Estimation of Body Fluid Volumes Using Tetrapolar Bioelectrical Impedance Measurements

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Mathematical equations using tetrapolar bioelectrical resistive (R) and reactive (Xc) impedance measures were developed and crossvalidated to predict total body water (TBW) and corrected bromide space (CBS) in two independent samples ($n = 110$). Height^2 per low R was the best predictor of TBW ($R = 0.96$) and CBS ($R = 0.92$). When the influence of TBW was removed from CBS and dependent variables, height^2 per low Xc was the best predictor ($R = 0.50$) of CBS. Double crossvalidation of each sample showed that observed and predicted TBW ($R = 0.978$ and 9.986) and CBS (0.937 and 0.907) were significantly related ($p < 0.001$), and there was no difference ($p > 0.05$) between the values. The lines representing the relationships between observed and predicted values had regression coefficients not different than the line of identity. Data from both samples were combined to give a representative multiple regression equation to predict TBW and CBS. This study establishes the validity of the tetrapolar bioelectrical impedance method to assess body fluid volumes in humans.

THE HUMAN BODY consists of four principal chemical components—water, protein, fat, and bone mineral (4). In routine assessment of body composition, fat and fat-free (e.g. the combination of water, protein, and bone mineral ash) masses are estimated using indirect methods (11). However, water, representing the largest single constituent of the body (55–60% body weight), remains a compositional variable that is troublesome to measure.

Traditional approaches for the estimation of total body water (TBW) rely upon determinating isotope dilution spaces using tritium or deuterium (16,17), and a variety of analytical methods to quantify these tracers in physiological fluids (6,11). Oxygen-18 also has been used as a tracer to measure TBW; it does not overestimate TBW, as do the hydrogen isotopes, because of the isotope exchange with labile hydrogen atoms in the body (21,22). Because the isotope dilution approach is costly and time consuming, routine determination of TBW is limited.

A more practical method of TBW assessment is the use of bioelectrical impedance plethysmography. This approach is based upon the principle that an applied electrical current is conducted by the body fluids and electrolytes. Thomasset and his coworkers first used this method to develop mathematical models to predict TBW and extracellular fluid volume estimated as bromide dilution space (3,25,26). Using two needle electrodes placed subcutaneously on the dorsal surfaces of one hand and the opposite foot, a 100 μA, A.C. current at two frequencies (1 kHz and 100 kHz) was introduced into the body. The measured low-frequency impedance was related to the bromide space, and the high-frequency impedance was related to TBW. Technical limitations generally restricted the application of this two-electrode method (2,10).

Recently, a tetrapolar bioelectrical impedance plethysmograph was used as a noninvasive method to assess TBW (7,9). This approach was unique because, rather than using multiple frequencies and measuring impedance, it introduced a constant frequency current (50 kHz and 800 μA, A.C.) and determined the geometric components of the measured impedance using $Z = \sqrt{R^2 + Xc^2}$, where Z is impedance, R is resistance, and Xc is reactance. By using phase-sensitive electronics, R is the sum of the in-phase electrical vectors and Xc is the sum of the out-of-phase electrical vectors. Also, four surface electrodes rather than two needle electrodes were used.

The tetrapolar bioelectrical impedance method was

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used to describe changes in impedance variables after alterations in body fluid volume. Resistance (R), reactance (Xc), and impedance (Z) were determined for two groups of patients with fluid accumulation before and after treatment (15,24). After fluid loss, Xc demonstrated the greatest relative change (50% increase) whereas R and Z exhibited the lowest relative change (10–15% increase). The significance of these changes in bioelectrical impedance variables relative to body water compartments has not been determined. However, because the treatments used to eliminate the excess fluids resulted in a loss of TBW, presumably by reducing fluid volume in the extracellular compartment, it is hypothesized that the individual impedance variables may be useful predictors of TBW and the extracellular or bromide space.

The present study was undertaken to develop and to crossvalidate equations using bioelectrical impedance variables to predict TBW and corrected bromide space (CBS) in independent samples of volunteers. We also sought to determine the importance of Xc as an independent predictor of CBS.

