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A comparison of bioimpedance methods for detection of body cell mass change in HIV infection

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Earthman, Carrie P., James R. Matthie, Phyllis M. Reid, Ingeborg T. Harper, Eric Ravussin, and Wanda H. Howell. A comparison of bioimpedance methods for detection of body cell mass change in HIV infection. *J. Appl. Physiol.* 88: 944–956, 2000.—The maintenance of body cell mass (BCM) is critical for survival in human immunodeficiency virus (HIV) infection. Accuracy of bioimpedance for measuring change (Δ) in intracellular water (ICW), which defines BCM, is uncertain. To evaluate bioimpedance-estimated ΔBCM, the ICW of 21 weight-losing HIV patients was measured before and after anabolic steroid therapy by dilution (total body water by deuterium − extracellular water by bromide) and bioimpedance. Multiple-frequency modeling- and dilution-determined ΔICW did not differ. The ΔICW was predicted poorly by 50-kHz parallel reactance, 50-kHz impedance, and 200 − 5-kHz impedance. The ΔICW predicted by 500 − 5-kHz impedance was closer to, but statistically different from, dilution-determined ΔICW. However, the effect of random error on the measurement of systematic error in the 500 − 5-kHz method was 12–13% of the average measured ΔICW; this was nearly twice the percent difference between obtained and threshold statistics. Although the 500 − 5-kHz method cannot be fully rejected, these results support the conclusion that only the multiple-frequency modeling approach accurately monitors ΔBCM in HIV infection.

human immunodeficiency virus; acquired immunodeficiency syndrome; weight loss; extracellular water; intracellular water

A MEASUREMENT of the central, energy-exchanging mass of working tissue has long been sought as a standard for assessing nutritional status (40). The core reference for the central tissues of the body has, in the past, been identified as fat-free mass (FFM). However, the FFM is too broad, including extracellular water (ECW) and the structural bone matrix, which are largely involved with support and not direct oxidative energy turnover. On the other hand, body cell mass (BCM) consists of cellular components minus ECW and support tissue. The ability to measure BCM would provide a reference basis for the measurement of oxygen consumption, caloric requirements, basal metabolic rate, and work performance (51). From an ideal point of view, the intracellular water (ICW) most closely approximates the BCM (40, 51). This is because acute changes in body protein occur mainly in the cellular compartment (23), and changes in body protein are generally accompanied by changes in ICW (2).

The depletion of BCM and involuntary weight loss are common in human immunodeficiency virus (HIV) infection and are closely associated with morbidity and mortality (1, 26, 42). In fact, BCM loss has been observed to precede overt weight loss even in the early stages of HIV infection (42), and a critical level of BCM must be maintained for survival (26). Therefore, the ability to accurately monitor changes in BCM is essential to the successful treatment of individuals with HIV infection and acquired immunodeficiency syndrome. BCM can be estimated from total body potassium (TBK) measured with whole body counting, from ICW measured by dilution methods, or from total body nitrogen measured by neutron activation (16). However, whole body counting and neutron activation are expensive, and dilution methods are tedious and time consuming.

Theoretically, bioimpedance measurements can be used to estimate BCM noninvasively by measuring ICW; however, there is considerable debate concerning the best bioimpedance method to use. One group of investigators believes that ECW, ICW, and total body water (TBW) are best predicted using a bioimpedance spectroscopy (BIS) approach (11, 58). Others continue to advocate the approach first proposed by Thomasset in 1963 (53; see also Refs. 14, 19). Although various frequency combinations have since been used, Thomasset proposed the use of a fixed, single low-frequency (1-kHz) bioimpedance approach to measure ECW and a fixed, single high-frequency (100-kHz) bioimpedance approach to measure TBW. The ICW was computed as TBW minus ECW. A third group of investigators proposes that BCM can be adequately measured by a fixed, single-frequency 50-kHz measurement of impedance (Z) (43) or reactance (X) transformed into parallel X (X_p) (7, 25, 31). Whereas the latter two approaches rely on the derivation of prediction equations for TBW, ECW, and ICW using statistical methods, BIS provides a more direct measure of body water components. In

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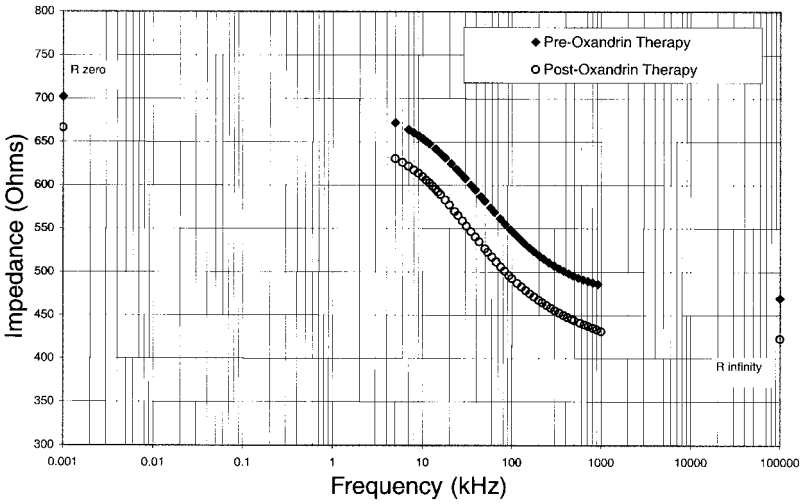

Fig. 1. Frequency vs. impedance (Z) measured on 1 subject pre- and post-Oxandrin therapy. Resistances (R) at 0 frequency (R_0) and infinity (R_∞) are represented as 0.001 and 100,000 kHz, respectively. R_0 and R_∞ were derived by modeling.

fact, BIS is the technique from which all underlying theories of the bioimpedance method evolved and implies fitting measured spectral data to a biophysical model (44).

Basic theoretical and analytic bioimpedance principles. Biological cell membranes behave as capacitors (C_m), and Z is frequency dependent (9) (Fig. 1). With direct current or at the zero frequency, there is theoretically no conduction through biological cells, and Z is purely resistive (R) and a function of ECW [R at 0 frequency (R_0) or R_E]. With alternating current, C_m charges and discharges the current at the rate of the frequency; therefore, as frequency increases, the amount of ICW measured increases. At some infinitely (∞) high frequency (>10,000 kHz or 10 MHz; Fig. 1), the charge and discharge of current through the cells become so fast that the effects of C_m become insignificant, Z becomes purely resistive (R_∞), and both ECW and ICW are fully measured. Once R_0 or R_E and R_∞ are determined, ICW resistance (R_I) can be computed as $1/R_I = 1/R_\infty - 1/R_0$ (Fig. 2). At R_0 and R_∞, the overall Z is independent of C_m; whereas, at the middle or characteristic frequency (f_c), the dependence on the value of C_m is at a maximum (Fig. 2). The f_c can also be defined as the frequency of maximum X (9). The f_c is an important term because it is computed from R_E, R_I, and C_m and thus changes with changes in ECW, ICW, or the cell membranes, respectively (30). The equation for f_c is $1/[2\pi C_m(R_E + R_I)]$.

Comparison of BIS and single-frequency approaches. Some studies comparing the BIS and single-frequency approaches report no advantage of BIS in predicting ECW, TBW, and ICW (43). These conclusions, however, are based on the prediction of absolute volume using correlation, standard error of estimate (SEE), and bias statistics. It is well known that ECW, ICW, and TBW are highly intercorrelated and that Z measured at any frequency can equally predict the absolute volume of each body water compartment (11, 43, 58). This intercorrelation would be expected because ECW and ICW are generally tightly regulated and comprise TBW. Detection of any systematic error (E_{sys}) that would impair the measurement of change in fluid compartments by a particular bioimpedance method would be obscured by the high intercorrelation of the variables when only absolute volume is predicted. For this reason, the ability to measure change should be the test of the validity of a bioimpedance method. Furthermore, correlation and SEE statistics should not be used to assess change, because neither of these statistics is sensitive to scaling; thus the predicted change could be significantly different from the actual change. For example, the correlation and SEE for the set of numbers 10, 15, 20, and 25 compared with the set 5, 7.5, 10, and 12.5 would be a perfect 1 and 0, respectively, despite the 50% difference between the sets.

