Comparison of isotope dilution with bioimpedance spectroscopy and anthropometry for assessment of body composition in asymptomatic HIV-infected and HIV-uninfected breastfeeding mothers\textsuperscript{1–3}

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ABSTRACT

Background: The effect of breastfeeding on the nutrition of HIV-infected (HIV+) mothers is unknown. Simple, valid methods are needed for body-composition assessment of HIV+ women.

Objective: We compared the ability of bioimpedance spectroscopy (BIS) and anthropometry with that of isotope dilution ($^2$H$_2$O) to measure fat-free mass (FFM) and fat mass (FM) in HIV+ and HIV-uninfected (HIV−) breastfeeding South African mothers.

Design: Total body water (TBW) content of 68 lactating mothers (20 HIV+, 48 HIV−) was measured 10 wk after delivery by using BIS and $^2$H$_2$O to measure FFM and FM. Anthropometric measurements included body mass index (BMI; in kg/m$^2$), midupper arm circumference (MUAC), and 4 skinfold thicknesses.

Results: TBW, FFM, and FM measurements determined by BIS were correlated with $^2$H$_2$O measurements in HIV+ ($r = 0.664, 0.621$, and 0.872, respectively; $P < 0.01$) and HIV− ($r = 0.876, 0.868,$ and 0.932, respectively; $P < 0.001$) mothers. TBW measured by BIS was greater than that measured by the $^2$H$_2$O method in both HIV+ (1.8 L) and HIV− (1.5 L) women; FM or FFM did not differ significantly by method. BMI, MUAC, and all skinfold-thickness measurements correlated strongly ($r > 0.62, P < 0.001$) with FM measured by $^2$H$_2$O in both groups. BMI and MUAC correlated ($r > 0.64, P < 0.001$) with FFM in HIV− mothers but not in HIV+ mothers.

Conclusions: In HIV+ and HIV− breastfeeding mothers, BIS provides an estimate of body composition comparable to that obtained with the $^2$H$_2$O method. BMI and MUAC are useful in predicting FM in both groups but are not valid measures of FFM in HIV+ mothers.


KEY WORDS HIV, breastfeeding, body composition, South Africa, fat mass, fat-free mass, anthropometry, isotope dilution, women, AIDS, lactation, bioimpedance spectroscopy

INTRODUCTION

Despite concerns about the risk of transmitting HIV to infants via breastfeeding (1) and the unknown effect of breastfeeding on the health and nutrition of HIV-infected [HIV-positive (HIV+)] mothers (2), the use of breast milk replacements is often considered unacceptable, unaffordable, or unsafe (3–6). Because it is likely that breastfeeding will remain the norm among HIV+ mothers in most of sub-Saharan Africa, it is important to determine whether breastfeeding may be detrimental to their nutrition and health. To assess nutritional status in these women, valid, inexpensive, and easily utilized field methods of measuring body composition are needed.

The stable isotope deuterium oxide ($^2$H$_2$O) is a reference technique for measuring total body water (TBW). After the ingestion and equilibration of a known dose of $^2$H$_2$O in the body’s water compartments, $^2$H$_2$O concentration serves as a marker for TBW from which fat-free mass (FFM) and fat mass (FM) are derived. This technique is a safe and well-established standard method for assessing body water compartments even during pregnancy (7), but it is cumbersome to use in the field. Thus, validation of a simpler method is desirable.

Bioelectrical impedance analysis (BIA), using a single-frequency measurement of resistance and reactance, has been shown to provide reliable estimates of FFM and FM in adults (8–10). It is an easy, reliable, and portable technique for measurement of body composition that is applicable for fieldwork and is less expensive than the $^2$H$_2$O method. However, chronic
illness and infection can cause shifts in body water compartments (11, 12) thereby introducing error in the measurement of body cell mass and making the use of single-frequency BIA in infected populations potentially problematic. To overcome this concern, multiple-frequency bioimpedance, known as bioimpedance spectroscopy (BIS), is an alternative to BIA. TBW and extracellular water (ECW) measurements made by using BIS (BIS) may be more accurate in populations in which there are potential alterations in fluid distribution, as seen with HIV infection and during lactation (13, 14).

In healthy HIV-uninfected [HIV-negative (HIV−)] pregnant and lactating women in the United States, measurements of TBW BIS correlated well with measurements of TBW made by using isotope dilution (2H2O) (15). Multifrequency BIA has also been used to monitor changes in body water compartments during pregnancy (16). It is recommended that the validity of impedance measures be confirmed as applicable for specific populations (10), and thus it is necessary to validate this method in HIV+ breastfeeding African mothers.

The primary objective of this study was to determine the validity of BIS and anthropometric measurements to measure body composition in HIV+ and HIV− women as compared with the values obtained with the reference stable isotope dilution method. The second objective was to describe the body composition of HIV+ lactating women in rural South Africa.

