

Validity of impedance-based predictions of total body water as measured by ^2H dilution in African HIV/AIDS outpatients

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Measurements of body composition are crucial in identifying HIV-infected patients at risk of malnutrition. No information is available on the validity of indirect body composition methods in African HIV-infected outpatients. Our first aim was to test the validity of fifteen published equations, developed in whites, African-Americans and/or Africans who were or not HIV-infected, for predicting total body water (TBW) from bioelectrical impedance analysis (BIA) in HIV-infected patients. The second aim was to develop specific predictive equations. Thirty-four HIV-infected patients without antiretroviral treatment and oedema at the beginning of the study (age 39 (SD 7) years, BMI 18.7 (SD 3.7) kg/m², TBW 30.4 (SD 7.2) kg) were measured at inclusion then 3 and 6 months later. In the resulting eighty-eight measurements, we compared TBW values predicted from BIA to those measured by ^2H dilution. Range of bias values was 0.1–4.3 kg, and errors showed acceptable values (2.2–3.4 kg) for fourteen equations and a high value (10.4) for one equation. Two equations developed in non-HIV-infected subjects showed non-significant bias and could be used in African HIV-infected patients. In the other cases, poor agreement indicated a lack of validity. Specific equations developed from our sample showed a higher precision of TBW prediction when using resistance at 1000 kHz (1.7 kg) than at 50 kHz (2.3 kg), this latter precision being similar to that of the valid published equations (2.3 and 2.8 kg). The valid published or developed predictive equations should be cross-validated in large independent samples of African HIV-infected patients.

Bioelectrical impedance: ^2H dilution: HIV: Body composition: Validation: Prediction

Malnutrition is likely to be a key factor in determining the progression from asymptomatic infections to AIDS. Wasting is considered a major clinical diagnosis criterion for AIDS and is a frequent complication of the disease^(1,2). Wasting is present in about 20–30% of HIV patients at the time of diagnosis and in 80% of patients at advanced stages of the disease⁽³⁾. Measurements of body composition are then crucial in identifying HIV-infected patients at risk of malnutrition. Kotler *et al.*⁽⁴⁾ showed that measurement of body weight alone failed to identify dramatic losses in body cell mass, a compartment closely correlated with survival in AIDS^(5–7). Water is the major component of the body and is an essential medium of the body's internal environment. Total body water (TBW) is constantly maintained in normal subjects and it is frequently measured to evaluate body composition, a sensitive indicator of health and nutritional status⁽⁸⁾. Bioelectrical impedance analysis (BIA) is a proven method for body composition studies^(9,10). BIA is often used to estimate body fat and muscle, but it is important to remember that it utilises

impedance measurements of water contained within tissue, which gives an assessment of TBW. BIA is therefore an indirect method from which body composition is predicted using statistical relations derived in similar populations against a reference method.

Prediction formulas for body composition are population-specific^(11–14). Therefore existing equations, obtained mainly in Western subjects, might be inappropriate for African subjects, although we recently showed that an equation derived from black subjects was no more valid than those derived from whites for predicting TBW from BIA in African women⁽¹⁵⁾. Furthermore, equations developed in normal healthy subjects may not be appropriate for patients with disease, and so it is essential that the BIA equations to be used in HIV-infected patients be validated specifically for that group. Few such validation studies have been published; one used ^3H dilution as the reference method and investigated both healthy and HIV-infected white, black and Hispanic subjects from the USA⁽¹⁶⁾, whilst two studies used ^2H dilution as the reference

Abbreviations: BIA, bioelectrical impedance analysis; RMSE, root mean square error; TBW, total body water.

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method in a small group of patients with AIDS^(17,18). The usefulness of these published equations in other African populations remains unknown.

The aim of the present study was to test the validity of fifteen published BIA-based prediction equations of TBW in African HIV-infected patients, since this is particularly important for nutritional status assessment. TBW was measured by using the ²H dilution technique^(19–21) along with BIA measurements, performed one to three times during a 6-month duration follow-up in a group of thirty-four Senegalese outpatients. The second objective was to develop specific prediction equations in our sample in order to compare their precision with that of valid published equations. To the best of our knowledge this is the first time that the validity of BIA-based TBW predictions has been tested in HIV-infected African patients.

