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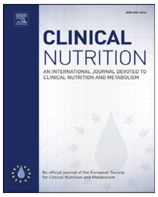

Original article

# Agreement between laboratory methods and the 4-compartment model in assessing fat mass in obese older Hispanic-American adults

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## SUMMARY

*Background/objectives:* Densitometry (Siri's and Brožek's equations), hydrometry (by the deuterium dilution technique), and dual-energy X-ray absorptiometry (DXA) are three methods for estimating body composition. However, because they are all based on certain assumptions, they may not be applicable to aged and obese subjects due to changes in their body composition. Hence, the validity of these “laboratory methods” could be affected in obese older people. The main aim was to assess the agreement between densitometry, hydrometry, and DXA with the 4-compartment (4C) model to estimate fat mass (FM) in obese older Hispanic-American adults. As secondary goals, we explored whether the bias in densitometry and hydrometry results could be improved by modifying the assumptions regarding fat-free mass (FFM) density and hydration factor (HF) values, respectively. In the case of DXA, we explored the factors that contribute to bias.

*Subjects/methods:* This is a cross-sectional study based on a sample of 171 obese subjects aged $\geq$60 years from 3 regions of northern Mexico. Body composition was assessed by the 4C model as the reference method and by all three laboratory methods. Agreement of the latter with the 4C model was probed by Bland and Altman analysis, a paired sample t-test, and simple linear regression analyses. In addition, the mean FFM density estimated in this sample, and HF values (published previously) of 0.737 and 0.753 for obese older Hispanic-American men and women, respectively, were considered as ethnic- and gender-specific values. These values were used to modify the densitometric and hydrometric equations in order to improve their bias. Finally, we tested whether the hydration status and indirect markers of adiposity are contributing factors to the bias of DXA using multiple linear regression analysis.

*Results:* Siri's equation overestimated FM by 0.57 kg ($p < 0.01$), while Brožek's equation, hydrometry, and DXA underestimated it by 1.24 kg, 0.89 kg, and 0.79 kg ($p < 0.01$), respectively, compared to the 4C model. The bias in the densitometry and hydrometry results was eliminated by substituting the ethnic- and gender-specific values into the equations. Finally, we found that hip circumference contributes to the bias in DXA.

*Conclusion:* The densitometry, hydrometry, and DXA methods are not interchangeable with the 4C model for assessing fat mass in obese, older Hispanic-American adults. The lack of agreement could indicate that the assumptions of each method do not apply to this population.

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## 1. Introduction

Obesity is considered a serious public health problem in Mexico and worldwide [1], due to its association with morbidity [2,3], mortality [4], and physical disability in older people [5]. Recently, fat mass (FM) adjusted by height squared —the fat mass index (FMI, kg/m$^2$)— was proposed and recommended as a more accurate tool than body mass index (BMI, kg/m$^2$) for diagnosing obesity, monitoring weight loss, and assessing risk-associated factors [6]. Implementing the FMI, however, requires valid body composition methods to estimate FM. The most common laboratory methods (hereinafter, "lab methods") used to assess body composition are densitometry, hydrometry, and dual-energy X-ray absorptiometry (DXA), but all three of these techniques estimate body composition based on certain assumptions.

Densitometry assumes FM and fat-free mass (FFM) densities of 0.9007 g/cm$^3$ and 1.100 g/cm$^3$, respectively, while hydrometry assumes a hydration factor (HF) of 0.732 [7]. These assumed FFM density and HF values, however, could be affected by both the changes in body composition that are part of the aging process and conditions of obesity, mainly in relation to total body water (TBW) and bone mineral content (BMC) [8—13]. DXA, in turn, assumes that measurements are not influenced by body thickness [14], though it is now well-recognized that body thickness above 25 cm —as in cases of obesity— can affect them [15]. Therefore, the validity of these lab methods for estimating body composition in obese older subjects could be subject to question.

An alternative approach for assessing body composition that is free of assumptions is the four-compartment (4C) model. This model considers the aqueous and mineral weight fractions to estimate FM [16] because, as mentioned above, TBW and BMC components change with age and obesity. The 4C model is an attractive option for assessing body composition in obese older adults, an done that has shown a high correlation (r = 0.98) with neutron activation analyses (the *in vivo* reference method) in estimates of FM [16]. One challenge to implementing the 4C model in some developing countries is that it requires substantial infrastructure. Due largely to this, densitometry, hydrometry, and DXA are used there as reference methods for validating predictive equations [17,18] and evaluating the health risks associated with body composition in older adults [19]. For these reasons, it is important to determine whether these lab methods are interchangeable with the 4C model for estimating body composition.

According to our research, densitometry [20—24] and hydrometry [20,22] appear to be valid methods for assessing FM in older adults, comparable to the 4C model, but results by DXA are contradictory [20—22,25]. It is important to note that earlier studies were conducted in older people with wide BMI ranges, so a critical assessment of the validity of FM measurements in obese older people is still lacking. The main aim was to assess the agreement between densitometry, hydrometry, and DXA with the 4C model to estimate FM in obese older Hispanic-American adults. As secondary goals, we explored whether the bias in densitometry and hydrometry results could be improved by modifying the assumptions regarding FFM density and HF values, respectively. In the case of DXA, we explored the factors that contribute to bias.

