Bioelectrical impedance analysis, hydrometry and hydrodensitometry for body composition assessment in adult Colombian women

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Abstract. Bioelectrical impedance analysis (BIA) is a noninvasive method for assessment of body composition with better accuracy, and less inter-observer error than anthropometry. Despite these advantages, reported BIA equations may not be suitable for populations other than where they were developed. The aim of this study was to develop equations for single-frequency (SF) BIA and bioelectrical impedance spectroscopy (BIS) using a three compartment (3C) model as the criterion method to predict total body water (TBW) and fat-free mass (FFM) in South-American adult women. A cross-sectional observational study was conducted using a convenience sample (n=40). SF-BIA and BIS prediction equations were developed using forward- stepwise multiple regression with TBW by \(\text{D}_2\text{O}\) and FFM by 3C model as dependent variables; and weight, resistance and impedance index (stature\(^2\)/resistance) as independent variables. A cross-validation was conducted in a randomly split subsample (n=20). Four final equations were developed. There were no differences between SF-BIA and BIS equations for TBW (p=0.68) and FFM (p=0.66). The cross-validation showed a strong association and broadly meets the limits of agreement with the 3C reference method. The equations have an excellent goodness-of-fit to predict TBW and FFM. Validation of these equations in populations of different ages and ethnicities is warranted.

1. Introduction
Overweight and obesity prevalence and their associated morbidities in developing countries are increasing [1, 2]. Intervention programs can produce changes in body composition. An analysis of its components (total body water (TBW), fat-free mass (FFM) or fat mass (FM)) enables the evaluation of such interventions [3, 4]. Ideally, measurement of these changes should be assessed by accessible and accurate methods [5].

Bioelectrical impedance analysis (BIA) is a noninvasive method for human body composition assessment, with minimal risk and higher accuracy than anthropometry [6] which uses prediction equations validated against reference methods of two or multiple compartments (2C or MC) models.
Despite its advantages, each BIA equation has been developed for a specific population; which has produced many equations. These have primarily been developed in Caucasian populations with few developed in other races or ethnicities [7, 8]. However, BIA equations may be appropriate [9] or inappropriate [10-13] for populations other than those for which they were developed.

A previous investigation showed that available equations were not valid for a South-American population [14]. A SF-BIA equation at 50 kHz was developed to predict FFM in a South-American population (n=30) that used a 2C model with hydrodensitometry (HD) as the reference method [15]. This equation has a coefficient of determination ($R^2$) of 0.72, reliability of 95%, statistical power of 80% and a standard error of estimate (SEE) of 2.48 kg. Our hypothesis now is that it is possible to improve these results by including a three compartment (3C) model and BIS and by increasing the sample.

The aim of this study was to develop SF-BIA and BIS equations for FFM and TBW prediction using a 3C model with hydrometry using, deuterium oxide dilution $D_2O$ and HD as reference methods, in South- American adult women.

2. Subjects and Methods

A cross-sectional observational study was conducted using a convenience sample of 40 volunteers, with 83% statistical power [7, 8, 16]. A cross-validation was performed in a subsample of 20 subjects chosen at random. The procedures were approved by the Bioethics Committee of the Universidad de Caldas (Colombia) and the Institutional Review Board of the Institute for Health Sciences of St. Luke’s-Roosevelt Hospital Center (USA). Volunteers signed an informed consent form.

2.1. Subjects

Volunteers were recruited among female students at the Universidad de Caldas (Manizales, Colombia). Inclusion criteria were: be healthy, 18 to 24 years of age, have a body mass index of between 18.5 and 29.9 kg/m$^2$, non-smoking, non-pregnant or lactating and willing to be immersed in water. Exclusion criteria were having dental braces, metallic prosthesis, pacemaker or silicone implants, having had bariatric surgery or be taking diuretics. Inclusion and exclusion criteria were verified by physical examination, self assessment and an interview with a physician. Each subject reported in the following state: no alcohol consumption within the last 48 hours, no vigorous exercise within the last 12 hours, and having fasted for 12 hours [17]. The phase of the menstrual cycle was taken into account [18]. Measurements were completed during a single visit.