SUBJECTS AND METHODS

Subjects

This study was conducted in government health clinics in the field area of the Africa Centre for Health and Population Studies in a rural, northern part of KwaZulu Natal Province, South Africa. Mothers were identified for the study when they came to clinic for their infants’ immunizations. Inclusion criteria were delivery 4 to 20 wk previously, maternal age ≥15 y, current breastfeeding of the infant, and arrival at clinic by 0900 to allow sufficient time for the full range of measurements. Exclusion criteria were current acute illness that would influence hydration status (eg, diarrhea or fever) and admission to the hospital in the previous 2 wk.

Mothers who were eligible and who agreed to participate provided written informed consent and agreed to HIV counseling and testing. HIV testing was anonymous, and mothers returned in 2 wk to obtain their results from the HIV counselor. Thus, at the time of body-composition measurement, HIV status was not known by subjects or study staff. Mothers were enrolled from November 2001 to April 2002, and all measurements were made on the day of enrollment.

The Ethics Committee of the Nelson R Mandela School of Medicine at the University of KwaZulu Natal, Durban, South Africa, and the Human Subjects Committee of the University of California, Davis, Davis, CA, approved the study.

Measurements

Isotope dilution method

Ideally, the isotope dilution method is conducted in a controlled environment, but that was not possible in this rural setting. Most of the women attending the clinic walked, from varied distances, to the clinic while carrying their infant. To ensure adequate and consistent hydration status, all women were encouraged to drink water ad libitum during the 30 min before the 2H2O administration. The women then provided a baseline urine specimen, after which they drank 20 g 2H2O mixed in 100 mL water. The women were asked to void 2 h after consuming the 2H2O dose; this urine was discarded, because the isotope is not equilibrated in the 2-h sample. Three hours after the 2H2O dose, a postequilibrium urine specimen was collected for measurement of the 2H2O concentration. A second urine collection 4 h after the dose was not possible in this setting, but, in other studies that we have conducted in pregnant and postpartum women (15) and in AIDS patients receiving anabolic therapy (17), we found that 3-h postdose samples were similar to 4-h postdose samples. During the 3-h equilibration period, each subject drank 500 mL bottled water but did not consume any other foods or fluids. Urine specimens were stored in screw-top containers with additional wrapping to prevent evaporation and leakage and maintained at −70 °C until they were processed at the Western Human Nutrition Research Center in Davis, CA. Urine was processed by vacuum sublimation to obtain a pure water sample. The 2H2O concentration was measured by fixed-filter infrared spectrometry. The concentration of 2H2O in urine was calculated with the following equation:

\[
\text{2H}_2\text{O enrichment} = \frac{3\text{-h 2H}_2\text{O} - \text{baseline 2H}_2\text{O}}{0.5 \text{ L} (900)}
\]

That concentration was used to calculate the TBW by using the following dilution formula:

\[
\text{TBW} = \frac{2\text{H}_2\text{O} \times 0.96 \times 0.944}{900 \times 0.96 \times 0.944 - 0.5 \text{ L}}
\]

Bioimpedance spectroscopy

BIS uses multiple measurements of resistance and reactance through a range of frequencies (5–1000 kHz) and is analyzed as a complex impedance plane from which the resistance of both TBW and ECW can be ascertained (20, 21). Tissues containing water and electrolytes are more conductive than are bone and fat, and the volume of conductive tissue can be estimated from its resistance. BIS measures resistance at low and high frequencies and provides estimates of intracellular water (ICW) and ECW. The BIS 4000 ANALYZER software (version 1.00d; Xitron Technologies, San Diego, CA) estimated the volume of ECW and ICW by measuring resistance and reactance at 25 frequency measurements between 5 and 1000 KHz. The spectra data were fitted to the Cole-Cole suspension model by using nonlinear curve fitting. ECW and ICW were calculated by the software from each subject’s resistance with the use of equations based on the Hanai mixture theory (22). More thorough theoretical discussion of this method can be found in the report by De Lorenzo et al (23). The software’s proprietary, iterative, successive approximation technique was used to obtain the best possible fit to the standard cell-suspension model. The programmed resistance
coefficients provided by the manufacturer (ie, 206 for ECW, 797 for ICW) were used. The BIS instrument was calibrated each morning before use. BIS measurements were taken ≈2.5 h after consumption of the isotope drink, immediately after the mother emptied her bladder, and as soon as possible after breastfeeding. All BIS measurements were taken ≥5 h after the last meal was consumed. The impedance electrode pads were placed on the dorsal surface of the left hand and foot at the distal metacarpals and metatarsals, and the sensing electrodes were placed between the distal prominence of the radius and ulna and the lateral and medial malleoli of the ankle.