Subjects and methods

Subjects

The research was part of a longitudinal study conducted in the outpatient reference centre of people living with HIV/AIDS (Centre de Traitement Ambulatoire) of Dakar, Senegal, West Africa. The study included thirty-four HIV-positive patients (fourteen men and twenty women) at different stages according to Centre for Disease Control classification⁽²²⁾. At the beginning, patients were excluded from taking part in the study if they were hospitalised or bed-ridden, undergoing anti-retroviral therapy or if they had been diagnosed with oedema or a progressive psychiatric disease. The ethics committee of the University of Dakar and the Senegalese Ministry of Health approved the study. Patients were informed of the study objectives and procedures and their written consent was obtained. Anthropometry, BIA measurements and isotope dilution were performed on the same morning after an overnight fast. The thirty-four patients were measured at inclusion. Three months later, at the second visit, thirty of the thirty-four patients were measured again, and twenty-four measured on a third occasion at a final visit 6 months after inclusion. Therefore, twenty-four subjects had three measurements, six had two measurements and four had only one measurement, a total of eighty-eight measurements in all. During the follow-up, all the patients received their usual monthly care, which is about diagnosis and treatment of opportunist infections, diarrhoea, gastrointestinal disease, anaemia, anorexia and, if necessary, the patients receive antiretroviral treatment.

Anthropometry

Anthropometric measurements were performed using standard procedures⁽²³⁾. Measurement of height to the nearest millimetre was made using a height board (Seca, Germany). Body weight was measured with electronic scales (Calor, France) with a precision of 100 g. The measurement was made without clothing or shoes.

²H dilution

Before dosing, a saliva sample (pre-dose) was collected into a clean, sterile and dry tube for the determination of natural ²H/¹H abundance. The patient then drank a standard dose of

30 g ²H-labelled water (99.8% purity; Cambridge Isotope Laboratories Inc., Andover, MA, USA) followed by 50 g local tap water. Subjects were instructed to remain in a quiet state during the equilibration period. Food or water was not permitted after drinking the dose until the end of the sample collection. Post-dose saliva samples (5 ml) were collected from each subject at 2, 3 and 4 h after drinking the dose. The samples (pre- and post-dose) were centrifuged (Sigma 2-16K centrifuge; Laborzentrifugen GmbH, Germany) at 11 000 rpm immediately after collection and the supernatant was retained for analysis. All the samples were stored frozen at –80°C. ²H/¹H enrichment of the saliva samples was measured by isotope ratio mass spectrometry using methods described elsewhere⁽²⁴⁾.

A preliminary survey indicated that maximum enrichment of body water occurred at 3 h and subsequently this time-point was used for the calculation of the ²H dilution space (D_{space}) using the following formula:

$$D_{\text{space}} \text{ (kg)} = \frac{(18.02/10^3) \times (\text{Dose}/20.02)}{\text{Enrichment}/10^6}$$

where Dose is in g and Enrichment is expressed as molar ppm. TBW was calculated assuming a 4% hydrogen exchange with non-aqueous compartments in the body⁽²⁵⁾.

Bioelectrical impedance analysis

BIA was performed using a multifrequency analyser (Xitron 4000B; Xitron Technologies, CA, USA). Measurements were made on the left side of the body. The multifrequency device uses two pairs of electrodes with one electrode in each pair acting to administer the current and the other detecting the voltage drop. Electrodes were placed on the hand, wrist, foot and ankle of each subject following a standardised procedure⁽⁸⁾. Before measurement, subjects were supine with their arms and their thighs apart for 15 min. The resistance (R) and impedance (Z) values of measurement, both provided by the device, were reported to the nearest 0.1 Ω from a digital display. The accuracy of the instrument was tested before the measurements using a 422 Ω standard resistor purchased with the analyser. From the spectrum of fifty measurements (5–1000 kHz) only BIA values obtained at the usual 50 kHz frequency (R_{50} and Z_{50}) and at a higher 1000 kHz frequency (R_{1000} and Z_{1000}), which is thought to better reflect whole-body conductor volume^(10,26), were used in the present study. Indeed, data obtained at 50 kHz were needed as most of the published equations used the traditional frequency of 50 kHz⁽¹⁰⁾. We therefore chose this frequency to develop predictive equations so that firstly, the equation can be used by all BIA users, and secondly to be able to compare their precision with that obtained with the published equations we tested. In addition, as some devices allow BIA measurements to be made at a wide range of frequencies and it has been shown that at a very high frequency (1000 kHz) total conduction through the cell membrane occurs, allowing the quantification of TBW⁽²⁷⁾, we chose this additional frequency to develop new equations. We note that Paton *et al.*⁽¹⁸⁾ showed in HIV-infected patients that the prediction of TBW using 1000 kHz was better than at 50 kHz. The impedance index was calculated by scaling by height squared (height^2/R or

Table 1. Published bioelectrical impedance analysis-based equations tested for prediction of total body water (TBW) in the present study on HIV-infected African outpatients