MATERIALS AND METHODS

Subjects: Volunteers were 110 healthy females and males aged 20–73 years. Each volunteer gave written informed consent after receiving verbal and written descriptions of the purpose and procedures of this investigation. All experimental procedures were approved by the Institutional Review Board of the University of North Dakota School of Medicine.

Each subject came to the laboratory between 0700–0800 hours after an overnight fast. Standing height, without shoes or socks, was measured to the nearest 0.1 cm with a stadiometer (Harpenden, Pembrokeshire, U.K.) mounted on a wall. Body weight, with the subject in minimal clothing, was determined on a calibrated scale (Toledo Scale Co., model 2831, Worthington, OH) accurate to ±0.2 kg.

Body fluids: Total body water was assesed by deuterium dilution, and CBS was estimated by bromide dilution. Subjects consumed a mixture containing 10 g of 99.8% deuterium oxide (ICN Biomedical, Cambridge, MA), 0.15 $\text{ml} \cdot \text{kg}^{-1}$ of a 3% (w/v) sodium bromide (Sigma, St. Louis, MO), and 300 ml of distilled-deionized water. Venous blood specimens were obtained before and 4 h after ingestion of the tracers. All urine was collected during the equilibration period.

Plasma and urine samples were analyzed for deuterium oxide concentrations after sublimation using infrared spectroscopy (8). The analytical precision and error of this method are 2.5% (8). Bromide (Br) concentrations in physiological fluids were assayed using fluorescent excitation analysis with a precision of 3% and an accuracy of 4.2% (18).

Body water was calculated using the retained deuterium oxide dose and the 4-h plasma deuterium oxide concentration:

$$\text{TBW (L)} = \frac{(\text{D}_2\text{O dose given} - \text{D}_2\text{O in urine})\ \text{mg}}{(\text{4-h plasma D}_2\text{O})\ \text{mg} \cdot \text{ml}^{-1}} \qquad \text{(Eq. 1)}$$

Corrected bromide space also was calculated using the dilution principle, and including the factors suggested by Bell (1):

$$\text{CBS(L)} = \frac{(\text{Br dose given} - \text{Br in urine})\ \text{mEq}}{(\text{4-h plasma Br})\ \text{mEq} \cdot \text{L}^{-1}} \times 0.90 \times 0.95 \times 0.94 \qquad \text{(Eq. 2)}$$

where 0.90 = fraction of administered bromide that remains extracellular, 0.95 = Donnan equilibration factor, and 0.94 = fraction of water in plasma.

Plasma sodium and potassium concentrations were determined using flame photometry (model #443, Instrumentation Laboratory, Inc., Lexington, MA). Plasma chloride concentrations were assayed using an adaptation of the method of Schales and Schales (20). Plasma osmolality was measured by vapor pressure depression using thermocouple hygrometry (model #5500, Wescor Inc., Logan, UT).

Bioelectrical impedance: Determinations of R and Xc were made using a four-terminal impedance plethysmograph (RJL Systems, model 101, Detroit, MI). Measurements were made in the morning after an overnight fast. Each volunteer, clothed but without shoes or socks, was supine on a cot made of nonconductive materials. Aluminum foil spot electrodes (Contact Products, No. M6001, Dallas, TX) were positioned in the middle of the dorsal surfaces of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively, and also medially between the distal prominences of the radius and the ulna and between the medial and lateral malleoli at the ankle. Specifically, the proximal edge of one detector electrode was in line with the proximal edge of the ulnar tubercle at the wrist, and the proximal edge of the other detecting electrode was in line with the medial malleolus of the ankle. The current-introducing electrodes are placed a minimum distance of the diameter of the wrist or ankle beyond the paired detector electrode. A thin layer of electrolyte gel was applied to each electrode before application to the skin. An excitation current of 800 μA, A.C., at 50 kHz was introduced into the volunteer at the distal electrodes of the hand and foot, and the voltage drop was detected by the proximal electrodes. Measurements of R and Xc were made using electrodes placed on the ipsilateral and contralateral sides of the body as shown in Fig. 1.

Statistical analyses: Subjects were randomly assigned to either a model or a validation group. Multiple regression analysis was applied to identify the best predictors of TBW and CBS in each group. The regressions were conducted in a stepwise manner (19) using the independent variables of height, weight, age, gender (dummy coded as male = 1 and female = 0), and all impedance measures.