Anthropometric measurements

Height, weight, midupper arm circumference (MUAC), and skinfold-thickness measurements were obtained by 1 of 2 registered dietitians who were crosstrained to ensure low interexaminer variation. Maternal height without shoes was measured to the nearest 0.1 cm by using a stadiometer (Scales 2000, Durban, South Africa), and weight without shoes and in light clothing was measured to the nearest 100 g by using a frequently standardized electronic digital scale (Scales 2000). MUAC was measured with a fibreglass tape, and skinfold thickness measurements were taken with Harpenden calipers (Harpenden, Trowbridge, United Kingdom) according to standardized methods at 4 body sites: triceps, biceps, subscapular, and suprailiac. Two independent measurements were taken at each body site on the left side, and if the difference between the 2 measurements was >3 mm, a third measurement was obtained. The mean measurements of each site were used in the analysis. If 3 measurements were taken, the mean of the closest pair was used. BMI was calculated as body weight (kg)/height (m²). The sum of the skinfold-thicknesses measurements was calculated as the sum of the measurements taken at all 4 body sites. Midupper arm muscle circumference was calculated as MUAC (cm) – [triceps skinfold thickness (mm) × 0.314]. The age-appropriate Durnin-Womersley equation for females was used to estimate body composition for all mothers from skinfold-thickness measurements (24, 25).

HIV viral load and CD4 cell count

Fresh venous blood specimens were processed at the Africa Centre virology laboratory. CD4+ cell counts from venous blood were measured within 24 h by using a FACScan (Becton Dickinson, Temse, Belgium) and a 3-color antibody cocktail of CD3/CD4/CD8. Serologic HIV testing was performed with a broad-based HIV-1/HIV-2 whole lysate screening enzyme-linked immunosorbent assay (ELISA) (Vironostica; Organon Teknika, Boxtel, Netherlands) to determine HIV status; this was followed by a confirmatory multiple-peptide ELISA (Murex Wellcozyme HIV 1 + 2 GAC ELISA; Murex Corporation, Dardford, United Kingdom) for all serologic test–positive mothers. Total HIV RNA was isolated from plasma by using guanidinium-silica methods (NucliSens Isolation Kit; Organon Teknika) and an automated extractor (Organon Teknika). The NucliSens HIV-1 QT assay has a quantitative range from 40 to >500,000 copies/mL plasma.

Statistical analysis

For demographic and body-composition variables, differences between HIV+ and HIV− mothers were tested for significance by using the chi-square test for categorical variables and the Student’s t test for continuous variables. Before examining the relations between different measures of body composition, we examined each of the individual values to exclude extreme outliers. With respect to the TBW measurement from any of the mothers, if the difference between the 2H2O and BIS results was >3 SDs, that value was considered to be an outlier and was removed from further analysis.

Pearson product-moment correlations and paired t tests were used to measure the bias, significance, and degree of correlation between 2H2O and BIS measurements of TBW, FFM, and FM in HIV+ and HIV− mothers. Repeated-measures analysis of variance (ANOVA) was used to ascertain whether differences between groups (HIV+ and HIV−) and assessment methods (2H2O and BIS) were significant and whether the 2-factor group × method interaction for all body-composition variables was significant. Agreement between results of the 2 methods in HIV+ and HIV− mothers was evaluated by using the Bland-Altman model (26), in which the difference between the measurements obtained with the 2H2O and BIS methods for each woman was plotted against the mean of the methods.

Pearson product-moment correlations were used to determine the correlation between the 2H2O dilution measurement of FFM and FM (FFM2H2O and FM2H2O, respectively) and anthropometric measurements in HIV+ and HIV− mothers. To develop a regression equation to predict FFM2H2O and FM2H2O from anthropometric measurements, measurements from 60% of both the HIV+ and HIV− mothers were randomly selected for use. All anthropometric measurements for these mothers were entered stepwise to determine the best equation, which was then cross-validated by using measurements from the remaining 40% of mothers. These results were then compared with FFM2H2O and FM2H2O by using regression and paired t tests. Because there was good agreement between the cross-validation groups when the anthropometric measurements of all mothers were used, we used linear regression models to develop equations to predict FFM2H2O and FM2H2O for HIV+ and HIV− mothers separately. Height, weight, MUAC, and each skinfold-thickness measurement were the independent variables. All analyses were performed with SPSS software (version 11.0; SPSS Inc, Chicago, IL).

RESULTS

Sixty-eight mothers were enrolled in the study, 20 of whom were HIV+. Four of the 48 HIV− mothers were excluded from analysis because of implausible BIS measurement (negative values) due to improper electrode placement (ie, the electrode placements were reversed). Two HIV+ mothers were excluded because of incomplete isotope dilution procedures (one could not drink the full solution, and the other could not urinate at the 3-h time point), and one HIV+ mother was removed as an outlier (2H2O method indicated 3.4 kg FM, which is incompatible with human physiology).