2.2. Laboratory environmental conditions

Temperature and relative humidity were monitored by a thermo-hygrometer 13307 ($\pm0.1^\circ C/\pm1\%RH$, DeltaTrak, France) and controlled by electric heater FMH416 and a dehumidifier BMD100 (Bionaire, USA). Atmospheric was measured using a barometer K4 ($\pm0.1$ mmHg, Konustar, USA).

2.3. Anthropometry

All participant emptied their bladder before measurements were taken. They wore underwear, a disposable hospital gown and were barefoot. Weight (PP2000; $\pm0.1$ kg, Icob-Detecto, A&D Co, Japan) and height (Heightronic-235; $\pm1$ mm, Seca, Hamburg, Germany) were measured in triplicate by the same operator using standard protocols [19].

2.4. BIA

Whole body tetrapolar wrist-ankle BIA was measured using the Hydra 4200 (Xitron, San Diego, CA) and 292-STE electrodes (Impedimed, USA) using standard protocols [17]. Frequency, resistance and reactance raw data and reciprocal calculations of impedance and phase at spectrum of fifty programmed frequencies were obtained by Hydra-S-Data-Acquisition-Utility (version 1.0e) software. The resistance at 50 kHz was used for SF-BIA and the infinite-resistance for BIS was obtained by Cole-Cole modeling software Hydra-Utility (version 2.2).
2.5. 2C and 3C body composition reference methods
The percentage FM (%FM) was obtained using a 2C model [20] and a 3C model [21], where body density (\(d_b\)) was measured by Hydrodensitometry (HD) and TBW by \(D_2O\) dilution. The FM was calculated as FM= Weight\(^{(%FM)}\) and FFM=Weight–FM.

2.6. TBW by hydrodynamics (\(D_2O\))
Each subject ingested \(\pm 10\) g \(\pm 0.002\) g \(D_2O\) (ICON, Summit, NJ) after a baseline blood sample (\(~7\) mL) was collected. Three hours after \(D_2O\) ingestion, a second blood sample (\(~7\) mL) was also collected. Plasma was extracted, tightly sealed in a glass tube (Fisher, cat#14-959-1A) and frozen (\(-20\) ºC) before transportation. The dose concentrations in the plasma samples were quantified on the SF-infrared-spectrophotometer (Nicolet 380; Thermo Electron, Madison, WI) after lyophilization [22]. TBW volume was calculated by dividing the dose by the net \(D_2O\) concentration in the samples. The accuracy for TBW measurement has been reported at \(\pm 1.0\%\) [23]. FFM by TBW was calculated using age and gender hydration factor for human FFM [24].

2.7. Body density (\(db\)) by Hydrodensitometry (HD)
The \(d_b\) was calculated by Archimedes’ principle as:

\[
d_b = \frac{W_a}{[(W_a-W_w)/d_w]-(R_v+0.1)}
\]

Where, \(W_a\) is weight in air, \(W_w\) is weight in water, \(d_w\) is water density, \(R_v\) is residual lung volume and \(0.1\) L is gastrointestinal volume correction. The \(W_w\) was measured ten times using the electronic scale PROGAN-1500SS (±0.001 kg, Prometalicos, Colombia) after maximal exhalation in a tank filled with water at 35.0 ± 2.0 ºC. Participants were in a seated position and submerged wearing a fitted swim suit and a belt-ballast of 5.5 kg to aid submersion [25]. The \(R_v\) was measured outside the tank three to six times with Quark PFT-2 (±0.01 L, COSMED, Italy) lung function equipment using the nitrogen washout technique [26]. The final \(W_w\) and \(R_v\) were the smaller average of two measurements whose difference was less than 0.1 kg or 0.1 L respectively.

