precision was assessed utilizing the  $R^2$  and RMSE values from the regression procedure. If the  $R^2$  value was higher than 0.70 and the RMSE value lower than 5.0, the equation was considered precise for estimating FM.
5. Agreement analyses: agreement between the new equations and the 4C model was evaluated by Bland and Altman's plots [41]. The difference in FM and the average FM

**Table 1.** General characteristics, body composition, anthropometric, and BIA variables in older Hispanic adults with excess adiposity by sex.

Variables	Men (n = 136)	Women (n = 250)	Total (n = 386)
Age, years	67.9 ± 5.9	68.3 ± 6.6	68.2 ± 6.3
Weight, kg	80.4 ± 11.9*	71.2 ± 9.9	74.4 ± 11.5
Height, m	1.69 ± 0.1*	1.55 ± 0.1	1.60 ± 0.1
BMI, kg/m <sup>2</sup>	27.9 ± 3.2	29.6 ± 3.6*	29.0 ± 3.6
FM, kg	27.1 ± 6.9	32.3 ± 6.9*	30.5 ± 7.4
FFM, kg	53.3 ± 7.6*	38.9 ± 4.9	43.9 ± 9.1
FMI, kg/m <sup>2</sup>	9.4 ± 2.1	13.4 ± 2.8*	12.0 ± 3.2
Mid-arm, cm	31.7 ± 2.7	32.5 ± 3.9*	32.2 ± 3.5
Hip, cm	100.0 ± 6.5	106.4 ± 9.4*	104.2 ± 9.0
Calf, cm	36.5 ± 3.2*	35.5 ± 3.3	35.8 ± 3.3
Triceps, mm	15.4 ± 5.2	23.3 ± 6.5*	20.5 ± 7.2
Biceps, mm	8.2 ± 4.4	13.9 ± 6.3*	11.9 ± 6.3
Subscapular, mm	20.9 ± 6.3	22.5 ± 6.9*	21.9 ± 6.7
Suprailiac, mm	16.3 ± 7.4	23.6 ± 7.6*	21.0 ± 8.3
Sum 4 skinfold, mm	60.3 ± 17.1	83.9 ± 22.2*	75.4 ± 23.4
Resistance, Ω	488.9 ± 64.3	577.3 ± 73.3*	546.1 ± 81.9
Reactance, Ω	51.4 ± 42.3	52.9 ± 49.9	52.4 ± 47.3
Ht <sup>2</sup> /R, cm/Ω	59.8 ± 9.2*	42.3 ± 6.0	48.5 ± 11.1

The mid-arm, hip, and calf variables are circumferences. The triceps, biceps, subscapular, and suprailiac variables are skinfold. The sex comparison was based on a two-sample independent *t*-test.

BMI body mass index, FM fat mass, FFM fat-free mass, FMI fat mass index, Ht<sup>2</sup>/R height square/resistance ratio.

\**p* ≤ 0.05.

between each new equation and the 4C model were considered as the dependent and independent variables, respectively. Limits of agreement (LOA) were calculated using ±2 standard deviations (SD) from the mean value of the dependent variable. Agreement between methods (equation and the 4C model) was considered when the mean value of the dependent variable was not different from zero according to a paired *t*-test (*p* > 0.05). To ascertain whether this bias remained, regardless of adiposity levels (independent variable), the homogeneity of the dependent variable was assessed by simple linear regression analysis using the *p* value of the beta ( $\beta$ ) parameter. Thus, homogeneity was considered at a *p* value >0.05 of the  $\beta$  parameter. All analyses were run in the STATA/SE 12.0 statistical program (StataCorp LP, TX, USA).

## RESULTS

A total of 386 (136 men, 250 women) older Hispanic adults aged 60–90 with excess adiposity were included. FMI ranges were 9.4–24.8 kg/m<sup>2</sup> (corresponding a BMI range of 21.2–42.5 kg/m<sup>2</sup>) and 6.3–15.4 kg/m<sup>2</sup> (corresponding a BMI range of 21.5–41.1 kg/m<sup>2</sup>) for women and men, respectively. According to these data, 204 and 182 subjects were classified in the excess fat and obesity categories, respectively. Table 1 shows that the mean values of body weight, height, FFM, calf circumference, and Ht<sup>2</sup>/R or resistance index were higher in men than women (*p* ≤ 0.05). In contrast, the means of BMI, FM, FMI, mid-arm, and hip circumferences, the four skinfolds and their sum, and *R* values were all significantly higher in the women than the men (*p* ≤ 0.05).