Study participants ranged in age from 15 to 40 y (median: 24 y), had 1–8 previous pregnancies (median 2), and had delivered between 4 and 19 wk previously (median: 10.1 wk). Less than half of the women had access to piped water or electricity in their homes. HIV+ mothers had more formal education than did HIV− mothers (8.5 ± 3.4 and 6.1 ± 3.0 y, respectively; P = 0.009), but otherwise the 2 groups were not significantly different (Table 1).
TABLE 1
Characteristics of study subjects by HIV status

<table>
<thead>
<tr>
<th></th>
<th>HIV-positive subjects</th>
<th>HIV-negative subjects</th>
<th>P&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.7 ± 3.9&lt;sup&gt;†&lt;/sup&gt;</td>
<td>23.6 ± 6.6</td>
<td>0.130</td>
</tr>
<tr>
<td>Previous pregnancies (n)</td>
<td>2.2 ± 1.4</td>
<td>2.2 ± 1.7</td>
<td>0.910</td>
</tr>
<tr>
<td>Gestation (wk)</td>
<td>38.4 ± 2.6</td>
<td>38.5 ± 2.8</td>
<td>0.830</td>
</tr>
<tr>
<td>Time after delivery (wk)</td>
<td>9.7 ± 3.1</td>
<td>10.5 ± 3.9</td>
<td>0.476</td>
</tr>
<tr>
<td>Education (y)</td>
<td>8.5 ± 3.3</td>
<td>6.1 ± 3.0</td>
<td>0.009</td>
</tr>
<tr>
<td>Uncomplicated vaginal delivery (%)</td>
<td>83</td>
<td>91 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>House built of cement block (%)</td>
<td>59</td>
<td>48 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>Water piped to yard or home (%)</td>
<td>41</td>
<td>3 7 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>Open-pit latrine or no toilet (%)</td>
<td>77</td>
<td>75 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Wood as primary cooking fuel (%)</td>
<td>50</td>
<td>50 ± 8.01</td>
<td></td>
</tr>
<tr>
<td>Paid employment (%)</td>
<td>18 ± 3.3</td>
<td>11 ± 6.0</td>
<td>0.800</td>
</tr>
</tbody>
</table>

<sup>1</sup> A t test was used to compare subject groups. Percentages were compared by chi-square test.
<sup>2</sup> ± SD (all such values).

The median viral load in the HIV+ mothers was 25 000 RNA copies/mL (log 4.4), and values varied from nondetectable to 170 000 copies/mL (log 5.23). The median CD4+ cell count was 631/μL (range: 179–1229), and just one subject had <200 cells/μL. Because the South African programs to prevent mother-to-child HIV transmission had not yet begun, none of the study participants had received antiretroviral drugs.