Although it has been reported that the Kotler et al. (25) X_p equation detected direction of change in 89% of the subjects with a BCM change (ΔBCM) $\geq$5%, the accuracy of the prediction of the actual ΔBCM was not reported. A method that predicts direction of change (i.e., positive or negative) but is in error by 50% would have little clinical value. To assess change, multiple

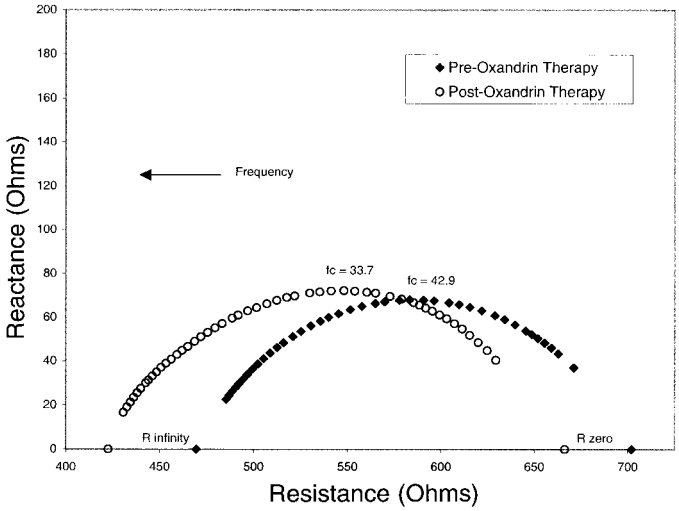

Fig. 2. R vs. reactance (X) measured on 1 subject pre- and post-Oxandrin therapy. R_0 and R_∞ were derived from modeling. X at R_0 and R_∞ was set at 0. Characteristic frequency (f_c) is frequency of maximum X.

measurements must be made, and this greatly increases the effect of random error. At the same time, that which is being measured (change) is significantly less than absolute volume, often by a factor of 10 (e.g., 2 vs. 20 liters). To accurately measure ICW change (ΔICW) in an individual, a method must have small E_{sys} and random errors. How well a method measures what it purports to measure is the major uncontrollable contributor to E_{sys}. Thus the closer the theory underlying a method matches reality, the smaller the E_{sys} and the better the measurement of change.

Van Loan et al. (55) recently reported that, after gonadal hormone replacement therapy in HIV-positive men, the BIS method accurately predicted change in FFM (ΔFFM) compared with the estimated accretion of lean tissue from nitrogen balance measured daily for 21 days with the use of 24-h urine and fecal collections. It was also reported that the BIS method predicted ΔFFM better than did dual-energy X-ray absorptiometry or deuterium (^{2}H) dilution. Because the cellular compartment would have retained most of the increased nitrogen (23), the results of this study strongly suggest that the BIS method is sensitive to ΔBCM. Given that BCM is most closely related to ICW, the aim of the present study was to determine which bioimpedance method would provide the most accurate estimate of ΔICW compared with ΔICW measured by dilution (difference between ^{2}H dilution for TBW and bromide dilution for ECW). It was hypothesized that BIS would provide valid measures of ICW (BCM) and would estimate ΔICW with better accuracy than would other bioimpedance methods. To accomplish this aim, measures of ICW by bioimpedance were compared with criterion dilution measures of ICW in patients with HIV infection receiving anabolic steroid therapy for treatment of weight loss.

SUBJECTS AND METHODS

Study design. This study was part of a larger clinical evaluation of HIV-infected individuals who were undergoing treatment for weight loss with the anabolic steroid oxandrolone (Oxandrin, BTG Pharmaceuticals, Iselin, NJ). Oxandrolone is an orally administered testosterone derivative (2-oxa analog of 17α-methyldihydrotestosterone) that was approved by the FDA more than 30 years ago "as adjunctive therapy to promote weight gain after weight loss following extensive surgery, chronic infections, or severe trauma, and in some patients who without definite pathophysiologic reasons fail to gain or to maintain normal weight" (3). Dose-dependent anabolic effects of oxandrolone in normal healthy subjects were demonstrated by decreased nitrogen excretion and increased protein synthesis with an anabolic potency 6.3 times that of methyltestosterone (17). Oxandrolone has been used successfully to treat growth failure (Turner's syndrome) in children (48) and has been safely used to treat protein-energy malnutrition in alcoholic liver disease (4, 5, 35, 36). Oxandrolone has also been shown to significantly increase body weight (Wt) and muscle function after burn injury (12).

In the present study, measurements of TBW and ECW were taken on 21 subjects at baseline and post-Oxandrin therapy. Time on the study averaged 20 wk; only four subjects deviated from this mean, with final measurements at 6, 28, 35, or 40 wk.

Subjects. Subjects were recruited from an outpatient clinic with a population of ~600 HIV-infected patients. Any individual with a history of weight loss, who was receiving standard antiretroviral therapy and whose primary care physician prescribed Oxandrin therapy, was recruited to participate in the study. Twenty-one subjects (20 men, 1 woman) between the ages of 27 and 56 yr, with a mean age of 41 ± 7.7 yr, completed this study. Of these, 20 subjects (19 men, 1 woman) were maintained on an Oxandrin dose of 20 mg/day, whereas one subject's dose was reduced to 10 mg/day after 1 mo. At the time of baseline assessment, the subjects had experienced an average weight loss of 9%. Ethnic distribution of the subject population was 72% (15 subjects) Caucasian, 14% (3 subjects) Hispanic, and 14% (3 subjects) African-American. The study protocol was approved by the Human Subjects Committee of The University of Arizona, and written, informed consent was obtained from all participants.

Procedures. Subjects came to the Metabolic Monitoring Laboratory at The University of Arizona for comprehensive assessment of body composition before initiation and after termination of Oxandrin therapy. To standardize testing conditions and to avoid acute changes in hydration status, subjects were instructed to abstain from vigorous exercise for at least 12 h and to abstain from alcohol and caffeine consumption for 48 h before assessment. Subjects reported to the laboratory on test days in the morning, after an 8- to 12-h fast.

Anthropometric assessment. At each visit, Wt was measured to the nearest 0.1 kg with a digital platform scale [Kubota model K-10–300L-A, Chugai Boyeki (America), Commack, NY]. Subjects were instructed to wear the same lightweight clothing at each visit. Standing height (Ht) was measured to the nearest 0.5 cm with a stadiometer (Narragansett Machine, Providence, RI).

Dilution volume. Criterion estimates of TBW and ECW were derived from ^{2}H and bromide dilution, respectively. While still in a fasted state and after baseline urine and venous blood samples were collected, subjects were given a weighed dose of deuterium oxide (^2H_2O; 99.8 atom percent; Isotec, Miamisburg, OH) equivalent to 0.15 g/kg TBW. To calculate the dose, an estimate of TBW was obtained by single-frequency bioimpedance analysis by using the Kushner et al. (29) equation. Immediately afterward, the subjects drank a measured dose of a sodium bromide (NaBr; Sigma Chemical, St. Louis, MO) solution, providing 1.0 ml of 3% (wt/vol) solution/kg body wt. Dose solutions were administered from paper cups and were followed by a 30-ml wash with deionized, distilled water. A 3-h equilibration period, during which subjects did not ingest anything, was allowed for ECW determination by bromide dilution. A second blood sample was drawn at 3 h postbromide dosing, and a light snack was provided immediately after the second blood draw. At 5 and 6 h after dosing with ^2H_2O, urine samples were collected for TBW determination by ^{2}H dilution. Blood samples were collected in 10-ml tiger-top vacutainer tubes and centrifuged at 3,000 rpm for 20 min to separate serum. Two 2.5-ml aliquots of each serum sample and three 5-ml aliquots of each urine sample were stored frozen at −80°C in airtight cryogenic vials until analysis.

Frozen urine samples, diluted dose (1:5 wt/wt), and tap water were packed on dry ice in a sealed, insulated biomailer, shipped overnight, and analyzed for ^{2}H enrichment by the National Institute of Diabetes and Digestive and Kidney Diseases laboratory in Phoenix, AZ. After treatment with activated charcoal, enrichment of ^{2}H was determined by using an isotope ratio mass spectrometer (model Delta S;

Finnigan MAT, Bremen, Germany) and hydrogen equilibration with Pt catalyst (250 μm diameter of 3% by weight Pt beads; Shoko, Minatoku, Tokyo, Japan), as described by Coplen and Harper (10). The reproducibility of this method is superior to the zinc reduction method. With 28 runs, the 841 δ‰ standard measured 840.1 ± 1.9 (SD) δ‰ and the −73 δ‰ standard measured −72.9 ± 1.4 δ‰. Appropriate dilutions of the doses were also used as internal controls. Stock dose solution was prepared twice during the study; *dose 1* was measured as 759.8 ± 1.1 (SD) δ‰ (n = 20) and *dose 2A* was 733.5 ± 1.4 δ‰ (n = 21). The TBW was calculated by using a two-point method as described by Schoeller et al. (47) using the ^{2}H enrichment of the urine sample collected 5 h postdose that had, in all cases, greater ^{2}H enrichment than did the sample collected at 6 h.