## 2. Methods

### 2.1. Subjects

This study included subjects aged ≥60 years with obesity according to the FMI cut-off points published by Kelly et al. [6] (>9.0 kg/m$^2$, >13.0 kg/m$^2$ for men and women, respectively), since FMI is considered a better marker of adiposity than BMI. The FM values used to calculate FMI were obtained by the 4C model. Volunteers were recruited from 2016 to 2019 through ads in local newspapers, announcements on radio and social networks, flyers, and visits to homes and clubs in three cities in northern Mexico: Hermosillo, Sonora, Ciudad Juárez, Chihuahua, and Monterrey, Nuevo León. Though no consensus has been reached on a suitable sample size for agreement studies in cross-sectional designs [26], the literature recommends at least 100 subjects [27]. Reports suggest that a sample size of 163 subjects provides a nominal power of 0.90 and an estimated power of 0.9003 [28]. In any case, our sample size was sufficient to detect significant differences in results.

Subjects were non-institutionalized, non-indigenous, non-athletes, with stable weight- (±3 kg) over the prior 3 months and no history of heart attack, heart disease, type 2 diabetes, kidney or liver diseases, or cancer, according to self-reports. Also, they were free of physical disability according to the Lawton and Brody scale [29], and cognitive dysfunction by the Pfeiffer scale [30]. Haematocrit values were indicative of good hydration status [31]. All volunteers were found to be free of type 2 diabetes by oral tolerance glucose tests [32]. Exclusion criteria were: subjects aged <60 years, no obesity (FMI ≤9.0 kg/m$^2$, ≤13.0 kg/m$^2$ for men and women, respectively), institutionalization, indigenous people, unstable weight, history of heart attack or heart disease, diabetes, kidney or liver failure, cancer, physical disability, cognitive dysfunction, and altered hydration status. Finally, athletes and volunteers whose height exceeded the dimensions of the DXA bed were excluded.

### 2.2. Design

This is a cross-sectional study based on a non-probabilistic sample carried out from 2016 to 2019 in the three aforementioned cities in northern Mexico. In a preliminary screening to identify potential volunteers, apparently healthy subjects by self-report and volunteers with some controlled diseases that do not affect body composition (e.g. hypertension, dyslipidaemia) were invited for an initial clinical assessment to apply the inclusion criteria. All subjects who met these criteria then underwent body composition measurements under fasting conditions. The study was conducted following the same protocol at three institutions: the Centro de Investigación en Alimentación y Desarrollo (CIAD), A.C., the Universidad Autónoma de Ciudad Juárez (UACJ), and the Universidad Autónoma de Nuevo León (UANL). The protocol was approved by the Ethics Committees of the CIAD (CE/008/2014), UACJ (CBE.ICB/023.10—14), and UANL (15-FaSPyN-SA-19). All volunteers provided and signed their informed consent. Details of the body composition measurements recorded are described below.

### 2.3. Body fat by the 4C model as the reference method

To estimate FM by the 4C model we measured body density (Bd) and determined the aqueous weight (*A*) and mineral weight fractions (*M*). The following three procedures were used to obtain these variables:

1. Bd was assessed by air displacement plethysmography (BodPod® Body Composition System, Life Measurement Instruments, Concord, CA, USA) according to the protocol published by Alemán-Mateo et al. [24]. 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, it was calculated the determination coefficient (R$^2$) between the FM estimated by considering both TGV values

(predicted and measured), and the $R^2$ found in the present study was of 0.96. The BodPod® system was calibrated daily in accordance with the manufacturer's guidelines.

2. The *A* was calculated from the ratio of TBW in kg to body weight in kg:

$$A = \frac{TBW}{body\ weight}$$

TBW was measured using the stable isotope of deuterium oxide ($D_2O$, 99.8 atom percent, Lot. No. 14G-316, Cambridge Isotope Laboratories, Inc., USA). $D_2O$ quantification in saliva samples was measured by two protocols: 66 samples were measured with the protocol reported by Aleman et al. [24] using isotope ratio mass spectrometry (IRMS; DELTA PLUS, Thermo Finnigan, Bremen, Germany), while 105 samples were measured with the protocol reported by the International Atomic Energy Agency (IAEA) [33] using Fourier transform infrared spectrophotometry (FTIR; 8400S, Cat No. 206-72400-92, Shimadzu Corporation, USA).

3. The *M* was calculated from the ratio of total body mineral mass (TBMM) in kg to body weight in kg:

$$M = \frac{TBMM}{body\ weight}$$

TBMM was calculated by multiplying the BMC in kg x 1.279 (the sum of bone and cell mineral content). 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 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 analysed according to the protocol published previously by Aleman-Mateo et al. [24]. In addition, the regions of interest (ROI) were marked manually by a qualified technician, following the procedures described by Ramos et al. [34], using Encore software (LU43616ES©2015, GE Healthcare Lunar).