2.8. Statistical analysis
Mean and standard deviation were used to evaluate demographic characteristics. Significant differences between validation and cross-validation groups and between FFM by 2C, FFM by 3C and FFM by TBW were evaluated. The SF-BIA and BIS prediction equations were developed using forward-stepwise multiple regression analysis (n=40, a=0.01) with TBW by \(D_2O\) and FFM by 3C as dependent variables; and weight, resistance and impedance index (stature\(^2\)/resistance) as independent variables [16, 27]. Infinite-resistance for BIS (bioelectrical impedance spectroscopy) was obtained by Cole-Cole modeling Hydra-Utility software included in our bioelectrical impedance spectrometer. A cross-validation between SF-BIA and BIS equation results was conducted in a randomly split subsample by \(R^2\) and SEE (standard error of estimate), significant difference and agreement (n=20). Significant differences were evaluated by paired Student 2-sided t-test (p<0.01) and agreement by the method of Bland and Altman [28]. Equations’ goodness-of-fit was evaluated by \(R^2\) (≥0.60), reliability (≥99%), statistical power (≥80%), Fisher’s variance (≤1%), Mallows index (\(C_P=C_P±1\)) and SEE (≤1.5 L or ≤2.0 kg). All analyses were performed using XLSTAT software (version 2013.1.01, Addinsoft). The reactance at 50 kHz (Xc50) was not included as an independent variable in the design of the equation because the coefficient of determination (\(R^2\)) with TBW-\(D_2O\) was 0.128 (very poor) and with FFM-3C it was 0.130 (very poor). Then, when \(R_{so}\) was included in the equation, next to the other independent variables, \(R^2\) decreased and SEE increased.
3. Results
There were 40 subjects in the validation group and 20 randomized subjects in the cross-validation group. Subject characteristics for validation and cross-validation groups (Table 1) showed normal distribution. There were no significant differences within subjects and between groups, FFM by 2C and FFM by TBW, or FFM by 2C and FFM by 3C. The $R^2$ between dependent variables (TBW by $D_2O$ or FFM by 3C) and independent variables (impedance index, weight or resistance) was calculated. To decide if resistance should be included in the models, its effective contribution was determined. The impedance index had stronger $R^2$ (0.85) than resistance (-0.47). Therefore, four final TBW and FFM prediction equations including impedance index by SF-BIA and BIS in the model were selected (Table 2).

Figure 1A shows a Bland and Altman plot of TBW by SF-BIA against TBW by BIS with a mean close to zero (-0.02 L) and a balanced limit of agreement (-0.51L to 0.48L). Figure 1B shows similar results for FFM (-0.02 kg and -0.47 kg to 0.44kg).

For cross-validation, simple regressions between the reference methods and SF-BIA and BIS were performed. Results were: $R^2=0.84$, SEE= 1.34 L, $p<0.0001$; $R^2=0.85$, SEE=1.33, $p<0.0001$ for TBW (L) by $D_2O$ vs by SF-BIA and by BIS respectively. For agreement between FFM by 3C vs SF-BIA and vs BIS, FFM (kg) $R^2=0.90$, SEE= 1.59 L, $p<0.0001$; and $R^2=0.91$, SEE= 1.58 L, $p<0.0001$ respectively. The $R^2$ 1.1% to 1.2% were higher and SEE were 0.6% to 0.8% lower for BIS compared to SF-BIA.

There were no significant differences between reference methods and the final equations for TBW ($p=0.66$) and FFM ($p=0.70$) by SF-BIA, and for TBW ($p=0.50$) and FFM ($p=0.53$) by BIS. Figures 2A and 2B show the Bland and Altman plots for TBW by SF-BIA and TBW by BIS, both compared against $D_2O$, where the means are close to zero (-0.06 L and -0.05 L) and the limits of agreement are balanced (-1.65 L to 1.53 L and -1.60 L to 1.50 L) respectively. Figures 2C and 2D showed similar results for FFM by SF-BIA and FFM by BIS, both compared against the 3C model. Means are close to zero (-0.11 kg and -0.10 kg) and the limits of agreement are balanced (-1.83 kg to 1.62 kg and -1.82 kg to 1.63kg).

