Development of bioelectrical impedance analysis-based equations for estimation of body composition in postpartum rural Bangladeshi women

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Abstract

Equations for predicting body composition from bioelectrical impedance analysis (BIA) parameters are age-, sex- and population-specific. Currently there are no equations applicable to women of reproductive age in rural South Asia. Hence, we developed equations for estimating total body water (TBW), fat-free mass (FFM) and fat mass in rural Bangladeshi women using BIA, with 2H2O dilution as the criterion method. Women of reproductive age, participating in a community-based placebo-controlled trial of vitamin A or β-carotene supplementation, were enrolled at 19.7 (SD 9.3) weeks postpartum in a study to measure body composition by 2H2O dilution and impedance at 50 kHz using multi-frequency BIA (n 147), and resistance at 50 kHz using single-frequency BIA (n 82). TBW (kg) by 2H2O dilution was used to derive prediction equations for body composition from BIA measures. The prediction equation was applied to resistance measures obtained at 13 weeks postpartum in a larger population of postpartum women (n 1020). TBW, FFM and fat were 22.6 (SD 2.7), 30.9 (SD 3.7) and 10.2 (SD 3.8) kg by 2H2O dilution. Height2/impedance or height2/resistance and weight provided the best estimate of TBW, with adjusted R2 0.78 and 0.76, and with paired absolute differences in TBW of 0.02 (SD 1.33) and 0.00 (SD 1.28) kg, respectively, between BIA and 2H2O. In the larger sample, values for TBW, FFM and fat were 23.8, 32.5 and 10.3 kg, respectively. BIA can be an important tool for assessing body composition in women of reproductive age in rural South Asia where poor maternal nutrition is common.

Key words: Body composition: Total body water: Bioelectrical impedance analysis: 2H2O dilution: Prediction equations: Women: Reproductive age: South Asia

Poor maternal nutritional status may be one reason for high rates of infant low birth weight in South Asian countries13, where women of reproductive age are likely to suffer from macro- and micronutrient deficiencies12,3. Low BMI at the onset of pregnancy has been associated with adverse birth outcomes in a variety of settings4,5, but more specifically birth weight has been positively associated with maternal fat-free mass (FFM) content measured following pregnancy in Indian15 and Mexican16 mothers. Conversely, excess body fat has been linked to increasing rates of metabolic syndrome and cardiovascular risk, now observed even in rural populations of South Asia7. Because specific components of body composition appear to be linked to two of the major public health problems of South Asia, adequately characterising body composition in populations in this region of the world is critical.

Body composition in field settings is often assessed using weight, height, circumference, or skinfolds as proxies for direct measures of fat and lean mass components. Using weight, height or BMI (kg/m2) alone will not distinguish fat and FFM compartments, which play different metabolic roles. Moreover, there is evidence that individuals of Asian descent have more body fat for a given BMI than Caucasian populations8–10. Skinfolds may provide regional information on fat depots and can be used to estimate whole-body composition, and waist circumference reflects central fat deposition.

Abbreviations: BIA, bioelectrical impedance analysis; FFM, fat-free mass; MF-BIA, multi-frequency bioelectrical impedance analysis; R50, resistance at 50kHz; RMSE, root mean square error; SF-BIA, single-frequency bioelectrical impedance analysis; TBW, total body water; Z50, impedance at 50kHz.

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However, more sophisticated body composition methods are not readily applicable in field settings on a large scale (e.g. \(^2\)H\(_2\)O dilution) or are completely laboratory or clinic-based (dual energy X-ray absorptiometry, air displacement plethysmography, etc).

Bioelectrical impedance analysis (BIA), in contrast, provides a potentially field-applicable means for assessing body composition in large, community-based epidemiological studies. It is safe, easy to use, non-invasive, portable and inexpensive. In single-frequency BIA (SF-BIA), a single current, typically 50 kHz, is applied to the body. The resistance of the body to the current is inversely proportional to the amount of body water and, thus, FFM content. Additionally, with multifrequency BIA (MF-BIA), a current at several frequencies is applied that allows for the distinction between inter- and intra-cellular fluid\(^{11}\).

