

Development of Prediction Equations for Body Composition in Indian Children Using Bioelectrical Impedance Analysis

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ABSTRACT

We aimed to develop a BIA equation to predict fat free mass (FFM) using deuterium oxide ($^2\text{H}_2\text{O}$) dilution as the reference method for children. Seventy seven children (36 boys and 41 girls), aged 6 to 24 month participated in the study. The best fit FFM prediction equation included weight, sex and length²/resistance (adjusted $R^2=0.83$ and root mean square error of 0.49 kg). Applying »leave-one-out« method the mean of the differences of FFM in kg and % body weight were nearly zero with 95% confidence intervals of ± 0.108 kg between those derived by $^2\text{H}_2\text{O}$ dilution and by the validated equation. PRESS statistic residual was 473g (≈ 0). The best fit equation for FFMkg was: $\text{FFMkg} = 0.72 + 0.30 \times (\text{weight}) + 0.16 \times (\text{sex}) + 0.52 \times (\text{Length}^2 / \text{resistance})$ (Length in cm, weight in kg, resistance in Ω , sex: boys=1, girls=0). The derived predictive equation has good precision and is useful for community based studies on body composition in South Asian children.

Key words: Fat free mass, Deuterium oxide, Young children, Total Body Water, Bioelectrical Impedance.

INTRODUCTION

Epidemiological studies have shown that low birth weight (LBW), restricted weight and height gain in infancy and, early rapid weight gain in childhood are associated with chronic adult diseases like coronary heart disease (CHD), diabetes, and metabolic syndrome¹⁻³. These studies used body weight, length, and length adjusted weight indices such as body mass index (BMI) and ponderal index for assessing growth. These measurements however do not distinguish between fat free mass (FFM) and fat mass (FM). We contend that FFM and FM measurements may help further define and better understand their relationship with chronic adult diseases. We therefore need better practical and reliable tools to measure FFM and FM in children in South Asia. Low Birth Weight rate is high in South Asian countries⁴. In India LBW rate is 30%, underweight 43%, wasting 20%, and stunting 48% in under five children^{5,6}. On the other hand developing countries like India are reporting high rates of CHD and type 2 diabetes in recent years that have become leading causes of morbidity and mortality^{7,8} and these epidemics are expected to intensify⁹.

Methods based on anthropometry (such as height or length and weight) have the inherent limitations in that they do not critically distinguish between FFM and FM¹⁰. The BIA method is based on the fact that only water (containing electrolytes) in the human body can conduct electricity and is well suited to assess total body water (TBW). Fat is devoid of water and restricts flow of current through it. In a steady state condition TBW correlates well with FFM. Modern BIA equipment is easily carried to the field for large community based studies. We have earlier evaluated three published equations based on BIA for prediction of TBW in children using the deuterium oxide dilution ($^2\text{H}_2\text{O}$) technique as the reference method¹¹. Of the three methods even the best fit one by Fjeld and colleagues¹² gave the average TBW values 2.5% lower than the reference $^2\text{H}_2\text{O}$ dilution method in young children from India. Therefore in the present study, we have developed new equations based on BIA for the prediction of FFM in children in India using the $^2\text{H}_2\text{O}$ dilution technique as the reference method. The derived best fit equation was validated by the cross-validation or »leave-one-out« method as described by Lohman and colleagues¹³. Press (prediction of sum of squares) statistics was also used for validation¹⁴.

Subjects and Methods

Subjects

The demographic and anthropometric details of the study children have been reported earlier^{13,15}. In short, the study was conducted in apparently healthy children from among the urban middle class and low middle class people attending a well baby clinic of a large charitable government hospital in the city of Kolkata, India where free service is provided. We used a purposive sample of children with a wide range of BMI percentile for age. Subjects represented the vast majority of children in countries in South Asia⁵. The well baby clinic is held once a week. Each week we interviewed the first 4 mothers who registered their children in the weekly clinic and we aimed to recruit up to two children each week. The recruitment was spread over a period of one year. A total of eighty six children were evaluated and nine who could not complete the ²H₂O dilution test were excluded. Among them 29% were underweight, 17% were wasted and 14% were stunted. They are similar to national average in India⁵. A total of eighty six children were evaluated and nine who could not complete the ²H₂O dilution test were excluded. The eligibility criteria for inclusion in the study were age 6–24 months of either sex, absence of illness during the preceding 1 month, absence of gross congenital anomalies and chronic diseases, and parents' willingness to participate. The study was conducted according to the Declaration of Helsinki. The purpose and procedures were explained to the parents, particularly the ²H₂O stable isotope use and the BIA technique and written informed consent was obtained from the parents and anonymity of the subjects was strictly preserved. The parents were assured that they are free to withdraw their child anytime during the study. They were also assured that withdrawal will in no way affect the care of the child. The study was approved by the Ethical Review Committee of the Society for Applied Studies, Kolkata.

