Received 17 November 2004, accepted after revision 27 February 2005

A bioelectrical impedance analysis equation for predicting total body water and fat-free mass in children with Human Immunodeficiency Virus-1 in the pre-HAART and HAART eras

TH Joffe[1], S Welle[2], R Roubenoff[3], SL Gorbach[1], GA Weinberg[4], C Duggan[5], L Furuta[5], J Nicchitta[6], TM Lipinczyk[6,7] and TL Miller[6,7]

[1]Department of Family Medicine and Community Health, Tufts School of Medicine, Boston, MA; [2]University of Rochester School of Medicine, General Clinical Research Center, Rochester, NY; [3]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; [4]University of Rochester School of Medicine, Division of Pediatric Infectious Diseases, Rochester, NY; [5]Children's Hospital, Division of Gastroenterology and Nutrition, Harvard Medical School, Boston, MA; [6]University of Rochester School of Medicine, Division of Pediatric Gastroenterology, Rochester, NY; [7]Miller School of Medicine at the University of Miami, Division of Pediatric Clinical Research, Miami FL, USA.

Bioelectrical impedance analysis (BIA) is commonly used to measure body composition, however limited studies of its usefulness in children with the human immunodeficiency virus (HIV) -1 infection exist. The objective of the study was to provide a BIA equation for predicting body composition in outpatient pediatric HIV populations, to compare performance of our equation to published equations derived from both non-HIV and HIV-positive pediatric populations and to evaluate performance of our equation developed in the pre-highly active antiretroviral (HAART) era, in a separate HIV-positive pediatric population on HAART. Total body water (TBW) by deuterium dilution and BIA measures from 30 HIV-positive pediatric subjects in the pre-HAART era were used to develop an equation for estimating body composition. We evaluated 18 published pediatric BIA equations in our subjects using Bland Altman analysis, and the performance of our model in a separate HIV-positive pediatric population on HAART with dual energy X-ray absorptiometry (DXA) measures. Using multivariate techniques, we developed a predictive equation for TBW using height^2 and resistance in children off HAART that correlated well (r=.95) with FFM measures obtained by DXA in children receiving HAART. A number of published BIA equations developed in healthy children also provided good estimates of TBW or FFM in our subjects. In conclusion: We provide a new BIA equation for estimating body composition in children on or off HAART. Thus BIA measures in HIV-infected children without clinically apparent lipodystrophy are not affected by HAART, although fat distribution cannot be well-defined by BIA. Published models derived from HIV populations do not always out-perform those derived from healthy subjects.

Key words: Bioelectrical impedance analysis, total body water, dual energy X-ray absorptiometry, body composition, HIV infection, children.

Introduction

Bioelectrical impedance analysis (BIA) is used routinely in the clinic and field to estimate total body water (TBW) and fat-free mass (FFM) in the living individual. It measures the opposition of body tissues to the flow of a small single (typically 50 kHz) or multi frequency alternating current. Two vectors, resistance and reactance, are measured from which impedance is calculated and body composition estimated. Resistance is a measure of the resistance of the tissues themselves to the current and reactance is a function of opposition to the additional capacitance of membranes, tissue interfaces and nonionic tissues. Measured resistance, when expressed as ht^2/resistance, is related to the volume of the conducting

Address for correspondence: Tracie L. Miller, MD, Division of Pediatric Clinical Research, Department of Pediatrics (D820), University of Miami School of Medicine, PO Box 016820, Miami, FL 33101, USA.
Tel: (305) 243-1422 Fax: (305) 243-8475
E-mail: tracie.miller@miami.edu

medium (body water). This relationship allows body composition to be predicted assuming that the hydration of the FFM is known or assumed.

BIA has been shown to estimate body composition with sufficient precision for use in clinical investigation and practice and has been useful for studying body composition and changes in body composition over time in adult patients with the human immunodeficiency virus (HIV)-1 [1,2]. Since changes in body composition are pervasive in pediatric HIV-1 [3,4] and often predict clinical outcomes [5,6], simple, non-invasive tools to measure body composition are needed to track children serially.

In this study, we developed a BIA equation for estimating body composition in HIV-1 infected children using TBW (derived from deuterium) while evaluating the performance of a number of BIA equations from the literature. In addition, we evaluated the efficacy of our equation for predicting body composition in another population of HIV-positive children for whom we had dual energy X-ray absorptiometry (DXA) measures and BIA measures in the HAART era. As body composition and fat distribution of HIV-1-infected children have changed from the pre-HAART to HAART era [7–9], our goal was to develop a widely applicable BIA equation to track body composition changes in children on or off HAART. Routine acquisition of easily obtainable and valid measures in the clinical setting enables body composition changes, induced by HIV-specific treatment regimens, to be tracked.

Subjects and methods

Subjects

Our reference population consisted of 30 HIV-1-infected children whose data were used to develop our equation. These children were enrolled as part of a prospective, longitudinal study on growth and nutrition in pediatric HIV between 1996 and 1999 at the Children's Hospital AIDS Program (CHAP), Boston, MA and the University of Rochester Pediatric HIV Program, Rochester, NY. These included 14 males and 16 females, all perinatally-HIV-1-infected, between the ages of three and 13 years who underwent both BIA and TBW measures prior to initiating HAART therapy. The ethnic distribution of the group included 18 Hispanic, eight black, non-Hispanic, three white, non-Hispanic and one child of mixed ethnicity. Our validation population included 14 perinatally-HIV-1-infected children plus one child infected through blood products (eight males and seven females) studied in the HAART era. These children were aged four to 19 years and underwent simultaneous BIA and DXA testing between 2001 and 2003 at the Children's Hospital AIDS Program (CHAP), Boston, MA. The ethnic distribution of this group included six white, non-Hispanic, one Asian, five black, non-Hispanic and three Hispanic children. The diagnosis of HIV-1 infection in all children was confirmed by repeatedly positive serum enzyme-linked immunosorbant assay (ELISA) in conjunction with Western Blot assays and repeatedly positive HIV-1 RNA or DNA polymerase chain reaction (PCR). Five children in the validation group had abdominal adiposity and two children had a buffalo hump, although none of the children had classic clinical features of lipodystrophy with extremity and facial wasting. No children received enteral or parenteral nutrition during the study period and no clinically obvious edema was present in the subjects. All children in the reference (TBW) and the validation (DXA) populations were measured once, thus all values are unique. None of the participants in the validation population were part of the reference population in which we developed our predictive equation. The Institutional Review Boards at both institutions reviewed and approved the research protocol and parental consent was obtained before participation in the study.