### Sample division

The total sample was split randomly into two similar groups, each with 193 subjects, to first develop (Group 1) and then validate

(Group 2) the predictive equations for FM. Table 2 shows that the general characteristics, body composition, anthropometric, and BIE variables did not differ statistically (*p* > 0.05) between the groups. Therefore, the two groups are homogeneous, or equal, as required by the cross-validation protocol.

### Selection of a potential predictor variable

According to simple linear regression (*p* ≤ 0.2), the potential predictor variables statistically associated with FM were age in years, sex (women = 0, men = 1), weight in kg, BMI in kg/m<sup>2</sup>, the mid-arm, hip, and calf circumferences in cm, and each skinfold and their sum in mm. In the case of FFM, age in years, sex (women = 0, men = 1), weight in kg, height in meters, BMI in kg/m<sup>2</sup>, the mid-arm, hip, and calf circumferences in cm, *R* in Ω, and the Ht<sup>2</sup>/R in cm<sup>2</sup>/Ω, or resistance index, were found to be statistically associated (Table 3).

### Generation and selection of the equations

Table 4 shows the optimal models, or equations, obtained in Group 1 by the 'all-possible-subsets' regression procedure that fulfilled the criteria (see the Statistical analysis section).

Model 1 thus included weight, sex, and BMI as predictor variables for FM. According to the results of the multiple linear regression analysis, Eq.#1 for FM in kg is:

$$FM = (0.409 * \text{weight}) - (8.063 * \text{sex}) + (0.549 * \text{BMI}) - 12.899$$

where weight is in kg, sex (women = 0 and men = 1), and BMI in kg/m<sup>2</sup> is the body mass index. This equation from Group 1 had an *R*<sup>2</sup> value of 0.79, an RMSE value of 3.45, and a Cp value of 4.1.

Concerning model 2, the predictor variables included for FFM were sex, weight, the Ht<sup>2</sup>/R ratio, and *R*. Results of the multiple linear regression analysis show that Eq.#2 for FFM in kg is:

$$FFM = (5.607 * \text{sex}) + (0.283 * \text{weight}) + (0.455 * \text{Ht}^2/\text{R}) + (0.015 * \text{R}) - 9.071$$

**Table 2.** Comparison of general characteristics, body composition by the 4C model, anthropometric, and BIE variables between the development and validation groups.