Numerous studies have reported that BIA reliably predicts total body water (TBW) or FFM, and a variety of equations to estimate these outcomes from BIA have been established, as summarised elsewhere\(^{11}\). However, to ensure the validity of such prediction equations, they should be derived under population-specific (e.g. sex, age and ethnicity) conditions by comparing bioelectrical impedance measures to more direct measures of TBW or FFM. Relatively few studies\(^{12-16}\) have been conducted to establish body composition equations from BIA in Asian populations. Furthermore, these equations are probably not applicable to rural South Asian women with poor nutritional status, whose body size is considerably different from that of women reported elsewhere\(^{17}\).

In the present study, we developed an equation to predict TBW from BIA measures in postpartum women in rural Bangladesh, using \(^2\)H\(_2\)O dilution as the criterion method. We then applied this equation to a larger population of postpartum women in the same community to demonstrate its utility for determining body composition in this population.

**Experimental methods**

**Study subjects**

The present study was conducted within a large, randomised, community-based trial to evaluate the impact of vitamin A and \(\beta\)-carotene supplementation on all-cause, pregnancy-related maternal and infant mortality\(^{18}\). The trial was conducted in ninety unions in the District of Gaibandha in northwestern Bangladesh from August 2001 to February 2007. Details of the main trial and its more intensive substudy have been provided elsewhere\(^{18,19}\). Briefly, of 596 individual ‘sectors’, small geographical regions that functioned as randomisation units, thirty-two contiguous sectors were designated for enhanced, home-based biochemical and anthropometric assessments that occurred shortly following pregnancy ascertainment (early pregnancy), in late pregnancy, and at approximately 3 months following the end of pregnancy, i.e. after a birth or after pregnancy termination due to abortion, miscarriage or stillbirth. These assessments were carried out by five specially trained teams and included anthropometry and SF-BIA. Postpartum data were collected on women from the main study from June 2003 until March 2007. Weight of the woman was measured with a scale accurate to 0·2 kg (SECA Electronic Scale 890; UNICEF). Height was measured using a stadiometer to the nearest 0·1 cm, and skinfolds and mid-upper arm circumference were measured using standardised techniques. A single-frequency, portable bioelectrical body composition analyser (Quantum II; RJL Systems) at 50 kHz was used to obtain resistance and reactance measurements from women as they lay supine on a non-conducting surface, as described previously\(^{13}\).

Beginning in the autumn of 2005, the postpartum visit also served as an opportunity to recruit women for a more intensive body composition study, which took place from spring of 2006 to early 2007. A subset of lactating women at the 3-month postpartum visit, selected in part based on their proximity to main roads to facilitate bringing them to a central location, was invited to participate in a clinic-based study to develop body composition equations for resistance (using SF-BIA; RJL Systems) or impedance (using MF-BIA, Quadscan 2000; BodyStat Limited). The purpose of this study was to generate equations to predict TBW (kg), using \(^2\)H\(_2\)O dilution as the referent method, as will be described next. Further, the prediction equation for TBW was applied to the larger population of postpartum women in the community with successful pregnancy outcomes (i.e. living infant) and valid resistance measures at 3 months postpartum to examine body composition in the larger population.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board in the USA and the Bangladesh Medical Research Council in Bangladesh. Verbal consent was obtained from all participants. Consent was witnessed and formally recorded.

**Body composition study procedures**

Women who consented to participate in the body composition study were brought in groups of five to eight, with their infants and a family member, to a central facility by vehicle in the morning after an overnight fast. A uniform breakfast was served upon arrival. A pre-weighted sari (median weight 300 g) was provided to each woman in order to measure body weight with uniform clothing. Before the initiation of any procedures, women were asked to breastfeed their infants and to void urine. Weight was measured to the nearest 0·2 kg (SECA Electronic Scale 890; UNICEF), and height was measured to the nearest 0·1 cm using a locally made fixed stadiometer modified with a level affixed to the cross-bar to help position subjects along the Frankfort plane. Baseline saliva samples were collected using sterile cotton cylinders (Salivette tubes; Sarstedt AG and Company) 2 h following breakfast to determine the natural \(^1\)H enrichment for each participating woman. Doses of enriched \(^2\)H\(_2\)O (99·8 % reported \(^2\)H enrichment; Cambridge Isotope Laboratories, Inc.) had been prepared the night before the study for each woman based on her body weight obtained during the home visit. A 0·15 g/kg body weight dose\(^{20}\) was sealed in an individually labelled plastic cup.
Individually labelled doses of $^2$H$_2$O were carefully administered to each woman, with the exact dose consumed calculated by difference in the weight of the dose cup and its cap before and after dosing. Following dosing, 75 ml of water was provided as a rinse to ensure that the total dose was swallowed. Follow-up saliva samples were collected 3 h after dose administration. During the 3 h equilibration period, women were not allowed to consume food or liquid and were requested to allow us to take their infants’ weight before and after breast-feeding and measure the volume of any urine voided to account for any loss of body fluids.