Methods

Total body resistance and reactance were measured in the supine position using a single-frequency 50-kHz tetra polar four terminal impedance analyzer (RJL Systems, Detroit, MI, USA). Measures were taken in the morning following an overnight fast. Current-injector electrodes were placed just below the phalangeal-metacarpal joint in the middle of the dorsal side of the right hand and below the metatarsal arch on the superior side of the right foot. Detector electrodes were placed on the posterior side of the right wrist, midline to the pisiform bone on the medial (fifth phalangeal) side with the foot semi flexed. Resistance and reactance have a reproducibility of ± 0.31% [10].

Height, weight, mid-arm muscle circumference (MAC), and triceps skinfold thickness (TSF) were measured by registered dieticians trained and standardized in anthropometry. Weight (recorded to the nearest 0.01 kg) and standing height (recorded to the nearest 0.01 cm) were measured by recommended techniques [11]. BMI was calculated as weight/height^2 (kg/m^2). TSF was measured with Lange skin calipers (Lange, Cambridge MD USA) and MAC was measured using standard techniques [12]. An average of three measurements were taken. TSF and MAC were used to derive arm muscle circumference (AMC), a measure of muscle mass [12]. Age- and sex-adjusted percentiles for TSF and AMC were derived from the Ten State Nutrition Survey for infants and children [13]. Weight, height and BMI were expressed as Z scores specific for age and gender and were calculated from EpiInfo [14].

TBW was estimated by deuterium dilution (^2H_2O). Children were fasted overnight and an initial baseline urine sample was obtained in order to measure naturally occurring deuterium. The child was then given 0.2 grams deuterium per kilogram of body weight orally. The container was washed with 30 milliliters of water and the child consumed that additional amount. After the administration of deuterium, the child con-

tinued to fast for an additional 1.5 hours to allow absorption of the isotope. Two spot urine samples were collected at least three hours after ingestion. This technique has a reproducibility of repeat measures for TBW of ±3% and an intra class correlation coefficient of 0.98 [15].

TBW was defined as the deuterium dilution space, based on the difference between the baseline and subsequent deuterium enrichment. Deuterium enrichment was determined by reducing cryogenically distilled urine water to hydrogen gas by reaction with zinc at 460°C and then measuring $^2HH\text{-}H_2$ ratios in an isotope ratio mass spectrometer (SIRA 12, VG Isogas, Middlewhich, Cheshire, UK) using the methods outlined by Welle et al [16]. All samples were assayed in triplicate and mean values given.

BIA derived resistance and reactance measures were used to estimate total body impedance where Impedance = $(Resistance^2 + Reactance^2)^{1/2}$. Deuterium dilution space was used to estimate TBW. For consistency with most of the equations we evaluate here, no correction for non-aqueous exchange of deuterium within the body was made. TBW was converted to FFM (fat-free mass) by dividing the water content of fat-free tissue by the age and sex-specific hydration fractions given by Forbes [18].

All DXA measurements were made in the posterior-anterior position using a Hologic QDR 4500 scanner (Hologic, Inc., Bedford, MA, USA) with a switched pulse dual-energy X-ray tube generating X-rays at 100kV and 140 kV. Delphi W software (Hologic Inc., Bedford, MA, USA) was used. A series of transverse scans were made from the head to toe at 2 mm intervals, as the child was lying supine. Data were

Table 1. Published equations for estimating TBW and FFM evaluated with our BIA, TBW and DXA data.

Author(s) and reference	Equation	N	Age	Study location	TBW or FFM measurement & collection methods
Total body water:					
Horlick et al [10]	TBW = 0.725 + 0.475 Ht^2/R + 0.140 W	1291	4–18	New York, USA	Deuterium saliva
Arpadi et al [22]	Ln(TBW) = 1.65 + 0.05 Ht^2/R	20	4–11	New York, USA	^{18}O urine
Gregory et al [23]	TBW = 0.79 + 0.55 Ht^2/I	28	7–16	Dundee, Scotland	Deuterium
Danford et al (24)	TBW = 1.84 + 0.45 Ht^2/R + 0.11 W	37	5–9	Illinois, USA	Deuterium, saliva (children) ^{18}O, urine (infants)
Kushner et al [25]		81	3 mo–9 yrs	Illinois, USA & Lima, Peru	Deuterium
Equation 1	TBW = 0.700 Ht^2/R – 0.32				
Equation 2	TBW = 0.593 Ht^2/R + 0.065 W + 0.04				
Davies et al [26]	TBW = -0.50 + 0.60 Ht^2/I	26	5–17	Dundee, Scotland	Deuterium
Davies & Gregory (28)	TBW = 0.13 + 0.58 Ht^2/I	54	5–17	Dundee, Scotland	^{18}O
Fjeld et al (31)	TBW = 0.76 + 0.18 Ht^2/I + 0.39 W	44	3mo–3yrs	Lima, Peru	Deuterium saliva
Leman et al [32]	TBW = 1.67 + 0.35 Ht^2/R + 0.24 W – 0.74 S	39	5–18	Nigeria	Deuterium
Fat-free mass:					
Horlick et al [10]	FFM = (3.474 + 0.459 Ht^2/R + 0.064 W)/ (0.769 – 0.009 A – 0.016 S)	1291	4–18	New York, USA	^{18}O
Goran et al [15]	FFM = (0.59 Ht^2/R + 0.065 W + 0.04 / (0.769 – 0.0025 A – 0.19 S)	31	4–6	Vermont & Arizona USA	Densitometry
Deurenberg et al [27][1]	FFM = 0.430 * 10^4 * Ht^2/I + 0.354 W + 0.9 S	64	8–11	Wageningen, Netherlands	Total body potassium
Cordain et al [29]	FFM = 6.86 + 0.81 Ht^2/R	30	9–14	Colorado, USA	DEXA, total body potassium
De Lorenzo et al [30]	FFM = 2.33 + 0.588 Ht^2/I + 0.211 W	35	7–13	Rome, Italy	Deuterium Respiratory water
Houtkooper et al [33]	FFM = 0.61 Ht^2/R + 0.25 W + 1.31	94	10–14	Ohio & Arizona, USA	Deuterium saliva
Schaefer et al [34]	FFM = 0.65 Ht^2/I + 0.68 A + 0.15	112	3–19	Heidelberg, Germany	40K whole body potassium counter