Variables	Group 1			Group 2		
	Men (n = 68)	Women (n = 125)	Total (n = 193)	Men (n = 68)	Women (n = 125)	Total (n = 193)
Age, years	67.8 ± 5.8	68.2 ± 6.7	68.1 ± 6.4	68.0 ± 6.0	68.4 ± 6.5	68.3 ± 6.3
Weight, kg	80.4 ± 13.2	71.2 ± 9.8	74.4 ± 11.9	80.5 ± 10.5	71.2 ± 10.1	74.5 ± 11.1
Height, m	1.69 ± 0.1	1.55 ± 0.1	1.59 ± 0.1	1.69 ± 0.1	1.55 ± 0.1	1.60 ± 0.1
BMI, kg/m <sup>2</sup>	27.9 ± 3.5	29.7 ± 3.6	29.1 ± 3.6	27.9 ± 2.8	29.5 ± 3.7	28.9 ± 3.5
FM, kg	27.3 ± 7.5	32.6 ± 6.9	30.7 ± 7.6	26.9 ± 6.2	32.1 ± 7.0	30.3 ± 7.2
FFM, kg	53.1 ± 8.0	38.6 ± 4.8	43.7 ± 9.2	53.6 ± 7.2	39.1 ± 4.9	44.2 ± 9.1
FMI, kg/m <sup>2</sup>	9.5 ± 2.3	13.6 ± 2.8	12.1 ± 3.3	9.3 ± 1.9	13.3 ± 2.8	11.9 ± 3.1
Mid-arm, cm	31.5 ± 2.8	32.9 ± 4.2	32.4 ± 3.8	31.9 ± 2.6	32.1 ± 3.5	32.0 ± 3.2
Hip, cm	100.2 ± 7.2	106.1 ± 10.1	104.0 ± 9.6	99.9 ± 5.8	106.7 ± 8.7	104.3 ± 8.4
Calf, cm	36.5 ± 3.5	35.4 ± 3.1	35.8 ± 3.3	36.5 ± 2.9	35.5 ± 3.5	35.8 ± 3.4
Triceps, mm	15.1 ± 4.6	23.5 ± 6.3	20.5 ± 7.0	15.7 ± 5.8	23.1 ± 6.6	20.5 ± 7.3
Biceps, mm	7.7 ± 3.8	14.0 ± 6.4	11.8 ± 6.4	8.7 ± 4.9	13.7 ± 6.2	11.9 ± 6.2
Subscapular, mm	20.3 ± 5.8	22.7 ± 7.2	21.9 ± 6.8	21.5 ± 6.7	22.4 ± 6.6	22.0 ± 6.6
Suprailiac, mm	16.9 ± 7.3	23.7 ± 7.5	21.3 ± 8.1	15.7 ± 7.6	23.5 ± 7.7	20.8 ± 8.5
Sum 4 skinfold, mm	58.7 ± 14.3	85.2 ± 22.3	76.3 ± 23.5	61.5 ± 19.1	82.5 ± 22.2	74.5 ± 23.4
Resistance, Ω	500.8 ± 66.9	574.6 ± 66.8	548.6 ± 75.5	476.9 ± 59.6	580.1 ± 79.6	543.7 ± 88.1
Reactance, Ω	51.7 ± 33.3	52.6 ± 40.2	52.3 ± 37.8	51.2 ± 49.9	53.0 ± 58.2	52.4 ± 55.3
Ht <sup>2</sup> /R, cm <sup>2</sup> /Ω	58.3 ± 9.7	42.2 ± 5.5	47.9 ± 10.6	61.3 ± 8.4	42.4 ± 6.6	49.1 ± 11.6

The mid-arm, hip, and calf variables are circumferences. The triceps, biceps, subscapular, and suprailiac variables are skinfolds. Data were examined using a two-sample independent t-test; there was no significant difference ( $p > 0.05$ ) between groups.

BMI body mass index, FM fat mass, FFM fat-free mass, FMI fat mass index, Ht<sup>2</sup>/R height square/resistance ratio.

**Table 3.** Association of potential predictor variables with FM and FFM in older Hispanic adults with excess adiposity (Group 1, n = 193).

Variables	Fat mass in kg		Variables	Fat-free mass in kg	
	β value	p value		β value	p value
Age, years	-0.23	<0.01	Age, years	-0.31	<0.01
Sex, male	-5.25	<0.01	Sex, male	14.5	<0.01
Region	0.45	0.5	Region	0.98	0.23
Weight, kg	0.4	<0.01	Weight, kg	0.59	<0.01
Height, m	-0.37	0.95	Height, m	78.9	<0.01
BMI, kg/m <sup>2</sup>	1.69	<0.01	BMI, kg/m <sup>2</sup>	0.49	<0.01
Mid-arm, cm	1.13	<0.01	Mid-arm, cm	0.34	0.05
Hip, cm	0.52	<0.01	Hip, cm	0.09	0.19
Calf, cm	1.05	<0.01	Calf, cm	1.33	<0.01
Triceps, mm	0.6	<0.01	Resistance, Ω	-0.08	<0.01
Biceps, mm	0.56	<0.01	Reactance, Ω	<0.01	0.98
Subscapular, mm	0.42	<0.01	Ht <sup>2</sup> /R, cm <sup>2</sup> /Ω	0.78	<0.01
Suprailiac, mm	0.44	<0.01			
Sum 4 skinfold, mm	0.19	<0.01			

The mid-arm, hip, and calf variables are circumferences. The triceps, biceps, subscapular, and suprailiac variables are skinfolds. β value from simple linear regression analysis.

FM fat mass, FFM fat-free mass, BMI body mass index, Ht<sup>2</sup>/R height square/resistance ratio.

where sex (women = 0, men = 1), weight is in kg, Ht<sup>2</sup>/R in cm<sup>2</sup>/Ω denotes the height squared/resistance, and R represents resistance in Ω at 50 kHz. This equation from Group 1 had an R<sup>2</sup> value of 0.89, an RMSE value of 3.08, and a Cp value of 6.4.