A double crossvalidation (5) of the prediction of body water compartments was performed by comparing the measured water variables in the model group with the predicted values calculated using the equation derived in the validation group and the data from the model group, and vice versa. Measured and predicted values also were compared using the dependent *t* test (23).

Partial correlation analysis (5) was used to determine the importance of Xc in predicting CBS. In this ap-

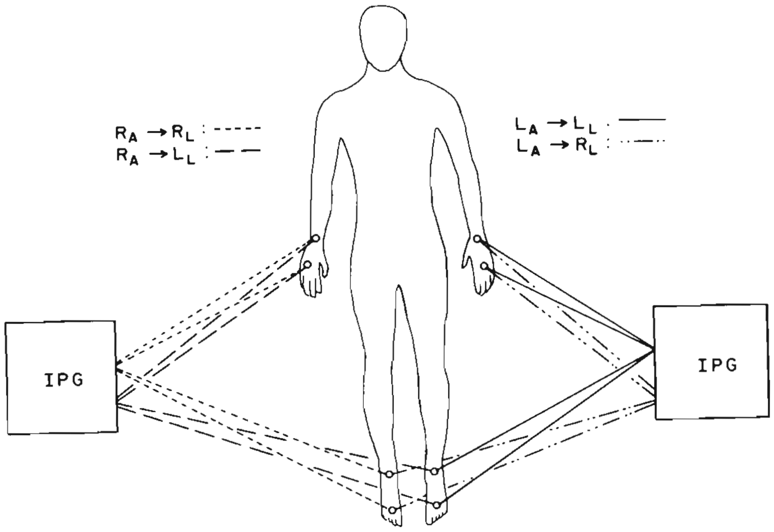

Fig. 1. Diagram of electrode arrangements used with the tetrapolar impedance plethysmograph (IPG). R = right, L = left, A = arm, L = leg.

proach, the influence of TBW is first removed from CBS and independent variables, and then the best predictors of CBS are determined. Comparisons of correlation coefficients were made using the Z transformation (23).

RESULTS

The physical characteristics of the volunteers are summarized in Table I. A wide range was found in age, TBW, CBS, and impedance measures. On the average, the males had larger body fluid volumes and lower R and Xc values than the females. Individual plasma electrolyte concentrations and osmolalities were within the range of normal values for our laboratory.

Prediction models: The multiple regression equations derived for the estimation of TBW in both groups are presented in Table II. Similar variables were identified as predictors of TBW in each sample of volunteers. The single best predictor of TBW was height squared over resistance ($Ht^2 \cdot R^{-1}$), where R is the lowest value observed among all electrode placements.

The models for the prediction of CBS are shown in Table III. As in the prediction model for TBW, $Ht^2 \cdot R^{-1}$ was the best predictor of CBS. Another significant ($p < 0.05$) predictor of CBS was $Ht^2 \cdot Xc^{-1}$. Interestingly, both R and Xc were the lowest of the four measured values for these variables.

Crossvalidation of the impedance method: Shown in Fig. 2 (upper panel) is the relationship between TBW measured in the model group and TBW predicted using the equation derived in the validation group and the impedance values from the model group. The data are distributed along a line whose slope is similar to 1 ($F = 0.17$, $p = 0.68$) and whose intercept is not different ($F = 0.38$, $p = 0.54$) than zero. There was no significant ($t = -1.0$, $p = 0.30$) difference between measured (36.6 ± 1.1 L; mean ± S.E.M.) and predicted (36.4 ± 1.1 L) values.

Fig. 2 (lower panel) also shows the strong relationship between TBW values measured in the validation group and predicted using the equation derived in the model group. The slope ($F = 0.36$, $p = 0.55$) and intercept ($F = 0.64$, $p = 0.43$) of the line are similar to the line of identity. Measured (36.9 ± 1.2 L) and predicted (37.1 ± 1.2 L) TBW values were similar ($t = 0.99$, $p = 0.33$).

