Total Body Water Measured by ^{18}O Dilution and Bioelectrical Impedance in Well and Malnourished Children

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ABSTRACT. Total body water (TBW) is an indicator of fat-free mass and thus of nutritional status but cannot be measured readily in children in developing countries who are at greatest risk of becoming malnourished. We therefore developed equations to predict ^{18}O TBW from bioelectrical impedance (Z), wt, and ht in well and malnourished infants and children whom we considered characteristic of children evaluated in nutritional surveillances in Peru. Children 3 to 30 mo of age, whose wt were 3.4 to 14.4 kg, which was -2.8 to $+1$ SD wt-for-ht, were randomly assigned to group I ($n = 30$) to develop equations to predict TBW or to group II ($n = 14$) to cross-validate the predictive equations. Mean TBW measured by ^{18}O dilution was 4.8 ± 1.2 kg in group I, and 5.6 ± 1.7 kg in group II. TBW ranged from 57 to 78% of body wt ($65 \pm 6\%$) in group I and from 56 to 80% ($64 \pm 6\%$) in group II, indicating no statistically significant differences in body composition. The following equation was developed and cross-validated: $\text{TBW, kg} = 0.48 + 0.68 \text{ ht}^2/\text{Z}$; standard error estimate = 0.36; $r = 0.98$. A slight improvement was achieved by the addition of body wt ($\text{TBW, kg} = 0.76 + 0.18 \text{ ht}^2/\text{Z} + 0.39 \text{ wt}$; standard error estimate = 0.23; $r = 0.99$). (*Pediatr Res* 27: 98–102, 1990)

Abbreviations

Z, impedance
R, resistance
Xc, reactance
TBW, total body water
FFM, fat free mass
L, conductor length
I, injector electrodes
E, detector electrodes
SEE, standard error estimate

as a valid means of measuring TBW (1–3) and FFM (4–6) in adults. A limited number of studies have been done to test the validity of the method in measuring body composition during late childhood (7–9). However, the method's validity for measuring body composition in malnourished infants and young children has not been established and thus, whether it would be a reliable method in nutritional surveillances of children in developing countries is uncertain.

The bioelectrical method for measuring TBW vol is based on the principle that Z to the flow of an applied electrical current is related to conductor length and cross-sectional area when signal frequency and conductor configuration are constant (3). The relationship is shown in equation 1.

$$V = \rho(L^2/Z) \quad (1)$$

where Z is $(R^2 + Xc^2)^{1/2}$, R is resistance, Xc is reactance, V is TBW vol, L is conductor length, and ρ is volume resistivity in ohm-cm and is a constant (3). R and Xc are measured after introducing an insensible current through one pair of electrodes (I) attached to the skin and then reading the voltage drop between a pair of detector electrodes (E). The method is practical, non-invasive, and can be implemented with little training.

The purpose of this study was to test the hypothesis that the bioelectrical impedance method accurately measures TBW in well-nourished children and in protein-energy malnourished children. Predictive equations were developed and were subsequently cross-validated in a separate group of comparable children.

MATERIALS AND METHODS

Subjects. Sixty-five children were admitted to the study who were either from peri-urban communities or from a population receiving inpatient care for protein-energy malnutrition. Criteria for admission to the study were 3 to 36 mo chronologic age, body wt from -3 to $+1$ SD wt-for-ht, afebrile, and free of diarrhea on the day of the study and for 7 or more d prior. All children admitted met these criteria. After explanations, parents or guardians gave written consent to their children's participation. The study was approved by the Ethical Review Committee of the Instituto de Investigación Nutricional in Lima, Peru, where the studies were done.

Two of the 65 children were eliminated from the study because they produced liquid stools after isotope administration, leaving doubts as to whether the full dose had been absorbed, and data from an additional 19 children were excluded because protocol requirements for urine collections were not met. Of the remaining 44 children 30 were randomly assigned to group I to develop the predictive equation and 14 were randomly assigned to group II to cross-validate it.

Protocol. Inpatient and outpatient protocols were identical

Rapid methods for nutritional surveillance of children are important in public health and nutrition programs. In surveillances of children in developing countries, methods for estimating caloric reserves are needed because caloric deficits may compound effects of infectious diseases and of temporary food shortages. The most practical method is anthropometry but its reliability in estimating body fat stores is limited.