**Body composition**

Mean body-composition measurements made with the <sup>2</sup>H<sub>2</sub>O method did not differ significantly by HIV status (Table 2). Of the body-composition measurements made with the BIS method, only FFM<sub>BIS</sub> and ICW<sub>BIS</sub> differed significantly by HIV status.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>HIV-positive subjects</th>
<th>HIV-negative subjects</th>
<th>P&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>66.8 ± 9.2&lt;sup&gt;†&lt;/sup&gt;</td>
<td>62.9 ± 11.9</td>
<td>0.228</td>
</tr>
<tr>
<td>TBW&lt;sub&gt;2H2O&lt;/sub&gt; (L)</td>
<td>29.5 ± 3.1</td>
<td>27.7 ± 3.4</td>
<td>0.072</td>
</tr>
<tr>
<td>TBW&lt;sub&gt;BIS&lt;/sub&gt; (L)</td>
<td>31.2 ± 3.0</td>
<td>29.2 ± 3.9</td>
<td>0.056</td>
</tr>
<tr>
<td>FFM&lt;sub&gt;2H2O&lt;/sub&gt; (kg)</td>
<td>40.2 ± 4.3</td>
<td>37.8 ± 4.7</td>
<td>0.072</td>
</tr>
<tr>
<td>FFM&lt;sub&gt;BIS&lt;/sub&gt; (kg)</td>
<td>41.7 ± 4.2</td>
<td>38.8 ± 5.2</td>
<td>0.045</td>
</tr>
<tr>
<td>Percentage FFM&lt;sub&gt;2H2O&lt;/sub&gt; (%)</td>
<td>60.8 ± 7.0</td>
<td>61.0 ± 6.4</td>
<td>0.920</td>
</tr>
<tr>
<td>Percentage FFM&lt;sub&gt;BIS&lt;/sub&gt; (%)</td>
<td>62.9 ± 6.1</td>
<td>62.8 ± 5.9</td>
<td>0.742</td>
</tr>
<tr>
<td>FM&lt;sub&gt;2H2O&lt;/sub&gt; (kg)</td>
<td>26.6 ± 7.6</td>
<td>25.1 ± 8.7</td>
<td>0.532</td>
</tr>
<tr>
<td>FM&lt;sub&gt;BIS&lt;/sub&gt; (kg)</td>
<td>25.2 ± 6.8</td>
<td>23.9 ± 7.9</td>
<td>0.542</td>
</tr>
<tr>
<td>Percentage BF&lt;sub&gt;2H2O&lt;/sub&gt; (%)</td>
<td>39.2 ± 7.0</td>
<td>39.0 ± 6.4</td>
<td>0.920</td>
</tr>
<tr>
<td>Percentage BF&lt;sub&gt;BIS&lt;/sub&gt; (%)</td>
<td>37.2 ± 6.1</td>
<td>37.4 ± 5.6</td>
<td>0.862</td>
</tr>
<tr>
<td>ECW&lt;sub&gt;BIS&lt;/sub&gt; (L)</td>
<td>14.2 ± 1.4</td>
<td>13.7 ± 1.8</td>
<td>0.318</td>
</tr>
<tr>
<td>Percentage ECW&lt;sub&gt;BIS&lt;/sub&gt; (%)</td>
<td>45.5 ± 3.3</td>
<td>47.0 ± 2.3</td>
<td>0.099</td>
</tr>
<tr>
<td>ICW&lt;sub&gt;BIS&lt;/sub&gt; (L)</td>
<td>17.1 ± 2.2</td>
<td>15.5 ± 2.3</td>
<td>0.020</td>
</tr>
<tr>
<td>Percentage ICW&lt;sub&gt;BIS&lt;/sub&gt; (%)</td>
<td>54.5 ± 3.3</td>
<td>53.0 ± 2.3</td>
<td>0.100</td>
</tr>
</tbody>
</table>

<sup>1</sup> TBW, total body water; BIS, bioimpedance spectroscopy; FFM, fat-free mass; FM, fat mass; ECW, extracellular water; ICW, intracellular water; 2H2O<sub>-</sub> measurement by the <sup>2</sup>H<sub>2</sub>O method; BIS<sub>-</sub> measurement by the BIS method.
<sup>2</sup> A t test was used to compare subject groups.
<sup>3</sup> ± SD (all such values).

When FFM<sub>BIS</sub> and ICW<sub>BIS</sub> were calculated as a percentage of body weight, however, there was no difference by HIV status. As was found by using the <sup>2</sup>H<sub>2</sub>O method, HIV+ mothers had greater TBW<sub>BIS</sub>, a difference that was of marginal significance (P = 0.056). ECW<sub>BIS</sub> compartments for both HIV+ (weight: 21.3%; TBW: 45.5%) and HIV− (weight: 21.8%; TBW: 47.0%) mothers are within normal limits (27) and did not differ significantly by HIV status.

None of the anthropometric measurements differed significantly between groups (Table 3). Although the HIV+ mothers weighed slightly (3.8 kg) more than did the HIV− mothers, the difference was not significant. Four mothers (5.9% of HIV+ and 6.8% of HIV− mothers) had BMIs <19.5, and 5 mothers (11.8% of HIV+ and 6.8% of HIV− mothers) had BMIs >30.5 (P = 0.82).

### Comparison of methods

#### The BIS and <sup>2</sup>H<sub>2</sub>O methods

TBW, FFM, and FM measured with the BIS method were significantly correlated with those measured with the <sup>2</sup>H<sub>2</sub>O method in both HIV+ (r = 0.664, 0.621, and 0.872, respectively; P < 0.01 for each) and HIV− (r = 0.876, 0.868, and 0.932, respectively; P < 0.001 for each) mothers. The <sup>2</sup>H<sub>2</sub>O and BIS methods did not differ in their ability to measure FFM and FM in HIV+ and HIV− mothers (Figure 1), and there was no significant difference between the 2 groups in slope (P > 0.60) or intercept (P > 0.70). In contrast, TBW was significantly different by method (P < 0.001), but there was no significant group effect.

Compared with the <sup>2</sup>H<sub>2</sub>O method, the BIS method overestimated TBW by a mean of 1.8 L (P < 0.05) in HIV+ mothers and 1.5 L in HIV− mothers (P < 0.001). Although the difference was not significant, the BIS method tended to overestimate FFM by 1.4 kg in HIV+ and by 0.9 kg in HIV− mothers and to underestimate FM by 1.4 kg in HIV+ and by 1.2 kg in HIV− mothers (P > 0.53) (Table 4). These differences are shown in Bland–Altman plots (Figure 2). Also evident in these plots is the distribution of values both above and below the mean, which indicates a lack of systematic bias in methods for the 2 groups.
With the use of analysis of covariance to adjust for either weight or BMI, there was no significant effect of HIV status on FFM or FFMBIS results (data not shown). After adjustment for CD4 count or viral load in the HIV mothers only, there was no effect on FFM or FFMBIS results (data not shown).