Because the equations crossvalidated in each group of subjects, the TBW and impedance data from each sample were combined and analyzed to yield a common multiple regression prediction equation:

$$\begin{aligned} \text{TBW (L)} &= 0.377\ \text{Ht}^2/\text{R} \\ &+ 0.14\ \text{Weight} - 0.08\ \text{Age} + 2.9\ \text{Gender} + 4.65 \\ R^2 &= 0.975,\ \text{SEE} = 1.50 \end{aligned} \qquad \text{(Eq. 3)}$$

where R is the lowest value in ohms measured among the four electrode placements, Ht is in centimeters, weight is in kilograms, age is in years, gender is 1 = male and 0 = female, and SEE is standard error of the estimate.

The relationship between measured and predicted CBS values is not as strong ($r = 0.937$ and 0.907 vs. 0.978 and 0.986; $p < 0.001$) as the relationships observed among the TBW data. Fig. 3 shows the slightly more variable CBS data. The relationship between CBS measured in the model group and predicted using the equation developed in the validation group using the impedance data from the model group is given in Fig. 3

TABLE I. VOLUNTEER CHARACTERISTICS.

	Model Group		Validation Group	
	Females	Males	Females	Males
N	28	25	31	26
Age, yr	45.1 ± 2.6*	29.4 ± 2.4	44.2 ± 2.6	39.6 ± 2.5
Height, cm	163.3 ± 1.4	175.7 ± 1.5	163.2 ± 1.2	176.4 ± 1.4
Weight, kg	65.6 ± 1.6	78.6 ± 2.4	67.0 ± 2.8	83.7 ± 2.6
TBW, L	30.9 ± 0.7	43.6 ± 1.0	30.0 ± 0.7	44.9 ± 1.1
CBS, L	13.6 ± 0.4	18.3 ± 0.5	13.7 ± 0.4	19.0 ± 0.5
Sodium$^+$, mM	141 ± 0.6	141 ± 0.5	140 ± 0.5	140 ± 0.6
Potassium$^+$, mM	4.4 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.3 ± 0.1
Chloride$^+$, mM	103 ± 0.6	103 ± 0.6	103 ± 0.5	103 ± 0.8
Osmolality, m osm • kg^{-1}	283 ± 1.0	282 ± 1.0	280 ± 1.0	282 ± 1.0
Resistance^{++}, ohm	539.1 ± 12.3	444.2 ± 8.7	562.8 ± 9.5	434.7 ± 7.1
Reactance^{++}, ohm	58.7 ± 1.6	57.2 ± 1.5	59.0 ± 1.5	56.8 ± 1.1

* Values are mean ± S.E.M.

$^+$ Values are plasma concentrations

$^{++}$ Lowest value observed across electrode placements

TABLE II. MULTIPLE REGRESSION EQUATIONS TO PREDICT TOTAL BODY WATER (TBW) USING BIOELECTRICAL IMPEDANCE MEASURES.

	Model Group				Validation Group		
	Predictor	R^2	SEE		Predictor	R^2	SEE
X_1	$Ht^2 \cdot R^{-1}$*	0.922	2.12	X_1	$Ht^2 \cdot R^{-1}$	0.938	2.19
X_2	Gender	0.936	1.93	X_2	Weight	0.955	1.87
X_3	Weight	0.946	1.80	X_3	Age	0.965	1.67
X_4	Age	0.966	1.61	X_4	Gender	0.972	1.51
	$TBW = 0.372X_1 + 3.05X_2 + 0.142X_3 - 0.069X_3 + 4.98$				$TBW = 0.374X_1 + 0.151X_2 - 0.083X_3 + 2.94X_4 + 4.65$		

* R = lowest resistance value measured among electrode placements; R^2 = coefficient of determination; SEE = standard error of estimate

(upper panel). The slope of the line is similar to 1 ($F = 0.48$, $p = 0.49$), and the intercept is not different than zero ($F = 0.53$, $p = 0.47$). Measured (15.6 ± 0.4 L) and predicted (16.0 ± 0.4 L) CBS values were not different ($t = 1.37$, $p = 0.18$).

Similarly, Fig. 3 (lower panel) presents the linear relationship between CBS values measured in the validation group and predicted using the regression equation developed in the model group. The regression coefficients of this line were found to be not different (slope = 1, $p = 0.87$; intercept = 0, $p = 0.85$) than those of the line of identity. There was no difference ($t = -1.57$, $p = 0.12$) between measured (16.0 ± 0.5 L and predicted (15.8 ± 0.5 L) CBS values.