Frozen serum samples were shipped overnight to the Pennington Biomedical Research Center, Baton Rouge, LA, for bromide analysis. Serum bromide enrichment was determined by the HPLC method of Miller and Cappon (38) by using an HPLC (model 1090M; Hewlett Packard, Palo Alto, CA) equipped with autosampler and diode-array detector. The analytic precision of the bromide measurements by this technique is ~2% (38). The coefficient of variation (CV) for the bromide assay in this study was ~5%. The ECW was calculated by using a correction of 10% for nonextracellular distribution and 5% for Donnan equilibration (39). The ICW was estimated as the difference between TBW and ECW. The accuracy of a dilution-determined ICW volume is unknown because ECW is impossible to measure directly. Nonetheless, the bromide dilution method for ECW is estimated to have an accuracy of 5% (54). Although in animal studies there is found to be some variability in the ^{2}H dilution method for TBW, it is reported to be accurate within 1.5% (22). Most laboratories do not report accuracy, but rather mean intra-assay CV, which is an indication of repeatability (43). For estimating the propagation of ICW error, we assumed the ^{2}H and bromide dilution methods to have accuracies of 1.5 and 5%, respectively.

The pre-Oxandrin therapy dilution-determined fluid compartments were as follows: TBW, 39.9 liters; ECW, 17.4 liters; and ICW, 22.5 liters (Table 1). A 1.5% error for ^{2}H dilution-measured TBW is 0.60 liter, and a 5% error for bromide dilution-measured ECW is 0.87 liter. Squaring and adding these two terms ($0.60^2 + 0.87^2$) equals 1.12. The square root of 1.12 is 1.06. Because volume change is being assessed, 1.06 was multiplied by 1.414 (square root of 2) and is 1.50 liters. This is a 6.7% error in 22.5 liters of ICW, assuming that the errors in the dilution methods are not correlated.

Bioimpedance measurements. After the measurement of Wt and Ht and ingestion of the ^2H_2O and NaBr doses, fasting subjects assumed a supine position for 30 min. The effects of orthostatic fluid shifts on Z can be considerable (i.e., 3% on recumbence, an additional 2% after 10 min, and an additional 4% after 4 h) (28). Thus the duration of recumbency before bioimpedance measurements are performed will affect the prediction of absolute volume, but, for estimation of fluid volume changes, the important thing is that repeated measurements be performed at identical time points during the recumbent period. This error was minimized by taking all measurements at the same 30-min mark after recumbence. With the use of the standard wrist-to-ankle measurement protocol (27), a Z measurement was made with a multiple-frequency bioimpedance device (model 4000B or 4200; Xitron Technologies, San Diego, CA). The multiple-frequency devices were confirmed to be in calibration according to manufacturer instructions. Three patients had baseline measurements taken with the 4000B device. All subsequent measurements were performed with the newer 4200 device. Comparison of R and X measurements by the two devices for seven subjects and 35 data points (frequencies 5, 50, 100, 200, and 500 kHz) showed no significant differences. Data were transmitted directly from the analyzer to a personal computer and controlled by the software programs supplied with the devices. All measurements were taken on the right side of the body by using disposable electrodes (IS4000; Xitron Technologies).

To evaluate the BIS method, Z and phase (θ) spectral data were fit to the Cole model (9) by using nonlinear least squares curve-fitting software supplied with the devices. The Cole model consists of R_E, R_I, C_m, and exponent α. Because it is not possible to measure Z at sufficiently low or high frequencies, the ends of the semicircle that is formed when R and X are plotted are extrapolated to R_0 and R_∞ by using mathematical curve-fitting techniques (Fig. 2). Then, as discussed, R_I is computed. In practice, R_E, R_I, C_m, α, and time delay (T_d) are simultaneously computed. The term C_m is related to the thickness of the cell membrane (9). Exponent α is a Cole model term that represents the suppression of the semicircle below the R axis (Fig. 2) (9). The suppressed semicircle is thought to be a result of a distribution effect caused by the different sizes and shapes of cells (49). The term T_d is used to account for any error introduced by a frequency-invariant time delay and allows for an improved fit to the Cole model. The T_d is caused by the interaction between contact R, stray capacitance, and transmission line effects. A detailed review of T_d and the method by which Z data are fit to the Cole model has been published by De Lorenzo et al. (11). The ECW and ICW volumes were predicted by using the values of Cole model terms R_E and R_I in equations developed by Xitron Technologies from mixture theory (11, 34). The TBW was calculated as ECW plus ICW. The resistivity constants used to predict ECW and ICW were developed by De Lorenzo et al. These constants (scalars) were selected because they previously predicted the expected sized ECW and TBW spaces [i.e., a TBW-to-Wt ratio of 0.60 and an ECW-to-TBW ratio (ECW/TBW) of 0.40 for healthy young men] (11). It is important to note, however, that differences in dilution methods makes calibrating the scaling of the Z method (zero bias) extremely difficult (11, 32, 57, 59). Fortunately, any bias between methods has less of an effect on volume change because it is smaller than absolute volume by a factor of 10.

The Z-to-body water volume relationship is not 1:1; therefore, reliability tests should be applied to both Z measurements and predicted water volume. Chumlea et al. (8) described reliability analyses using estimates of interobserver mean absolute difference (MAD) and technical error (TE). The MAD was computed as the difference between the mean of Z measurements taken at two time points by one observer and the mean of corresponding measurements by a second

Table 1. *Subject characteristics*

	Pre-Oxandrin Therapy	Post-Oxandrin Therapy
Height, cm	175.4 ± 6.6	175.4 ± 6.6
Weight, kg	68.1 ± 10.0	70.4 ± 10.2*
TBW, liters	39.9 ± 4.5	42.7 ± 5.5*
ECW, liters	17.4 ± 2.3	17.8 ± 2.6
ICW, liters	22.5 ± 3.5	24.9 ± 4.0*
ECW/TBW	0.44 ± 0.04	0.42 ± 0.04

Values are means ± SD for 21 human immunodeficiency virus positive (HIV+) subjects. TBW, total body water criterion determined by deuterium dilution; ECW, extracellular water criterion determined by bromide dilution; ICW, intracellular water criterion estimated as TBW − ECW. *Significantly different from pre-Oxandrin therapy, $P < 0.001$ by repeated-measures ANOVA.

observer. The TE provides an estimate of the magnitude of the error for individual measurements and was computed as the square root of the sum of $d^2/2N$, where d is the difference between paired measurements for an individual and N is the number of individuals. Because the same-day interobserver MAD and TE have been reported to be only 1% for R up to 1 MHz and X up to 100 kHz for the Xitron 4000B device (8), no further analyses of precision were performed. For the BIS method, no differences ($P > 0.05$; paired t-test) were found for repeat measurements taken on 10 healthy subjects (with no change in electrodes) or on a simulated electronic circuit (34). The CV for the BIS method was 1.3% for ECW and 1.9% for TBW after repeat measurements (with repositioned electrodes) taken using the Xitron 4000B device on 29 patients with edema and 11 patients with gastrointestinal cancer (20). Because the present study used a new multifrequency bioimpedance device (Xitron 4200), additional measurements were taken to assess the MAD, TE, and CV. A total of 90 measurements was obtained by taking two consecutive measurements each day over 5 consecutive days (4 consecutive days for 3 subjects) plus the day following a weekend on eight subjects (6 men, 2 women; ages 26–50 yr) for the computation of MAD. To compute TE, the *day 1* and *day 2* measurements were paired for each subject. The CV for ECW, ICW, and C_m was calculated by using the first measurement for each subject on each day (45 measurements). To simulate field conditions, the subjects performed the measurements on each other after instructions were given on the first day. Measurements were taken at the same time each day to minimize biological variation. Subjects were asked to wear similar clothing each day and were instructed to refrain from eating large meals for several hours before the test and to void immediately beforehand. Ht was measured on the first day, and Wt was measured daily. Bioimpedance measurements were obtained as described above at the 4- to 6-min mark after the recumbence. The raw data were fit to the Cole model, and ECW and ICW volumes were computed.