Once calculated, Bd, *A*, and *M* were incorporated into the following equation to estimate FM as a percentage (%FM) [35]:

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

The percentage FM was then converted to FM in kg using this equation:

$$FM = (\%FM * body\ weight) / 100$$

Finally, FFM in kg was calculated from the difference in body weight derived from the BodPod® scale and FM, both in kg.

### 2.4. Body fat by densitometry

This trial was designed to explore the agreement of two densitometric equations with the 4C model for estimating FM. The densitometric equations were published by Siri [36] and Brožek et al. [7], where Bd values were measured by BodPod® following the protocol mentioned above [24]:

$$\text{Siri's equation: } \%FM = \left(\frac{4.95}{Bd} - 4.50\right) * 100$$

$$\text{Brozek's equation: } \%FM = \left(\frac{4.570}{Bd} - 4.142\right) * 100$$

Finally, the %FM generated by each equation was converted to FM in kg by the equation:

$$FM = (\%FM * body\ weight) / 100$$

### 2.5. Body fat by hydrometry

The FM in kg by hydrometry was calculated from the difference between the body weight derived from the BodPod® scale in kg and FFM in kg. The latter was derived from the classic hydrometric equation considering an HF of 0.732, where TBW in kg was measured by the deuterium dilution technique:

$$FFM\ (kg) = \left(\frac{TBW}{0.732}\right)$$

### 2.6. Fat mass by DXA

To explore agreement between DXA and the 4C model, FM in kg from the DXA-reports was recorded. To ensure that FM values from whole DXA scans were included, only subjects whose body size did not exceed the dimensions of the DXA bed were analysed in the DXA validation process.

### 2.7. Exploration of the bias in the densitometry and hydrometry methods

To explore the bias related to the assumptions of the densitometry and hydrometry methods, we modified the associated equations considering the mean FFM density and HF values estimated for our sample, respectively. The FFM density value was calculated using Baumgartner et al.'s equation [35], which considers FFM as the sum of aqueous, protein, and mineral content:

$$FFM\ density = (1.0063 * aFFM + 0.3292 * mFFM + 0.7463 * pFFM)^{-1}$$

where *aFFM* is the aqueous fraction of FFM (TBW in kg/FFM in kg), *mFFM* is the mineral fraction of FFM (TBMM in kg/FFM in kg), and *pFFM* consists mostly of protein with a small amount of glycogen:

$$pFFM = 1 - (aFFM + mFFM)$$

The FFM used was the value derived from the 4C model. Next, the mean FFM density values by gender were used to replace the 1.100 g/$cm^3$ values through an algebraic procedure applied to Siri's and Brožek's equations to estimate FM by these modified densitometric equations.

In the case of the hydrometry method, the HF value of 0.732 was replaced in the hydrometric equation with the ethnic- and gender-specific HF values of 0.737 and 0.753 for obese older Hispanic-American men and women, respectively, published by González-Arellanes et al. [37]. FFM was then estimated using the modified equations:

$$\text{Men}: FFM = \left(\frac{TBW}{0.737}\right)$$

$$\text{Women}: FFM = \left(\frac{TBW}{0.753}\right)$$

Finally, FM in kg was calculated from the difference between body weight in kg and FFM in kg derived from these modified hydrometric equations.

### 2.8. Other measurements

Anthropometric variables such as body weight, height, BMI, waist and hip circumferences, waist-to-hip index (WHI), bicipital, tricipital, subscapular, and suprailiac skinfolds, and the sum of skinfolds (tissue thickness) were measured as well. Body weight in kg was recorded on a BodPod® digital scale. Standing height in meters was measured to the nearest 0.1 cm using a stadiometer (SECA 264, Germany). BMI (kg/m$^2$) is the ratio of body weight to height squared. Waist, hip, and calf circumferences were measured in centimetres using a flexible steel tape measure (0–200 ± 0.01 cm, Rosscraft, Canada). WHI is the waist-to-hip ratio, also measured in centimetres. The thickness of four skinfolds (triceps, biceps, subscapular, suprailiac) was measured in millimetres with a Harpenden skinfold calliper (0–80 ± 0.2 mm, model HSB-BI, Burgess Hill, England). The sum of these four skinfolds in mm was also calculated.

All measurements were obtained with subjects barefoot, under fasting conditions, and with minimal clothing. Measures were taken twice in 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 [38]. The scales and stadiometers employed were calibrated daily using 20-kg dumbbells and a 1-m-long metal bar, respectively. The calliper was calibrated as per the manufacturer's guidelines. Note that the body weight used in all procedures was measured by the BodPod® scale.

### 2.9. Exploration of bias in the DXA method

In order to examine potential factors related to hydration status and adiposity that could contribute to the differences in FM between DXA and the 4C model, a multiple linear regression analysis was carried out. Hydration status (HF and haematocrit values), and indirect markers of adiposity (BMI, waist, hip, and calf circumferences, WHI, skinfolds, and the sum of skinfolds) were considered as potential contributing factors. First, we selected those variables with a p-value ≤ 0.2 by univariate analysis. Subsequently, the selected variables were entered into the stepwise selection (pe ≤ 0.05 and pr ≥ 0.051). Finally, the preliminary model was assessed for linearity, normality, homoscedasticity, and collinearity.