### Table 1. Subject characteristics for validation and cross-validation groups.

| Variables                  | Validation group (n=40) † | Cross-validation group (n=20) † |
|----------------------------|----------------------------|---------------------------------|
| Age (y)                    | 21.2 ± 1.7 (18.1 – 24.6)  | 21.4 ± 1.7 (18.8 – 24.6)       |
| Weight (kg)                | 54.1 ± 6.0 (43.1 – 67.7)  | 54.3 ± 7.0 (43.1 – 67.7)       |
| Height (cm)                | 156.8 ± 4.0 (148.7 – 166.3) | 156.8 ± 4.4 (148.7 – 166.3)  |
| BMI (kg/m²)                | 22.0 ± 2.3 (18.6 – 27.6)  | 22.1 ± 2.5 (18.8 – 24.6)       |
| Body density (kg/L)        | 1.0 ± 0.0 (1.0 – 1.06)    | 1.0 ± 0.0 (1.019 – 1.0)        |
| TBW (L)                    | 28.5 ± 2.4 (23.7 – 34.3)  | 28.8 ± 2.6 (23.7 – 34.3)       |
| FFM by TBW (kg) §          | 39.0 ± 3.3 (32.4 – 46.9)  | 39.3 ± 3.6 (32.2 – 46.9)       |
| FFM by 3C model (kg)       | 38.9 ± 3.0 (32.3 – 45.2)  | 39.3 ± 3.5 (32.3 – 45.2)       |
| FFM by 2C model (kg)       | 39.3 ± 3.3 (31.8 – 46.0)  | 39.7 ± 4.2 (31.8 – 46.0)       |
| R50 (Ω)                    | 646.1 ± 53.9 (546.6 – 774.7) | 640.4 ± 56.6 (546.6 – 774.7)  |
| Rinf (Ω)                   | 528.4 ± 42.0 (450.9 – 594.2) | 532.1 ± 43.6 (457.1 – 584.4)  |

### Table 2. SF-BIA and BIS equations for TBW and FFM including resistance in the models.

| Method | Equation |
|--------|----------|
| SF-BIA | $\hat{\text{TBW}} = 0.712 \text{ stature}^2/ \text{R50} + 0.092 \text{ weight} + 0.021 \text{ R50} – 17.216$ |
|        | $\hat{\text{FFM}} = 0.774 \text{ stature}^2/ \text{R50} + 0.204 \text{ weight} + 0.025 \text{ R50} – 18.081$ |
| BIS    | $\hat{\text{TBW}} = 0.582 \text{ stature}^2/ \text{Rinf} + 0.100 \text{ weight} + 0.026 \text{ Rinf} – 17.673$ |
|        | $\hat{\text{FFM}} = 0.630 \text{ stature}^2/ \text{Rinf} + 0.212 \text{ weight} + 0.031 \text{ Rinf} – 18.477$ |
Figure 1. Bland-Altman plots for agreement between SF and BIS equations for estimation of (A) TBW and (B) FFM. BIS, bioelectrical impedance spectroscopy; FFM, fat-free mass; SF, single-frequency bioelectrical impedance analysis; SD, standard deviation; TBW, total body water.

Figure 2. Bland and Altman plots for agreement of measured TBW by D$_2$O and FFM by 3C against estimated TBW and FFM via SF and BIS equations. (A) TBW by D$_2$O vs. TBW by SF; (B) TBW by D$_2$Ovs.TBWbyBIS; (C) FFM by 3C vs. FFM by SF; (D) FFM by 3C vs.FFM by BIS. Abbreviations used: 3C, 3-compartment model; BIS, bioelectrical impedance spectroscopy; D$_2$O, deuterium oxide dilution; FFM, fat-free mass; SF, single-frequency bioelectrical impedance analysis; TBW, total body water.