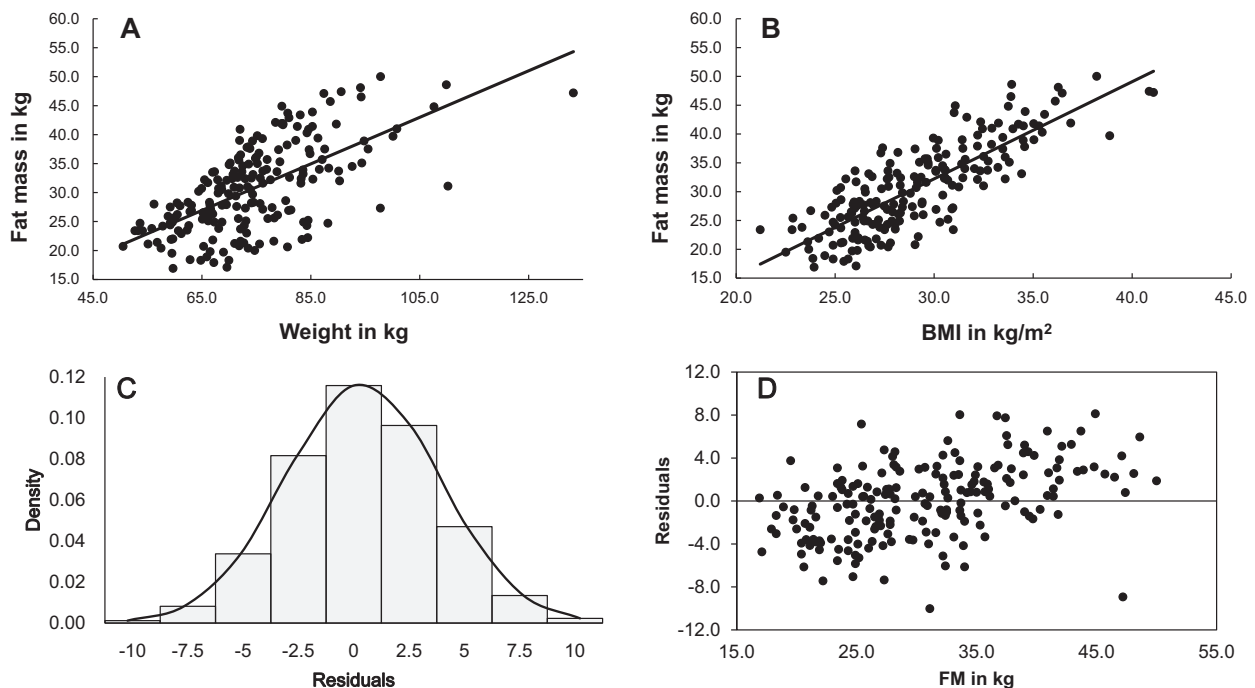
### Model evaluation

It is important to note that each quantitative predictor variable in Eq.#1 and Eq.#2 had linearity with FM and FFM, respectively (Figs. 1 and 2). In addition, the normality and homoscedasticity

**Table 4.** Evaluation of models based on “all-possible-subsets” regression procedures (Group 1,  $n = 193$ ).

Models	Variables	$\beta$ value	$p$ value	VIF	$R^2$	RMSE	$C_p$	Intercept
Model 1 for FM in kg					0.79	3.45	4.1	-12.899
	Weight, kg	0.409	<0.01	3.71				
	Sex, male	-8.063	<0.01	2.18				
	BMI, kg/m <sup>2</sup>	0.549	<0.01	2.38				
Model 2 for FFM in kg					0.89	3.08	6.4	-9.071
	Sex, male	5.607	<0.01	2.63				
	Weight, kg	0.283	<0.01	2.21				
	Ht <sup>2</sup> /R, cm/ $\Omega$	0.455	<0.01	8.39				
	R, $\Omega$	0.015	<0.01	3.31				

FM fat mass, FFM fat-free mass, VIF variance inflation factor,  $R^2$  coefficient of determination from the model, RMSE root mean square error,  $C_p$  Mallows's  $C_p$  value, BMI body mass index, Ht<sup>2</sup>/R height square/resistance ratio, R resistance.