During the isotope equilibration period, MF-BIA was measured in all women, while SF-BIA was also measured in a subset of women at the same visit to allow us to apply prediction equations to resistance data already collected in the field. When both MF- and SF-measurements were taken, they were done so one after the other so that the study conditions were identical. Using the MF-BIA instrument and 2 × 7 cm electrodes recommended by the manufacturer, impedance ($Z$) was recorded at 5, 50, 100 and 200 kHz. Resistance and reactance in Ohms ($\Omega$) at 50 kHz were assessed for SF-BIA using 1 × 4 cm electrodes provided by the manufacturer. Analysers were tested in the morning on each day of use with standard resistors at 500 $\Omega$ supplied by the manufacturers, with acceptable limits of 496–503 $\Omega$ for the MF-BIA unit and 495–505 $\Omega$ for the SF-BIA unit. Only resistance ($R_{50}$) from SF-BIA and impedance at 50 kHz ($Z_{50}$) from MF-BIA were used for developing body composition prediction equations.

Cotton cylinders saturated with saliva were centrifuged for 5 min at 6000rpm to precipitate saliva, which was kept at −20°C until analysis. Baseline and post-equilibration saliva samples, along with an aliquot of $^2$H$_2$O from each of two bottles of $^2$H$_2$O stock from which the doses were prepared, were transported to Saint John’s Research Institute, Bangalore, India for the analysis of $^3$H enrichment by Zn reduction followed by dual-inlet MS (Europa Scientific) (21). Each sample was analysed in duplicate and the mean was used for analysis. Repeated analysis for natural background samples gave a CV of 0·02%.

**Body composition calculations**

TBW (kg) was calculated using data on dose enrichment and weight and the change in the enrichment of $^3$H in maternal saliva over the equilibration period, using the molar weight of $^2$H$_2$O of 20·0274 g/mol and of pure water of 18·0153 g/mol (22), and accounting for 4% hydrogen exchange (21). After determining TBW either via $^2$H$_2$O dilution or from BIA prediction equations, FFM (kg) was determined assuming hydration of FFM was 0·732 (23,24). Fat mass (kg) was determined as the difference between total body weight (kg) and FFM (kg). All these body composition components were also expressed as a percentage of total body weight.

**Statistical analysis**

Differences in subject characteristics between the women selected for body composition assessment and those of the larger population of women with a living infant from a singleton birth, complete data for weight, height and resistance, and a valid resistance measure of $R_{50}$ $>400\Omega$ were examined by $t$ test or $\chi^2$ analysis, with differences considered significant at $P<0·05$. Within the body composition group, differences in women who had only impedance measured $v$. those with both impedance and resistance were also examined by $t$ test. Comparability of impedance and resistance among women with both measurements was examined by correlation analysis and paired $t$ test.

We used TBW (kg) derived from $^2$H$_2$O dilution as the dependent variable to develop regression equations to predict TBW from resistance or impedance using forward stepwise regression, with height$^2/Z_{50}$ or height$^2/R_{50}$, weight and age as predictor variables. Other anthropometric variables were also initially examined. For comparing the models, we sought the highest adjusted $R^2$ values, and lowest root mean square error (RMSE), a measure of precision (25). The precision and robustness of the prediction equations were also assessed by calculating the PRESS statistic as part of an internal cross-validation strategy (25,26). The PRESS statistic is calculated as the square root of the sum of the squared prediction residuals divided by the total number of observations, with a lower PRESS statistic indicative of better fit of the prediction equation. The prediction residuals were obtained by fitting the regression equations with actual TBW as the dependent variable and height$^2/Z_{50}$ (or $R_{50}$) and weight as independent variables for all but one observation sequentially, obtaining the predicted value of the excluded observation, and calculating the prediction residual as the observed – predicted value for TBW (25,26). Scatterplots of predicted $v$. actual measures of TBW and the predicted residuals against predicted TBW were generated.