The interday, interobserver TE with the use of the Xitron 4200 device, with electrode repositioning, was 0.072 liter for ECW and 0.110 liter for ICW. The average MAD for daily consecutive measurements was 0.022 liter for ECW and 0.066 liter for ICW. The mean MAD for the entire period was 0.56 liter (0.28 − 1.02 liter) for ECW and 1.08 liter (0.76 − 1.50 liter) for ICW. Relative to the dilution-determined ECW and ICW volumes obtained in this study (Table 1), this represents 2.8 and 3.8% maximum variation (including normal biological variation) in the ECW and ICW, respectively. The interday, interobserver CVs for ECW and ICW measurements with electrode repositioning were 1.28% (0.013 liter) and 1.72% (0.017 liter), respectively.

To evaluate the validity of the single low-frequency method for predicting ECW volume, the following 5-kHz equations were used: the Deurenberg et al. (14) Z [$ECW_{5Z(D)}$], Hannan et al. (20) R [$ECW_{5R(H)}$], and Segal et al. (50) R [$ECW_{5R(S)}$]. The ECW was also estimated by subtracting the predicted ICW from the predicted TBW by using the Kotler et al. (25) 50-kHz X_P [$ICW_{50X(K)}$] and Z [$TBW_{50Z(K)}$] equations, respectively. To evaluate the single high-frequency method for predicting TBW, the following equations were used: $TBW_{50Z(K)}$ (25), Deurenberg et al. (14) 100-kHz R [$TBW_{100R(D)}$], Segal et al. (50) 100-kHz R [$TBW_{100R(S)}$], Hannan et al. (20) 200-kHz R [$TBW_{200R(H)}$], and Hannan et al. (19) 500-kHz R [$TBW_{500R(H)}$]. For the three subjects whose baseline measurements were made using the 4000B device, R measured at 204 and 488 kHz was used to compute TBW. Because the average differences for seven subjects between R measured at 200 and 204 kHz and between R measured at 488 and 500 kHz were only 0.3 ± 0.5 and 0.8 ± 0.7%, respectively, no further analyses were performed.

To evaluate the single-frequency method for predicting ICW, the ECW predicted by the 5-kHz equations was subtracted from the TBW predicted by the 100-, 200-, and 500-kHz equations. Two published 50-kHz equations developed specifically from subjects with HIV infection were also evaluated (25, 43). For the $ICW_{50X(K)}$ method, X was transformed as suggested into X_P and used in published male and female exponential equations to predict TBK (25). To obtain ICW volume, predicted TBK was divided by 150 mmol. This is valid because potassium is primarily distributed in ICW, and the relation between TBK and BCM is through the TBK-to-ICW relationship (40). The Paton et al. (43) 50-kHz Z equation [$ICW_{50Z(P)}$] was also used. For 17 subjects and 38 measurements, no difference was observed between R and X ($\alpha = 0.05$; $P = 0.063$ and $P = 0.139$, respectively) measured at 50 kHz by the Xitron 4000B or 4200 devices and the RJL model 101A device (RJL Systems, Clinton Township, MI).

Data analysis. To evaluate the fit to the Cole model, the total weighted least squared error was computed by the modeling routine. The fit error is expressed as a ratio between conformance to model to accuracy specification of the device. A ratio of 1 would indicate that conformance to model was equal to device performance. The expected accuracy at each measured frequency is established as a pair of arrays (Z and θ). The error ratio is then established at each modeling point by dividing the modeling error by the corresponding stored array error. Additional statistical analyses were carried out by using SPSS version 8.0 software (SPSS, Chicago, IL). For descriptive statistics, means and SDs were computed. Repeated-measures ANOVA was used to compare pre- and post-Oxandrin therapy Wt and dilution-determined TBW, ECW, and ICW volumes.

Pearson's product moment correlation (r) and SEE were computed for absolute ECW, ICW, and TBW volumes measured by dilution and predicted by the various Z methods. It is established that the various Z methods equally predict absolute volume (43, 58) and that an accurate prediction of absolute volume does not validate a method for predicting volume change. In addition, the various Z equations have been calibrated to different dilution methods that yield differently sized ECW and TBW spaces (11, 32, 57, 59). Therefore, any bias between methods could simply be due to differences in the dilution method used for calibration. Because correlation and SEE statistics are insensitive to scaling (32), these are appropriate statistics for evaluating the similarities between equations for predicting absolute volume. No further statistical analysis was performed on absolute volume data.

Volume changes were calculated by subtracting baseline volumes from the volumes obtained post-Oxandrin therapy. Pearson's product moment correlation for fluid volume change by dilution and each bioimpedance method, the average change, and the root mean squared error (RMSE) were computed (41). The RMSE was computed by subtracting the average error of the measured change ($E_{avg\ chg}$; the difference between the predicted and measured average change) from the error of each individual measurement, squaring each value, and then computing the mean and its square root. The RMSE computed in this way serves as a measure of random error in each measure of change. For an infinitely large sample size, $E_{avg\ chg}$ is equivalent to the E_{sys} of a method. For all other sample sizes, to estimate E_{sys} in a method, the effects of random error on the measurement of E_{sys} must be determined. This was accomplished by dividing the RMSE by the square root of the sample size ($RMSE/\sqrt{n}$), and then adding

and subtracting this term from $E_{avg\ chg}$ ($E_{avg\ chg} \pm RMSE/\sqrt{n}$). The E_{sys} likely falls within the range of values produced. Two-tailed paired *t*-tests were used to determine whether estimated average ΔECW, ΔTBW, and ΔICW for each method compared with the dilution method were statistically different. Because the changes in fluid volumes in some subjects were found to be similar in magnitude (100%) to the random measurement error, a 90% confidence level ($\alpha = 0.10$) was used to determine statistical significance. This reduced the probability of making a type II error and increased the power of the experiment (41). An α of 0.05 was used for comparing instruments because the uncertainty was expected to be far less (1%). The null hypothesis was that the test mean was not different from the criterion (dilution) mean.

Bland-Altman plots were not constructed because determining both the random error and E_{sys} is a more in-depth analytic approach rather than a simple visual analysis. However, to evaluate how well each method predicted ΔICW at the individual level, the difference between predicted ΔICW by the various Z methods and the dilution method was computed for each subject, and the results were categorized as ≤1.0, 1.5, 2.0, 2.5, or 3.0 and >3.5 liters. The total percent represented by each category and the cumulative percent were computed. This presentation of the data is useful because the prediction accuracy of the individual data points can be categorized.

RESULTS

Study findings of fluid compartment change after Oxandrin therapy indicate that both TBW and ICW increased considerably ($P < 0.0005$; repeated-measures ANOVA), whereas ECW did not change (Table 1). Consequently, ECW/TBW decreased. The data fit the Cole model with high precision as evidenced by the low total least squares fit error ratio (Table 2). The fit error ratio for the pre-Oxandrin therapy data does not include the three subjects measured at baseline with the Xitron 4000B device. The routine that was used to model the 4000B data does not display a fit error ratio. However, the fit was rated as good, which means that the fit error ratio equaled 1, and the correlation of fit for these three subjects was >0.997. Consistent with theory, R_E was greater than R at 5 kHz and continued to decrease with increasing frequency. Interestingly, C_m increased as would likely occur with an increase in intracellular hydration (44). The data used for evaluating the various bioimpedance equations are shown in Table 2.

Table 2. *Bioimpedance characteristics of the subjects*

	Pre-Oxandrin Therapy	Post-Oxandrin Therapy
	Cole model	
Fit error ratio	0.28 ± 0.06	0.29 ± 0.05
R_E, Ω	614.02 ± 68.44	588.24 ± 66.99
R_∞, Ω	406.17 ± 43.24	377.64 ± 41.61
R_I, Ω	1,214.73 ± 221.48	1,070.16 ± 177.55
C_m, nF	2.24 ± 0.54	2.58 ± 0.53
T_d, ns	1.24 ± 3.31	0.98 ± 1.08
α	0.66 ± 0.02	0.66 ± 0.01
f_c, kHz	41.47 ± 7.44	39.29 ± 5.92
	Single frequency	
R-5 kHz, Ω	588.0 ± 62.8	557.3 ± 61.6
R-50 kHz, Ω	501.1 ± 52.2	469.2 ± 49.4
X-50 kHz, Ω	59.4 ± 10.7	59.4 ± 10.1
Z-50 kHz, Ω	504.7 ± 52.5	473.0 ± 49.8
R-200 kHz, Ω	449.9 ± 47.3	418.6 ± 44.8
R-500 kHz, Ω	429.2 ± 45.3	398.2 ± 43.1

Values are means ± SD for 21 HIV+ subjects. R_E, Cole model term representing resistance ECW; R_∞, resistance at infinite frequency; R_I, Cole model term representing resistance ICW; C_m, Cole model term representing cell membrane capacitance; T_d, frequency-invariant time delay; f_c, characteristic frequency or frequency at maximum reactance. R-5 kHz, resistance at 5 kHz; R-50 kHz, resistance at 50 kHz; X-50 kHz, reactance at 50 kHz; Z-50 kHz, impedance at 50 kHz; R-200 kHz, resistance at 200 kHz; R-500 kHz, resistance at 500 kHz.