Equation	Subjects in which equations were developed and country	BMI		Age (years)†		RMSE†	Reference
		Mean	SD	Mean	SD or range		
Developed in subjects including HIV-infected patients							
(A) M: $TBW = 0.58(ht^{1.62}/Z_{50}^{0.70})(1.0/1.35) + 0.32(wt) - 3.66$ F: $TBW = 0.76(ht^{1.99}/Z_{50}^{0.58})(1.0/18.91) + 0.14(wt) - 0.86$	206 M (105 HIV-infected + 101 normal); USA					7.59	Kotler <i>et al.</i> ⁽¹⁶⁾
(B) $TBW = 0.79(ht^{1.81}/Z_{50}^{0.66})(1.0/4.53) + 0.20(wt) - 5.65$	126 F (29 HIV-infected + 97 normal); USA 332 black, white, hispanic (206 M + 126 F) (134 HIV-infected + 198 normal); USA			40		8.20 8.39	Kotler <i>et al.</i> ⁽¹⁶⁾
(C) $TBW = 0.37306(ht^2/Z_{50}) + 0.228728(wt) - 0.163312(Age) + 10.94$	33 male HIV-infected patients; UK	21.4	3.4	37	7	2.17	Paton <i>et al.</i> ⁽¹⁸⁾
(D) $TBW = 0.309030(ht^2/Z_{1000}) + 0.217489(wt) - 0.158662(Age) + 11.81$	33 male HIV-infected patients; UK	21.4	3.4	37	7	2.09	Paton <i>et al.</i> ⁽¹⁸⁾
(E) $TBW = 1.041667(TBW_{R_{JL}}) - 4.375‡$	10 M + 1 F HIV-infected patients; Netherlands	20.6	2.0	45	11.3	1.35	Sluys <i>et al.</i> ⁽¹⁷⁾
Developed in non-HIV-infected whites or white and black subjects							
(F) M: $TBW_{R_{JL}} = 0.3963(ht^2/R_{50}) + 0.143(wt) + 8.40$ F: $TBW_{R_{JL}} = 0.3821(ht^2/R_{50}) + 0.1052(wt) + 8.3148$							RJL Systems
(G) $TBW = 4.96 + 0.42(ht^2/R_{50}) + 0.13(wt) + 3.34(gender)$ [F = 0, M = 1]	20 M + 20 F; USA					1.37	Kushner & Schoeller ⁽³³⁾
(H) $TBW = 0.830 + 0.714(ht^2/R_{50})$	20 M + 20 F; USA					2.50	Kushner & Schoeller ⁽³³⁾
(I) M: $TBW = 1.203 + 0.176(wt) + 0.449(ht^2/R_{50})$ F: $TBW = 3.747 + 0.113(wt) + 0.45(ht^2/R_{50})$	574 white M + 138 black M; USA 875 white F + 214 black F; USA				12–94 12–94	3.8 2.6	Sun <i>et al.</i> ⁽³⁴⁾
Developed in non-HIV-infected black subjects							
(J) $TBW = 4.563 + 0.621(ht^2/R_{50})$	43 black M + 45 black F; USA				30.8 (M), 30.8 (F)	2.06	Zillikens & Conway ⁽³⁵⁾
(K) $TBW = -9.212 + 0.576(ht^2/R_{50}) + 0.128(Xc_{50}) + 0.107(wt)$	43 black M + 45 black F; USA				30.8 (M), 30.8 (F)	1.70	Zillikens & Conway ⁽³⁵⁾
(L) M: $TBW = 0.257(ht^2/R_{50}) - 0.068(age) + 0.229(wt) + 0.162(ht) - 12.02$ F: $TBW = 0.282(ht^2/R_{50}) - 0.034(age) + 0.160(wt) + 0.135(ht) - 9.53$	88 black M; USA 94 black F; USA	26 27		49 51	18–94 18–94	3.1 2.4	Wang <i>et al.</i> ⁽³⁶⁾
(M) $TBW = 0.562(ht^2/R_{50}) + 0.081(wt) + 1.3$	89 black M and F; USA	18–50			14–53	Not given	Schoeller & Luke ⁽³⁷⁾
(N) $TBW = 1.93 + 0.47(ht^2/R_{50}) + 0.13(wt) - 1.20(gender)$ [F = 1, M = 0]	42 black M + 50 black F; Nigeria	19.8	5.2	27.2	5–65	1.7	Leman <i>et al.</i> ⁽³⁸⁾
(O) $TBW = 0.51 + 0.64(ht^2/R_{50})$	42 black M + 50 black F; Nigeria	19.8	5.2	27.2	5–65	2.0	Leman <i>et al.</i> ⁽³⁸⁾

F, females; ht, height (cm); M, males; R, resistance (Ω); RMSE, root mean square error; wt, body weight (kg); Xc, reactance (Ω); Z, impedance (Ω).

† In the sample in which the equation was developed.

‡ $TBW_{R_{JL}}$ is obtained by using the prediction of the RJL analyser (equation F).

Impedance-based predictions of total body water

height²/Z, cm²/Ω) as this is a crude approximation to the volume of the conductor (i.e. the body) and should therefore be highly correlated with laboratory estimates of TBW⁽²⁶⁾.