Anthropometric measurements and the $^2$H$_2$O method

All individual skinfold-thickness measurements and their sum, BMI, MUAC, and midupper arm muscle circumference were significantly ($r > 0.62, P < 0.001$) correlated with FM in both HIV+ and HIV− mothers; BMI and MUAC had the highest correlation with FM. These same indicators were significantly ($r > 0.64, P < 0.001$) correlated with FFM only in HIV− mothers (Table 5). Compared with the $^2$H$_2$O method, the Durnin-Womersley equations significantly overestimated FFM ($P < 0.05$) and correspondingly underestimated FM significantly ($P < 0.05$) in both groups of mothers (Table 4).

As indicated in the description of statistical analyses, the initial equations used to predict FFM and FM from anthropometric equations were based on a subsample of 60% of the population and were cross-validated in the remaining 40%. The correlations between the results obtained with the $^2$H$_2$O method and those obtained with the newly developed equations for this population were strong and significant for FFM ($r = 0.915$) and FM ($r = 0.859$), which suggested that these equations were appropriate for use in this population. Therefore, we pooled the dataset to develop separate versions of a more robust equation for use in HIV+ and HIV− mothers (Table 6). The results from the new

![Figure 1: Regression between deuterium ($^2$H$_2$O) and bioimpedance spectroscopy (BIS) to measure fat-free mass (FFM) and fat mass (FM) by HIV status.](image)

| TABLE 4 | Correlations between body-composition measurements obtained by using the $^2$H$_2$O method, the bioimpedance spectroscopy (BIS) method, and equations derived from anthropometric measurements$^1$ |
|---------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| Method  | Body-composition measurements                               | Correlation with $^2$H$_2$O method                          | Bias$^2$                                                   | Precision$^3$                                                   |
|         | HIV+ subjects ($n = 17$)                                    | HIV− subjects ($n = 44$)                                    | HIV+ subjects                                             | HIV− subjects                                             | HIV+ subjects                                             | HIV− subjects                                             |
|         | TBW$_{2H2O}$                                              | 29.5 ± 3.1$^d$                                             | 27.7 ± 3.4                                                | −1.8                                                     | −1.5                                                     | 2.5                                                       | 1.9                                                       |
|         | TBW$_{BIS}$                                               | 31.2 ± 3.0                                                | 29.2 ± 3.9                                                | 0.664$^d$                                                | 0.876                                                    | −1.4                                                     | −0.9                                                     | 3.7                                                       | 2.2                                                       |
|         | FFM$_{2H2O}$                                              | 40.2 ± 4.3                                                | 37.9 ± 4.7                                                | 0.621$^d$                                                | 0.868                                                    | −5.0                                                     | −5.5                                                     | 3.2                                                       | 2.8                                                       |
|         | FFM$_{BIS}$                                               | 41.7 ± 4.2                                                | 38.8 ± 5.2                                                | 0.718                                                    | 0.862                                                    | −2.2                                                     | −2.6                                                     | 3.3                                                       | 1.7                                                       |
|         | FFM$_{Durnin}$                                            | 45.2 ± 4.2                                                | 43.4 ± 5.5                                                | 0.682                                                    | 0.857                                                    | −2.2                                                     | −2.6                                                     | 3.3                                                       | 1.7                                                       |
|         | FFM$_{Natal}$                                             | 40.9 ± 3.2                                                | 38.1 ± 4.5                                                | 0.682                                                    | 0.857                                                    | −2.2                                                     | −2.6                                                     | 3.3                                                       | 1.7                                                       |
|         | FM$_{2H2O}$                                               | 26.6 ± 7.6                                                | 25.1 ± 8.7                                                | 0.872                                                    | 0.932                                                    | 1.4                                                       | 1.2                                                       | 3.7                                                       | 3.2                                                       |
|         | FM$_{BIS}$                                                | 25.2 ± 6.8                                                | 23.9 ± 7.9                                                | 0.912                                                    | 0.965                                                    | 5.0                                                       | 5.5                                                       | 3.2                                                       | 2.8                                                       |
|         | FM$_{Durnin}$                                             | 21.6 ± 6.2                                                | 19.8 ± 6.8                                                | 0.935                                                    | 0.894                                                    | 0.0                                                       | −0.2                                                     | 2.7                                                       | 4.2                                                       |
|         | FM$_{Natal}$                                             | 25.9 ± 7.1                                                | 24.6 ± 8.7                                                | 0.935                                                    | 0.894                                                    | 0.0                                                       | −0.2                                                     | 2.7                                                       | 4.2                                                       |

$^1$ TBW, total body water; FFM, fat-free mass; FM, fat mass; $^2$H$_2$O; determined by using the $^2$H$_2$O method; BIS, determined by using the BIS method; Durnin, determined by using Durnin-Womersley skinfold-thickness, age-appropriate equations; Natal, results of the regression equation developed from full data set of HIV-positive and HIV-negative mothers as shown in Table 6. Paired $t$-tests were used for all comparisons.