The bioelectrical impedance method has been well described

Received April 20, 1989; accepted August 8, 1989.

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Supported by Agency for International Development, PSTC 936-6.344 to J.F.T. and by NIH Grant DK26678.

except that children from the community were brought by a fieldworker to the outpatient clinic between 0600 and 0730 h on the morning of the study and inpatients were studied during 1 d of hospitalization. Basal urine samples for measurement of natural ^{18}O abundance were obtained on the morning of the study, body wt was recorded (precision ± 5 g) and ^{18}O was given orally as H_2^{18}O by syringe. Plastic collectors for urine were attached and replaced immediately following each void. Urine collections were continued until samples from at least three postdose voids were obtained, the last no less than 5 h postdose. Samples were frozen (-20°C) until ^{18}O analysis by isotope ratio mass spectrometry. Bioelectrical resistance and reactance were measured after attaching nonpolarizing disposable skin electrodes to the surfaces of the hands, feet, shoulder, and hip, as described below. An *ad libitum* feeding of milk was then given at 1–1.5 h postdose. This feeding was weighed (precision ± 1 g) and the water equivalent calculated as 96% (10). Ht (crown-heel length) was measured using a stadiometer with a sliding footboard (± 0.5 cm).

TBW by ^{18}O -dilution. Oxygen-18 dilution spaces were measured as previously described (11). Briefly, within 30 min after obtaining baseline urine samples, oral doses of 0.08 g pure ^{18}O were given as 11.34 atom percent excess hydrogen normalized water (Mound Laboratories, Miamisburg, OH). Differences in isotopic enrichments of $<3\%$ between consecutive urine samples were required to demonstrate isotopic equilibration. The $^{18}\text{O}/^{16}\text{O}$ isotope ratio analyses were performed as previously described (12) using differential isotope ratio mass spectrometry (Nuclide 3-60 RMS, State College, PA). TBW was assumed to be 99% of measured dilution spaces (13).

Bioelectrical impedance. Bioelectrical R and Xc were measured with a tetrapolar impedance analyzer (RJL Systems, Detroit, MI) which applies a 50 kHz oscillating current of 800 μA . Clothing was removed except for dry underwear or diapers. The skin at the sites where disposable adhesive-backed electrodes (RJL) were to be attached was cleaned with alcohol. No direct contact was made with the child's skin during the measurements, which were made while children were tranquil, and supine, with both arms extended from the torso at about 45° , and legs approximately in parallel with the arms. R and Xc were recorded as the respective means of five consecutive measurements made in immediate succession. Z was calculated as $(R^2 + Xc^2)^{1/2}$ (3).

In general, bioelectrical impedance studies have used a right-sided ipsilateral configuration of electrode placement, where current-I are placed on the dorsal surfaces of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints. E are placed medially between the distal prominence of the radius and ulna and between the medial and lateral malleoli at the ankle (14). A preliminary test was done to determine whether placement of the electrodes at these anatomical sites resulted in measurement errors from electrode interaction due to the relatively short distance between these sites in infants and young children. To make this test, electrodes were placed with the inner edges touching, then separated by moving the E electrode proximally at increments of 0.5 cm until up to 7 cm separated the inner edges. R was recorded at 0 and at each 0.5-cm increment. In keeping with the results from this preliminary study (Fig. 1), the minimum distance used to separate inner edges of the electrode pairs was 3 cm.

Whole body R and Xc were measured by attaching I and E electrodes using the anatomical sites just described unless <3 cm separated the inner edges of the electrodes. In that case, the proximal electrode was moved to achieve an inner-electrode distance of 3 cm. Measurements were recorded for transmission pathways between the right hand and right foot, left hand and right foot, and left hand and left foot. Comparisons of TBW by ^{18}O dilution and ht^2/Z were made, where Z was either from the right-sided ipsilateral configuration, the mean of left and right ipsilateral measurements, or the lowest (right-right, left-left, or

Fig. 1. Bioelectrical resistance expressed as percent of lowest measurement (y) versus interelectrode distance (x) (mean and SD, $n = 10$).

right-left) measurements. L^2/Z was also evaluated as a predictor of TBW, where L is the distance between midpoints of the right ipsilateral E electrodes. Inasmuch as most of the TBW is contained within the torso, which has a more homogenous configuration than the whole body, we also hypothesized that Z in this region alone might give a more accurate prediction of TBW. Therefore, TBW measured by ^{18}O dilution was predicted from L_t^2/Z_t , where Z_t was measured only in the torso by positioning electrodes on the shoulder and on the hip. L_t was measured between midpoints of the electrode pairs. Body wt was also evaluated as a predictor of TBW.