The CBS and impedance data from both groups were combined. A common multiple regression line was developed:

$$CBS\ (L) = 0.189\ Ht^2/R + 0.052\ Weight - 0.0002\ Ht^2/Xc + 1.03$$
$$R^2 = 0.884,\ SEE = 1.01 \qquad \text{(Eq. 4)}$$

where R and Xc are the lowest values in ohms among the four electrode placements, Ht is in centimeters, and weight is in kilograms.

Partial correlation analysis: Because TBW was highly correlated ($r = 0.90$, $p < 0.0001$) with CBS, the effect of TBW was removed from CBS and from candidate predictor variables of CBS using partial correlation analysis. The results show that Ht, Ht^2, low R, and $Ht^2 \cdot$ low R^{-1} ($r = -0.03$, -0.03, -0.12, and 0.14, respectively) are less important ($p < 0.0001$) predictors of CBS than are low Xc and $Ht^2 \cdot$ low Xc^{-1} ($r = -0.45$ and 0.50, respectively). Furthermore, $Ht^2 \cdot$ low Xc^{-1} was a better ($t = 3.21$; $p < 0.005$) predictor of CBS than low Xc.

DISCUSSION

The use of bioelectrical impedance methodology to assess conductive fluid volumes is not new. The pioneering work was performed by Thomasset and his colleagues who developed a bipolar subcutaneous needle electrode method that introduced an alternating current at two frequencies longitudinally across the body (3,25,26). They derived equations relating the low frequency Z to $Ht^2 \cdot$ bromide space^{-1} ($n = 65$; $r = 0.71$) and the high frequency Z to $Ht^2 \cdot TBW^{-1}$ ($n = 44$; $r = 0.93$). Although this early work identified the potential of using impedance plethysmography to estimate water volumes, this bipolar approach has not gained widespread acceptance. Factors related to the use of needle electrodes, including subject discomfort and altered impedance values associated with the use of two electrodes, are practical disadvantages of this technique (2,10). These limitations led to the development of the four electrode method.

Hoffer and his coworkers (2) used four surface electrodes placed on the dorsal surfaces of the right hand and left foot and introduced 100 μA, A.C. at 100 kHz. Body water, estimated using tritium dilution space, was correlated with $Ht^2 \cdot Z^{-1}$ in normal volunteers ($n = 29$; $r = 0.92$) and patients ($n = 34$; $r = 0.93$) with congestive heart failure.

Subsequently, other investigators used the tetrapolar method of Nyboer (e.g., 800 μA at 50 kHz; 13) to determine Z and its geometric components, and to relate them to TBW. In a sample of 37 males, $Ht^2 \cdot R^{-1}$, where R is the lowest observed among four longitudinal electrode arrangements, was highly correlated with TBW ($r = 0.95$) estimated as deuterium space (9). In a preliminary trial involving 25 females and 25 males aged

TABLE III. MULTIPLE REGRESSION EQUATIONS TO ESTIMATE CORRECTED BROMIDE SPACE (CBS) USING BIOELECTRICAL IMPEDANCE MEASURES.

	Model Group				Validation Group		
	Predictor	R^2	SEE		Predictor	R^2	SEE
X_1	$Ht^2 \cdot R^{-1}$*	0.858	1.17	X_1	$Ht^2 \cdot R^{-1}$	0.828	1.40
X_2	Weight	0.874	1.08	X_2	Weight	0.863	1.20
X_3	Ht^2/Xc	0.899	0.98	X_3	Ht^2/Xc	0.890	1.03
	$CBS = 0.212X_1 + 0.024X_2 + 0.0022X_3 + 0.59$				$CBS = 0.177X_1 + 0.063X_2 - 0.0017X_3 + 1.80$		

* R and Xc are lowest resistance and reactance values, respectively, among electrode placements; R^2 = coefficient of determination; SEE = standard error of estimate