$^2$ The mean value by which the reference method ($^2$H$_2$O) varies from the values obtained with the BIS method or anthropometric prediction equations.

$^3$ The SD of the difference from the $^2$H$_2$O method.

$^d$ ± SD (all such values).

$^c$ Correlation between these 2 groups, $P = 0.06$. 

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equations were well correlated with those obtained with the $^2$H$_2$O method, but the bias between methods (or the difference between the results from the anthropometric equation and from the reference standard) was much smaller than when the Durnin-Womersley equations were used.

**DISCUSSION**

We have shown that BIS provides a reliable estimate of body composition in HIV+ and HIV− breastfeeding mothers. Results obtained with the BIS method correlated well with those obtained with the $^2$H$_2$O method. There was no significant difference between methods in the measurement of FFM or FM. Although the TBW estimate obtained with BIS was larger than that obtained with the $^2$H$_2$O method in both HIV+ and HIV− mothers, the difference was fairly small (5–6%) and was considered acceptable. It is difficult to ascertain which of the methods is the correct estimate in this field setting.

**Comparison of methods**

*The BIS and $^2$H$_2$O methods*

Correlations between the BIS and $^2$H$_2$O methods were significant in both groups but stronger in the HIV− mothers. The difference between HIV+ and HIV− mothers in this study may
be due to differences in the women’s body composition, or they may be an artifact of the larger sample size and broader range of values in the HIV− group. Grinspoon et al (28) found that the correlation of FFM results obtained with dual-energy X-ray absorptiometry and BIA was greater (but not significantly so) in HIV− women than in HIV+ women. One explanation for this difference maybe that the quality of FFM in HIV+ persons is likely to be different from that in the HIV− persons who are usually studied for the development of body-composition reference values (ie, by the $^{2}\text{H}_2\text{O}$ method) (29). Although the HIV+ mothers in this study were asymptomatic and most were not immunocompromised, it is possible that some of these women may have had changes in body water compartments, and, thus, more time was needed for the $^{2}\text{H}_2\text{O}$ to equilibrate into TBW in their bodies.

The difference between the results from $^{2}\text{H}_2\text{O}$ and BIS methods to measure TBW in all mothers may be partially due to the less-than-full equilibration of the $^{2}\text{H}_2\text{O}$ at the 3-h time point for the postdose urine sample from lactating women. In previous studies of pregnant and postpartum women in our laboratory and of AIDS patients, we found that 3-h postdose samples were similar to 4-h postdose samples and that they provided reasonable estimates of TBW in a 4-compartment model. Therefore, we believed the 3-h postdose time point for a urine sample was appropriate. Each woman was encouraged to breastfeed before the TBW$_{\text{BIS}}$ measurement, but the larger effect may have been on the TBW$_{\text{2H2O}}$ estimate. It was not possible to repeatedly weigh the infant to adjust the TBW$_{\text{2H2O}}$ estimate for milk production on the basis of infant weight gain. The possible lack of full equilibration of $^{2}\text{H}_2\text{O}$ would result in TBW results that were lower than the BIS results, as was found in this study. In retrospect, because of lactation, a longer time may be needed for equilibration of isotopic tracers. Wong et al (30) suggested that an equilibration time of ≥6 h is needed in lactating women. Such a long equilibration time will also result in a greater nonaqueous $^{2}\text{H}_2\text{O}$ exchange, greater insensible losses through respiration and urine production, and the need to keep the lactating subject hydrated. Therefore, trade-offs between benefits and detriments must be made when designing study procedures in a field setting.

The reasonable intermethod difference (26) for each group increased our confidence that BIS accurately measured FFM and FM in both groups of mothers. The bias and precision between methods in our study were comparable to those found in other studies that compared methods of assessing body composition (29, 31).

Maintenance of FFM and FM is considered important for health and for the attenuation of HIV disease progression (11, 32). Accurate measurements of FFM and FM are therefore important in the clinical assessment of HIV+ persons. The $^{2}\text{H}_2\text{O}$ method is not possible for routine use in poor areas of Africa for methodologic and technical reasons. In contrast, BIS is simple to perform with adequate operator training, can be operated on battery power in almost any setting, is quick and without patient discomfort, and requires only one person to take the measurement. The major initial cost is the BIS instrument; the recurrent cost of the gel electrode pads and alcohol wipes is minimal compared with the costs of other laboratory-based methods.