The equation for TBW from resistance measures was applied to the larger population from whom the body composition study women were derived, and body composition data were explored in relation to age and BMI category by ANOVA.

Statistical analyses were performed using STATA version 10 (Stata Corporation), although the PRESS residuals and statistic were obtained using the PROC REG procedure with PRESS Statistics in SAS version 9.2 (SAS Institute, Inc.).

**Results**

A total of 147 women successfully completed the body composition assessments, of which eighty-two had both resistance and impedance measures taken. Data for one woman had been dropped due to implausibly high TBW assessed by $^2$H$_2$O dilution. The larger sample of women in the main trial sub study ($n$ 1020) was similar to the women in the body composition study in that they also had a living infant and complete and valid data for weight, height and resistance at the time of the 3-month postpartum home visit. Data from the 3-month postpartum home visit comparing the women in
the $^2$H$_2$O dilution study and the larger sample are shown in Table 1. Women participating in the body composition study were significantly younger, weighed less, had lower skinfold and mid-upper arm circumference measures and had higher resistance values at 3 months postpartum. Additionally, body composition study participants were more likely to be primiparous (66 vs. 45 %; P<0·0001). Despite these differences, both groups revealed women of reproductive age with high rates of underweight and short stature.

The subgroup of eighty-two women in the body composition study who had the resistance measure was similar in most characteristics to the larger group of 147 (Table 2), although they had somewhat higher impedance values than the sixty-five women who had data for impedance values alone (P=0·01). Median length of time between the home visit and the body composition assessment was 1·9 weeks among all women.

**Table 1. Characteristics of postpartum women participating in the body composition study at the time of enrolment in early pregnancy and their comparability to the larger group of women** (Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Body composition study (n = 147)</th>
<th>Non-body composition study (n = 1020)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>Time postpartum (weeks)</td>
<td>13·3</td>
<td>2·0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20·8</td>
<td>5·3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>41·7</td>
<td>6·1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>148·4</td>
<td>5·1</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>18·9</td>
<td>2·1</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>22·2</td>
<td>2·0</td>
</tr>
<tr>
<td>Triceps skinfold (mm)</td>
<td>9·7</td>
<td>3·3</td>
</tr>
<tr>
<td>Subscapular skinfold (mm)</td>
<td>12·2</td>
<td>4·4</td>
</tr>
<tr>
<td>$R_50$ (Ω)</td>
<td>729</td>
<td>71</td>
</tr>
</tbody>
</table>

MUAC, mid-upper arm circumference; $R_50$, resistance at 50 kHz.

The variance in TBW when it alone was in the models (not shown) was minimal (−14·3 (SD 12·7) for impedance-resistance; 0·57 (SD 0·49) when expressed as the denominator of height$^2$) relative to a range of 580–998Ω for $Z_{50}$ and $R_{50}$ and 20–44 cm$^2$/Ω for height$^2$/Z$_{50}$ and height$^2$/R$_{50}$, respectively, observed in these women. Thus, coefficients for the prediction equation for TBW were similar whether using impedance or resistance. Regression parameters for the stepwise prediction equation development process are shown in Table 3 for impedance and resistance measures, as variables were added into the prediction equations. Age did not contribute significantly in the prediction of TBW and was therefore ultimately excluded from both models. The final equations were

\[
\text{Impedance based: } TBW = 4·573 + 0·177 \times W^2 + 0·351 \times Ht^2/Z_{50},
\]

\[
\text{Resistance based: } TBW = 4·297 + 0·190 \times W^2 + 0·349 \times Ht^2/R_{50},
\]

where TBW is in kg; Ht, height in cm; W, weight in kg. The total variance in TBW that was explained by the prediction models was 76 and 78% using impedance and resistance, respectively. Body weight explained 65 and 68% of the variance in TBW when it alone was in the models (not shown) and weight was highly correlated with $Ht^2/Z_{50}$ ($r$ 0·76) and $Ht^2/R_{50}$ ($r$ 0·77), both P<0·0001. The RMSE for TBW for the final equations were 1·34 and 1·30 kg, respectively (Table 3).

**Application of bioelectrical impedance analysis equations**

Mean TBW, FFM and fat mass obtained using the final developed equations and those obtained by $^2$H$_2$O dilution are shown in Table 4. Predicted TBW using the equation for $Z_{50}$ was 0·02 (SD 1·33) kg higher than actual TBW, and predicted TBW using $R_{50}$ differed by 0·00 (SD 1·28) kg from actual TBW, both near zero and neither different by paired t test.