Statistical analysis. Linear and multiple regression analyses (15) were applied to the data to select the combination of variables which would give the lowest standard error of the estimate, but which would not rely on cumbersome techniques. Coefficients from regression equations developed in group I were used to predict TBW in the cross-validation study in group II. Correlation coefficients from equations 2, 3, and 4 were compared in X^2 analyses (15). Confidence limits on mean and individual predicted values were calculated as described (15). Predicted TBW was compared with TBW measured by ^{18}O dilution by paired t tests. The accuracy of the BIA method in healthy and malnourished children was tested by comparing correlations between ^{18}O TBW and TBW predicted by equation 2, 3, and 4 for the well-nourished and malnourished subsets of the cross-validation group (15). Results with a $p > 0.05$ were not considered statistically significant.

RESULTS

Selected characteristics of children in group I and II are listed in Table 1. Group II children were slightly older ($p = 0.05$) and taller ($p = 0.05$) than those in group I; however, the small absolute differences are probably not physiologically important in the present context. Nutritional status estimated by wt/ht was not statistically different between groups I and II. Characteristics of the groups studied are comparable with those encountered in surveillance programs of children in developing countries.

Mean TBW measured by ^{18}O dilution in the 30 children in group I was 4.80 ± 1.22 kg, which was equivalent to $64.8 \pm 6.1\%$ of body wt (range 57 to 78%). In group II, TBW measured by ^{18}O dilution was 5.58 ± 1.69 kg, which was equivalent to $64 \pm 5.9\%$ of body wt (range 56 to 80%). TBW as a percent body wt was not statistically different between the groups ($p > 0.5$) indicating comparable body composition.

Data for selected bioelectrical measurements for groups I and II are presented in Table 2. Correlation coefficients and standard errors of the estimates from linear regressions of selected potential

Table 1. Selected characteristics of children

	<i>n</i>		Age (mo)	Wt (kg)	Ht (cm)	W/H SD*
Develop	30	Range	3–30†	3.9–11.6	54.5–83.8†	–2.4–+0.9
		Mean (SD)	13.1 (6.9)	7.6 (2.3)	69.4 (8.5)	–0.9 (0.8)
Validate	14	Range	6–30	3.4–14.4	58.3–97.0	–2.8–+0.1
		Mean (SD)	18.3 (7.3)	8.9 (3.0)	76.7 (11.0)	–1.4 (1.0)

* SD from mean wt-for-ht.

† $p < 0.05$.

Table 2. Selected bioelectrical data

	Mean	SD	Minimum	Maximum
Z_r (ohms)*				
Group I†	777	74	650	907
Group II	831	98	681	981
Ht^2/Z_r				
Group I	6.36	1.71	3.32	9.74
Group II	7.34	2.29	3.46	12.24
TBW predicted (kg)‡				
Group I	4.74	1.14	2.70	7.00
Group II	5.39	1.53	2.80	8.68
TBW by ^{18}O (kg)§				
Group I	4.80	1.22	2.81	7.38
Group II	5.58	1.69	2.76	9.11
Z_{low} (ohms)				
Group I	758	71	635	892
Group II	819	93	667	937
Z_{mean} (ohms)¶				
Group I	774	72	646	906
Group II	832	91	674	960

* Impedance, whole body right ipsilateral measurements.

† Group I ($n = 30$) to develop equations; group II ($n = 14$) to cross-validate equations.‡ TBW, kg = $0.67 \text{ ht}^2/Z_r + 0.48$, equation 2.§ TBW, kg measured by H_2 ^{18}O dilution.

|| Impedance, lowest of ipsilateral or bilateral whole body measurements.