A = Age in years; Ht = height (cm); W = weight (kg); S = sex where males =1 and females=0; TBW = total body water (L); I = impedance; R = resistance derived from bioelectrical impedance analysis; Arpadi et al [22] include HIV-positive subjects only; Horlick et al [10] include 54 HIV-positive subjects. Deurenberg et al [27] where height in meters; males = 1 and females = 0. The authors refer to impedance as R in their paper. [1]Estimated from body density.

collected over an area of 200 cm x 60 cm and expressed as grams of fat, grams of lean tissue mass and/or percent lean body mass.

Statistical analysis

The relationship between BIA measures and deuterium-derived TBW was modeled, using least squares linear regression. We then added additional variables to the model to determine whether they improved the accuracy of the prediction. These variables included weight, age, sex, triceps skinfold thickness and mid-arm circumference. The best-fit criteria were based on maximizing the correlation coefficient and significance of the constant and coefficient, while minimizing the standard error of the estimate (SEE). All analyses were carried out using SPSS for Windows (SPSS Inc., Chicago, IL, USA) where significance was based on 95% confidence limits.

In addition, the performances of 18 BIA models from the literature (listed in Table 1) were evaluated using our BIA and TBW data. These models were derived using a variety of methods measuring either TBW or estimating FFM (see Table 1). The evaluation of model performance included a Bland Altman [19, 20] assessment of the agreement between predicted TBW or FFM using the published equations and observed TBW or FFM as measured or estimated in our study.

Using the methods of Bland and Altman [19, 20], error was calculated as predicted TBW or FFM minus observed TBW or FFM estimated from deuterium dilution. Percentage error was calculated as [(predicted – observed TBW or FFM)/(observed TBW or FFM) * 100]. The mean percent error reflects bias in estimates where a positive bias represents over-estimation in TBW or FFM by a model and negative bias represents under-estimation. Loss of precision was calculated as [(variance of difference between predicted and observed TBW or FFM)/(variance of observed TBW or FFM)] * 100, based on equal sample sizes for both predicted and observed variables. Loss of precision gives an indication of the increase in sample size required to compensate for the use of predicted rather than criterion measures in an experimental or epidemiological study [21].

In addition to Bland Altman analysis, we evaluated whether the bias in estimates was constant or varied as a function of TBW or FFM by determining whether the slope describing bias in relation to the mean of criterion and estimate body composition measures deviated significantly ($P \leq 0.05$) from zero.

The same methods as described above were applied to data from our validation study. We used the sex-specific predictive equations utilizing height and resistance derived in our reference study (equations 2 and 3 listed in Table 3) to estimate total body water from BIA in our validation study. Forbes' [18] age and sex-specific hydration fractions were used to estimate FFM from TBW. FFM estimated by DXA was then used as the criterion value for the purposes of the Bland Altman analyses in which we compared DXA derived measures of FFM with BIA derived estimates of FFM. In addition, the performance of two previously published pediatric equations [10,22] was evaluated in the DXA validation population as these equations represent the only other published equations including HIV-positive pediatric subjects. TBW estimates from Arpadi et al [10] were converted to FFM estimates utilizing the hydration fractions of Forbes [18], while FFM derived from the Horlick et al [10] equations was estimated directly with no correction for hydration fraction required.

Following Bland and Altman [19, 20], we plotted the difference (bias) between the predicted and observed TBW or FFM value against the average of the two values [(predicted TBW or FFM – observed TBW or FFM from deuterium)/2] for visual assessment of the limits of agreement and bias between predictive models and our data.

Results

Patient characteristics

The clinical characteristics of the two study groups are shown in Table 2. The two populations differed statistically in a number of ways, based on independent sample T-tests with limits set at 95% confidence intervals. Children in the reference population were on average younger than those in the validation population, with a mean age difference of five years. In keeping with their younger age, height, weight, TBW and FFM resistance, reactance and impedance measures were lower in the reference versus validation study participants. The children in the validation group were all on HAART therapy whereas none of the children in the reference population were on HAART. Although not statistically significant, CD4 counts in the validation population were 26% higher than in the reference population. CDC stages in the reference population were as follows: eight children stage A; 17 children stage B, five children stage C. In the validation group, three children were stage A, seven children were stage B, five children were stage C. In the validation study group, weight Z score, height Z score and triceps and mid-arm circumference percentiles were also higher, albeit not significantly, than those of the reference study participants. The reference study participants had BMI Z scores that did not differ statistically from the validation study participants (mean BMI Z score = –0.21 and –0.28 respectively). Therefore, after controlling for age differences, the reference and validation populations did not differ in nutritional status.