### 2.10. Statistical analyses

Normal distribution was tested by the skewness and kurtosis tests for normality and by normality plot (histogram). Gender differences in the general characteristics and body composition variables were compared by a two-sample independent t-test. To explore whether the magnitude of the differences in FM between densitometry using Siri's equation and the 4C model (dependent variable) are related to the individual FFM components (mineral, aqueous, residue) and/or density FFM values (independent variable), correlations were evaluated by Pearson's correlation test.

The accuracy of each lab method (densitometry, hydrometry, and DXA) was tested by agreement analysis based on a Bland and Altman plot [39]. In this plot, the difference in the FM and the average FM between each laboratory method and the 4C model were considered as the dependent and independent variables, respectively. Limits of agreement (LOA) were calculated using ±2 standard deviations (SD) of the mean value of the dependent variable. To determine whether the mean differences in FM between the lab methods and the 4C model were significant ($p > 0.05$), we performed a paired two-sample t-test, which is adequate for detecting significant bias, or lack of agreement, between the methods evaluated. To ascertain whether this bias remained regardless of adiposity levels (independent variable), we assessed the homogeneity of the dependent variable by simple linear regression (β parameter). All analyses were run in the STATA/SE 12.0 statistical program (StataCorp LP, TX, USA).

## 3. Results

Table 1 shows the age and some anthropometric and body composition variables of the 171 obese older adults (112 women, 59 men), aged 60–88 years. All subjects were considered obese by their mean BMI and FMI values (31.6 ± 3.4 and 14.3 ± 3.0, respectively). Results showed that the obese older men had higher mean values for body weight, height, TBW, BMC, TBMM, FFM, Bd, and FFM density than the obese older women ($p \le 0.05$), while the latter had higher BMI, FM (in kg and %), FMI, and HF ($p \le 0.05$).

### 3.1. Validation procedures

There was no agreement between the densitometry method using Siri's equation and the 4C model in the FM assessments in obese older Hispanic-American adults. On average, Siri's equation overestimated FM by 0.57 kg compared to the 4C model ($p < 0.01$), and this bias was distributed homogeneously according to the results of a β- value of 0.003 kg and a p-value of 0.91 from the simple lineal regression analysis over the entire range of average FM values between methods (Table 2, Fig. 1-A). This means that the overestimation was consistent in obese subjects regardless of their adiposity levels, compared to the 4C model.

Likewise, there was no agreement between the densitometry method using Brožek's equation and the 4C model in the FM assessments in this study population. On average, Brožek's equation underestimated FM by 1.24 kg compared to the 4C model ($p < 0.01$), but this bias was not distributed homogeneously over the entire range of average FM values between the two methods. A β- value of −0.066 kg and a p-value of 0.01 were obtained from the simple lineal regression analysis (Table 2, Fig. 1-C). This distribution suggests that the underestimate of FM by Brožek's equation tends to be higher in obese subjects with high adiposity levels than in obese subjects with lower adiposity levels, compared to the 4C model.

Regarding the comparison between the hydrometric method using the classic HF value of 0.732 and the 4C model for estimates of FM in the study population, results showed a lack of agreement, as

**Table 1**
Age and anthropometric and body composition characteristics in obese older adults by gender.

| Variables | Men ($n = 59$) | Women ($n = 112$) | Total ($n = 171$) |
|---|---|---|---|
| Age, years | 67.8 ± 5.8 | 69.2 ± 6.4 | 68.7 ± 6.2 |
| Weight, kg | 86.5 ± 11.5* | 77.5 ± 8.9 | 80.6 ± 10.8 |
| Height, m | 1.69 ± 0.06* | 1.54 ± 0.06 | 1.59 ± 0.1 |
| BMI, kg/m$^2$ | 30.0 ± 3.0 | 32.4 ± 3.2* | 31.6 ± 3.4 |
| Body density, gr/cm$^3$ | 1.015 ± 0.007* | 0.989 ± 0.006 | 0.999 ± 0.014 |
| TBW, kg | 39.8 ± 5.6* | 29.7 ± 3.9 | 33.2 ± 6.6 |
| BMC, kg | 2.99 ± 0.4* | 2.23 ± 0.3 | 2.49 ± 0.5 |
| TBMM, kg | 3.7 ± 0.5* | 2.7 ± 0.4 | 3.1 ± 0.6 |
| FFM$_{4C}$, kg | 54.0 ± 7.1* | 39.4 ± 4.6 | 44.5 ± 8.9 |
| FM$_{4C}$, % | 37.5 ± 3.6 | 48.9 ± 3.6* | 45.0 ± 6.5 |
| FM$_{4C}$, kg | 32.5 ± 5.7 | 38.0 ± 5.9* | 36.1 ± 6.4 |
| FMI, kg/m$^2$ | 11.3 ± 1.6 | 15.9 ± 2.3* | 14.3 ± 3.0 |
| FFM density, gr/cm$^3$ | 1.099 ± 0.010* | 1.095 ± 0.012 | 1.097 ± 0.012 |
| HF | 0.737 ± 0.033 | 0.753 ± 0.034* | 0.747 ± 0.035 |

BMI = body mass index, TBW = total body water, BMC = bone mineral content, TBMM = total body mineral mass, FFM$_{4C}$ = fat-free mass by the 4C model, FM$_{4C}$ = fat mass by the 4C model, FMI = fat mass index by the 4C model, HF = hydration factor; *$p < 0.05$. The between-gender comparison was tested by a two-independent sample t-test.