Sample size

Our plan was to develop simple prediction equations for measuring FFM for large scale use, based on BIA method. The reference method is based on ²H₂O (a stable isotope) dilution. For an ideal prediction equation, the difference in the measured quantity between the reference method and the method under study should be zero. The sample size depends on the magnitude of deviation from zero and its confidence interval that will be acceptable for the purpose the method is to be used and therefore can only be indicative. The comparisons are paired i.e. the results from the two methods on each subject will be compared. The degree of deviation of the mean of the differences from zero is of interest for our study. We have calculated the sample size to detect 1.5% or more deviation from zero difference with a standard deviation of 2%. To detect this degree of deviation with 95 percent confidence and 90 percent power we needed a sample of 71 subjects in each group¹⁶. Since the same subject will provide data for FFM

by both reference method and the comparison methods the sample size remains the same that is 71 subjects. We added 10 percent to this number to give a sample size of 78 subjects.

Bioelectrical Impedance analysis

Bioelectrical Impedance Analysis measures impedance of the body to a small electric current. The generic theoretical model treats the body as a single cylinder, with measurements made between electrodes placed manually on the wrist and ankle. Adjustment of bioelectrical data for height allows estimation of TBW. The theoretical relation¹⁷ is as follows:

$$V \propto L^2/Z$$

where V is the volume of the body water in the subject, Z is impedance, and L is the length of the subject. Water (containing electrolytes) in the body conducts electricity; fat restricts it. Cell membranes also resist electricity. The resistance by the cell membranes being negligible at 50 KHz the impedance index (i.e. Length²/resistance) is a good predictor of TBW and has been used in our study. Impedance to the flow of current in body tissues is a function of resistance (R) and reactance (Xc). Cell membranes act as small capacitors and thus offer a reactive resistance (i.e. reactance) to the flow of current. Based on electrical theory, current at relatively higher frequencies passes through both extracellular and intracellular fluid and can provide an index of TBW. Thus BIA method can distinguish between non-fat body mass (FFM) and fat mass (FM)¹⁸.

Impedance and resistance were measured with a multi-frequency BIA (Xitron model 4000b; Xitron technologies Inc. San Diego, USA) using a single frequency of 50 kHz [19,20]. We described the methods in detail in our earlier reports¹¹.

Briefly children with dry light clothes lay supine^{19,20} with arms apart from the body and legs separated so that the thighs did not touch. After cleaning the skin contact area with alcohol, one pair of electrodes (foil disposable 5 cm² ECG electrodes) was placed on the dorsal surface of the right hand at the distal metacarpal joints and between the distal prominence of the right radius and ulna. Another pair was placed at the distal metatarsal joints and between the lateral malleoli of the right foot. Each BIA measurement was taken 3 times and the average value was used.

Anthropometric measurements

The anthropometric measurements were made using recommended protocols^{13,21} and are briefly described. The measurements were taken on the same day the children underwent ²H₂O dilution and BIA procedures. Each anthropometry measurement was taken 3 times and the average value was used.

Length: For children less than two years of age recumbent length was measured with a wooden measuring board²¹. The board was made sufficiently broad to cover the shoulder blades. The reading was taken to the nearest 0.1cm. The board was designed and tested by a physical anthropologist of the organization for precision.

Weight: Weight was measured nude, using an electronic platform balance (Avery India limited, model no. L111A) with a precision of 10 gm. The balance was checked regularly for accuracy using standard weights.