The same investigator performed all measurements. The degree to which repeated BIA measurements yield the same value, i.e. the reproducibility of measurement made by the investigator, was obtained from replicate measures taken over a short timescale. The reproducibility was estimated in eighty-eight replicates from the subjects in the present study. The differences among duplicates were calculated as absolute values. The technical error of the measurement ($\sqrt{\sum(\text{intra-observer difference})^2/2 \times \text{number of duplicates}}$), and the percentage of reliability (technical error \times 100/overall mean of the measurements) were calculated.

Bioelectrical impedance analysis-based published prediction equations

Published equations we tested here can be divided into three groups according to the HIV disease status or the ethnicity of the population in which the equations were developed (Table 1).

Equations in the first group had been developed in subjects comprising HIV-infected patients. We used the sex-specific equations validated by Kotler *et al.*⁽¹⁶⁾ (A) in a large sample of white, black and Hispanic subjects that included HIV-infected individuals, and also a sex-independent equation (B) from the same study. These formulas had been previously used in African adults with pulmonary tuberculosis^(28–30). These formulas have also been tested for validity in a small group of male HIV-infected patients⁽¹⁸⁾ in a study that also provided two predictive equations, one equation using BIA measurements at the usual frequency of 50 kHz (C), and the other one using BIA at high frequency (1000 kHz) (D). Sluys *et al.*⁽¹⁷⁾ predicted TBW by using the formula provided by the manufacturer RJL Systems in HIV-infected patients; moreover from their results it was possible to derive another prediction equation that we tested here (E).

Secondly, we tested prediction equations developed in non HIV-infected subjects including only whites or African-American subjects. As several studies^(17,31,32) used only the RJL equation to estimate body composition in HIV-infected patients, we also tested the sex-specific RJL equations (F). We also tested the two TBW prediction equations⁽³³⁾ recently reported to be valid for assessing TBW in Africans⁽¹⁵⁾ (G, H), and a recent sex-specific TBW prediction equation developed in a multicentre study in a large race-combined sample⁽³⁴⁾ (I).

The third group included equations developed in non-HIV-infected black subjects (J, K, L, M, N, O)^(35–38).

All the equations, which are presented in Table 1, use impedance and anthropometric measurements that are easily performed in the field. Some detailed information about their initial development and validation is also given in Table 1: their precision, reported from the articles cited in reference, is given by the root mean square error (RMSE, see later) which indicates whether the predictive equations fitted well to the data from which they were generated.

Blood analysis

Lymphocyte count (CD4/CD8) was determined by Immunocytometry (Becton Dickinson FACS Count Immunocytometry

Systems, CA, USA) in total blood using FACS count kits reagent (Becton Dickinson, BD Bioscience).

Statistical analyses

Statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and the SAS system for Windows release 9.0 (SAS Institute Inc., Cary, NC, USA).

The total data used for the comparison comprise measurements performed up to three times per subject during a 6-month follow-up. Consequently, the different analyses described took into account the within-subject visits dependencies using relevant estimates of between-subject variances using specific procedures for subject-level clustering (SAS 'surveyreg' procedure, 'cluster' statement). This was performed for all analyses either to assess validity by modelling difference between methods as a function of their mean as recommended by Bland & Altman or for estimating regression equations of prediction.

Firstly, equations from the literature to predict TBW from BIA were applied to our sample of African HIV-infected outpatients and the results compared to TBW measured by ²H dilution. The success of the prediction equations for TBW was tested using a general linear model⁽³⁹⁾. The correlations between predicted and reference values of TBW were assessed. However, a high correlation does not mean that the methods agree, and it was therefore necessary to use the Bland–Altman approach^(40,41) to analyse bias and error values and relationships between bias and mean of the two methods. The bias was the difference between measured and predicted values of TBW and the error was the standard deviation of the bias⁽⁴⁰⁾. The dependency of the bias on the mean of measured and predicted values^(40,41) was tested using correlation analysis. The limits of agreement, calculated as bias \pm 1.96 error (i.e. 95 % CI of the individual difference), were used to test agreement between the two methods (measurement and prediction of TBW values). For cross-validation purposes (i.e. when testing the predictive power of an equation for data not used in the equation's development) the pure error (accuracy) statistic⁽⁴²⁾ was used. This is defined as the root mean square of the differences between predicted and measured data.

$$\text{Pure error} = \sqrt{\frac{\sum(\text{predicted value} - \text{observed value})^2}{\text{number of observations}}}$$

The smaller the pure error, the greater the accuracy of the tested equation. There is no formal criterion that can be applied to pure error to indicate successful cross-validation, but the pure error should be similar to the RMSE (precision) obtained using the data from which the predictive equation was derived. The RMSE statistic is defined as the square root of the sum of the squared deviations between prediction and observation, divided by the number of observations minus the number of parameters use for prediction, which should be minimised.