**Table 2. Anthropometric and bioelectrical impedance characteristics of all women in the body composition study, and in the subgroup in whom resistance was measured** (Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Body composition study participants (n = 147)</th>
<th>Resistance measurement subgroup (n = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>Time postpartum (weeks)</td>
<td>19·7</td>
<td>9·3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>41·7</td>
<td>6·1</td>
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<td>Height (cm)</td>
<td>148·7</td>
<td>5·2</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>18·5</td>
<td>2·1</td>
</tr>
<tr>
<td>$Z_{50}$ (Ω)</td>
<td>729</td>
<td>75</td>
</tr>
<tr>
<td>Height$^2$/Z$_{50}$ (cm$^2$/Ω)</td>
<td>30·7</td>
<td>4·1</td>
</tr>
<tr>
<td>$R_{50}$ (Ω)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Height$^2$/R$_{50}$ (cm$^2$/Ω)</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

$Z_{50}$, impedance at 50 kHz; $R_{50}$, resistance at 50 kHz.
This gives a 95 % limit of agreement (mean ± 2SD) of −2.64 to 2.64 for predicted relative to actual TBW by impedance and −2.56 to 2.56 kg for TBW for resistance; that is, for 95 % of women with a resistance measure, predicted measures were within 2.56 kg of actual measures of TBW. Among just the eighty-two women for whom both resistance and impedance were measured, predicted TBW using the equation for Z50 was 0.01 (SD 1.28) kg lower than actual TBW, with a 95 % limit of agreement (mean difference of −0.01 (SD 0.17) kg, P = 0.71).

The correlation coefficient in TBW determined via 2H2O dilution and BIA using Z50 was 0.87 (P < 0.001). There was some over-prediction of TBW, and thus consistent with a slight over-prediction of TBW, and thus derived prediction equation in some women compared to their actual TBW. The PRESS statistic (square root of the sum of squared prediction residuals divided by n) for impedance was 1.35 kg, while that for resistance was 1.33 kg.

Body composition parameters in the wider population using the prediction equation are shown in Table 5. Values across the entire population were similar to those observed among the body composition study participants. One woman whose resistance value was 418 Ω, near the cutoff for exclusion as an outlier, had a negative value for fat mass, consistent with a slight over-prediction of TBW, and thus FFM, and subsequent under-prediction of fat mass at the low extreme of the available data.

Associations of body composition measures with age and BMI are also explored in Table 5. Measures of height2/resistance at 50 kHz (ZR50) were lowest in the youngest women (P < 0.01 for ANOVA), while body fat was highest in 20–30-year-old women whether expressed as mass or percentage of total body weight. While TBW (kg), FFM (kg) and fat mass (kg) increased with increasing BMI, the percentage of body fat relative to total body weight increased with increasing BMI while the percentage of body FFM declined.

Table 3. Equation development for predicting total body water from height2/impedance at 50 kHz (Z50, n 147) or height2/resistance at 50 kHz (ZR50, n 82): regression parameters

<table>
<thead>
<tr>
<th>(β Coefficients and 95 % confidence intervals)</th>
<th>Height2/Z50 (cm²/Ω)</th>
<th>Weight (kg)</th>
<th>Age (years)</th>
<th>Intercept (kg)</th>
<th>β</th>
<th>95 % CI</th>
<th>β</th>
<th>95 % CI</th>
<th>Adj R²</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>0.551</td>
<td>0.492, 0.611</td>
<td>–</td>
<td>–</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
<td>95 % CI</td>
<td>Adj R²</td>
<td>RMSE</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.351</td>
<td>0.269, 0.433</td>
<td>0.177</td>
<td>–</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
<td>95 % CI</td>
<td>Adj R²</td>
<td>RMSE</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.356</td>
<td>0.274, 0.437</td>
<td>0.180</td>
<td>0.125, 0.236</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
<td>95 % CI</td>
<td>Adj R²</td>
<td>RMSE</td>
</tr>
</tbody>
</table>