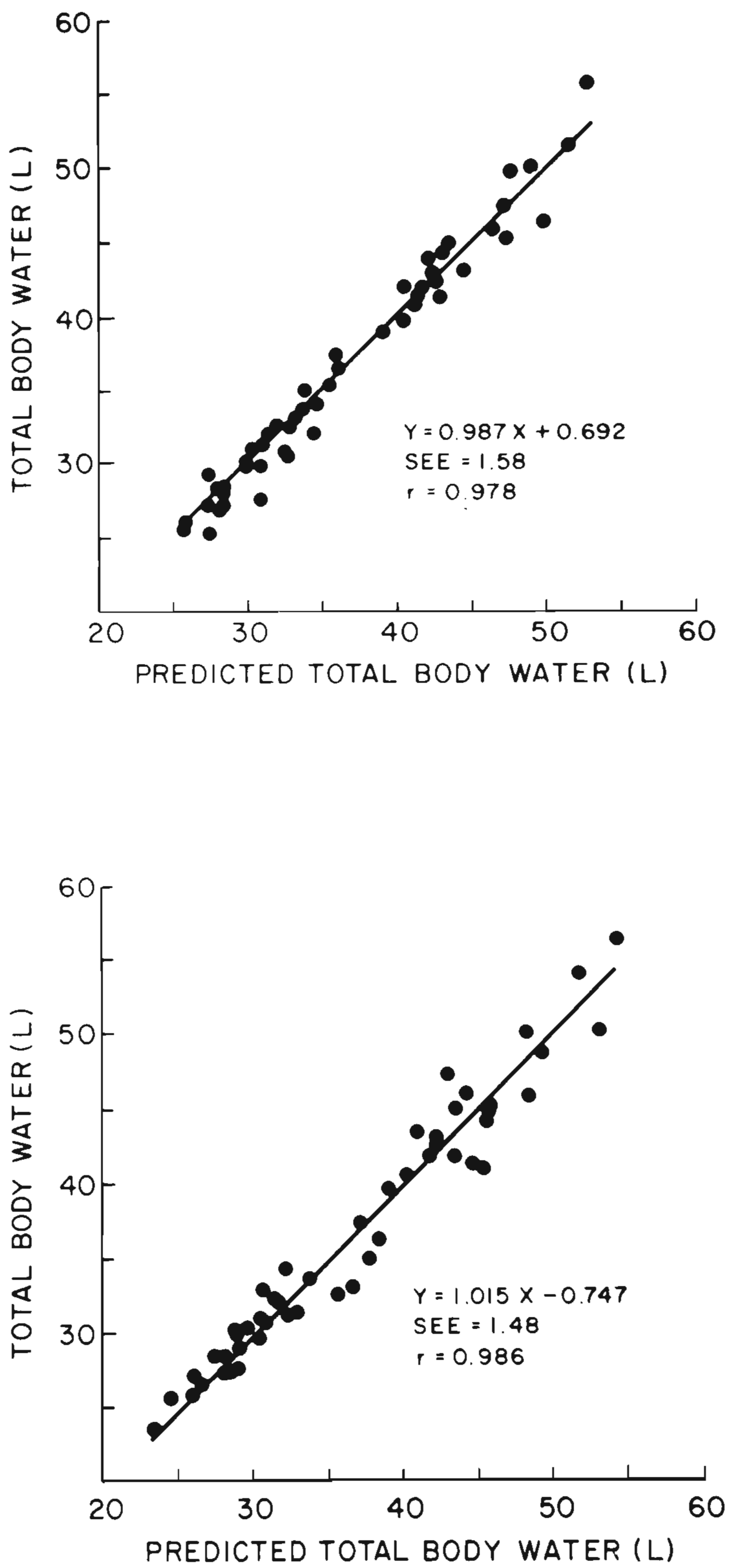

Fig. 2. Relationships between total body water values predicted from an equation developed in the validation group and values measured by isotope dilution in the model group (upper panel), and vice versa (lower panel).