²H₂O dilution method

²H₂O, water with a stable isotope of hydrogen was used and was described earlier¹⁵. This measurement took place between 09.00 and 14.00 hours. Because of their age the children were not fasted prior to the test. A vast majority of them were breast-fed (88%). As expected, the children were normally hydrated (as evaluated clinically) at the time of the test. Children's pre-dose saliva sample was collected by a disposable syringe. We took a measured volume of ²H₂O (99.9%, sigma, St Louis, MO) approximately equivalent to 50 mg/kg body weight in a disposable container (²H₂O specific gravity = 1.107). We weighed the ²H₂O dose using a precision balance with an accuracy of 0.1 mg and used in calculating TBW. The empty container was rinsed with two consecutive lots of 15 ml of distilled water and the child drank both lots from the same disposable syringe. This 50 ml of ingested water was adjusted for in calculating the total body water. Time was allowed to equilibrate the ²H₂O into the body fluid. No food or fluid including breast milk and water was permitted during the equilibration period of 3 hours. At the end of the equilibration period the post-dose saliva sample was collected and analyzed for deuterium using a dual-inlet mass spectrometer (Europa Scientific, Crewe, UK), using the zinc reduction technique. The results of ²H₂O concentration in the saliva samples were used for calculating the TBW¹⁵.

The following equations were used²²:

$$\text{TBW in kg} = (\text{TBW in moles} \times \text{molecular weight of H}_2\text{O (i.e., 18.0153)})/1000$$

$$\text{TBW in moles (N}_2) = (F_1 \times N_1) \div (F_2 \times 1.041)$$

$$F_1 = \text{dose of } ^2\text{H}_2\text{O in atom percent} \times 10^6/100 - 150 \text{ ppm of } ^2\text{H}_2\text{O}$$

$$= (99.9 \times 10^6/100) \text{ ppm} - 150 \text{ ppm} = (999000 - 150) = 998850 \text{ ppm}$$

$$150^* = \text{the average concentration of } ^2\text{H}_2\text{O in naturally occurring water.}$$

$$F_2 = \text{ppm after dose} - \text{ppm before dose of } ^2\text{H}_2\text{O}$$

$$N_1 = \text{Dose of } ^2\text{H}_2\text{O in gram/ molecular weight of } ^2\text{H}_2\text{O (i.e., 20.0274)}$$

In using deuterium for estimating TBW, it is to be noted that this isotope undergoes some exchange with non-aqueous hydrogen and a correction factor of 1.041 was used. The FFM was calculated as TBW divided by an age and sex specific hydration factor for FFM and for this age group that ranged from 0.807 to 0.77 for boys and 0.807 to 0.780 for girls²³.

All the tests were carried out on the same day according to the following sequence. The anthropometric measurements were made first. Then the BIA estimation followed by ²H₂O tests were carried out.

Statistical Analysis

Statistical programs the Epi Info, version 6²⁴ and Stata version 11.2²⁵ were used. Multiple linear regression analyses were applied to the data to select the combination of variables which would give the lowest Root Mean Square Error (RMSE) and highest adjusted R². The candidate predictor variables for the development of a regression equation for estimating Fat Free Mass in kg (FFMkg) included length, weight, age, sex and whole body resistance and reactance from BIA. Initially higher order polynomial terms were included in a regression to test for nonlinearity. This was done univariately for each independent variable with FFMkg derived by ²H₂O dilution method as the dependant variable. Both analyses showed that the linear model was appropriate. All possible two way interactions were tested and if any were statistically significant were considered for evaluation in the model. We used bootstrap estimates for the standard errors of the regression co-efficient.

The best fit equation derived by regression analyses was first examined on the children on whom the equation was developed. Validation was accomplished by cross-validation or a »leave-one-out« method as described by Lohman and colleagues¹³. In this method one data point at a time is left out, the model is fit to the remaining data points and then is applied to the excluded point to determine how well the excluded point is predicted (using ²H₂O dilution method as reference). The average of the prediction errors, each point being left out once, is the cross validated measure of the prediction error. Statistical theory shows that this estimate is approximately equivalent to an independent sample validation²⁶. The differences in the FFMkg for each child of the cross validation set between those derived by the developed equation and by ²H₂O dilution were evaluated for their deviation from zero by paired t-test. For a good agreement, we expect that the range, mean of the difference \pm two standard error to indicate 95% limits of agreement within which group means will lie 95% of the time when applied on independent population groups²⁷. We also derived the PRESS statistic by taking the square root of the sum of squares of the residuals divided by the total number of observations. We followed the procedure described by Sun and colleagues¹⁴. Validation by this method is also similar to applying the equation on an independent sample.

Results

The study children came from middle to low middle class community in a metropolitan city in India. They represent the average children in this age group in India. The BMI percentile for age was between 5th and 85th percentile of WHO standard in 81% of the children. Eighty-six children participated in the study. In nine of them, the ²H₂O procedures were incomplete because of vomiting, or being fed during the equilibration period. They were dropped from the study. The study children were predominantly breast-fed (88%).