**Fig. 1** Evaluation of Eq.#1. Plots **A** and **B** show the linearity assessment between FM by the 4C model and the weight and BMI, respectively. Plot **C** and **D** shows the normality and homoscedasticity assessment of the residuals, respectively.

assumptions of the residuals of both equations were verified (Figs. 1 and 2). Finally, an absence of collinearity of all regressors in each equation was observed ( $VIF < 10$ , Table 4).

#### Accuracy at the group level

Results of the two-way ANOVA (Table 5) for Group 2 show that the comparisons of FM in kg by the 4C model with both equations had no significant effect for the term method ( $p = 0.61$ ). Therefore, these equations are accurate at the group level for estimating FM in older Hispanic adults with excess adiposity. The ANOVA analyses for both equations showed an effect for the term sex ( $p \leq 0.05$ ) that indicates—or confirms—that FM is higher in women than men.

#### Accuracy at the individual level

Results of the regression analysis for Group 2 show clearly that the slope ( $\beta = 0.99$ , close to one) in both equations differed statistically ( $p < 0.05$ ) from zero. The intercept values were not statistically different ( $p > 0.05$ ) from zero (Table 5). These results

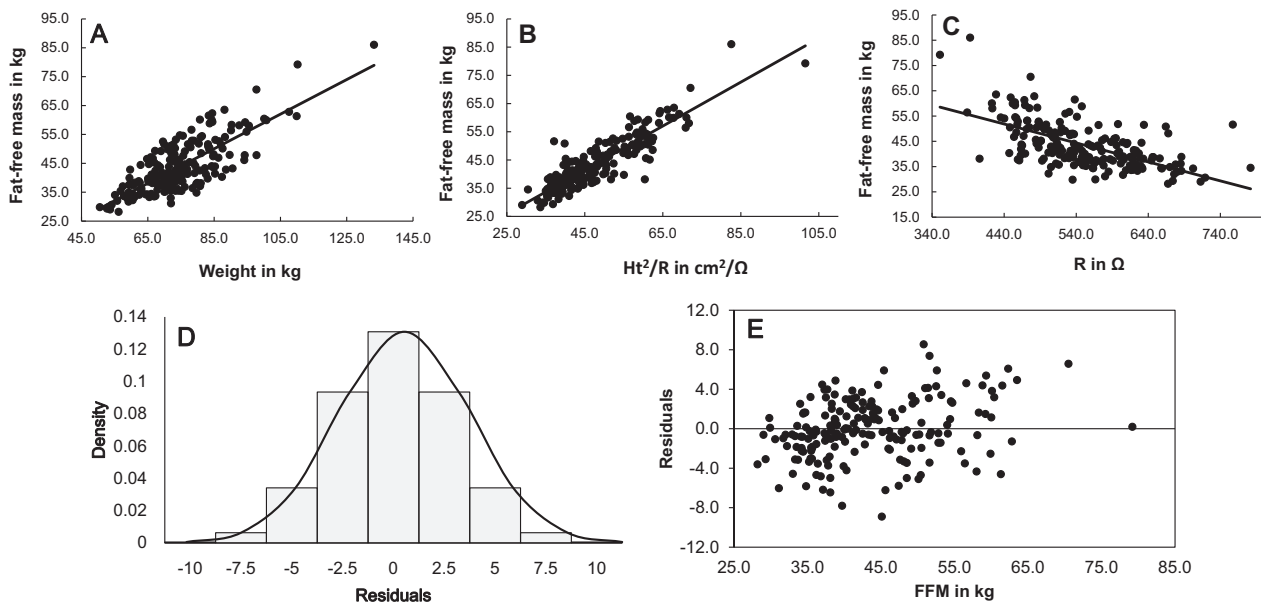
suggest that both equations are accurate for estimating FM at the individual level when compared to the 4C model.

#### Precision

Based on the previous regression analysis for Group 2, the  $R^2$  and RMSE values permit the inference that both equations were precise in estimating FM when compared to the 4C model. Eq.#1 explained 76% of the variance in FM by the 4C model, and the estimates had an RMSE of 3.49 kg. Eq.#2 provided the most precise estimates of FM in the total sample because it explained 81% of the variance in FM by the 4C model, and the estimates had an RMSE of 3.10 kg.