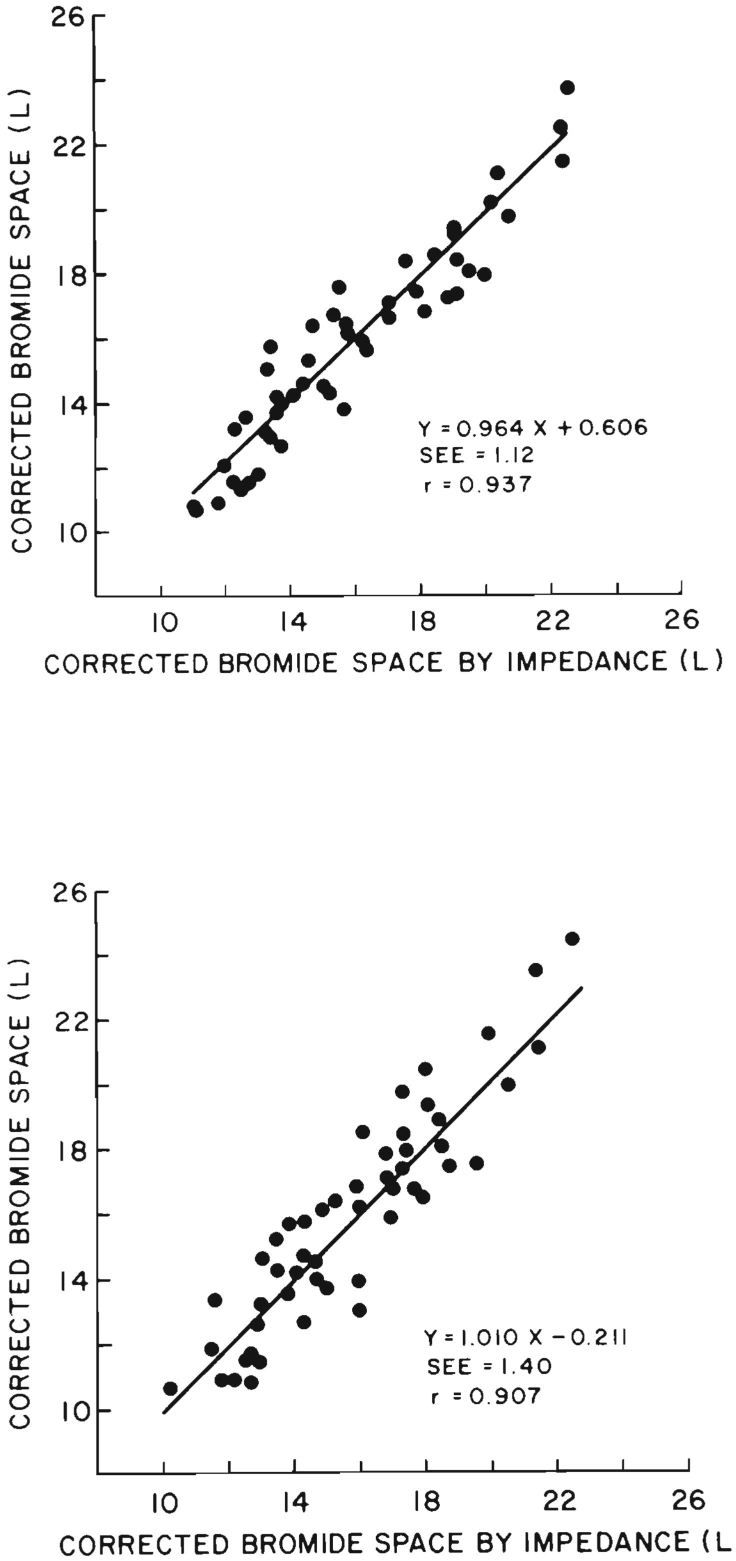

Fig. 3. Relationships between corrected bromide space values predicted from a model derived in the validation group and values determined by isotope dilution in the model group (upper panel), and vice versa (lower panel).

18–55 years, TBW was best predicted by $Ht^2 \cdot \text{low } R^{-1}$ (10). Multiple regression analysis also identified weight, age, and gender as significant predictors of TBW ($R^2 = 0.96$; SEE = 2.1).

Kushner and Schoeller (7) similarly derived an impedance model to predict TBW. Using impedance measures obtained on the right side of the body, $Ht^2 \cdot R^{-1}$ was best correlated with TBW (r = 0.97; SEE = 2.50) in 40 females and males. The best prediction equation was 0.42 $\text{height}^2 \cdot R^{-1}$ R + 0.13 weight + 3.34 gender + 4.96 ($R^2 = 0.98$; SEE = 1.57). When this equation was used to predict TBW in a group of 18 obese females and males, there was no difference between predicted and measured TBW values.