Prediction of absolute body water volume. As reported previously, the prediction of absolute ECW, TBW, and ICW volume was similar for all methods tested. Because all 5-kHz ECW equations provided similar predictions, only the $ECW_{5R(H)}$ equation (20) is reported. Similarly, the two 100-kHz TBW equations evaluated (14, 50) resulted in predictions similar to those obtained using 50 and 200 kHz. Thus only the predictions from the $TBW_{50Z(K)}$ (25), $TBW_{200R(H)}$ (20), and $TBW_{500R(H)}$ (19) equations are reported. The single-frequency equations used for the predictions being reported are shown in Table 3. Correlation and SEE for ECW estimates by all methods ranged from 0.78 to 0.92 and 1.04 to 1.70 liter, respectively (Table 4). Subtraction of the $ICW_{50X(K)}$-predicted ICW from the $TBW_{50Z(K)}$-predicted TBW produced the best correlation and SEE for ECW (Table 4). For the prediction of absolute TBW volume, correlation and SEE for all methods ranged from 0.90 to 0.97 and 1.15 to 2.09 liter, respectively (Table 4). The $TBW_{50Z(K)}$ equation predicted TBW with the highest correlation and lowest SEE.

The prediction of absolute ICW volume was similar for all five methods evaluated, with correlation and SEE ranging from 0.76 to 0.88 and 1.70 to 2.31 liters, respectively (Table 4). Although the $ICW_{50Z(P)}$ equation was developed for men, it is unlikely that its use for the one female subject in this study altered the overall results. This would be particularly true for change data because the measurement of change is far less sensitive to scaling differences between methods.

Prediction of body water volume change. The correlation of ΔECW was similar for the BIS and $ECW_{5R(H)}$ methods and slightly higher for the $TBW_{50Z(K)} - ICW_{50X(K)}$ approach (Table 5). The bromide-determined ΔECW was small, and all methods detected this. The ΔECW predicted by BIS was different ($P = 0.022$) from that estimated by the dilution method. All methods exhibited E_{sys}, because zero (a perfect answer) was not a possible outcome. For every method, the error was of positive polarity (Table 5; Fig. 3).

The correlation of ΔTBW was similar for all methods, ranging from 0.73 to 0.82 (Table 5). However, for the $TBW_{50Z(K)}$ and $TBW_{200R(H)}$ approaches, the predicted ΔTBW was different ($P = 0.008$ and $P = 0.003$, respectively) from that determined by the dilution method. In addition, the random error (RMSE) was almost as large as the ΔTBW predicted by these methods (Table 5). There was no difference between the ΔTBW predicted by BIS and $TBW_{500R(H)}$ ($P = 0.109$ and $P = 0.429$,

Table 3. *Equations used to predict total body water, extracellular water, and intracellular water from single-frequency bioimpedance data*

Reference	Pneumonic	Equation	Subject Population	Criterion Method
		Total body water		
Kotler et al. (25)	$TBW_{50Z(K)}$	(*1*) $0.58[(Ht^{1.62}/Z^{0.70})(1.0/1.35)] + 0.32\ Wt - 3.66$	Healthy and HIV+ men, diverse ethnicities	^3H dilution
Hannan et al. (20)	$TBW_{200R(H)}$	(*2*) $0.239\ (Ht^2/R_{200}) + 0.189\ Wt + 2.97\ S + 5.46$	Patients with gastrointestinal diseases	^3H dilution
Hannan et al. (19)	$TBW_{500R(H)}$	(*3*) $0.399\ (Ht^2/R_{500}) + 0.114\ Wt + 5.69$	Male and female surgical patients	^3H dilution
		Extracellular water		
Hannan et al. (20)	$ECW_{5R(H)}$	(*4*) $0.178\ (Ht^2/R_5) + 0.0688\ Wt + 3.77$		^{77}Br
Kotler et al. (25)	$TBW_{50Z(K)} - ICW_{50X(K)}$	Eq. (*1*) − Eq. (*5*)		
		Intracellular water		
Kotler et al. (25)	$ICW_{50X(K)}$	(*5*) $\{0.76[(Ht^{1.60}/X_P^{0.5})(59.06)] + 18.52\ Wt - 386.66\}/150$		TBK
Paton et al. (43)	$ICW_{50Z(P)}$	(*6*) $0.265731\ (Ht^2/Z_{50}) + 0.111448\ Wt - 2.59$	HIV+ men	$^2H_2$ dilution (TBW) and bromide dilution (ECW)
Hannan et al. (20)	$TBW_{200R(H)} - ECW_{5R(H)}$	Eq. (*2*) − Eq. (*4*)		
Hannan et al. (19)	$TBW_{500R(H)} - ECW_{5R(H)}$	Eq. (*3*) − Eq. (*4*)		

Ht, standing body height in cm; Wt, body weight in kg; R_{200}, R_{500}, R_5: resistance at 200, 500, 5 kHz, respectively; Z_{50}, impedance at 50 kHz; X_P, parallel reactance; S, value = 1 for men or 2 for women; ^3H, tritium; ^2H, deuterium; TBK, total body potassium; $TBW_{50Z(K)}$, TBW predicted by Z at 50 kHz (25); $TBW_{200R(H)}$, TBW predicted by R at 200 kHz (20); $TBW_{500R(H)}$, TBW predicted by R at 500 kHz (19); $ECW_{5R(H)}$, ECW predicted by R at 5 kHz (20); $TBW_{50Z(K)} - ICW_{50X(K)}$, ECW predicted by the difference between 50-kHz Z-predicted TBW and a 50-kHz parallel X-predicted ICW (25); $ICW_{50X(K)}$, ICW predicted by X_P at 50 kHz (25); $ICW_{50Z(P)}$, ICW predicted by Z at 50 kHz (43); $TBW_{200R(H)} - ECW_{5R(H)}$, ICW predicted by the difference between 200-kHz R-predicted TBW and 5-kHz R-predicted ECW (20); $TBW_{500R(H)} - ECW_{5R(H)}$, ICW predicted by the difference between 500-kHz R-predicted TBW and 5-kHz R-predicted ECW (19, 20).

respectively). The RMSE and the predicted average change were better for the $TBW_{500R(H)}$ approach compared with the BIS method (Table 5). Additionally, $TBW_{500R(H)}$ had less E_{sys} than did the other methods, because only for this method was zero (a perfect result) a possible outcome (Table 5; Fig. 3).

The correlation of ΔICW was similar for all methods, ranging from 0.59 to 0.68 (Table 5). The RMSE was also similar among methods. Only the ΔICW predicted by BIS did not differ ($P = 0.570$) from the dilution method (Table 5). In addition to being statistically different from criterion, ICW predicted by $ICW_{50X(K)}$, $ICW_{50Z(P)}$,

Table 4. *Extracellular water, total body water, and intracellular volumes predicted by various bioimpedance methods*

Method	Pre-Oxandrin Therapy			Post-Oxandrin Therapy		
	Mean ± SD	*r*	SEE	Mean ± SD	*r*	SEE
		Extracellular water				
Br, liters	17.4 ± 2.3			17.8 ± 2.6		
BIS, liters	17.0 ± 2.1	0.78	1.49	17.8 ± 2.3	0.78	1.70
$ECW_{5R(H)}$, liters	17.9 ± 1.7	0.80	1.43	18.6 ± 2.0	0.79	1.66
$TBW_{50Z(K)} - ICW_{50X(K)}$, liters	20.3 ± 2.9	0.89	1.09	20.9 ± 3.2	0.92	1.04
		Total body water				
^2H, liters	39.9 ± 4.5			42.7 ± 5.5		
BIS, liters	40.4 ± 6.0	0.90	2.03	43.9 ± 6.9	0.93	2.09
$TBW_{50Z(K)}$, liters	41.9 ± 5.0	0.97	1.15	43.8 ± 5.4	0.97	1.37
$TBW_{200R(H)}$, liters	37.7 ± 3.7	0.97	1.19	39.4 ± 4.2	0.96	1.58
$TBW_{500R(H)}$, liters	42.4 ± 4.7	0.95	1.38	45.0 ± 5.5	0.95	1.81
		Intracellular water				
$^2H - Br$, liters	22.5 ± 3.5			24.9 ± 4.0		
BIS, liters	23.4 ± 4.5	0.81	2.06	26.1 ± 4.9	0.92	1.65
$ICW_{50X(K)}$, liters	21.6 ± 2.8	0.88	1.70	22.9 ± 3.0	0.92	1.60
$ICW_{50Z(P)}$, liters	21.4 ± 3.0	0.76	2.31	22.8 ± 3.4	0.84	2.26
$TBW_{200R(H)} - ECW_{5R(H)}$, liters	19.8 ± 2.1	0.87	1.74	20.8 ± 2.3	0.93	1.54
$TBW_{500R(H)} - ECW_{5R(H)}$, liters	24.5 ± 3.0	0.84	1.93	26.4 ± 3.6	0.89	1.87

Br, ECW criterion determined by bromide dilution; BIS, bioimpedance spectroscopy-predicted ECW, TBW, or ICW (11); ^2H, TBW criterion determined by deuterium dilution; $^2H - Br$, ICW estimated by the difference between the TBW and ECW criterions; SEE, SE of estimate.