$$\text{RMSE} = \sqrt{\frac{\sum(\text{predicted value} - \text{observed value})^2}{(\text{number of observations} - \text{number of parameters} - 1)}}$$

Secondly, when developing the predictive equation for isotope-derived TBW data from BIA data, body weight, age

and sex, the approach of Guo & Chumlea⁽⁴²⁾ was used. Briefly, the impedance index was used as the primary independent variable, and then three secondary variables (weight, age, sex) were tested as additional predictors, each one being added alone, or in combination with the others. The optimal combination was selected by consideration of the correlation between predicted and observed data (which should be maximised), and by the RMSE statistic. Due to the small number of observations in our dataset, instead of the usual split sample approach for cross-validation we used a ten-fold approach. In this method the data are divided evenly into ten groups. At each stage nine of these groups are combined and used to optimise the prediction equation (precision of the prediction). Then TBW was predicted for the remaining group (the validation set), and the pure error calculated (accuracy of the prediction). This was repeated with data being predicted for all ten groups, and the combined pure error calculated. In case that the accuracy was similar to the precision, there was a justification in expanding the regression model to the whole data set ($n = 88$), prediction and validation sets combined.

For all analyses, the first type error rate was set at 0.05.

Results

Bioelectrical impedance analysis measures reproducibility

Absolute values of the difference between replicates of R_{50} ranged from 0 to $2\ \Omega$, mean difference 0.52 (SD 0.47 Ω). Absolute values of the difference between replicates of R_{1000} ranged from 0 to $2.7\ \Omega$, mean difference 0.04 (SD 0.57). The percentage reliability was good: 0.08 % for R_{50} and R_{1000} .

Subjects' description

Data were obtained from thirty-four subjects (fourteen men and twenty women). Mean age, body weight and height were 39 (SD 7) years, 54.1 (SD 12.2) kg and 169.8 (SD 10.6) cm. The mean BMI was 18.7 (SD 3.7) kg/m². Most of the patients had BMI less than 18.5 kg/m² (ten men and eleven women), in whom five and four were severely malnourished (BMI < 16 kg/m²). Overall, the mean of TBW measured by the ²H dilution method was 30.4 (SD 7.2) kg. Patients with both types of virus, HIV-1 and HIV-2, were represented; however, HIV-1 was more prevalent than HIV-2. According to the Centre for Disease Control classification, the majority of the patients (71 % of the men and 50 % of the women, 59 % overall) were at advanced stage of the disease (stage B), with eight having onset of AIDS (stage C) at the beginning of the study. The median CD4 lymphocytes count of the patients was 251 (interquartile range 95–411) with fourteen having CD4 < 200 cells/mm³. After 3 and 6 months follow-up, any significant difference was observed in the CD4 count, median CD4 251 (interquartile range 158–392) and 275 (interquartile range 98–485), respectively. At inclusion, 3 and 6 months, the most common pathologies affecting the patients were gastroenteritis (in eight, six and three patients, respectively), prurigo (in five, five and four patients, respectively), oral candidiasis (in six, six and two patients, respectively), diarrhoea (in two, two and zero patients, respectively) and tuberculosis (in two, one and one patients, respectively). None of the

patients had clinical signs of peripheral oedema or dehydration during the entire period of the study. The patients were not also clinically lipodystrophic.

Validity of published prediction equations

Comparisons between the TBW values measured by ²H dilution and those from each of the predictive equations are given in Table 2. All correlations between predicted and reference values of TBW were high. All the equations, except equation K, gave acceptable error values that are comparable with prediction errors for TBW from previously reported BIA^(33,43). The higher error value of 10.4 kg was observed for equation K that includes reactance and body weight as additional predictors along with the impedance index, whereas the single-predictor equation J developed from the same sample showed a lower error value of 3.2 kg (Table 2). The bias value was not different from zero for the two equations I and M which also yielded a good accuracy, i.e., low pure error value. Among the other thirteen equations, TBW was mainly overestimated. The lower absolute value for significant bias (0.97 kg) was observed from equation B developed in a large sample that included HIV patients. The higher absolute value for the bias (4.3 kg) was observed from an equation (D) developed in HIV patients but with a small sample size (Table 1). Interestingly equation D used BIA measures performed at a high frequency. The other higher absolute value for bias (4.1 kg) was from equation L developed in non HIV-infected African-Americans. The equations showing the highest bias and/or error values (C, D, H, J, K, L, O) had large limits of agreement, yielding unacceptable potential bias at the level of the individual (Table 2). Three equations (A, H, K) showed a significant correlation between the bias and the mean of measured and predicted TBW values.