The results of the present study confirm the previous observations that $Ht^2 \cdot \text{low } R^{-1}$ is a valid predictor of TBW. The double crossvalidation method found no deviation from the identity relationship when the TBW values measured in one sample were compared with values predicted using the multiple regression equation derived in the other sample, and vice versa. This method showed nearly identical correlation coefficients (r = 0.978 and 0.986) and similar errors of predicting TBW (SEE = 1.58 and 1.48) when regression equations, derived from independent samples, were used to estimate TBW. Also, no differences were found between measured and predicted TBW. The data from both samples were combined to give a common model to predict TBW. The multiple regression equation to predict TBW derived in the present study is similar to the model developed by Kushner and Schoeller (7).

Another fluid compartment that is infrequently assessed is the extracellular fluid space. Because all tracers used to estimate this compartment are known to be imperfect, only an approximation of this volume can be made. Some investigators attempted to relate impedance measures to estimates of this fluid volume.

Using a low-frequency current (1 kHz), Jenin *et al.* (3) reported the first relationship (r = 0.71) between bromide space, or extracellular volume, and Z. This low-frequency signal was used because it was thought not to penetrate the cell membrane, and to be conducted only by fluid and electrolytes in the extracellular space (13).

Without developing a relationship, Nyboer and his coworkers (15,24) provided evidence that changing body fluid volume affects impedance variables. Among patients with excessive fluid accumulation associated with renal disease and congestive heart failure, dehydration therapy resulted in significant ($p < 0.01$) increases in Z, R, and Xc. Particularly noteworthy was the disproportionate change in Xc (50%) relative to R and Xc (10–15%).

Based upon this finding, we conducted a pilot study to develop a relationship between impedance variables and bromide space (10). In a sample of 50 females and males, a multiple regression equation including $Ht^2 \cdot \text{low } R^{-1}$, weight, Xc, and age was derived to predict bromide space ($R^2 = 0.90$; SEE = 1.0).

The present study extended those findings about the use of impedance variables to estimate bromide space. Among the independent samples, similar regression equations were developed which, when crossvalidated, yielded linear relationships that were not different than the line of identity. Also, the measured and predicted CBS values were similar. Interestingly, the predictive accuracy (SEE) of the crossvalidation trials expressed as a percentage of the mean CBS is similar to the accuracy of the analytical method for determining bromide (about 5%).

Consistent with the descriptive findings of others (15,24), Xc was an important predictor of CBS. Even though $Ht^2 \cdot \text{low } R^{-1}$ was the best single predictor of CBS, this relationship was dependent upon TBW. When the influence of TBW was controlled among CBS and all independent variables using partial regression analysis, the strength of the association between CBS and $Ht^2 \cdot \text{low } R^{-1}$ was diminished and the relationships between CBS and low Xc and $Ht^2 \cdot \text{low } Xc^{-1}$ were enhanced. These findings establish the statistical importance of low Xc in models estimating CBS.

The biological significance of reactive impedance is not known. Nyboer (13) hypothesized that it is an electrical index of cell membrane function and, hence, may be useful in predicting extracellular space. McDougall and Shizgal (12) related Xc, measured along the right side of the body, to extracellular mass estimated by radiosodium dilution space in 64 normal and malnourished patients. The actual biological meaning of Xc remains to be proven.

In our prediction models, low R and Xc values are specified. This indicates that these impedance variables must be determined along the four longitudinal axes of the body. As discussed elsewhere (10), arbitrary selection of electrode placement can result in an error in determining the low R and the low Xc values in more than 50% of those measured. Thus, impedance measurements must be made using the four transmission axes to obtain the appropriate R and Xc values for valid use of the TBW and CBS equations.

The findings of the present study indicate that specific measurements obtained using tetrapolar bioelectrical impedance are useful predictors of body fluid compartments in humans. These findings further suggest that this method, which is safe and noninvasive, can facilitate the routine assessment of TBW and CBS. Although these findings are encouraging, they should be viewed as a necessary step in the development of the bioelectrical impedance method. Additional work is needed to validate this approach in individuals with altered fluid and electrolyte status; such work is currently in progress. More research also is required to compare the importance of reactive impedance derived using a single frequency with the multifrequency impedance values obtained over a large range, such as 1 kHz–1 MHz, in predicting body fluid compartments.

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