Table 3 shows four least squares regression models derived from our reference data using a variety of variables. We added height^2/resistance, weight, age, sex, triceps skinfold thickness and mid-arm circumference to the regression models. Equation 1 was the best predictive model for TBW in our study where 97.6% of the variation in TBW was explained by height^2/resistance and where TBW was predicted with a SEE of 0.82 l. Adding weight (equation 4), age

Table 2. Characteristics of TBW and DXA Study Groups

	TBW sample			DXA sample		
	n	mean	sd	n	mean	sd
Age (years)	30	7.7	3.0	15	12.7†	3.7
Height (cm)	30	118.5	17.4	15	148.2†	17.3
Height Z score (sd)	28	–1.10	1.25	14	–0.33	1.13
Weight (kg)	30	23.1	8.2	15	41.8†	15.5
Weight Z score (sd)	29	–0.74	0.94	14	–0.49	0.83
BMI Z score	30	–0.21	0.70	15	–0.28	1.20
Triceps skinfold (%)	29	33.52	31.07	15	38.53	27.97
MAC (%)	29	46.03	31.12	15	44.91	34.87
TBW (l)	30	14	5.2	15	22.9†	6.8
FFM (kg)	30	18.0	6.95	15	30.5†	9.3
Body fat (%)	29	16.8	6.5	15	20.3	8.2
Resistance (Ω)	30	752	96	15	673†	112
Reactance (Ω)	30	67.2	6.4	15	73.6	16.8
Impedance (Ω)	30	755	96	15	678†	112
Height^2/resistance (m/Ω)	30	19.8	8.4	15	34.4	11.1
CD4 count (cells/mm^3)	28	643	521	14	812	530

%, percentile; † significant difference (P≤0.05); TBW study group comprised of 57% males; DXA study group comprised of 53% males; resistance, reactance and impedance (W) from BIA; body fat calculated from deuterium (in TBW sample) and by DXA; MAC = mid-arm circumference.

Table 3. Equations derived for estimating TBW from BIA in HIV-1 infected children

Equation	R^2	n	SE of estimate	SE of constant	SE B
1 TBW = 1.957 + 0.608 Ht^2/R	0.976	30	0.817	0.389	0.018
2 Males only: TBW = 2.522 + 0.588 Ht^2/R	0.992	14	0.561	0.350	0.015
3 Females only: TBW = 1.112 + 0.647 Ht^2/R	0.952	16	0.961	0.713*	0.034
4 TBW = 0.892 + 0.347 Ht^2/R + 0.271 W	0.982	30	0.725	0.501*	0.091 0.092

TBW = total body water (L); Ht = height in cm; W = weight (kg); $P<0.0001$; *non significant ($P>0.05$) based on two-tail Pearson.

and sex improved the model only slightly, while the addition of triceps skinfold and mid-arm circumference measures did not improve the model (data not shown for age, sex, triceps skinfold and mid-arm circumference).

Table 4 lists the statistics for agreement between the published prediction equations in Table 1 and TBW or FFM in our subjects. Statistics include the correlation expressed as a percentage between TBW or FFM and the predicted value, the standard error of the estimate (SEE), descriptive statistics for the bias, including the 95% confidence intervals of the mean in bias, percentage error statistics and loss of precision as a percentage. It also includes information on whether the slope describing the relationship between the bias and the mean of the two methods deviates significantly from zero, representing a non-constant bias.

With the exception of the Arpadi et al [22] and Goran et al [15] models for predicting FFM, the correlations between predicted and observed TBW and FFM values for these models were all very high, ranging from 94 to 99.2%, indicating good linear relations for these predictive models. The Arpadi et al [22] and Goran et al [15] models produced the highest loss of precision percentages as in both cases, the models performed most poorly in males.

When we constructed Bland Altman plots (ie bias in TBW or FFM estimate in relation to mean estimate based on both methods) several models were associated with fitted least squares regression lines that deviated significantly from zero [15, 22–25]. The remaining models were associated with slopes that did not deviate significantly from zero. Some models produced clear differences in bias between the sexes [15,22,28,30,31].

We selected the best-fit models by determining which maximized the correlation coefficient between estimate and criterion values while minimizing the SEE, mean percentage error, the range between lower and upper limits of agreement for error and percentage error, and minimizing loss of precision and positive or negative trends in bias. In addition we

Table 4. Correlations, SEE, error statistics and 95% confidence intervals associated with Bland Altman Analyses Assessing the Accuracy of Published Equations listed in Table 1 with our BIA, TBW and DXA Data.

			Error[1]				Percentage Error			
Reference	Corr. % R	SEE	Mean	95% CI	Lower limit	Upper limit	Mean	Lower limit	Upper limit	Loss of precision
Total body water:										
Arpadi et al [22]	92.7	1.97	1.65	–0.54, 3.84	–1.41	31.43	7.13	–11.86	97.42	129.5[a]
Males excl. 1 outlier	95.7	1.15	0.62		–1.41	7.05	4.02	11.86	41.42	18.9[a]
All males	96.5	1.66	2.66	–2.25, 7.58	–1.33	31.43	8.44	–10.19	97.42	195.3[a]
Females excl. 1 outlier	96.7	0.96	0.45		–1.33	7.05	1.60	–10.19	31.20	32.32[a]
All females	96.3	1.18	0.76	0.01, 1.51	–1.41	4.83	5.98	–11.86	41.42	10.98[a]
Gregory et al [23]	97.6	0.843	–2.21	–2.62, –1.81	–3.47	0.54	–17.17	–34.21	8.16	5.92[a]
Danford et al [24]	99.0	0.746	–0.72	–1.04, –0.40	–2.39	2.04	–4.22	–15.81	31.11	2.76[a]
Kushner et al [25]										
Equation 1	98.8	0.817	–0.41	–0.88, –0.05	–2.61	2.48	–4.59	–31.77	18.53	4.64[a]
Equation 2	98.9	0.777	–0.72	–1.04, –0.40	–2.41	1.26	–5.94	–29.31	19.19	2.77[a]
Davies et al [26]	97.6	0.843	–2.58	–2.94, –2.21	–3.85	–0.15	–20.86	–44.41	–2.24	4.88
Davies & Gregory [28]	97.6	0.843	–2.31	–2.68, –1.93	–3.54	0.25	–18.32	–38.80	3.84	5.15
Fjeld et al [31]	98.0	0.755	–0.54	–0.87, –0.20	–1.45	1.80	–3.49	–17.61	27.47	4.09
Horlick et al [10]	99.0	0.738	–0.64	–0.91, –0.37	–2.06	1.64	–4.59	–22.61	25.03	1.98
Leman et al [32]	99.2	0.682	–1.00	–1.26, –0.74	–2.59	1.08	–7.16	–21.74	16.46	1.81
Fat–free mass:										
Goran et al [15]	83.0	3.94	11.61	8.57, 14.65	–0.55	32.05	64.09	–5.17	142.6	135.7 [a]
Deurenberg et al [27]	98.9	0.78	–1.11	–1.46, –0.77	–2.06	1.57	–6.42	–19.34	18.58	1.23
Cordain et al [29]	98.8	1.10	4.58	4.18, 4.98	2.95	7.82	28.6	10.26	92.82	2.38
de Lorenzo et al [30]	97.9	1.06	0.59	0.13, 1.06	–0.94	3.69	4.84	–8.85	43.78	2.25
Houtkooper et al [33]	99.0	0.99	0.83	0.45, 1.21	–1.72	3.50	5.13	–12.35	41.57	2.13
Horlick et al [10]	99.1	0.96	2.20	1.81, 2.58	0.02	4.65	13.23	0.14	55.18	2.15
Schaefer et al [34]	97.3	1.2	–0.11	–0.72, 0.49	–2.99	2.73	–1.43	–28.01	15.04	3.80
DXA FFM:										
Our equation	95.1	3.19	–1.42	–3.13, 0.29	–8.43	6.06	–3.85	–20.22	21.91	9.52
Arpadi et al (22)	88.6	4.80	13.09	1.67, 24.50	–3.03	76.30	31.92	–11.19	149.0	425.78 [a]
Males	89.6	4.98	17.88	–4.47, 40.24	–3.03	76.30	41.94	–11.19	149.0	663.73 [a]
Females	94.8	3.36	7.60	–1.26, 16.47	–0.80	25.56	20.46	–3.21	65.65	98.98[a]
Horlick et al [10]	96.8	2.6	–0.53	–1.92, 0.87	–5.51	5.54	–1.37	–13.22	20.04	6.4