#### Agreement analysis

Eq.#1 and the 4C model showed agreement in assessing FM in the older Hispanic adults with excess adiposity in Group 2. The estimate of FM by Eq.#1 was similar to that of the 4C model because the differences in FM ( $0.34 \pm 3.5$  kg) between them did not differ from zero ( $p = 0.17$ ). In addition, the regression line



**Fig. 2** Evaluation of Eq.#2. Plots A, B and C show the linearity assessment between FFM by the 4C model and the weight,  $Ht^2/R$  and  $R$ , respectively. Plots D and E show the normality and homoscedasticity assessment of the residuals, respectively.

indicates that bias was not distributed homogeneously ( $\beta = -0.14$  kg,  $p \leq 0.01$ ) along the entire range of average FM values (Table 5 and Fig. 3A).

With respect to Eq.#2 and the 4C model, analyses showed agreement in assessing FM in the older Hispanic adults with excess adiposity in Group 2. The estimate of FM by Eq.#2 was similar to that of the 4C model since the difference ( $-0.25 \pm 3.1$  kg) between the two did not differ from zero ( $p = 0.26$ ). In addition, the regression line indicates that bias was not distributed homogeneously ( $\beta = -0.09$  kg,  $p \leq 0.01$ ) over the entire range of average FM values (Table 5 and Fig. 3B). However, the lack of homogeneity of the errors in both equations did not affect their agreement, since the bias was not different from zero.

## DISCUSSION

Nutritional status assessments must be included to achieve accurate body composition evaluations in older adults, but most methods available for analyzing body composition are not feasible—or are inaccessible—for clinical practice or population studies because implementation is expensive and requires complex infrastructure and trained personnel [42]. Developing a practical method for estimating FM in clinical medicine is essential for both improving assessments of nutritional status and monitoring treatment of obesity in older adults.

The main findings of this study are that the new equations designed and validated considering a direct marker of fat mass to classify the subjects with excess adiposity using the 4C model as the reference method allow the predictor variables to be measured easily and accurately in clinical practice. The FMI approach was utilized to classify subjects with excess adiposity because BMI is not a parameter of body composition and cannot distinguish between FM and FFM [10]. The 4C model, meanwhile, is recommended for estimating body composition in older adults because it considers independent measurements of the components of FFM (BMC and TBW) that change during the aging process [23]. Both new equations included sex and weight as predictor variables of body composition due to their association with FM and FFM. In general, older men have higher FFM but lower FM values than older women (Table 1). These differences in body composition are attributable to the action of sex steroid

hormones. Estrogen, for example, is important in the accumulation, metabolism, and distribution of body fat in women, while in men, testosterone plays an essential role in increasing FMM [43]. However, since weight is the sum of FM plus FFM, it is to be expected that it will be included in most equations as a predictor variable [26].

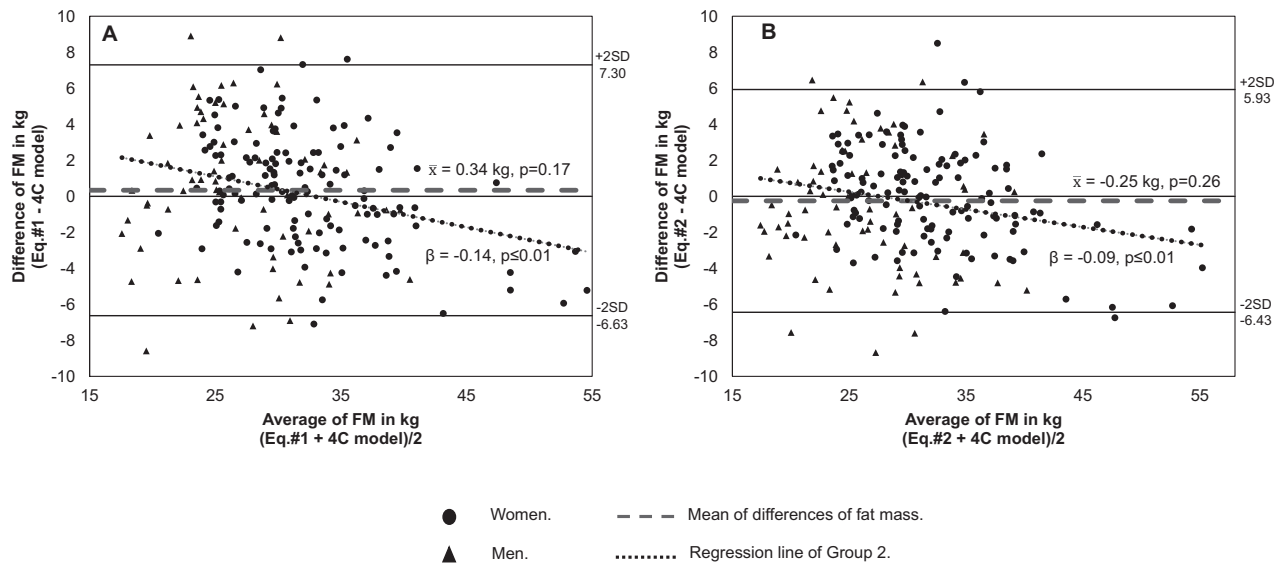
In addition to sex and weight, Eq.#1 included BMI as a predictor of FM. This parameter was proposed mainly as a “direct marker of FM” [44], and has been widely used to define obesity in clinical and epidemiologic studies [9, 45]. Published equations including BMI as the predictor variable explained 48–62% of the variance in FM in younger and older Mexican American adults [46]. Eq.#2, meanwhile, in addition to sex and weight, included  $Ht^2/R$  and  $R$  as predictors of FFM. The  $Ht^2/R$  index describes an empirical relation between impedance ( $Length^2/R$ ) and the volume of water [47], while  $R$  is the pure opposition of the conductor to alternating current. The field of body composition studies assumes that  $R$  is inversely proportional to the distribution of TBW and electrolytes [48]. Thus, the  $R$  value is crucial because older Hispanic adults with obesity have high hydration factor values ( $0.747 \pm 0.035$ ) due to excess adiposity [49].