Seventy seven children (36 boys and 41 girls) were studied; details are available in our earlier report¹¹. In India 42% people are below the poverty line²⁸ which is closely similar to our study population. Fifty six percent of the mothers had school education of six years or more. These features are comparable to the norms for the urban population in India. The mean, standard deviation (SD) and ranges for age, anthropometric variables and BIA variables for 77 subjects are presented in Table 1. The mean BMI ranged from 12.35 to 19.11 kg/m². The resistance values ranged from 641 to 1025 ohms. R² and RMSE of the candidate independent variables with FFM as dependent variables are presented in Table 2. The length²/resistance as a single independent variable had the highest R² value and the lowest RMSE of all the variables when regressed singly. The final set of equations was derived by selecting the independent variables from the preliminary equations and the final set of equations to estimate FFMkg are shown in Table 3. Comparing 5 models for the subjects, the regression model 5 has the lowest RMSE and the highest adjusted R² and was selected as the equation of our choice. This equation yielded RMSE of 0.4861. Length²/resistance was a highly significant pre-

TABLE 2
R² AND ROOT MEAN SQUARE ERROR (RMSE) VALUES FOR THE INDEPENDENT VARIABLES WITH FAT FREE MASS IN KG AS DEPENDENT VARIABLE DERIVED BY ²H₂O DILUTION.

Fat Free mass	R ²	RMSE
1. Length	0.65	0.690
2. Weight	0.73	0.599
3. Length ²	0.65	0.686
4. Length ² /Resistance	0.80	0.524
5. Age	0.29	0.980
6. BMI	0.002	1.163

dictor when used in the model with weight and sex variables (model 5). The prediction equation (model 5) for FFMkg is:

$$\text{FFMkg} = 0.72 + 0.30 \times (\text{Weight}) + 0.16 \times (\text{sex}) + 0.52 \times (\text{Length}^2/\text{resistance}) - 1$$

(Where, Length in cm, Weight in kg, Resistance in Ω, (sex): Boys=1, Girls=0).

Using this equation, the cross validation was accomplished by the »leave-one-out« method and the resulting mean, SD of the differences of paired values in FFMkg between those derived by ²H₂O dilution and the developed equation under study and 95% confidence interval of the differences are shown in Table 4. As stated earlier we were able to cross validate these new equations on the same children applying leave-one-out method. The mean of the differences of the FFMkg (<0.001) and as % body weight (0.007) using model 5 were very small.. The 95% confidence limits of the mean of the differences between FFMkg derived by ²H₂O and the predicted value by the vali-

TABLE 1
CHARACTERISTICS OF THE STUDY CHILDREN (n=77)

Variable	Boys (n=36)		Girls (n=41)	
	Mean±SD or number	Range	Mean±SD or number	Range
Age (m)	14.7±5.6	6.2–23.8	16.0±5.3	7.0–23.6
Weight (kg)	8.9±1.3	6.4–12.1	8.8±1.4	6.3–11.9
Length (cm)	75.9±5.7	65.5–86.5	75.6±6.3	63.8–86
BMI (kg/m ²)	15.5±1.6	12.3–19.1	15.3±1.0	13.5–17.8
¹ Resistance (50 KHz) (Ω)	780.2±96.6	641–1025	812.0±69.3	676–998
¹ Length ² /Resistance (cm ² /Ω)	7.5±1.2	5.0–11.2	7.1±1.3	4.8–9.8
² TBW (kg)	5.8±0.9	4.0–8.1	5.5±0.9	3.5–7.1
² TBW %	65.2±3.8	55.3–71.6	62.7±6.6	47.3–72.3
BMI-for Age<5 th percentile	9		3	
BMI-for Age≥5 th to<85 th percentile	24		38	
BMI-for Age≥85 th to<95 th percentile	2		0	
BMI-for Age≥95 th percentile	1		0	

¹using Bioelectrical Impedance analysis

²Derived by ²H₂O dilution (reference method)