Height2/R50 (cm²/Ω) | Weight (kg) | Age (years) | Intercept (kg) | β | 95 % CI | β | 95 % CI |
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>0.566</td>
<td>0.487, 0.645</td>
<td>–</td>
<td>–</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.349</td>
<td>0.240, 0.458</td>
<td>0.190</td>
<td>0.117, 0.263</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.351</td>
<td>0.241, 0.461</td>
<td>0.191</td>
<td>0.117, 0.266</td>
<td>β</td>
<td>95 % CI</td>
<td>β</td>
</tr>
</tbody>
</table>

Table 4. Comparability of body composition measures using 2H2O dilution and newly derived prediction equations based on impedance or resistance

<table>
<thead>
<tr>
<th>(Mean values and standard deviations)</th>
<th>Body composition study participants (n 147)</th>
<th>Resistance measurement subgroup (n 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2H2O dilution</td>
<td>Height2/Z50 prediction</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Total body water (kg)</td>
<td>22.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Total body water (%)</td>
<td>55.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>30.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>75.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>10.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>24.3</td>
<td>6.4</td>
</tr>
</tbody>
</table>

ZR50, impedance at 50 kHz; R50, resistance at 50 kHz.
The purpose of the present study was to develop prediction equations for body composition in rural South Asian women using bioelectrical properties of impedance and resistance to predict TBW assessed using $^{2}$H$_2$O dilution. We selected post-partum women from a large, community-based trial designed to examine pregnancy outcomes in whom to derive these equations. Body composition measures in these women will allow us to more fully explore the facets of nutritional status associated with pregnancy outcomes. Moreover, women in this study were typical of women of reproductive age in the area, who are generally undernourished as determined by weight, height, skinfolds and arm circumference. Thus, we expect these prediction equations to be broadly applicable for describing distributions of body composition parameters among women of reproductive age in the region.

We previously demonstrated the unique bioelectrical characteristics of this population, with the distribution of resistance shifted to higher values than those of women reported elsewhere, and we speculated that this shift was explained by lower body volumes of these undernourished women, estimated from their low heights and weights(17).

Among women in the body composition study, impedance and resistance were strongly correlated and had a similar range of values, but on average somewhat lower ($14 \Omega$) values were observed for impedance. The close association of these measures was expected, as impedance is related to resistance and reactance by phase angle (11) but comprised primarily (approximately 95%) of resistance (27). The somewhat lower mean values for impedance compared to resistance observed in this study were probably attributable to the differences in equipment and electrodes used. Nonetheless, while distinct prediction equations were generated for resistance and impedance, the 95% CI around the $\beta$ coefficients for each variable were overlapping, in agreement with the overall similarity of resistance and impedance across this group of women.

Both prediction equations provided excellent agreement with direct measures of TBW, and estimated TBW and its variance were nearly identical when compared between the eighty-two women in whom equations for impedance and resistance could both be applied. Moreover, both equations demonstrated a high $R^2$ and low RMSE as measures of fit and relatively low PRESS statistics in the cross-validation approach. Although there are no specific criteria against
which to evaluate these statistics(25), Sun et al.(26) reported an
$R^2$ of 0·79, but a RMSE and PRESS statistic of 2·6 litres, and
range of PRESS residuals of about ± 9 litres in women in the
US population in whom prediction equations were generated,
although greater homogeneity in the US than Bangladeshi
population may have contributed to higher RMSE and PRESS
statistics in the US compared to Bangladeshi setting. Individual
variability in the ability of the new equations to predict TBW
was consistent across the TBW distribution of this population.
Although bias in the measures for TBW was low using the new
prediction equations, BIA somewhat overestimated actual
TBW at the low end and underestimated TBW at the high
end of its distribution, explaining lower standard deviations
for distributions of body composition measures when assessed
by BIA compared to $^2$H$_2$O dilution. Thus, despite favourable
performance characteristics of these equations overall, users
must be mindful of the degree of error surrounding the use
of the equations to predict TBW of individuals.