**Table 2**
Analysis of bias in the lab methods against the 4C model for estimating FM.

| Techniques | FM in kg | Bias, kg | Lower LOA | Upper LOA | Distribution of errors (β- values) |
|---|---|---|---|---|---|
| 4C model | 36.1 ± 6.4 | | | | |
| Siri's equation | 36.7 ± 6.4 | 0.57 ± 2.1* | −3.68 | 4.82 | 0.003 |
| Modified Siri's equation | 36.1 ± 6.3 | −0.04 ± 2.1 | −4.28 | 4.20 | −0.019 |
| Brožek's equation | 34.9 ± 5.9 | −1.24 ± 2.1* | −5.38 | 2.91 | −0.066† |
| Modified Brožek's equation | 36.2 ± 6.3 | 0.11 ± 2.1 | −4.13 | 4.35 | −0.020 |
| Classic hydrometric equation | 35.2 ± 6.9 | −0.89 ± 2.0* | −4.91 | 3.12 | 0.082† |
| Modified hydrometric equation | 36.1 ± 7.1 | −0.02 ± 1.9 | −3.88 | 3.83 | 0.102† |
| DXA | 34.9 ± 6.6 | −0.79 ± 2.8* | −6.30 | 4.71 | 0.099† |

FM = fat mass, 4C = four-compartment, DXA = dual-energy X-ray absorptiometry. LOA = limits of agreement. Bias is the mean difference in fat mass between the lab methods and the 4C model. Regression coefficient of differences and average in fat mass as the dependent and independent variables, respectively, between methods. *p ≤ 0.05 by paired sample t-test indicates that bias is significant.†p ≤ 0.05 by simple linear regression indicates that the distribution of errors was not homogeneous.

hydrometry underestimated FM by 0.89 kg compared to the 4C model ($p < 0.01$). As in the previous case, this bias was not distributed homogeneously over the entire range of average FM values, since the β- and p values from the simple lineal regression were 0.082 kg and <0.01, respectively (Table 2, Fig. 1-E). This means that FM was underestimated in obese subjects with lower adiposity levels, while in obese subjects with higher adiposity levels FM was overestimated, compared to the 4C model.

The results of the comparison of FM by DXA and the 4C model also showed a lack of agreement, as DXA underestimated FM by an average of 0.79 kg compared to the 4C model ($p < 0.01$). Once again, the bias was not distributed homogeneously over the entire range of average FM values. Results of the simple lineal regression analysis showed a β- value of 0.099 kg of FM and a p-value of <0.01 (Table 2 and Fig. 1-G). In this case, there was a tendency towards underestimating FM in obese subjects with lower adiposity levels, but towards overestimating it in those with higher adiposity levels, compared to the 4C model.

### 3.2. *Exploration of the bias in the densitometry and hydrometry methods and the 4C model*

In the case of densitometry using Siri's equation, Pearson's correlation test showed that the magnitude of the differences, or bias, in FM between Siri's equation and the 4C model was positively-correlated with the aqueous fraction of FFM ($r = 0.92$, $p < 0.01$), but negatively-correlated with the mineral and residue fractions of FFM, and the FFM density value ($r = -0.45$, $r = -0.81$, and $r = -0.98$, respectively, $p < 0.01$). In addition, the mean FFM density values by gender in the study sample were 1.099 g/cm$^3$ and 1.095 g/cm$^3$ for obese older Hispanic-American men and women, respectively (Table 1). Therefore, we replaced only the assumed value of 1.100 g/cm$^3$ with the mean FFM density values in Siri's equation, obtaining the following two “Siri's equations modified by gender”:

$$\text{Men:}\%FM = \left(\frac{4.97}{Bd} - 4.523\right)*100$$

$$\text{Women}:\%FM = \left(\frac{5.054}{Bd} - 4.615\right)*100$$

The results of the analysis of densitometry using these modified Siri's equation showed agreement between the two methods in the study population, since the mean difference or bias between them was only −0.04 kg of FM; a result that is not statistically different from zero ($p = 0.82$). In addition, the bias between methods was distributed homogeneously over the entire range of average FM values, as confirmed by the β- value of −0.019 kg and the p-value of 0.47 from the simple lineal regression analysis (Table 2, Fig. 1-B). Therefore, the differences in FM between these two methods were maintained regardless of the adiposity levels.