4. Discussion

BIA might be a good option for body composition assessment in developing countries where access to reference methods is limited. This study presents four new BIA equations (two for SF-BIA and two for BIS) to predict TBW and FFM in adult women from Colombia South-America. According to international parameters, the four final equations have an excellent goodness-of-fit [29]. The cross-validation also showed an excellent SEE, a strong association and excellent limits of agreement with the reference methods.

Contrary to the findings of previous studies [30, 31] when 2C and 4C models were compared in adult women, our FM results showed no significant difference between 2C and 3C models. The apparent lack of agreement between these studies could be due to the different models used in each study. However, our results agree with Moon et al. [32] which compared 2C and 3 C models to estimate FM in adult women. Although our results provide data validating the use of the 2C model HD to FM assessment in our adult women, this should be used with caution for individual FM assessments because of the multiple assumptions involved in HD [33]. The 3C model is superior to the 2C model to estimate FM [34] because it controls the biological variability of body water [35]. Since, no significant difference between FFM by TBW and FFM by 2C was found it could be suggested that the HD protocol used in this investigation is sufficiently rigorous and reliable to use as part of the 3C model. Also, it must be acknowledged that
2C and MC models have higher costs and complexity and are less available compared to field methods such as skinfolds and BIA [29, 32, 36]. BIA methods have advantages and estimate TBW or FFM through equations validated against 2C or MC models [7, 8, 36]. BIA offers better accuracy [6], less inter-observer errors [37] than skinfolds and a good agreement with MC models if a specific population equation is available [5, 18, 38]. Nevertheless, most published equations have been developed for Caucasians [7, 8]. The validity of these equations in South-American populations has been questioned [14, 39]. In addition, Hispanic ethnicity may be associated with modestly higher levels of adiposity and slightly lower amounts of FFM [40, 41]. Nigam et al [42] also showed that an equation developed for Asians was not valid for Indian Asians. Thus, it is optimal to develop appropriate equations to allow the use of BIA, especially in Hispanic countries [18]. To our knowledge, these are the first BIA equations validated against a 3C model for South-American populations.

The use of a standardized BIA protocol, minimizing possible biological and environmental variations affecting BIA measurements and the homogeneous study group evaluated are strengths of this study [17, 8]. The homogeneity and normal distribution of the validation and cross-validation groups and their being no significant difference within subjects and between groups probably indicate that the randomization of the subsample was adequate.

After developing 8 equations, four were finally selected because their goodness-of-fit were better than models that did not include the resistance, which agrees with previous studies [43, 44, 45]. The four final TBW and FFM equations have a strong association with impedance index.

These results are in line with previous studies that have reported good agreement between SF-BIA and BIS compared to deuterium and bromide dilution, the superiority of BIS vs SF-BIA, and the potential of BIS for improving the BIA standardization method even when TBW is altered [46, 47].

The inclusion of D2O for the assessment of TBW is a strength of this study because there was an increase in R² and a decrease in SEE when comparing the final FFM SF-BIA equation of this study with a previous one [14]. Similar findings were reported by Macías et al [48].

Recently, some authors developed FFM BIA equations for specific ethnicities in developing countries, suggesting that the development of ethnic specific BIA equations is still needed [49, 50].

5. Conclusion
The new BIA equations developed in this study showed good agreement with the criterion method. They are only for adult women (18 to 24 years old) with a body mass index of between 18.5 and 29.9 kg / m² of our region. However, in the absence of more specific equations, they could be used in other South American populations, instead of relying on equations designed for Caucasians, Americans or other populations. The equations presented in this paper will help to increase the use of BIA in the region.

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Conflict of interest
No conflicts of interest are declared by authors.

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