Nonetheless, we demonstrated the utility of these equations
for women in this population-based study, in whom body
composition information is of great importance to enrich our
knowledge of nutritional status. When the prediction equation
using resistance was applied to the larger group of postpartum
women, FFM comprised approximately 76% and fat mass 24%
of body weight, similar to the body composition group despite
some differences in age and anthropometry between the
women selected and those not selected for the body compo-
sition study. Among the larger group of women, the highest
fat mass and percentage body fat occurred among 20–30-
year-old women. A tendency towards the highest fat mass in
the 20–30-year-old age group is consistent with baseline
characteristics that showed greater mean age and higher
weight, skinfolds and arm circumference among the women
who did not participate in the body composition study com-
pared to typically younger women who did. The lowest total
FFM was observed among the youngest study participants,
consistent with a hypothesis that these youngest women
may have still been acquiring lean body mass at the time
they became pregnant(17). A previous study of married adoles-
cents in this population using triceps and subscapular skin-
folds to derive percentage body fat showed that body fat
was approximately 19% of body weight, declining by 1·4%
at 6 months postpartum among young women who became
pregnant(26). Other studies of body composition in women
of reproductive age have shown percentage body fat to aver-
age over 30% among women of Asian descent(12,15,29,30),
although mean BMI in those studies typically averaged over
22 kg/m$^2$. One study in lactating women from India showed
persistently low body fat across lactation (6-month intervals
to 18 months postpartum) of approximately 28% body fat(31).
It is clear that women in the area of Bangladesh
under study here have considerably lower fat mass than
women of reproductive age elsewhere worldwide.

Regardless of age, data relating body composition com-
ponents to BMI show that TBW, FFM and fat mass all increase

![Fig. 4. Plot of prediction residuals (observed total body water (TBW) –
predicted TBW, where predicted TBW is derived from a regression equation
that excludes each observation once) v. corresponding predicted TBW (kg)
for the prediction equation using resistance at 50 kHz ($R_{50}$).]

Table 5. Application of the height$^2$/resistance at 50 kHz ($R_{50}$) prediction equation for body composition to the postpartum women not selected for the
body composition study

<table>
<thead>
<tr>
<th>Age (years)*</th>
<th>BMI (kg/m$^2$)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n...</td>
<td>&lt;18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>n...</th>
<th>&lt;20</th>
<th>20–30</th>
<th>&gt;30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Height$^2$/R$_{50}$</td>
<td>32·5</td>
<td>4·6</td>
<td>31·7</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>23·8</td>
<td>2·4</td>
<td>23·3</td>
</tr>
<tr>
<td>TBW (kg)</td>
<td>55·8</td>
<td>3·6</td>
<td>56·0</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>32·5</td>
<td>3·3</td>
<td>31·8</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>76·3</td>
<td>4·9</td>
<td>76·5</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>10·3</td>
<td>3·2</td>
<td>9·9</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>23·7</td>
<td>4·9</td>
<td>23·5</td>
</tr>
</tbody>
</table>

TBW, total body water; FFM, fat-free mass.

* Age group differences all significant by ANOVA, $P<0·0001$, except TBW (%) and FFM (%), $P=0·008$; for fat (kg), $P=0·0006$; for multiple comparison tests of height$^2$/R$_{50}$, TBW (kg), FFM (kg), women <20 differ from women 20–30 and >30 years; for TBW (%), FFM (%), fat (%), women 20–30 differ from women >30 years; for fat (kg), women 20–30 differ from women <20 and >30 years, $P<0·05$.† BMI group differences all significant by ANOVA ($P<0·0001$).
with increasing BMI. However, when expressed as a percentage of total body weight, only percentage body fat increased with increasing BMI. These findings demonstrate that BMI reflects body fat across the range of BMI observed in this study, extending findings in other studies that relate body fat to BMI to the low end of the BMI distribution\(^{32–34}\).

For our calculations, we assumed a hydration constant for FFM of 0.732 among all women. The accuracy of estimates of FFM and fat mass depend on the accuracy of that constant, which in reality may vary from 0.70 to 0.76, and may differ by site. The hydration of FFM and fat mass depend on the accuracy of that constant, K. J. S. engaged in study design, analysis and manuscript preparation; A. K. completed the \(^{2}\)H\(_{2}\)O assessments; H. A. oversaw field operations, subject recruitment and participation; A. A. S. and M. R. provided scientific and administrative oversight of the main trial and its intersection with this study; S. M. assisted with manuscript preparation and study organisation and management; L. S.-F. W. managed all trial data and supported the data analysis; A. B. L., as project scientist for the main trial, coordinated activities with IAEA and within Bangladesh and oversaw the study design; P. C. and K. P. W. were the principal investigators for the main trial and provided guidance throughout the research process. None of the authors has conflicts of interest to declare. The contributions of the JiViTA field and data management teams, Johns Hopkins collaborators, and the staff at Saint Johns Research Institute are gratefully acknowledged.

### References