In the case of Brožek's equations, we replaced the assumed value of 1.103 g/cm$^3$ (ratio of coefficients, 4.570/4.142) with the mean FFM density values estimated for our sample. It is important to note that we considered an assumed FM density value of 0.9007 g/cm$^3$ [7]. The following two “Brožek's equations modified by gender” were obtained:

$$\text{Men:}\%FM = \left(\frac{4.992}{Bd} - 4.542\right)*100$$

$$\text{Women:}\%FM = \left(\frac{5.076}{Bd} - 4.636\right)*100$$

The comparative analysis of densitometry using the modified Brožek's equations and the 4C model showed agreement in the study population. The mean difference or bias between these two methods was 0.11 kg of FM; once again, a value that does not differ from zero ($p = 0.52$). In addition, the bias was distributed homogeneously over the entire range of average FM values, since the β-value was −0.02 kg and the p-value was 0.44 (from the simple lineal regression analysis results, Table 2, Fig. 1-D). The bias between these two methods was also maintained regardless of adiposity levels.

With respect to the evaluation of hydrometry using the gender-specific HF values cited in the Methods section and the 4C model, results showed agreement in the study sample. The mean difference or bias between methods was just −0.02 kg of FM, which does not differ from zero ($p = 0.87$). In this comparison, however, the bias was not distributed homogeneously over the entire range of average FM values, as shown by the β- value of 0.102 kg of FM and the p-value of <0.01 (from the simple lineal regression analysis, Table 2, Fig. 1-F). Here, the distribution of the bias revealed differences, as the FM in obese subjects with lower adiposity levels was underestimated, while in subjects with higher adiposity levels it was overestimated, compared to the 4C model.

### 3.3. *Exploration of the bias between the DXA method and the 4C model*

The univariate analysis showed that the haematocrit, BMI, waist circumference, hip circumference, calf circumference, WHI, and tricipital, bicipital, suprailiac skinfold, and sum of 4 skinfolds values were all associated ($p \le 0.02$) with the difference in FM between DXA and the 4C model (Table 3). However, only hip circumference (independent variable) was determined to be significantly associated with the dependent variable by stepwise analysis. Results of the regression analysis showed that each 1-cm