Table 5. *Extracellular water, total body water, and intracellular water volume changes predicted by various bioimpedance methods*

Method	Change, liters	r	E_{avgchg}, liters	P ($\alpha = 0.10$)	RMSE, liters	RMSE/$\sqrt{n}$, liters	$+E_{sys}$, liters	$-E_{sys}$, liters
				Extracellular water				
Br	0.4 ± 1.2							
BIS	0.8 ± 0.9*	0.75	0.43	0.022	0.78	0.17	0.60	0.26
$ECW_{5R(H)}$	0.7 ± 0.7	0.73	0.30	0.114	0.82	0.18	0.48	0.12
$TBW_{50Z(K)} - ICW_{50X(K)}$	0.6 ± 0.9	0.79	0.22	0.182	0.72	0.16	0.38	0.06
				Total body water				
^2H	2.8 ± 2.4							
BIS	3.5 ± 2.3	0.73	0.65	0.109	1.71	0.37	1.02	0.28
$TBW_{50Z(K)}$	1.9 ± 1.5*	0.81	−0.94	0.008	1.45	0.32	−0.62	−1.26
$TBW_{200R(H)}$	1.7 ± 1.3*	0.82	−1.12	0.003	1.48	0.32	−0.80	−1.44
$TBW_{500R(H)}$	2.6 ± 2.0	0.82	−0.24	0.429	1.35	0.30	0.06	−0.54
				Intracellular water				
$^2H - Br$	2.4 ± 1.8							
BIS	2.7 ± 1.8	0.59	0.22	0.570	1.63	0.36	0.58	−0.14
$ICW_{50X(K)}$	1.3 ± 0.8*	0.64	−1.16	0.002	1.47	0.32	−0.84	−1.48
$ICW_{50Z(P)}$	1.4 ± 1.1*	0.63	−1.04	0.004	1.43	0.31	−0.73	−1.37
$TBW_{200R(H)} - ECW_{5R(H)}$	1.0 ± 0.7*	0.68	−1.42	0.000	1.47	0.32	−1.10	−1.74
$TBW_{500R(H)} - ECW_{5R(H)}$	1.9 ± 1.4*	0.67	−0.54	0.093	1.38	0.30	−0.24	−0.84

Change values are means ± SD. E_{avgchg}, the difference between the predicted and measured average change; RMSE, root mean squared error computed by subtracting the average error of the measured change from the error of each individual measurement, squaring each value, then computing the mean and its square root; E_{sys}, systematic error computed as $E_{avgchg} \pm RMSE/\sqrt{n}$. *Significantly different from criterion, $P < 0.10$.

and $TBW_{200R(H)} - ECW_{5R(H)}$ had a random error (RMSE) that was larger than the predicted ΔICW. The BIS method had far less E_{sys} than did all other methods. The $E_{avg\ chg}$ was small (0.22 liter), and only for the BIS method was zero a possible outcome (Table 5; Fig. 3). The predicted average ΔTBW by $TBW_{500R(H)}$ had an error of −0.24 liter, and the predicted average ΔECW by $ECW_{5R(H)}$ had an error of +0.30 liter. Because ICW is computed by the high- and low-frequency method as TBW minus ECW, the error in the predicted average ΔICW is determined by subtracting the ECW error from the TBW error. It is important to note that this resulted in virtually twice the error (−0.54 liter) in the predicted average ΔICW by $TBW_{500R(H)} - ECW_{5R(H)}$ (Table 5). As shown in Table 6, for all but the BIS and $TBW_{500R(H)} - ECW_{5R(H)}$ methods, approximately one-third or more of the predictions had an error as large as the mean criterion ΔICW of 2.4 liters (Table 5). The $ICW_{50X(K)}$, $ICW_{50Z(P)}$, and $TBW_{200R(H)} - ECW_{5R(H)}$ methods predicted ΔICW very poorly. Although the $TBW_{500R(H)} - ECW_{5R(H)}$ method performed slightly better than the other single-frequency methods, 33% of

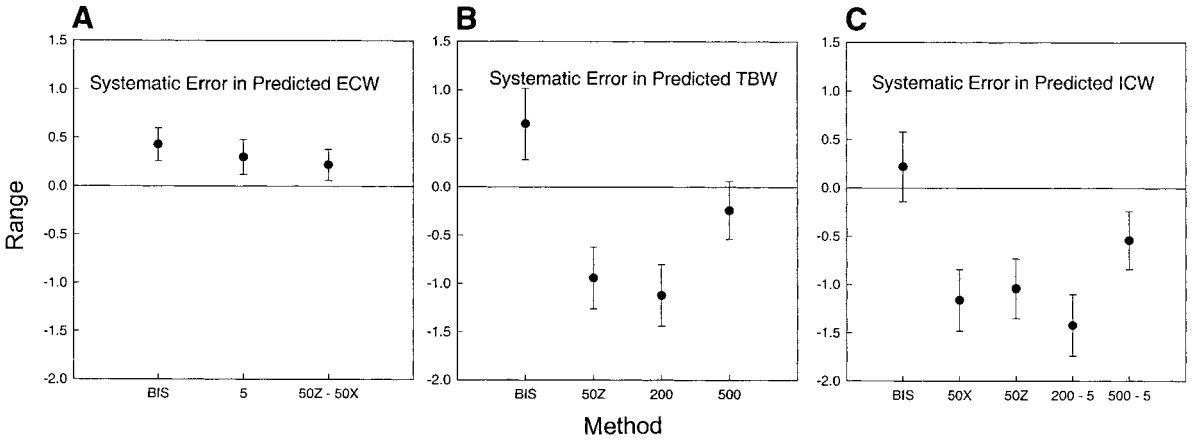

Fig. 3. Systematic error in the various Z methods for predicting extracellular water (ECW; *A*), total body water (TBW; *B*), and intracellular water (ICW; *C*). Methods are described as follows. *A*: BIS, bioimpedance spectroscopy-predicted ECW, TBW, or ICW (11); 5, ECW predicted by R at 5 kHz (20); 50Z − 50X, ECW predicted by difference between 50-kHz Z-predicted TBW and 50-kHz parallel X-predicted ICW (25). *B*: 50Z, TBW predicted by Z at 50 kHz (25); 200, TBW predicted by R at 200 kHz (20); 500, TBW predicted by R at 500 kHz (19). *C*: 50X, ICW predicted by parallel X at 50 kHz (25); 50Z, ICW predicted by Z at 50 kHz (43); 200 − 5, ICW predicted by difference between 200-kHz R-predicted TBW and 5-kHz R-predicted ECW (20); 500 − 5, ICW predicted by difference between 500-kHz R-predicted TBW and 5-kHz R-predicted ECW (19, 20).