Corr. % R = regression ` between predicted and observed TBW or FFM, expressed as a percent. P = 0.000 in all cases. SEE = standard error of estimate. CI=confidence interval of mean bias.

[1] Error = predicted TBW or FFM – observed TBW or FFM; percentage error =[(predicted – observed TBW or FFM)/observed TBW or FFM] * 100; Loss of precision = (variance of difference between predicted and observed TBW or FFM)/(variance of observed TBW or FFM) expressed as a percentage.

Error and lower and upper limits for error in liters for TBW and kg for FFM. Correlation, percentage error, and loss of precision in percentages.

[a] slope deviates significantly from zero, when bias in estimate is regressed against mean estimate of the two methods (representing non–constant bias in estimates)

evaluated the 95% confidence intervals for the mean bias. Based on these criteria and the results listed in Table 4, the Leman [32] and Horlick et al [10] BIA models estimated TBW in our sample with the highest accuracy (mean bias of –1 kg and –0.64 kg respectively, with loss of precisions <2%). Both models provided 96% confidence limits within about 0.5 kg. The Arpadi et al [22] model performed most poorly in our subjects, particularly in males, where it failed to provide a loss of precision value below 100%. The confidence intervals in this model had a range of 10 kg. Removal of one male outlier did not markedly improve the overall performance of the model. With the exception of the Arpadi model, all TBW equations slightly under-estimated TBW in our subjects but still provided fairly good estimates of TBW, with 95% of estimates within 1 kg of the mean bias.

Accurate BIA models for predicting FFM in our sample included all but Goran et al [15]. The Horlick et al [10], Houtkooper et al [33], de Lorenzo et al [30], Cordain et al [29] and Deurenberg et al [27] models, where correlation coefficients were between 98–99%, were the best models, all providing confidence limits within a kilogram. The Schaefer et al [34] model performed slightly less well. FFM in our sample was somewhat over-estimated by some models [15,29 and to a slight degree by 10,30,33] and underestimated by others [27, and to a lesser degree by 34]. Although some models performed less well than others, in every case criterion and estimated values for TBW or FFM were significantly correlated.

Assessment of our model with dual energy x-ray absorptiometry and children on HAART therapy

Based on Bland Altman analysis, our equations per-

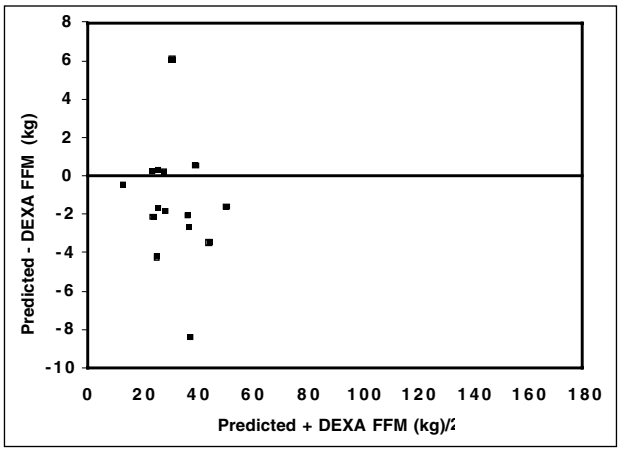

Figure 1. Bland Altman analysis showing bias in fat-free mass (FFM) predicted from our sex-specific equations listed in Table 2 and that determined from DXA in relation to mean FFM calculated from both methods.

formed well in the DXA subjects where they provided a non-significant bias in estimates of FFM when compared with DXA measures (Figure 1). Using our sex-specific equations, the mean difference in FFM was –1.4 kg, the minimum bias was –8.4 kg and the maximum bias was +6.1 kg with confidence intervals of –3.13 kg and +0.29 kg. There was a high correlation between estimates of FFM using our equation and those of DXA (R = 0.95, SEE = 3.2). When we fited a regression slope through these data (ie FFM bias in relation to the mean of DXA and our equation's estimates of FFM), it did not deviate significantly from zero, suggesting that the bias in estimates was both non-significant and constant across FFM.