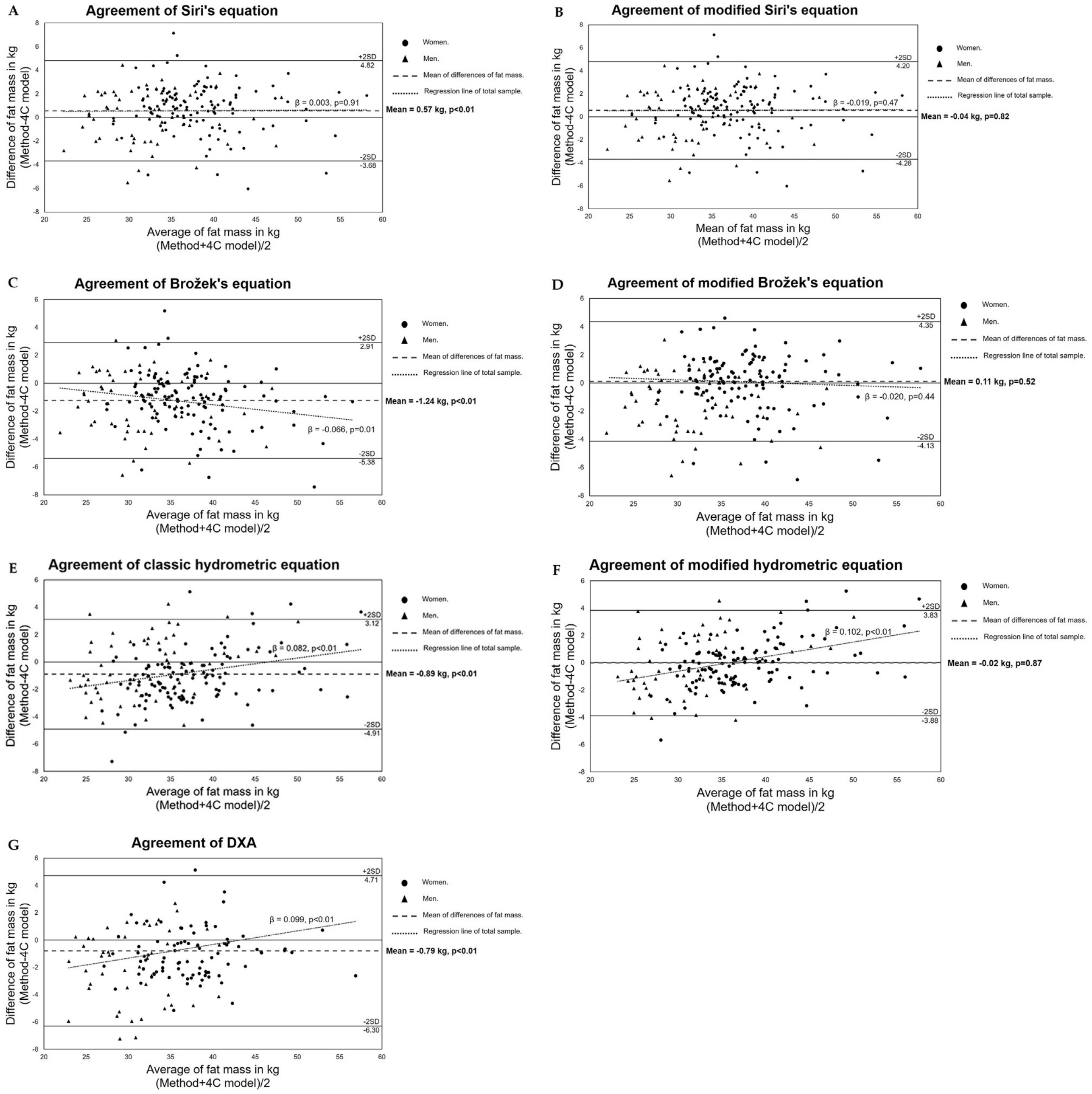

**Fig. 1.** Bland and Altman analysis of the agreement in FM between lab methods and the 4C model with the difference in FM between methods as the dependent variable and the average FM between methods as the independent variable. **A** is the agreement for FM by densitometry using Siri's equation. **B** is the agreement for FM by densitometry using the modified Siri's equation. **C** is the agreement for FM by densitometry using Brožek's equation. **D** is the agreement for FM by densitometry using the modified Brožek's equation. **E** is the agreement for FM by hydrometry using the classic HF value of 0.732. **F** is the agreement for FM by hydrometry using the modified hydrometric equation. **G** is the agreement for FM by DXA. Data are shown separately for men (solid triangle) and women (solid circle). See Table 2 for a summary of the statistical analyses.

increase in hip circumference significantly increased the difference in FM between methods by 0.104 kg ($p < 0.01$) in these obese older Hispanic-American adults (β- value of 0.104 kg of FM, p-value of <0.01).

In summary, Siri's equation overestimated FM by 0.57 kg ($p < 0.01$), while Brožek's equation, hydrometry, and DXA underestimated FM by 1.24, 0.89, and 0.79 kg ($p < 0.01$), respectively, compared to the 4C model. The biases in the densitometry and hydrometry methods were eliminated when the ethnic- and gender-specific values were used. Finally, the factor of hip circumference was found to contribute to the bias in DXA.

## 4. Discussion

The purpose of the present study was to contribute to the field of nutritional science by testing the accuracy of three lab methods used to assess FM in older obese people. According to our results, none of the methods tested (densitometry using Siri's and Brožek's equations, hydrometry by the deuterium dilution technique using an assumed HF value of 0.732, and DXA) are interchangeable with the 4C model for assessing body composition in obese older Hispanic-American adults. Specifically, the bias in the densitometry and hydrometry methods is related to the assumed FFM density

**Table 3**
Univariate analysis of potential contributing factors with the differences in FM between DXA and the 4C model in the study sample.

| Variables | Difference in FM in kg | |
|---|---|---|
| | β-values | p-values |
| *Hydration status* | | |
| Hydration factor | 6.08 | 0.26 |
| Haematocrit, % | −0.13 | 0.03 |
| *Indirect markers of adiposity* | | |
| BMI, kg/m$^2$ | 0.31 | <0.01 |
| Waist circumference, cm | 0.05 | 0.06 |
| Hip circumference, cm | 0.10 | <0.01 |
| Calf circumference, cm | 0.15 | 0.03 |
| WHI | −4.67 | 0.05 |
| Tricipital, mm | 0.11 | <0.01 |
| Bicipital, mm | 0.07 | 0.04 |
| Subscapular, mm | −0.01 | 0.76 |
| Suprailiac, mm | 0.07 | <0.01 |
| Sum 4 skinfold, mm | 0.02 | 0.02 |

FM = fat mass, BMI = body mass index, WHI = waist-to-hip index.

and HF values used, while in the case of DXA, the bias could be related to the factor of body thickness. Therefore, implementing the modified densitometric and hydrometric equations presented herein could improve the assessment of FM in obese older adults. Another suitable option could consist in applying the correction factor of −0.57 kg to FM measures obtained by Siri's equation.

Siri's equation overestimated FM by 0.57 kg in our sample of older obese adults in results similar to those published previously by Alemán-Mateo et al. [24], who reported an overestimate of 0.93 kg of FM in older Mexican people. In contrast, Goran et al. [20] found that Siri's equation underestimated FM by 0.1 kg in older Caucasian adults, but their studies were conducted with older subjects who had a wide BMI range. The distribution of bias by densitometry using Siri's equation in our sample was homogeneous, a finding that allows us to suggest the correction factor of −0.57 kg mentioned above. Brožek's equation underestimated FM by 1.24 kg, but because this was not distributed homogenously, no correction factor can be suggested.

Significantly, we found that the bias in the densitometry method is eliminated compared to the 4C model when the assumed FFM density value is replaced by ethnic- and gender-specific FFM values (Table 2, Fig. 1-B, 1-D). Over the past three decades, many researchers have evaluated whether the density values assumed in Siri's and Brožek's equations are valid for older people [38,41,42]. According to our results and those of Baumgartner et al. [35], the magnitude of the differences, or bias, between densitometry and the 4C model was significantly associated with the individual's FFM components [40]. The negative correlation ($r = -0.98$, $p < 0.01$) between FFM density values and bias suggests that densitometry tends to overestimate FM in subjects with lower FFM density values. These findings support the notion that the bias in densitometry is related to the assumed FFM density values used.