Table 6. *Difference between intracellular water volume change predicted by criterion and by various bioimpedance methods*

Difference From Criterion, liters	BIS			50 X			50 Z			200 − 5			500 − 5		
	No. of subjects	Percent of total	Σ Percent	No. of subjects	Percent of total	Σ Percent	No. of subjects	Percent of total	Σ Percent	No. of subjects	Percent of total	Σ Percent	No. of subjects	Percent of total	Σ Percent
≤1.0	9	42.9	42.9	7	33.3	33.3	5	23.8	23.8	6	28.6	28.6	10	47.6	47.6
≤1.5	5	23.8	66.7	3	14.3	47.6	7	33.3	57.1	3	14.3	42.9	1	4.8	52.4
≤2.0	3	14.3	81.0	4	19.1	66.7	3	14.3	71.4	1	4.8	47.6	7	33.3	85.7
≤2.5	2	9.5	90.5	2	9.5	76.2	2	9.5	81.0	4	19.1	66.7	2	9.5	95.2
≤3.0	1	4.8	95.2	5	23.8	100.0	3	14.3	95.2	5	23.8	90.5	1	4.8	100.0
>3.5	1	4.8	100.0	0	0.0	100.0	1	4.8	100.0	2	9.5	100.0	0	0.0	100.0

BIS, predicted change (Δ) in ICW (11); 50 X, ΔICW predicted by X_P at 50 kHz (25); 50 Z, ΔICW predicted by Z at 50 kHz (43); 200 − 5, ΔICW predicted by the difference between 200-kHz R-predicted TBW and 5-kHz R-predicted ECW (20); 500 − 5, ΔICW predicted by the difference between 500-kHz R-predicted TBW and 5-kHz R-predicted ECW (19, 20).

the predictions of ΔICW were only within 2.0 liters of criterion. The $TBW_{500R(H)} - ECW_{5R(H)}$ method either predicted ΔICW very well or only moderately. Such a disproportionate distribution of results suggests the emergence of error under certain conditions. On the other hand, the distribution of the BIS results suggests that this method is primarily affected only by random error.

DISCUSSION

R would be expected to change when ECW and ICW change, but it was of interest that C_m increased. In vitro studies have shown C_m to be inversely related to cell membrane thickness (9); therefore, an increase in C_m suggests swelling of the cells and thinning of the membranes (44). Such a finding appears consistent with the theory of Häussinger et al. (21) that an increase in cellular hydration acts as an anabolic proliferative signal. However, not only are there many factors that can affect C_m, but it can change when there is no change in ICW. For example, C_m increased 45% from pre- to posthemodialysis with virtually no change in ICW (32). De Lorenzo et al. (11) discussed the difficulties of using C_m to estimate ICW (BCM). Furthermore, its use appears unnecessary because ICW can be determined. Although repeat measurements of C_m on healthy adults and a simulated circuit showed no significant differences (34), the accuracy of determining C_m is dependent on the accuracy of the model fit. Evaluation of the accuracy of fit to the Cole model has been fully discussed by De Lorenzo et al. For repeated measures, the CV for C_m in this study was 5%.

Because it is difficult to relate f_c, defined as the frequency of maximum X, to a Z plot, it is useful to conceptualize f_c as defined by Schwan (49). He defined f_c as the frequency corresponding to Z at the midpoint between Z at frequencies zero and infinity (Fig. 1). At any frequency other than zero and infinity, the proportion of ICW volume measured varies with f_c, and f_c varies when ECW, ICW, or C_m change (30, 32). Thus predictions of ECW using anything other than R_0 should be less sensitive to ΔECW, because a portion of ICW will be included in the measurement. Theory (9), mathematical simulation, and initial in vivo results suggest that this is so (32). In this study, the BIS prediction of ECW was statistically different from the criterion. However, the random error effect on the measurement of E_{sys} was 0.17 liter or 43% of the measured average change of 0.40 liter (Table 5). Furthermore, the random error (RMSE) in each measure of change was, for all bioimpedance methods, larger (0.72–0.82 liter) than the average dilution ΔECW. Therefore, ΔECW was too small to draw any conclusions from the findings of this study (Table 5). It is important to consider that an $E_{avg\ chg}$ of 0.4 liter (Table 5; Fig. 3) represents only 2% of a total ECW volume of 17.5 liters (Table 1). The BIS method has been used successfully to predict ΔECW in comparison with bromide dilution (15), with net fluid balance during surgery (52), and with volume removed by ultrafiltration during dialysis (60). However, it needs to be clearly established whether it performs better than the fixed low-frequency (e.g., 5-kHz) approach. Gudivaka et al. (18) reported that only an equation using Cole model term R_E accurately predicted ΔECW. All predictions were corrected by 1.6% based on the assumption that change in plasma albumin affects the total ECW volume. Coincidentally, 1.6% was effectively the same as the magnitude of the under- and overprediction of ΔECW using the Xitron ECW equation. Because the ECW equation published by Xitron (34) corrects for mixture effects, the additional 1.6% correction applied by Gudivaka et al. was, in effect, a double correction.

The $TBW_{500R(H)}$ method predicted ΔTBW well; however, these results need to be qualified. The f_c values for the subjects in this study ranged from 30 to 66 kHz and are similar to the 40–60 kHz measured for healthy subjects (11). The range of f_c values obtained makes the difference between Z at 500 kHz and infinity small (Table 2; Fig. 1). Had the f_c values been higher, less ICW would have been measured, and the sensitivity of 500 kHz to ΔTBW would have been reduced. This is supported by the finding that the 50- and 200-kHz methods did not accurately predict ΔTBW. It has long been known that f_c changes considerably when tissue hydration changes (30). For example, f_c values >200 kHz have been reported in hemodialysis patients (11), and f_c values >500 kHz have been reported in young children with severe diarrheal disease (37). Although it is unsound to fit a theory to a result, the ratio of the

measurement frequency to f_c may be useful for exploring the cause for the results of this study. For a measurement frequency of 200 kHz and an f_c of 50 kHz, the ratio is 4 (200/50). This suggests that, to achieve the same results when f_c is 125 kHz, a measurement frequency of 500 kHz would be required. Following this reasoning, if f_c is 300 kHz, a measurement frequency of 1.2 MHz would be required to produce the same poor prediction of ΔTBW that 200 kHz yielded in this study. The fact that the 500-kHz equation (19) accurately predicted ΔTBW may be due in part to the similarity of f_c values for the subjects of this study (Table 2) to the 40- to 60-kHz values determined for healthy subjects (11). This suggests that a measurement frequency-to-f_c ratio of 10 (500/50) may be needed to accurately predict ΔTBW by using a single-frequency measurement. If this were so, a measurement frequency of 2 MHz would be needed if f_c were 200 kHz. However, using a very high single frequency to reduce the error introduced by f_c would still be problematic, because a twofold increase in frequency increases measurement error by a factor of 4. Thus a measurement at 1 MHz is four times less accurate than a measurement at 500 kHz. This partially explains why multiple-regression analyses provide the best TBW predictions at frequencies between 200 and 500 kHz and why accuracy decreases progressively at frequencies >500 kHz (13). Although the BIS method correctly predicted ΔTBW in this study, it did have E_{sys} (Table 5; Fig. 3). Because TBW is predicted as ECW plus ICW, the E_{sys} in the predicted TBW may have simply been carried over from ECW and ICW. The *P* value for BIS-predicted TBW was only 9% above the threshold for considering the results different, whereas the effect of random error on the measurement of E_{sys} was 0.373 liter or 13% of the measured average change of 2.8 liters (Table 5). Although the TBW predicted by BIS was not different from the criterion ($P = 0.109$), further research is needed to confirm these findings. An $E_{avg\ chg}$ of 0.65 liter is only a 1.6% error in 40 liters of TBW (Table 1).

Although it has recently been reported that a multiple-regression equation including X_P accurately predicted ΔICW in healthy, nonobese subjects (18), we found that the $ICW_{50X(K)}$ and $ICW_{50Z(P)}$ methods predicted ΔICW poorly. This discrepancy may be at least partly explained by the fact that Gudivaka et al. (18) used an α of 0.05 to test for differences between methods, which can increase the risk of a type II error in a situation in which sample sizes and the changes measured are small. An evaluation of how well a method performed is not possible without the actual *P* values for each method. Furthermore, from a physics standpoint, at any single frequency, the effect of a change in ECW, ICW, or C_m on X cannot be discerned; therefore, any prediction of ICW using X_P would be circumstantial (32). The ΔICW predicted by the fixed high- and low-frequency methods [$TBW_{200R(H)} - ECW_{5R(H)}$ and $TBW_{500R(H)} - ECW_{5R(H)}$] was also considered statistically different from the criterion. A poor single-frequency prediction of TBW or ECW would result in a poor prediction in ICW because it is computed as TBW minus ECW by these methods. In addition, a change in f_c causes errors in the predicted ECW and TBW in opposite directions, thereby magnifying ICW error (Table 5). When f_c increases, a fixed low frequency (5 kHz) becomes closer to zero, and a fixed high frequency (500 kHz) becomes further from infinity. The opposite would occur when f_c decreases (Fig. 2). In this study, the changes in f_c were very small, but f_c can change by as much as 50% (11). Such a large change in f_c would cause large errors in the predicted ΔICW. Although the ΔICW predicted by $TBW_{500R(H)} - ECW_{5R(H)}$ was different ($P = 0.093$) from that determined by criterion methods, it is important to consider these results carefully. The probability ($P = 0.093$) that the results achieved were true was only slightly (7%) below the significance level for considering the results equal. On the other hand, the effect of random error on the measurement of E_{sys} was 0.30 liter or 12–13% of the measured average change of 2.4 liters (Table 5). As the *P* value becomes smaller and the null hypothesis becomes more unreasonable, however, the point at which results are accepted or rejected is subjective. As such, the findings of this study do not lead to a firm rejection of the $TBW_{500R(H)} - ECW_{5R(H)}$ method.