In addition to our equation, the Horlick et al [10] equation estimated DXA FFM with good accuracy (Figure 2). This model outperformed most other published equations we tested in our reference group and

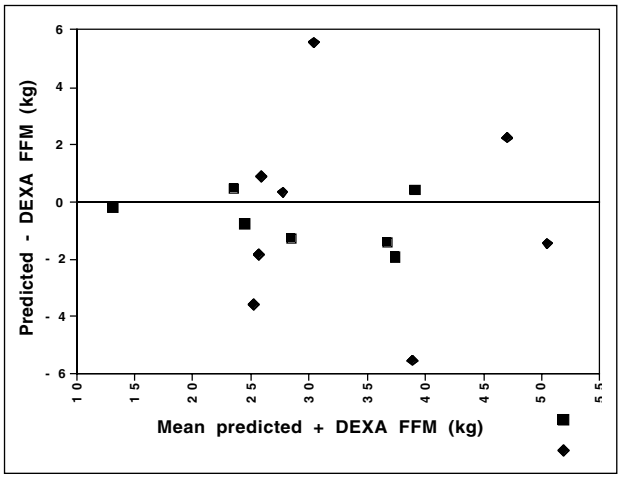

Figure 2. Bland Altman analysis showing bias in fat-free mass (FFM) predicted from Horlick et al's [10] equation listed in Table 1 and that determined from DXA in relation to mean FFM calculated from both methods.

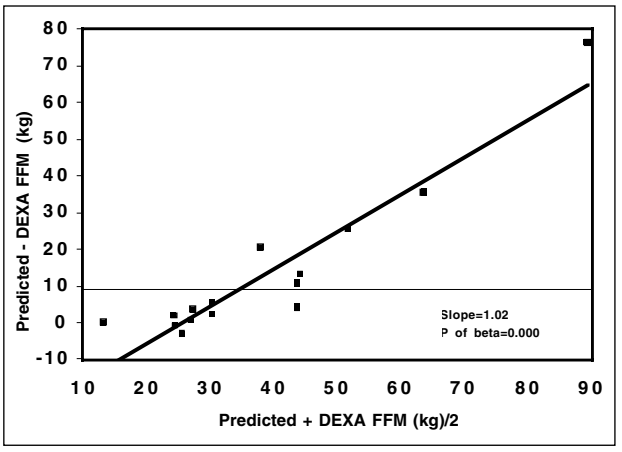

Figure 3. Bland Altman analysis showing bias in fat-free mass (FFM) predicted from the Arpadi et al. (22) equation listed in Table 1 (predicted TBW scaled to the age and sex-specific hydration fractions from Forbes (18) to estimate FFM) and that determined from DXA in relation to mean FFM calculated from both methods

performed equally well in our validation group where estimated FFM explained almost 97% of the variance in FFM by DXA, and where FFM estimates were on average only 0.5 kg below DXA measures, with 95% confidence intervals of –1.92 kg and 0.87 kg.

In contrast, the Arpadi equation [22] did not perform well in our DXA validation group where it over-estimated FFM with a non-constant bias and high percentage error (mean = 32%, minimum = –11.2%, maximum = 149%, 95% CI=1.67, 24.50 kg) and loss of precision (426%). A positive bias of 76.3 kg was seen in one male who accounted for the greater loss of precision in males (664%) as opposed to females (99%).

In addition, regression analysis demonstrated that bias between DXA and the Arpadi FFM estimates increased with increasing FFM. A least squares linear regression described the relationship between FFM estimate bias and the mean of the DXA and Arpadi FFM estimates where the slope of the regression line deviated significantly from zero (Figure 3). The Arpadi equation therefore performed similarly in both our reference and validation groups; children with TBW and DXA estimates of FFM. In both cases, the Arpadi equation over-estimated FFM and performed most poorly in individuals with high body FFM and in males in general.

Discussion

We provide a new equation for predicting body composition from bioelectric impedance analysis and total body water by deuterium dilution in HIV pediatric subjects and have evaluated the accuracy of the equation for predicting FFM from dual energy X-ray absorptiometry in a separate HIV pediatric population on HAART therapy. Our equation performed well in this group of HIV-positive children where the bias in

estimates was non-significant and constant. Our equation therefore may be used to estimate body composition in those HIV-positive children between the ages of 3 and 19 years with and without HAART therapy and who do not have clinically apparent lipodystrophy.

The use of BIA to accurately determine body composition has been fraught with difficulty and investigators have recommended its use only with age and disease-specific equations [35]. HIV in both adults and children has become two disorders; the disease prior to HAART therapy and the disease on HAART therapy. In contrast to the improved clinical condition of the patient on HAART, is the potentially adverse nutritional impact of HAART leading to the metabolic syndrome with its attendant cardiovascular and metabolic consequences. In children, lipodystrophy (the metabolic syndrome) is far less prevalent than in adults, although there is emerging evidence of sub clinical indicators of this syndrome in children [36]. Thus, knowledge of a well-performing BIA equation for children off and on HAART is important.

The interpretation of BIA in patients with clinically obvious lipodystrophy can be problematic, based on the assumptions and principles of this method [37]. Geometry of the individual (BIA works best in a cylinder), the size of the tissue, and the intrinsic electrical conductivity of the tissues (ie differentiating lean, the conducting tissue versus fat, the tissue which offers resistance) are basic assumptions in its interpretation. With clinical lipodystrophy, the abdominal adiposity changes the assumed cylindrical shape, as well as the composition of the abdominal organ (more fat), potentially making interpretation of BIA equations developed in the pre-HAART era challenging [38]. However, in our study, although these children were on HAART therapy, most did not have clinically apparent lipodystrophy. Our equation worked well in this group of children. Thus, we advocate the use of our equation in children on or off HAART who do not have clinically obvious lipodystrophy. However, we would caution its use in children with clinically apparent lipodystrophy, until there is further validation in this type of patient.