The validation procedure showed that the new equations are accurate at the group level because the estimates of FM did not differ from the reference method, as the ANOVA revealed. The new equations also proved to be accurate at the individual level because the intercept did not differ from zero, while the slope was different from zero, according to the regression procedure (Table 5). Regarding precision, Eq.#1 had  $R^2$  and RMSE values of 0.76 and 3.49 kg, respectively. These are considered acceptable in relation to the nature of anthropometric variables for predicting FM. Huerta and collaborators [30] reported an  $R^2$  value of 0.84 and a standard error of estimate (SEE) of 3.2 kg for their equation, also based on anthropometric variables. Regarding Eq.#2, we found an  $R^2$  value of 0.81 and an RMSE value of 3.10 kg, which are close to those reported by Sun and collaborators [28] for older women and men ( $R^2 = 0.85$  and  $0.90$ ;  $SEE = 2.8$  and  $3.7$  kg, respectively). Huerta’s and Sun’s equations may seem more precise than Eq.#1 and Eq.#2, respectively, for estimating FM compared to the 4C model, but these earlier equations are not specific for subjects with excess adiposity, so their precision could be affected when they are used with populations with this characteristic.

**Table 5.** Validation of the “new equations” against the 4C model to estimate fat mass in kg (Group 2,  $n = 193$ ).

Technique	Men ( $n = 68$ )	Women ( $n = 125$ )	Total ( $n = 193$ )	Intercept	Slope	$R^2$	RMSE	Bias, kg	Lower LOA	Upper LOA	Distribution of errors ( $\beta$ value)
The 4C model.	26.9 $\pm$ 6.2	32.1 $\pm$ 7.0	30.3 $\pm$ 7.2								
Anthropometric equation.	27.3 $\pm$ 5.7	32.4 $\pm$ 5.9	30.6 $\pm$ 6.3	-0.26 $\pm$ 1.25	0.99 $\pm$ 0.04*	0.8	3.49	0.34 $\pm$ 3.5	-6.63	7.3	-0.14**
Anthropometric-BIA equation.	26.2 $\pm$ 5.6	32.1 $\pm$ 6.0	30.0 $\pm$ 6.5	0.55 $\pm$ 1.05	0.99 $\pm$ 0.03*	0.8	3.1	-0.25 $\pm$ 3.1	-6.43	5.93	-0.09**

The comparison was between fat mass in kg by the 4C model and each equation separately. Accuracy at the group level was examined by a two-way (sex and method) analysis of variance; there was a significant effect of sex ( $p \leq 0.05$ , men vs. women), but no significant effect of method was observed ( $p > 0.05$ ). Accuracy and precision at the individual level was examined by regression procedure, where fat mass by the 4C model as the dependent variable, and fat mass by each equation as the independent variable; \* $p < 0.05$  significantly different from zero. According to paired sample t-test, no significant bias was observed ( $p > 0.05$ ). Regression coefficient ( $\beta$  value) was obtained from the differences and average in fat mass as the dependent and independent variables, respectively, between methods; \*\* $p \leq 0.05$  by simple linear regression, indicates that the distribution of errors was not homogeneous. 4C four-compartment, LOA limits of agreement, RMSE root mean square error.