Albeit the strong prediction of TBW using 500 kHz suggests otherwise, the prediction of ΔTBW using a single high-frequency measurement should be poor because the R_E and R_I differ by a factor of 3 (44). To predict TBW volume, it must be assumed that TBW resistivity is constant. Such an assumption seems tenuous considering that a simple change in the ECW/ICW can alter TBW resistivity.

These results support the BIS or modeling approach. The implication is that the BIS method predicted ΔBCM better than the other methods did solely because it is based on the Cole model. To explore if this were true, multiple-regression analysis was used to predict ICW pre-Oxandrin therapy by using variables Ht, Wt, and R_I. According to the common single-frequency model used, Ht^2/R, not R alone, should correlate to water volume; thus multiple regression was also performed by using variables Ht^2/R_I and Wt. Wt alone can be predictive of ΔICW, but it is often an additive term in published multiple-regression equations (25) and was therefore included. The resulting equations were used to predict ICW pre- and post-Oxandrin therapy. The ΔICW was computed, and two-tailed paired *t*-tests were performed (α = 0.10). The equation using Ht^2/R_I and Wt performed best. The predicted ICW volume was similar to that obtained from the other methods (Table 4) with pre- and post-Oxandrin correlations of 0.77 and 0.88 and SEEs of 2.27 and 1.99, respectively, but the prediction of ΔICW was poor ($P = 0.015$). We did not log transform R_I before running the regression analysis as suggested by Kotler et al. (25). It was not clear from the literature how to replicate the procedure, and it did not appear valid because X_P predicted ΔBCM poorly (Table 5). The implication of these findings is that a mathematical modeling approach that solves for R_0 and R_∞ and accounts for any mixture effects should be used (11, 33).

The underlying basis for the mixture theory equations developed by Xitron (33) is that the relationship between Z and body water should be explained scientifically rather than randomly by using multiple-regression analysis. Use of a physical model (the Cole model) is an important first step, but, according to theory, the Z-to-body water association is complex and nonlinear because of conductor (ECW and ICW) and nonconductor (fat and bone) interactions. As such, Cole model terms R_E and R_I are model terms and not simply ECW and ICW R values, respectively. To accurately predict ECW and ICW volume, mixture effects must be taken into account. The results of this study seem to support this premise, because the prediction of ΔBCM solely by the Cole model was poor. The mixture theory equations used in this study have been fully described (11). Gudivaka et al. (18) recently reported that the ICW mixture equation published by Xitron (34) predicted ΔICW poorly, and that only a multiple-regression equation, including Cole model term R_I, accurately predicted ΔICW. The equation tested was the original equation developed by Xitron, which assumes a linear relationship between ECW/ICW and TBW resistivity, whereas the present equation in the Xitron software, developed in 1993, assumes a nonlinear relationship. De Lorenzo et al. (11) discussed the differences between equations.

It is generally understood that FFM is not an ideal measure of BCM because it includes ECW and solids (40). Van Loan et al. (55) recently reported the successful prediction of ΔFFM using bioimpedance. The ΔFFM determined by BIS accurately reflected the nitrogen balance change in wasted acquired immunodeficiency syndrome patients after gonadal hormone therapy (55). The first successful prediction of ECW, TBW, and FFM with the use of BIS methods was reported in 1992 (34). The BIS FFM equation developed by Xitron is (D'_{ECW} V_{ECW}) + (D'_{ICW} V_{ICW}), where V_{ECW} and V_{ICW} are ECW and ICW volumes, and D'_{ECW} and D'_{ICW} are the apparent densities of the ECW and ICW and their associated materials, respectively. The D'_{ECW} (1.45 g/cm^3 for men, 1.48 g/cm^3 for women), and D'_{ICW} (1.31 g/cm^3 for men, 1.23 g/cm^3 for women) terms were adjusted by obtaining the best fit using FFM, predicted from dilution volumes, against the densitometrically determined FFM (56). The traditional single-frequency 50-kHz R-predicted TBW estimate of FFM (TBW/0.73) assumes that the ECW-to-ICW, protein-to-ICW, and water-to-bone relationships are fixed (59). The Xitron FFM method is theoretically less affected by changes in ECW/ICW and would better account for changes in body protein, assuming protein/ICW remained fixed. However, it is still dependent on assuming a fixed water-to-bone relationship. The above D'_{ECW} and D'_{ICW} coefficients are weighted so that the extracellular compartment influences the prediction more than the intracellular compartment does. This is probably because the early multifrequency devices made by Xitron predicted ECW better than ICW. With the use of the above constants, if ECW and bone do not change, the Xitron FFM method would reasonably predict ΔBCM. However, FFM will never be a valid measure of BCM, because a change in bone or water changes the assumed water-to-bone relationship and causes error. On the other hand, the BIS ICW-to-BCM relationship is only dependent on ICW/protein.

The BIS methodology used in this study has been successfully validated in other clinical populations (15, 22, 57), and there is evidence that ECW and ICW differences can be detected between patient groups (24, 57). Whereas these previous findings add strength to the findings of this study, it is unknown whether the results of this study are applicable to other populations. Hannan et al. (19) reported that the BIS method did not accurately predict ECW and TBW, but the potential cause was not discussed. Hannan et al. (personal communication) have observed f_c values approaching 1 MHz, and this is consistent with the recent report of Meyer et al. (37). With an f_c of 1 MHz, which is equal to the highest frequency measured, an accurate determination of R_∞ would be extremely difficult because there would be data on only one side of the semicircle to fit (Fig. 2). Even with these constraints, however, it appears that ECW can still be predicted accurately (15).

When f_c becomes very high, it is doubtful that either BIS or a fixed, single high-frequency measurement will accurately predict ΔICW. If an accurate bioimpedance measurement were possible over a range of frequencies up to 5 MHz, accurate computation of the Cole model might be possible when f_c is very high. Without consistent use of good protocol, the bioimpedance technique can also be adversely affected by inaccurate electrode placement, geometry differences, orthostatic fluid shifts, inappropriate limb abduction, changes in ion concentration, changes in core and skin temperature, and changes in vascular perfusion. Whereas many of the above factors can be controlled, there may be instances when they cannot. Clinicians should have a thorough understanding of the sources and magnitude of potential error in the Z method. A detailed description of the error sources in the Z method can be found in the papers by Kushner et al. (28) and Scharfetter et al. (45). Each clinical population presents unique limitations for the method. For example, when body water is not evenly distributed in the body segments, as in ascites, the wrist-ankle approach used in this study would not yield valid results (46). Furthermore, when changes in body water occur faster than equilibration can occur in the body segments, as would be the case with infusion and ultrafiltration, a segmental approach must be used (52, 60).

In conclusion, the results of this study indicate that the $ICW_{50X(K)}$, $ICW_{50Z(P)}$, and $TBW_{200R(H)} - ECW_{5R(H)}$ methods predict ΔICW poorly. The ΔICW predicted by $TBW_{500R(H)} - ECW_{5R(H)}$ was different from the criterion. However, the effect of random error on the measurement of E_{sys} was 12–13% of the average measured ΔICW with the use of this method. This was two times larger than the difference between the obtained and critical threshold statistics. Although the $TBW_{500R(H)} - ECW_{5R(H)}$ method predicted ΔICW with only marginal accuracy, it can be neither clearly rejected nor accepted

by the results of this study. However, because of potential variations in f_c and the considerable E_{sys} inherent in this method, the utility of the $TBW_{500R(H)} - ECW_{5R(H)}$ method is questionable. E_{sys} indicates a poor underlying basis that will not be corrected by simply regressing equations against larger data sets (6, 25). Random error decreases with sample size, but E_{sys} does not. The BIS mixture theory approach convincingly provided the best prediction of ΔICW (ΔBCM). This was particularly evident when individual results were compared. It can be concluded that the BIS measurement is useful as a field method for monitoring ΔBCM in HIV-infected populations.

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