In addition, we have shown that a number of BIA models derived from healthy pediatric populations perform well in predicting TBW and FFM in HIV-positive children who are relatively healthy. These include the models of Horlick et al [10], Leman et al [32], de Lorenzo et al [30] and Houtkooper et al [33]. The Horlick et al [10] model performed very well in estimating FFM in our validation group. Although this model was developed in both HIV-negative and positive children, it performed well in our HIV-positive DXA study subjects who were generally healthy with a mean BMI over 18 and mean BMI Z score of –0.28. This model was derived from the largest study population of all models assessed here (n=1291).The predictive equation of Arpadi et al [22] was derived in a population of HIV-positive children aged 4 to 11 years. It is, therefore, surprising that this model performed poorly in our subjects. The Arpadi model showed a clear performance difference between the sexes with markedly poorer performance in males and in individuals with high FFM in general. It is therefore likely that differences exist between the males in our studies, and several of those published, and those in the Arpadi population from which their predictive equation was derived. Unfortunately Arpadi et al [22] did not provide sex-specific descriptive statistics so it is not possible to directly compare our male subjects to determine if body composition differences are present. It is, however, unlikely that a simple body composition difference exists between our male subjects and those of Arpadi and colleagues as males in our reference and validation groups themselves differ in a number of key ways as shown in Table 2, while bias in estimates derived from the Arpadi et al [22] model were comparable in both our reference and validation group participants.

A possible explanation for the discrepancy between Arpadi estimates of TBW and FFM may be the difference in morbidity between the Arpadi population and our study groups. Mean CD4 count in the Arpadi et al [22] sample was 319 cells/mm^3 (sd=330) while it was significantly higher in our reference and validation groups at 643 cells/mm^3 (sd=521) and 812 cells/mm^3 (sd=530) respectively. Depleted CD4 counts in HIV-infected patients are often associated with diarrhea and secondary illness and may be associated with hydration status and body cell mass changes in patients as well as wasting [39,40]. While we are unable to compare CD4 counts between the sexes in the Arpadi study and our own, increased bias may arise from error in FFM estimates as a result of possible alterations in the hydration fraction of lean tissue in ill HIV-positive children. Here we used the sex and age-specific lean tissue hydration fractions given by Forbes [18]. However, it is not known whether these accurately reflect hydration of fat-free tissue in sick children. Given that males have proportionately more FFM than females, alterations in the hydration fraction may result in increased error in males.

Body composition differences between the sexes may then play a role in the sex-specific biases in estimates seen here using Arpadi's BIA equation to estimate FFM. This may, in part, help to explain why poorer model performance in males was not confined to the Arpadi models but included those of Goran [15], Gregory [23], Fjeld [31], Davies and Gregory [28], and de Lorenzo [30] which produced statistically significantly higher mean TBW and FFM biases in males.

Equally, possible differences in methodologies utilized to measure TBW or FFM, to collect samples, or to control for non-aqueous deuterium exchange within the body (see Table 1), may also contribute to error differences between equations and validation samples [41, 42]. In the case of the Arpadi equation, this is however unlikely to provide an explanation as bias margins markedly exceed error margins attributable to methodological differences between studies.

We have assessed the usefulness of BIA equations for predicting body composition in HIV-positive pediatric populations both prior to and following the introduction of HAART therapies. A key finding is the usefulness of BIA for estimating body composition both in the pre-HAART and HAART eras, in the absence of severe wasting, dehydration or clinical lipodystrophy. The equation we present will be useful to monitor body composition changes over time in children on and off HAART. As HIV-infected children demonstrate adverse nutritional consequences of antiretroviral therapy, a simple, non-invasive method to detect early changes in body composition is important. However, body fat distribution cannot be ascertained with this method. Moreover, the relative constancy in bias found here between BIA and TBW and BIA and DXA measures of body composition make correcting for bias in individual measures a relatively straightforward procedure. The applicability of this equation in developing countries where there are other factors that can alter body composition or in children with clinically apparent lipodystrophy has yet to be determined.

Acknowledgments—The authors would like to thank the patients, families and staff of the GCRC for making this study possible. Dr Roubenoff is now an employee of Millennium Pharmaceuticals, Cambridge, MA. This work was supported in part by NIDDK grant 1PO1DK45734, General Clinical Research Center grants MO1-RR00054, 5 M01 RR00044, 5 M01 RR02172 from the National Center for Research Resources, NIH and NIH grant 5 P30 DA013868-02