Development of prediction equations

As the two published equations that appeared valid in the present sample were not developed in HIV-infected patients, we developed further equations to predict TBW measurements from BIA measurements in African HIV patients and for comparison purpose with these valid equations. Two impedance indices, obtained at the two frequencies of 50 and 1000 kHz, were used. The first stage of the selection of predictors was performed on the total sample (eighty-eight measurements). For the two impedance indices, the equation that yielded the highest R^2 was one using body weight as the second predictor variable. Weight, but not age or sex, was significantly associated with TBW (R^2 0.90 and 0.94 for height²/ R_{50} and height²/ R_{1000} , respectively; $P < 0.0001$).

Global results from the ten-fold approach cross-validation are presented in Table 3. Addition of body weight as the second predictor to each of the two impedance indices improved the precision as well as the accuracy. The prediction using height²/ R_{50} and weight as predictor variables yielded an accuracy of 1.72 kg and those using height²/ R_{1000} and weight yielded an accuracy of 1.55 kg. Precision and accuracy were better when predictions used impedance measured at high frequency (Table 3). When developed from the whole data set ($n = 88$), the final models deemed to be applicable

Table 2. Comparison of total body water (TBW) measured by ²H dilution and predicted from bioelectrical impedance analysis by using the published equations, in the eighty-eight measurements performed in thirty-four HIV-infected African outpatients†

Published equation	Correlation coefficient between TBW measured and predicted*		Bias (kg)‡		P value	95% limits of agreement (kg)§	Pure error (kg)	Correlation between mean and difference of the two methods		P value
	Mean	SD	Mean	SD						
A	0.94		-1.70	2.38	<0.0001	-6.36, +2.96	2.92	0.35	0.0006	
B	0.93		-0.97	2.71	0.0011	-6.28, +4.34	2.86	0.11	0.31	
C	0.91		-3.06	3.01	<0.0001	-8.96, +2.84	4.28	0.29	0.26	
D	0.95		-4.34	2.21	<0.0001	-8.67, 0	4.87	0.092	0.52	
E	0.94		+1.32	2.47	<0.0001	-3.52, +6.16	2.79	0.10	0.35	
F	0.94		-1.69	2.47	<0.0001	-6.53, +3.15	2.98	0.22	0.050	
G	0.94		-1.51	2.56	<0.0001	-5.53, +3.05	2.96	-0.017	0.89	
H	0.91		-2.34	3.59	<0.0001	-9.38, +4.70	4.27	-0.33	0.0052	
I	0.94		+0.11	2.34	0.67	-4.48, +4.70	2.33	0.12	0.24	
J	0.91		-1.85	3.16	<0.0001	-8.04, +4.34	3.65	-0.032	0.79	
K	0.56		-3.21	10.39	0.0057	-23.57, +17.15	10.81	0.59	0.021	
L	0.95		-4.05	2.28	<0.0001	-8.52, +0.42	4.64	0.10	0.34	
M	0.93		-0.36	2.75	0.23	-5.75, +5.03	2.76	0.055	0.62	
N	0.94		+1.25	2.44	<0.0001	-3.53, +6.05	2.73	0.035	0.75	
O	0.90		+1.34	3.22	<0.0001	-4.97, +7.65	3.47	0.10	0.39	

* P < 0.05 for all.

† For details of subjects and procedures, see Subjects and Methods. For details of the published equations, see Table 1.

‡ Measured - predicted value (a negative value of the bias reflects an overestimation by the prediction equation).

§ 95% CI of the individual differences.

|| Pure error = $\sqrt{\sum(\text{measured} - \text{predicted})^2/n}$, where n is the number of measurements. The smaller the pure error the greater the accuracy.

in the case of African HIV patients, with two-predictor equations, are:

$$\text{TBW} = 0.3788(\text{Height}^2/\text{R}_{50}) + 0.2348(\text{Weight}) + 0.8152 (R^2 0.90; \text{precision } 2.28 \text{ kg; limits of agreement } -4.43, +4.43 \text{ kg})$$

$$\text{TBW} = 0.3756(\text{Height}^2/\text{R}_{1000}) + 0.1717(\text{Weight}) - 0.5756 (R^2 0.94; \text{precision } 1.72 \text{ kg; limits of agreement } -3.33, +3.33 \text{ kg}).$$

The two predictions showed a non-significant intercept.

Discussion

Water is the major component of fat-free mass and its contribution to body composition remains relatively stable through adulthood⁽⁴⁴⁾; however, changes in TBW can be associated with HIV infection. Early identification of malnutrition in HIV-infected patients requires the measurement of body composition. The validation of BIA, an inexpensive method for determination of TBW and potentially valuable in the context of low-income countries, is a requirement before it can be used to assess the improvement of nutritional intervention programmes. The effect of race-ethnicity on body composition has been well documented in healthy populations⁽⁴⁵⁾, and our team previously detailed and discussed the validity of BIA-based published equations for the prediction of TBW in Senegalese women⁽¹⁵⁾.