### Anthropometric measurements and the $^{2}\text{H}_2\text{O}$ method

Circumference and skinfold-thickness measurements are inexpensive, noninvasive techniques for measuring subcutaneous fat depots and estimating total body fat in this population. However, accurate measurements are dependent on appropriately trained observers whose measurements are standardized against each other, as was done in this study. In addition, given the relation between lipodystrophy and antiretroviral therapy, the anthropometric measurements have the advantage of measuring changes in the location of body fat stores. Our study found that all skinfold-thickness measurements, BMI, and MUAC were well correlated with FM$_{\text{2H2O}}$ in both HIV+ and HIV− mothers. It is interesting that none of the anthropometric measures was correlated with FFM$_{\text{2H2O}}$ in HIV+ mothers, whereas they were so correlated in HIV− mothers but less well than with FM. This is a finding similar to that of Grinspoon et al (28), who noted that BMI was not significantly correlated with FFM determined by dual-energy X-ray absorptiometry in 33 HIV-infected women and that skinfold-thickness measurements correlated least well to FFM.

In our study population, the Durnin-Womersley equation for estimating body composition tended to overestimate FFM and underestimate FM in both HIV+ and HIV− mothers; this finding is similar to that from a study of HIV+ men in Australia (33). Unfortunately, the race or ethnicity of the subjects used to develop the Durnin-Womersley equation is not reported. If most of the subjects were white, that may partially explain this difference between methods. Wagner and Heyward (34) reviewed studies of ethnic patterns of body composition and found that blacks have less subcutaneous fat in the limbs than in the trunk, and that they tend to carry more fat on the back and lateral portions of the trunk than do whites. Kotler et al (35) found that FM was significantly higher in HIV− African American women than in HIV− white American women, but the it did not differ significantly from that in HIV− African women from Zaire. This is consistent...
with our finding in this study that black mothers in KwaZulu-Natal have more FM than do mainly white breastfeeding mothers from the northern hemisphere (36–38). These ethnic or racial differences could produce systematic errors in the use of reference values of body composition that do not take ethnicity and race into consideration. Further studies are needed to investigate the influence of ethnicity and race on body composition, to validate anthropometric equations for predicting FFM and FM in African women, and to ascertain the best methods for accurately measuring FFM in HIV+ women.

**Effect of HIV on body composition**

Our study found no evidence of wasting in either HIV+ or HIV− breastfeeding mothers living in this region of South Africa. Indeed, the mean percentage body fat (37%) is higher than that in lactating women in other countries (28, 39). Compared with 46 HIV+ women (breastfeeding status unknown) in Zaire who were measured with BIA, the HIV+ mothers in the current study weighed 20 kg more and had 2 kg more FFM and 18 kg more FM (35). This difference may be partially explained by the general good health of the mothers we enrolled. None of the mothers were acutely ill, and only one had advanced immunosuppression (CD4 cell count: <200/μL). In contrast, most mother-to-child transmission prevention trials report that 12–15% of enrolled HIV+ women have CD4+ cell counts < 200/μL. Thus, we are unable to assess the effect of more advanced disease on the validity of the BIS method.

We are not aware of any studies describing the nutritional status of either HIV+ or HIV− breastfeeding mothers in South Africa. It is therefore difficult to ascertain whether the mothers measured in this study are representative. However, the study mothers were socioeconomically representative of the location and had demographic surveillance information consistent with that obtained from the Africa Centre Demographic Information System.

In summary, we have shown that BIS provides an estimate of body composition that is 105% to 106% of the estimate obtained with the H2O method, as measured under our study conditions. This is a reasonable approximation of the values obtained with the reference method, and, given our study conditions, we are not sure which method provides the truest estimation. Regardless of the true value, the relation does not vary for HIV+ and HIV− mothers. BIS is a simple field technique that is useful for assessing body composition and for comparing groups of HIV+ and HIV− women in this location. Breastfeeding mothers living in our study area appear to be adequately nourished in terms of body composition and anthropometry. Studies of women with advanced HIV disease are needed to more fully test the limits of the BIS method for accurate assessment of body composition.

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PCP was responsible for the study concept and design, field supervision, analysis and interpretation of results, and drafting of the manuscript. KHB, NCR, MDVL, and MLB contributed to the study design, interpretation of results, and manuscript revisions. MVL was also responsible for the supervision of H2O analysis. The manuscript was reviewed and approved by all authors. None of the authors had a personal or financial conflict of interest.

**REFERENCES**