References

1. Kotler DP, Burastero S, Wang J, Pierson Jr. RN. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. Am J Clin Nutr 1996; 64: 489S–497S.
2. Kotler DP, Thea DM, Heo Moonseong, Allison DB, Engelson ES, Wang J, Pierson Jr. RN, St Louis M, Keusch GT. Relative influences of sex, race, environment, and HIV infection on body composition in adults. Am J Clin Nutr 1999; 69(3): 432-9.
3. Miller TL, Evans S, Orav EJ, McIntosh K, Winter HS. Growth and body composition in children with human immunodeficiency virus-1 infection. Am J Clin Nutr 1993; 57: 588–92.
4. Arpadi SM, Horlick MN, Wang J, Cuff P, Bamji M, Kotler DP. Body composition in prepubertal children with human immunodeficiency virus type 1 infection. Arch Pediatr Adolesc Med 1998; 152: 688–93.
5. Miller, TL, Easley KA, Zhang W, Orav EJ, Bier DM, Luder E, Ting A, Shearer WT, Vargas JH, Lipshultz SE. Maternal and infant factors associated with failure to thrive in children with vertically transmitted human immunodeficiency virus-1 infection: The prospective, P^2C^2 human immunodeficiency virus multi-center study. Pediatrics 2001; 108: 1287–96.
6. Miller TL, Awnetwant EL, Evans S, Morris VM, Vazquez IM. Gastrostomy tube supplementation for HIV-infected children. Pediatrics 1995; 96: 696–702.
7. Miller TL, Mawn B, Orav EJ, Wilk D, Weinberg G, Nicchitta J, Furuta L, Cutroni R, McIntosh K, Burchett S, Gorbach SL. The effect of protease inhibitors on growth and body composition in HIV-infected children. Pediatrics 2001; 107: e77.
8. Arpadi SM, Cuff PA, Horlick M, Wang J, Kotler DP. Lipodystrophy in HIV-infected children is associated with high viral load and low CD4-lymphocyte percentage at baseline and use of protease inhibitor and stavudine. JAIDS 2001; 27: 30–34.
9. Brambilla P, Bricalli D, Sala N, et al. Highly active antiretroviral-treated HIV-infected children show fat distribution changes even in the absence of lipodystrophy. AIDS 2001; 15: 2415–22.
10. Horlick M, Arpadi SM, Bethel J, Wang J, Moye J, Cuff P, Pierson RN, Kotler D. Bioelectrical impedance analysis models for prediction of total body water and fat-free mass in healthy and HIV-infected children and adolescents. Am J Clin Nutr 2002; 76: 991–9.
11. Lohman TG, Roche AF, Marttorell R. Anthropometric standardization manual. Champaign, Ill: Human Kinetics Publishers, 1988.
12. Roberts SLW. Nutritional assessment manual. Iowa City, Ia: University of Iowa Hospital and Clinics, 1987.
13. United States Department of Health, Education and Welfare. Ten-state nutrition survey in the United States 1968–1970. Atlanta, Ga: Centers for Disease Control, 1972.
14. EpiInfo, version 6.04. Centers for Disease Control and Prevention, Atlanta, Georgia.
15. Goran MI, Kaskoun MC, Carpenter WH, Poehlman ET, Ravussin E, Fontvieille AM. Estimating body composition of young children by using bioelectrical resistance. J Appl Physiol 1993; 75(4): 1776–80.
16. Welle S, Forbes GB, Statt M, Barnard RR, Amatruda JM. Energy expenditure under free-living conditions in normal-weight and overweight women. Am J Clin Nutr 1992; 55: 14–21.
17. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am J Clin Nutr 1982; 35: 1169–75.
18. Forbes GB. Human body composition: growth, aging, nutrition, and activity. New York: Springer-Verlag, 1987.
19. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307–10.
20. Bland J, D Altman. Comparing methods of measurement: why plotting differences against standard method is misleading. Lancet 1995; 346: 1085–7.
21. Fleis J. The design of clinical experiments. New York: John Wiley & Sons, 1986: 1–14.
22. Arpadi SM, Wang J, Cuff P, Thornton J, Horlick M, Kotler D, Pierson RN. Application of bioimpedance analysis for estimating body composition in preburtal children infected with human immunodeficiency virus type 1. J Pediatr 1996; 129(5): 755–7.
23. Gregory JW, Greene SA, Scrimgeour CM, Rennie MJ. Body water measurement in growth disorders: a comparison of bioelectrical impedance and skinfold thickness techniques with isotope dilution. Arch Dis Child 1991; 66(2): 220–2.
24. Danford LC, Schoeller DA, Kushner RF. Comparison of two bioelectrical impedance analysis models for total body water measurement in children. Ann Hum Biol 1992; 19(6): 603–7.
25. Kushner RF, Schoeller DA, Fjeld CR, Danford L. Is the impedance index (ht2/R) significant in predicting total body water? Am J Clin Nutr 1992; 56(5): 835–9.

26. Davies PSW, Preece MA, Hicks CJ, Halliday, D. The prediction of total body water using bioelectrical impedance in well and malnourished children. Ann Hum Biol 1988; 15: 237–40.
27. Deurenberg P, van der Kooy, Paling A, Withagen P. Assessment of body composition in 8–11-year-old children by bioelectrical impedance. Eur J Clin Nutr 1989; 43: 623–9.
28. Davies PSW, Gregory JW. Body water measurements in growth disorders. Arch Dis Child 1991; 66: 1467(letter).
29. Cordain L, Whicker RE, Johnson JE. Body composition determination in children using bioelectrical impedance. Growth Dev Aging 1988; 52(1): 37–40.
30. de Lorenzo A, Sorge SP, Iacopino L, Andreoli A, de Luca PP, Sasso GF. Fat-free mass by bioelectrical impedance vs dual-energy X-ray absorptiometry (DEXA). Appl Radiat Isot 1998; 49(5–6): 739–41.
31. Fjeld CR, Freundt-Thurne J, Schoeller DA. Total body water measured by 18-O dilution and bioelectrical impedance in well and malnourished children. Pediatr Res 1990; 27(1): 98–102.
32. Leman CR, Adeyemo AA, Schoeller DA, Cooper RS, Luke A. Body composition of children in south-western Nigeria: validation of bio-electrical impedance analysis. Ann Trop Pediatr 2003; 23: 61–7.
33. Houtkooper LB, Lohman TG, Going SB, Hall MC. Validity of bioelectric impedance for body composition assessment in children. J Appl Physiol 1989; 66(2): 814–21.
34. Schaefer F, Georgi M, Zieger A, Scharer K. Usefulness of bioelectrical impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. Pediatr Res 1994; 35(5): 617–24.
35. Ellis KJ. Human body composition: in vivo methods. Physiol Rev 2000; 80: 649–80.
36. Brambilla P, Bricalli D, Sala N, Renzetti F, Manzoni P, Vanzulli A, Chiumello G, di Natale B, Vigano A. Highly active antiretroviral-treated HIV-infected children show fat distribution changes even in absence of lipodystrophy. AIDS. 2001 Dec 7; 15: 2415–22.
37. Foster KR, Lukaski HC. Whole-body impedance – what does it measure? Am J Clin Nutr 1996; 64(suppl): 388S–96S.
38. Hellerstein MK. The changing face of ADSI: translators needed. Am J Clin Nutr 1999; 70: 787–8
39. Kotler DP, Wang J, Pierson RN. Body composition studies in patients with the acquired immunodeficiency syndrome. Am J Clin Nutr 1985; 42(6): 1255–65.
40. Bedogni G, Severi S, Manzieri AM, Trunfio O, Poli M, Battistini N. Use of bioelectric impedance analysis (BIA) in children with alterations of body water distribution. Appl Radiat Isot 1998; 49: 619–20.
41. Schoeller DA, Dietz W, Van Santen E, Klein P. Total body water measurements in humans with 18O and 2H labeled water. Clin Nutr 1980; 33(12): 2686–93.
42. Pullicino E, Coward WA, Stubbs RJ, Elia M. Bedside and field methods for assessing body composition: comparison with the deuterium dilution technique. Eur J Clin Nutr 1990; 44(10): 753–76.