The assumptions underlying body composition measurements using BIA may not be valid in individuals with altered hydration. Hyperhydration and hypohydration can induce increased or decreased body resistance in estimating the amount of TBW^(46,47). In HIV-infected patients, water balance may be altered by dehydration or fluid retention causing a bias in TBW estimate. The most obvious sources of error are due to fluid overload, such as with severe cardiac, renal or hepatic diseases, hypoalbuminaemia, intravenous hydration, and fluid deficits from severe persistent diarrhoea or poor fluid intake. Side-effects associated with antiretroviral therapy such as fat redistribution syndrome (lipodystrophy) has also been reported as a confounding factor in the determination of lean body mass by BIA⁽⁴⁸⁾. Such clinical conditions were not present in our patients who were not clinically ill AIDS patients. Over-hydration of adipose tissue may occur in obese subjects but none of the patients was obese. Moreover, except for four subjects at 6 months, patients who remained in the longitudinal study did not receive antiretroviral therapy because the study was conducted before the widespread use of antiretroviral treatment in the Centre de Traitement Ambulatoire. Therefore TBW measurement in the present study was accurately estimated with BIA.

BIA has been used in European clinically stable AIDS patients⁽¹⁷⁾ and in a large cohort of American HIV patients⁽¹⁶⁾ to estimate TBW and to monitor change of nutritional status. Currently, there are limited data in the literature involving African subjects. The present study was specifically designed to test the validity of published BIA-based predicted equations

Table 3. Precision and accuracy of the predictive regression equations tested by using the ten-fold cross-validation procedure†

Response variable	Predictor variables	Precision in the sub-samples used to establish the equations		Accuracy from data held out Pure error (kg) (accuracy)
		R ² ‡	RMSE (kg) (precision)§	
TBW _{2H2O}	Height ² /R ₅₀	0.82	3.07	2.38
	Height ² /R ₅₀ , weight	0.90	2.27	1.72
	Height ² /R ₁₀₀₀	0.91	2.22	2.00
	Height ² /R ₁₀₀₀ , weight	0.94	1.72	1.55

2H₂O, ²H-labelled water R, resistance; RMSE, root mean square error; TBW, total body water.

† TBW = 0.5531(Height²/R₅₀) + 5.7928. TBW = 0.3783(Height²/R₅₀) + 0.2350(weight) + 0.8290. TBW = 0.4887(Height²/R₁₀₀₀) + 2.1944. TBW = 0.3755(Height²/R₁₀₀₀) + 0.1717(weight) - 0.5723.

‡ R² = proportion of the total variance in the response variable explained by the predictor variables.

§ RMSE = $\sqrt{\sum(\text{observed} - \text{predicted})^2 / (n - p - 1)}$, where *n* is the number of observations and *p* is the number of predictor variables.

|| Pure error = $\sqrt{\sum(\text{observed} - \text{predicted})^2 / n}$, where *n* is the number of observations.

of TBW in African HIV-infected patients and showed that the comparability of TBW determined by isotope dilution and BIA was dependent on a specifically derived BIA equation for the population. None of the equations developed in HIV-infected patients were valid, even if they were developed in a large sample (A, B) or they used sex-specific prediction (A). However, equations A and B include HIV patients and non-HIV subjects. Equations C and D developed in smaller populations of HIV-infected patients showed higher bias values and limits of agreement in the present study. We hypothesised that the bias observed could be due to the fact that the definition of the disease is not harmonised according to the studies. When compared with our subjects, most of the patients for whom equations C, D and E had been developed were at advanced stage or onset of the disease, which is known to accompany acute infection associated with abnormalities in water compartment. Now, the extracellular water: intracellular water ratio is a factor known to limit the applicability of predictive equations generated by BIA to populations with varying hydration⁽⁴⁹⁾ such as HIV/AIDS patients. Equations A and B, validated by Kotler *et al.*⁽¹⁶⁾ in a large sample of white, black and Hispanic subjects that included HIV-infected individuals, were also not valid in our sample. However, the equation I recently developed in a very large multicentre and race-combined population appeared to be valid in our sample. BIA-based predictive equations derived in healthy populations do not necessarily require modification for application to the diseased state. Similarly, as we previously reported, these equations may be independent of ethnicity⁽¹⁵⁾. One explanation could be that this hypothesis was generally reported for percentage body fat or fat-free mass estimations, whereas TBW requires more direct evaluation and fewer assumptions than the assessment of lean or fat compartments from BIA. Indeed, BIA uses impedance measurements of fluids contained within tissue. In equations developed specially in black subjects, only one equation (M) was valid in our sample; however, its accuracy was slightly lower than that of equation I. Finally, two equations developed in non-HIV-infected subjects showed a non-significant bias in our sample and can be considered as usable in African HIV-infected patients.