¶ Impedance, mean of right and left ipsilateral whole body measurements.

predictors (x) of total body water (y) are shown for group I in Table 3. Ht^2 and whole body impedance (Z), whether from the right side alone (ht^2/Z_r), from the lowest measurement of the right or left ipsilateral or bilateral measurements (ht^2/Z_{low}), or from the mean ipsilateral and bilateral measurements (ht^2/Z_{mean}), gave identical correlation coefficients and standard errors ($r = 0.95$ to 0.96 ; $SEE = 0.37$). Equation 2 predicts TBW (kg) from Z and height alone. Results are depicted in Figure 2.

$$\text{TBW, kg} = 0.67 (\pm 0.04) \text{ ht}^2/Z + 0.48 (\pm 0.26) \quad (2)$$

TBW (kg) can also be predicted from body weight (eq. 3) with a slightly smaller SEE than equation 2.

$$\text{TBW, kg} = 0.51 (\pm 0.02) \text{ wt} + 0.95 (\pm 0.17) \quad (3)$$

TBW predicted from the distance between the proximal electrode pairs on the wrist and the ankle (L_r) and whole body right ipsilateral Z (Z_r) was less precise (L_r^2/Z_r ; $r = 0.87$; $SEE = 0.45$), than predictors just described, but was more precise than the

Table 3. Correlation coefficients (SE of the estimates) for group I ($n = 30$)

	^{18}O -TBW (kg)
Ht^2/Z_r	0.95 (0.37)
Ht^2/Z_{low}	0.96 (0.37)
Ht^2/Z_{mean}	0.96 (0.37)
Wt	0.98 (0.27)
Torso $^2/Z_r$	0.61 (0.96)
Length $^2/Z_r$	0.87 (0.45)
Wt and ht^2/Z	0.98 (0.26)

Fig. 2. Comparison of ^{18}O -total body water with ht^2/Z in group I.

prediction using measurements made in the torso alone L_r^2/Z_r ; $r = 0.61$; $SEE = 0.96$).

A multiple regression equation combining ht^2/Z_r and body wt slightly improved the correlation coefficient (0.98) and reduced the SE (0.26) compared to those obtained when either variable was tested alone (equation 4). Equations 2, 3, and 4 did not differ statistically, however, in their abilities to predict TBW.

$$\begin{aligned} \text{TBW, kg} = & 0.18 (\pm 0.10) \text{ ht}^2/Z \\ & + 0.39 (\pm 0.07) \text{ wt} + 0.76 (\pm 0.19) \end{aligned} \quad (4)$$

Equations 2, 3, and 4 were applied to data from children in group II to cross-validate the predictions. Mean (SD) TBW measured by ^{18}O dilution in group II was 5.58 ± 1.69 kg. TBW predicted from equations 2, 3, and 4 were 5.39 ± 1.53 , 5.45 ± 1.50 , and 5.53 ± 1.55 kg, respectively. Correlation coefficients from regressions of ^{18}O -TBW and predicted TBW were 0.98 (equation 2), 0.98 (equation 3), and 0.99 (equation 4). The respective SEE were 0.36, 0.30, and 0.23 (Table 4). The predictions did not differ statistically. Cross-validation results from application of equations 2 and 4 are shown in Figures 3 and 4, respectively.

The 95% confidence intervals on mean TBW estimated by equations 2, 3, and 4 correspond to 4, 3, and 2%, respectively,

Table 4. Confidence intervals for mean and individual predictions of TBW in cross-validation study (group II, $n = 14$)

	r	SEE	95% Confidence limits on:			
			Mean (g)	TBW (%)	Individual (g)	TBW (g)
(equation 2) $0.67 \text{ ht}^2/\text{Z} + 0.48$	0.98	0.36	220	4	670	12
(equation 3) $0.51 \text{ wt} + 0.95$	0.98	0.30	168	3	670	12
(equation 4) $0.18 \text{ ht}^2/\text{Z} + 0.39 \text{ wt} + 0.76$	0.99	0.23	112	2	500	9

Fig. 3. Comparison of ^{18}O -total body water with body water predicted in cross-validation from $0.68 \text{ ht}^2/\text{Z} + 0.48$ (equation 2). $\blacktriangle$, malnourished ≤ 1 SD W/L; $\bullet$, well nourished. Inner dashed line is line of identity. Outer dashed lines indicate 95% confidence limits.

Fig. 4. Comparison of ^{18}O -total body water with body water predicted in cross-validation from $(0.39 \text{ wt}) + (0.18 \text{ ht}^2/\text{Z}) + 0.76$ (equation 4). $\blacktriangle$, malnourished ≤ 1 SD W/L; $\bullet$, well-nourished. Inner dashed line is line of identity. Outer dashed lines indicate 95% confidence limits.

of TBW measured by ^{18}O dilution (Table 4). The 95% confidence intervals on individual measurements of TBW correspond to 12, 12, and 9%, respectively.