The estimations of body composition by densitometry and hydrometry employ assumed values taken from chemical analysis performed on cadavers of non-obese, adult male Caucasians, which showed that the aqueous, protein, and mineral fractions of FFM were 0.738, 0.194, and 0.068, respectively [7]. In contrast, the values determined for these three factors in our sample were 0.747, 0.183, and 0.069, respectively. Therefore, a higher water fraction (the FFM component with the lowest density) could explain the lower FFM densities and higher HF values in obese older Hispanic-American adults when compared to those chemical analyses.

In the case of hydrometry, when the assumed HF of 0.732 was replaced with ethnic- and gender-specific HF values, the bias was eliminated. Although this bias was not distributed homogeneously (Table 2 and Fig. 1-E), we can recommend the hydrometry method because the underestimate of FM of 0.02 kg is so low as to be non-significant, compared to the 4C model. It is important to understand that the ethnic- and gender-specific HF values of 0.737 and 0.753 for older obese Hispanic-American men and women, respectively, are statistically-different from 0.732 [37], so these findings can explain the underestimate of 0.89 kg of FM when the assumed HF value of 0.732 was applied in our sample of obese older Hispanic-American adults. Goran et al. [20] found that hydrometry using the HF value of 0.732 generated a significant underestimate of 1.1 kg in older Caucasian men. They suggested that this significant bias was related to the HF value due to the fact that the specific mean HF value for older Caucasian men was 0.747 [20].

In our sample, DXA significantly underestimated FM (0.79 kg) compared to the 4C model. Similar underestimates of FM have been reported in older people with a wide BMI range by Goran et al. [20] (0.7 kg) and Clasey et al. [21] (−1.9% and 2.0% for women and men, respectively), but those differences did not reach the level of significance. The underestimate observed in our study sample could be due to increased tissue thickness caused by obesity since excess adipose tissue affects the attenuation of the energy beams emitted by the DXA device [41]. Our regression analysis found that circumference is a contributing factor to the difference in FM between DXA and the 4C model. Our findings suggest that increases in hip circumference due to obesity may well increase tissue thickness and affect the accuracy of DXA.

The significant under- and overestimates (bias) of FM measured by the three lab methods tested and compared to the 4C model fluctuated between −1.24 kg and 0.57 kg. To explore whether this small bias is clinically-significant for diagnoses of obesity, we calculated the prevalence of obesity based on the FMI using the FM in kg of each laboratory method, keeping in mind that all the older subjects (100%) in this study were classified as obese by their FMI using the FM in kg from the 4C model. In this secondary analysis, the prevalence of obesity by Siri's and Brožek's equations were 95.9%, and 89.5%, respectively, by hydrometry using the 0.732 value it was 87.1%, while the result of the DXA test was 88.9%. Therefore, the significant bias reported in the present study influenced the obesity classification of these older adults by FMI. The review published by DeCaria et al. [42] demonstrates the need for additional research to clarify the classification of obesity by a direct marker of adiposity in older people.

One important limitation of the present study is the validation process, a procedure that requires comparing the FM estimated by the method under validation with the true FM value determined by the direct method. However, we were unable to obtain true FM values by the direct method using *in vivo* methodologies [43]. To resolve this issue, the 4C model assumes minimum errors in measuring FM in older people [35], so it was taken as the reference method. Also, the average FM between the method under validation and the reference method is the best estimate of true FM values. Another limitation is that two methods to measure TBW were used (180 and 232 subjects by IRMS and FTIR, respectively). Jennings et al. [44] reported that there were no differences between these two approaches in terms of determining TBW in saliva samples. Regarding the DXA validation process, only 136 of our obese older subjects (89 women, 47 men) with a range of FMI of 9.1–24.8 kg/m$^2$, whose body size did not exceed the dimensions of the DXA bed were included. Therefore, these data cannot be applied to extremely obese older people.

## 5. Conclusion

Densitometry analyses using Siri's equation, Brožek's equation, hydrometry by the deuterium dilution technique, and DXA are not valid methods for assessing body fat in obese older Hispanic-American, since none of these methods showed agreement with the 4C model. The bias in the densitometry and hydrometry results was eliminated by substituting the ethnic- and gender-specific values into the equations. The assumed values of FFM density and HF may not be applicable in obese older subjects and will alter body composition estimations. In the case of DXA, the factor of body thickness due to obesity tissue was found to be related to the bias detected. Finally, because the biases determined in the present study are apparently small, additional studies are required to assess the specificity and sensibility of these lab methods in relation to classifying obesity in obese older adults.

## 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 (CE/008/2014), UACJ (CBE.ICB/023.10–14), and UANL (15-FaSPyN-SA-19). Informed written consent was obtained from all subjects.

## Consent for publication

Not applicable.

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## Authors' contributions

GAR, was responsible for designing the study protocol, conducting the field and laboratory studies, cleaning the data, data analysis, and the writing and editing of the manuscript. URR, RTA, ERJ, MERO, and 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 the study design, DXA measurements, analysis and interpretation of the data collected, and the writing and editing of the manuscript.

## Conflict of interest

The authors declare no competing interests.

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