**Fig. 3** Bland and Altman analysis of the agreement in FM between the “new equations” and the 4C model. The difference in FM between these methods was taken as the dependent variable, while the average FM between the methods was the independent variable. **A** is the agreement for FM by Eq. #1 based on anthropometric variables. **B** is the agreement for FM by Eq. #2 based on combining anthropometric and BIA variables. According to these results, the new equations are reliable, practical options for estimating FM in older Mexican adults with adiposity.

Finally, there was agreement between both equations and the 4C model in their respective estimates of FM. The mean difference in FM between Eq.#1 and the 4C model was 0.34 kg, a value similar to that reported by Huerta and collaborators [30] (0.3 kg). This bias did not differ statistically from zero ( $p > 0.05$ ). In the case of Eq.#2, the mean of the differences in FM was -0.25 kg, compared to Sun and collaborators’ [28] figures of -0.3 kg for men and -0.6 kg for women between their equations and the 4C model for estimates of FFM.

An important limitation of the present study is that true FM values cannot be obtained by the direct method using in vivo methodologies, so when comparing two indirect methods a poor result for validation might result due to the numerous assumptions that underlie these methods [50]. However, the 4C model assumes minimal errors in measuring the FM of older people [23], so it was taken as the reference method. A second limitation is that potential volunteers with extreme obesity were excluded because they did not satisfy the inclusion criteria. Another limitation is that three methods to measure BMC were used and differences among DXA systems have been reported. The GE Lunar iDXA technique, for example, underestimates BMC in adults

(range -0.04 to -0.06 kg, very low but significant) compared to the Lunar DPX-L and GE Lunar Prodigy methods [51]. However, for estimates of BMC high agreement has been found among the different DXA systems ( $R^2 = 0.85$  to 0.99). A final limitation is that two methods for measuring  $D_2O$  were used (148 and 238 subjects by IRMS and FTIR, respectively). In this regard, however, there are reports that no significant differences exist between the IRMS and FTIR methods for quantifying  $D_2O$  in saliva samples ( $616 \pm 70$  and  $612 \pm 68$   $\mu\text{mol/mol}$  by IRMS and FTIR, respectively) [52].

## CONCLUSIONS

Two predictive equations were developed and validated to estimate body composition in older Hispanic adults with excess adiposity using the 4C model as the reference method. The equation based on anthropometric variables included weight, sex, and BMI as predictor variables, while the one based on anthropometric and BIA variables combined included sex, weight,  $Ht^2/R$ , and  $R$  as predictor variables. According to the cross-validation protocol, both equations proved to be reliable for estimating body composition and are interchangeable with the 4C



model. Finally, these equations can be used in epidemiological and clinical studies, as well as clinical practice, to estimate body composition in older Hispanic adults with excess adiposity.

## DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### AUTHOR CONTRIBUTIONS

GAR was responsible for designing the study protocol, conducting the field and laboratory studies, cleaning, and data analysis, as well as for the writing and editing process of the manuscript; URR, RTA, ERJ, MERO, RE contributed to the study design and critically reviewed the manuscript. ERJ was also the main adviser on the statistical analyses applied; RSAE was the adviser on deuterium

determination by FTIR and critically reviewed the manuscript; PMBI contributed to the laboratory studies and critically reviewed the manuscript; AMH was the project leader and participated in study design, DXA measurements, analysis and interpretation of the data collected, and the writing and editing process of the manuscript.

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### COMPETING INTERESTS

The authors declare no competing interests.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted according to the guidelines laid down in the Helsinki Declaration, and all procedures involving human subjects were approved by the Ethics Committee of the CIAD, A.C. (CE/008/2014), UACJ (CBE.ICB/023.10-14), and UANL (15-FaSPyN-SA-19). Informed written consent was obtained from all subjects.

### ADDITIONAL INFORMATION

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