Considering that the development of new prediction equations is a difficult and expensive process in low-income countries, we suggest focusing research efforts on validating published equations. For example, the equations A or B tested here have been also tested in a group of HIV patients

and showed a significant bias of 1.65 and an error value of 2.54⁽¹⁸⁾ that is similar to our results. When evaluated using the data series from eleven patients with AIDS, the A, B and C equations gave bias values of -2.23 ($P < 0.0001$), -1.48 ($P = 0.0018$) and -2.16 ($P = 0.0016$), and error values of 1.02, 1.17 and 1.67⁽¹⁷⁾ that appear better when compared to the results in our sample.

As a result of the fact that neither of the two published equations we found valid had been developed in HIV-infected patients, we also developed predictive equations in the present study to both assess their accuracy in comparison with that of the valid published equations, and to contribute to future cross-validations in other groups as few studies exist for African patients. Multiple linear regression analysis established that weight, but not age or sex was significantly associated with TBW and incorporation of this independent variable, in addition to impedance index, gave equations with an accuracy of about 2.3 and 1.7 kg at the two BIA frequencies used. This compares favourably with previously reported equations for TBW prediction in normal subjects^(9,16). Based on the precision value (2.3 kg) or on the 95 % limits of agreement, the prediction model to estimate TBW we developed here showed only slightly improved potential usefulness when compared to the two valid published equations (2.3 and 2.8 kg) (comparison is possible only for BIA data obtained at 50 kHz). However, we showed that, when using BIA at 1000 kHz, the performance of predictive equations can be highly improved and become largely better than valid published equations.

A subjective rating system for evaluating the standard error of estimate for equations used to predict lean body mass in adults when validating a new method was developed by Houtkooper *et al.*⁽⁵⁰⁾. A standard error of estimate of <2.3 kg is regarded as 'very good'. We could therefore consider that the predictive equation using R₅₀ and weight generated from our sample showed good performance. It could now be applied in epidemiological studies or field studies in which only BIA can be easily measured. Moreover, studies that could use multifrequency BIA, allowing data to be obtained at 1000 kHz, can predict TBW with very good accuracy. When subjectively evaluated using the data series of the published equation E⁽¹⁷⁾, the equation we developed from our sample (at 50 kHz) showed a non-significant bias of -0.24 ($P = 0.45$) and an error value of 1.03 kg. It therefore appeared to be valid in this case. However, it has to be underlined that the sample size of the present study can clearly represent a limitation in

our conclusions. The predictions we developed should be cross-validated in other studies in large, independent samples in HIV African subjects. It is a necessary step to confirm that the present work could provide adequate data to support the general use of the developed predictive equations.

The present study underlines the potential importance of evaluating various published equations for the prediction of TBW, using data from other validation studies, which provide reference values. Data exchanges and tests between research teams would be very useful to obtain a strong validation and accurate prediction of body composition for HIV-infected patients. Such information sharing would benefit the expected application of ease-of-use and indirect methods in the follow-up of body composition in ill patients. Independent of the specificity from the original sample of a prediction, the degree of bias can be minimised with a choice of the most appropriate BIA equation. At present, and before advancements are obtained in such a topic, we could recommend that the equations developed in HIV patients using BIA at 1000 kHz from the present study, showing accuracy around 1.7 kg TBW, be used in HIV African patients.

Having now the possibility to assess TBW in HIV-infected African people, the further validation of the technique as well as a test of its ability to quantify changes in TBW are needed to determine the full applicability of BIA in nutritional evaluation and in monitoring nutritional support^(16,27). This is particularly important in the case of HIV infection in Africa. For example, weight loss starts early in HIV infection while CD4 counts are still above the range associated with opportunistic infections. On the other hand, indicators of clinical wasting may persist beyond the successful reduction of viral load and improvement in CD4 counts, showing the importance of the weight and body composition in evaluating the HIV evolution⁽⁵¹⁾. Moreover, the composition of the weight loss depends on the baseline body fat composition. Weight loss consists principally of fat loss in those HIV-infected patients with adequate fat stores⁽⁵¹⁾. Loss of lean body mass is a better predictor of survival than weight loss⁽⁷⁾. It should be therefore of great importance to be able to evaluate changes in body composition that occur as the disease advances in patients with untreated HIV infection or patients with pre-existing malnutrition, as is common in developing countries. Indeed, HIV-associated wasting continues to be of clinical concern⁽⁵²⁾ and is intimately related to survival and quality of life. Assessment of the efficacy of treatment interventions aiming to prevent or reverse the wasting syndrome ultimately relies on the accurate determination of body composition before and after implementation.

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