TABLE 3
REGRESSION MODELS FOR ESTIMATION OF FAT FREE MASS IN KG

Variable	Regression co-efficients (♣SE)				
	Model 1	Model 2	Model 3	Model 4	Model 5
Weight (kg)	0.72 (0.050)	0.54 (0.097)	0.53 (0.098)	0.52 (0.095)	0.30 (0.082)
Length (cm)		0.05 (0.022)			
Length ²			0.0003 (0.0001)	0.0003 (0.0001)	
Sex (Male=1, Female=0)				0.30 (0.130)	0.16 (0.113)
Length ² /Resistance (cm ² /Ω)					0.52 (0.089)
Constant	0.84 (0.450)	-1.28 (1.034)	0.61 (0.449)	0.51 (0.438)	0.72 (0.366)
R ²	0.74	0.75	0.75	0.77	0.83
Adjusted R ²	0.73	0.75	0.75	0.76	0.82
Root Mean Square Error	0.5993	0.5836	0.5833	0.5665	0.4861
Press statistics				0.5516	0.4733
(Press Residuals)				(≈0)	(≈0)
Mean difference	<0.001	<0.001	<-0.001	<0.001	<0.001
(Reference-predicted FFMkg)					

♣ – Standard error by Bootstrapping

dated equation (with one observation excluded one at a time) was ±0.108 only. For comparison we have also provided the mean differences in FFM and TBW in kg and as percent body weight between those derived by ²H₂O dilution and by each of the three published BIA equations (Table 4) for children. Published BIA based equations for body composition predict TBW which is then converted into FFM by adjusting for hydration factor. In our equation we integrated the hydration factor in the equation itself to predict FFM directly. This was done by using FFM as the dependent variable instead of TBW. Its application in future studies is expected to provide information on its usefulness. To compare with the published equations, we have also developed an equation to predict

TBW using the same procedure. Using leave-one-out method the TBW equation also gave a high level of predictive power (Table 4). The equation is given below

$$\text{TBWkg (Predictive equation)} = 0.79 + 0.24 \times (\text{Length}^2/\text{resistance}) + 0.34 \times (\text{weight}) + 0.21 \times (\text{sex})$$

(Where, Length in cm, Weight in kg, Resistance in Ω, (sex): Boys=1, Girls=0).

The mean differences in FFM and TBW between each of the published equations and ²H₂O dilution in the study population were substantially larger than those between ²H₂O dilution and the equation derived in this study. The same was true for prediction of TBW.

TABLE 4
DIFFERENCE IN FAT FREE MASS (FFM) AND TOTAL BODY WATER (TBW) (OF PAIRED VALUES) IN KG AND AS PERCENT OF BODY WEIGHT BETWEEN THOSE DERIVED BY ²H₂O DILUTION AND BY EACH OF THE THREE PUBLISHED BIA EQUATIONS AND BY THE NEW EQUATION DEVELOPED IN THIS STUDY⁴. (MEAN VALUES AND STANDARD DEVIATIONS AND 95% CONFIDENCE INTERVALS (CI) OF THE DIFFERENCE)

Difference between methods	FFM difference in kg (as % Body weight)				TBW difference in kg (as % Body weight)			
	Mean	SD	95% CI	P*	Mean	SD	95% CI	P*
D ₂ O – Fjeld ¹	0.22 (2.49)	0.52 (5.90)	0.10 to 0.33 (1.15 to 3.83)	0.0005 (0.0004)	0.11 (1.24)	0.50 (5.13)	0.007 to 0.207 (0.07 to 2.41)	0.04
D ₂ O – Bocage ²	0.45 (5.37)	0.51 (6.03)	0.33 to 0.56 (4.01 to 6.74)	<0.001	0.29 (3.52)	0.44 (5.36)	0.19 to 0.39 (2.31 to 4.74)	<0.001
D ₂ O – Kushner ³	0.99 (11.40)	0.50 (6.05)	0.87 to 1.10 (10.03 to 12.77)	<0.001	0.71 (8.26)	0.48 (5.96)	0.60 to 0.82 (6.91 to 9.61)	<0.001
D ₂ O – Predictive equation ⁴	<0.001 (0.007)	0.48 (5.57)	-0.11 to +0.11 (-1.26 to +1.27)	1.00 (0.99)	<0.001 (0.008)	0.42 (5.07)	-0.096 to +0.096 (-1.14 to +01.16)	1.000 0.99

FFM; fat free mass. TBW; total body water *The t-test evaluates the difference of the paired values from zero.

Fjeld¹; BIA based equations of Fjeld et al. [12], Bocage²; BIA based equations of Bocage [38], Kushner³; BIA based equations of Kushner et al [39], New Equation⁴; Results based on leave-one-out method.