Mean (SD) differences between ^{18}O TBW and predicted TBW were 190 g (± 367) by equation 1, 132 g (± 329) by equation 2, and 51 g (± 256) by equation 3. By paired t test, differences between ^{18}O TBW and TBW predicted by equations 2, 3, or 4 were not statistically significant ($p \geq 0.10$).

To compare the accuracy of the BIA method in well versus malnourished children, the correlation coefficients for these two groups between ^{18}O TBW and TBW predicted by equations 2, 3, or 4 were compared. None of the differences approached statistical significance, demonstrating that the equations predict TBW accurately for both well-nourished or malnourished children.

DISCUSSION

The bioelectrical impedance method accurately measured TBW in both well- and malnourished children. Addition of body wt to the predictive equation improved precision, but would require a calibrated scale, a resource not always available in nutritional surveillances in developing countries. Due to the fact that the ^{18}O dilution method has an accuracy of 1%, this validation study's results provide a more reliable predictive equation than previous studies in children that compared fat or caloric stores estimated from the bioelectrical method with anthropometric estimates of fat stores (9).

The cross-validation in 14 well- and malnourished children has shown that body water predicted from the bioelectrical method is accurate to within 4% of mean body water measured by isotope dilution. The addition of wt to the equation gives an accuracy of 2%. Thus, in absolute terms, the bioelectrical method using ht or ht and wt, measures body water volumes with accuracies that are biologically meaningful and applicable to nutritional surveillance of children at risk of becoming malnourished.

Previous studies in children have developed slightly different regression equations than the ones shown in equations 2–4. A comparison of findings is shown in Table 5. There are two primary reasons for the differences between previous and present findings. First, the previous studies were concerned with older children (7) and second, they measured body composition by total body potassium (8) or by hydrodensitometry (9).

The hydration coefficient of FFM varies according to age, gender, and nutritional status. The FFM of boys 9 to 18 mo old is approximately 77% water (16). Using this hydration coefficient as an example, the 95% confidence limit in body fat estimated from the bioelectrical method using equation 2 would correspond to 9 to 10% of body wt, or as commonly expressed by others, the 1 SD uncertainty would be 3 to 5% of body wt. These are twice as great as the corresponding values if body water is measured by isotope dilution. The difference in precision between the bioelectrical and isotope dilution methods could be biologically and clinically important. However, the bioelectrical method has the additional advantages of being inexpensive, completely noninvasive, and practical in field settings. The only other method that has similar advantages in terms of cost and practicality is anthropometry. Anthropometric analysis of body composition in children, however, is less accurate than we have shown the bioelectrical method to be. Thus, the bioelectrical method is superior to anthropometry in terms of precision, and is superior to isotope dilution in terms of cost and rapidity.

We found that equations based on wt alone or on bioelectrical impedance and height both give reliable predictions of TBW. Inasmuch as reliance on body wt alone would not detect changes in body water due to changes in hydrational status that occur in children with malnutrition, the bioelectrical method has a theoretical advantage over wt alone as a predictor of TBW.

Acknowledgments. The authors thank Drs. Francisco Sylvester, Francisco Oieda, Luis Faverio and the nursing staff of the Instituto de Investigación Nutricional for help in conducting the studies.

Table 5. Comparison of predictors of FFM and TBW based on bioelectrical impedance in studies of children of various ages and physiologic states

	Equation	<i>r</i>	SEE	Reference
FFM (kg)	$6.86 + 0.81 (ht^2/R)$	0.83	4.08	8
FFM (kg)	$-1.23 + 0.92 (ht^2/Z)$	0.88	4.02	9
FFM (kg)	$-1.38 + 0.96 (ht^2/Z)$	0.84	4.19	9
TBW (kg)	$-0.5 + 0.60 (ht^2/R)$	0.97	1.69	7
TBW (kg)	$0.48 + 0.68 (ht^2/Z)$	0.98	0.36	Present study
TBW (kg)	$0.76 + 0.18 (ht^2/Z) + 0.39 (wt)$	0.99	0.23	Present study

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