We graphically examined the performance of the final equation by plotting the predicted versus the observed values for FFMkg (Fig. 1 (a)). We also plotted the PRESS residuals versus the predicted values of FFMkg (Fig. 1 (b)). This equation showed a good precision; the predicted and observed values for FFMkg fell on or near the line of identity and the residuals are randomly scattered on or near the narrow band around zero for FFMkg (Fig. 1 (b)). PRESS residuals plotted against predicted values do not show any specific trend in their distribution around zero.

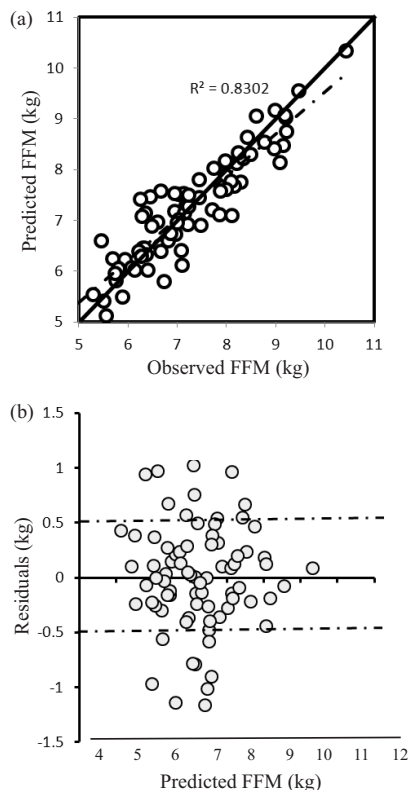


Fig. 1. Predicted (model 5) versus observed fat free mass and press statistics. (a) Predicted Fat Free Mass (FFM) in kg versus observed FFM in kg, the line of identity, and the regression line of predicted and observed FFM; (b) PRESS (prediction of sum of squares) residuals versus predicted FFM and the zero reference line in model 5.

Discussion

The purpose of this study was to develop prediction equations for FFM using BIA measurements that are suitable for South Asian children. Hardly any such equations are available in this age group in Asian children. We have earlier evaluated three published equations based on BIA measurements on children of this age group in India and all three equations consistently underestimated TBW and the derived FFM (Table 4) and are inadequate for predicting FFM in children in South Asia¹¹. The BIA is a simple and suitable method as an epidemiological tool and is the only practical method that estimates FFM and this newly

developed prediction equation should be suitable for this population.

In our search for relevant variables to develop the prediction equations for FFM, of all the independent variables length²/resistance had the highest R² and the lowest RMSE values (Table 2) when regressed individually on FFM derived by ²H₂O dilution. As expected, the best fit equation is provided by weight, sex, and length²/resistance. The 95% confidence intervals of the differences of paired values of FFM in kg and in percentage body weight between those derived by ²H₂O dilution and the best fit validated equation are within narrow ranges (i.e. ±108 gm. and ±0.007% respectively).

We used a stable isotope-dilution technique as the reference standard. Isotope dilution methods use a two component model to measure FFM and FM and are generally considered safe, reliable, accurate and feasible in infants and children²¹. To derive FFM from TBW one has to use age and sex-specific hydration factors such as those derived by multicomponent models²³. While the hydration of FFM changes with age and maturation, use of age and sex specific hydration factor minimizes errors associated with maturation. The hydration factors for FFM proposed earlier for 1 to 5 year old children give closely similar results^{29,30}. Measurement of TBW using ²H₂O dilution methods has been used as a reference method in many of the classic studies of body composition in children^{30–33}.

To summarize, we developed new BIA based prediction equations to directly measure FFM in infants and children in South Asia, using ²H₂O dilution as reference method. We validated the final equations by the cross-validation or, a »leave-one-out« method and also by the PRESS statistics. Using the »leave-one-out« method, the paired differences between FFMkg derived by the ²H₂O reference method and by the best fit BIA based equation developed by us provided a narrow 95% confidence interval (±108 gm) making it suitable for comparing groups such as in epidemiological studies. The PRESS statistics indicated that the performance of the cross-validated best fit FFM equation was good (Table 3). Unlike other published BIA based equations which predict TBW the new prediction equation directly predicts FFM. However, we also developed BIA based prediction equation to predict TBW and can be used to derive FFM.

Reference methods for deriving equations based on BIA are all indirect. Apart from isotope dilution method used in this study, densitometry (underwater weighing), total body potassium (TBK), dual energy X-ray absorptiometry (DXA), and multi-compartment models are used as reference methods. Each of them makes assumptions and therefore has limitations. They include hydration factor of FFM, a constant used for total body potassium and FFM ratio, and a constant density for FFM. DXA is software dependent and the results do not agree among manufacturers. While TBK is a reference method for body cell mass its usefulness in predicting FFM is limited. While the multicompartments models in theory can overcome some of the limitations of two compartment models as used in our study, the error terms associated with the measurement of each compartment may have unpredictable com-

bined error effect and may negate their putative advantages. Isotope dilution methods for TBW are considered reliable, accurate and feasible in infants and children²¹. TBW measurement as a reference method has been used in many well known body composition studies in children^{30,32–34}. BIA works well in healthy subjects and in patients with stable water and electrolyte balance with a validated BIA equation that is appropriate for age and sex. Clinical use of BIA with extreme overweight or with abnormal hydration is inappropriate. One other limitation of the study is that subjects could not be fasted prior to ²H₂O dilution study which may overestimate the TBW measurements. We expect this to be marginal given that there would be some water loss due to sweating and insensible water loss from the lungs and skin during 3 hours of the test.

We have used cross-validation (leave-one-out) method rather than independent validation. For the latter, the commonly used method for estimating generalization error is to reserve a part of the data as a »test« set which is not used for developing the prediction equation. The best fit equation is then applied to the test set to evaluate the generalization error. The disadvantage of this method for validation is that it reduces the sample size both for development and validation of the prediction equation. On the other hand cross validation (leave-one-out) method uses nearly the full data set and is considered markedly superior for small sample data sets^{35,36}. Present study carried out on children under 2 years is procedure-intensive. Considering the ethical issues related to the age and elaborate procedures the sample size was restricted to the one just adequate for addressing the research question. Further, leave-one-out cross validation is considered to work well for estimating generalization error for continuous error functions such as the mean squared error, as is the case in our study³⁷.

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BIA based equations are largely population specific and therefore any equation developed in our population needs validation for use on a different population group. In an earlier study we evaluated three selected BIA based equations developed in other countries (Peru, Lima and Jamaica) on children in India and even the best of the three underestimated TBW and FFM by –2.46% and 2.98% respectively compared with ²H₂O reference method¹¹.

This new equation should be suitable for epidemiological studies on similar population in South Asia. Its application on overweight children may require independent validation and the same applies for older children.

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Author Contribution

Authors: BS, a Research Fellow took part in study design, carried out the study procedures, took part in the analysis and in writing the manuscript. DM conceived and developed the study design and statistical analysis, supervised her work, took part in data analysis and interpretation and in writing the manuscript. None of the authors have any conflict of interest to declare.

Any enquiry on the data set availability should be addressed to the Director, Society for Applied Studies, Kolkata.

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RAZVOJ PREDVIĐANJA JEDNADŽBE ZA SASTAV TIJELA U INDIJSKE DJECE KORISTEĆI ANALIZU BIOELEKTRIČKE IMPEDANCIJE

SAŽETAK

Cilj nam je razviti BIA jednadžbe za predviđanje mase bez masti (FFM), koristeći otopinu deuterij-oksida ($^2\text{H}_2\text{O}$) kao referentnu metodu za djecu. Sedamdeset i sedamero djece (36 dječaka i 41 djevojčice), u dobi od 6 do 24 mjeseci sudjelovalo je u istraživanju. Najbolje predviđena jednadžba FFM-a uključuje težinu, spol i dužinu² / otpornost (korigirani $R_2 = 0,83$ i korijen srednje kvadratne pogreške 0,49 kg). Primjena metoda »izostavi-jedan« srednja razlika u FFM u kg i postotku tjelesne težine bilo je gotovo nula s 95% intervala pouzdanosti a ± 0.108 kg između onih koji ostvare otopinu $^2\text{H}_2\text{O}$ i validirane jednadžbe. PRESS statistika preostalo je 473g. Najbolja fit jednadžba za FFMkg je: $\text{FFMkg} = 0.72 + 0.30 \times (\text{težina}) + 0,16 \times (\text{spol}) + 0,52 \times (\text{Dužina}^2 / \text{otpor})$ (Dužina u cm, težina u kg, otpornost, spol: dječaci = 1, cure = 0). Izvedena prediktivna jednadžba ima dobru preciznost i korisna je studije zajednica o sastavu tijela djece u južnoj Aziji.