



# Estimation of Body Fat Mass from Bioelectrical Impedance Analysis in Indian Adults Aged 23 to 81 Years: A Comparison with Dual Energy X-ray Absorptiometry

<sup>1</sup>BR Patil, <sup>2</sup>DP Patkar, <sup>3</sup>SA Mandlik, <sup>4</sup>CD Kapse, <sup>5</sup>GD Jindal

## ABSTRACT

The purpose of this study was to validate a bioelectrical impedance analysis (BIA) equation for prediction of body fat mass (FM) against dual energy X-ray absorptiometry (DXA) in healthy Indian adults with large variations in body mass index and age. Healthy subjects (28 males and 85 females) were investigated by two methods: FM was measured by a dual energy X-ray absorptiometry and segmental bioelectrical parameters at various frequencies were measured by a commercial segmental multi-frequency BIA instrument. Total body parameters were derived from segmental bioelectrical parameters. As correlation was high and prediction error was low, a single equation was developed for FM as follows:  $FM = 15.45 + [0.0074 \times (R_{\text{body250}})] - (3.89 \times \text{sex})$ ; men = 1, women = 0) +  $(0.844 \times w) - [6938 \times (h^2/Z_{\text{body50}})] - (22.22 \times h) + [3 \times (X_{\text{body250}} - X_{\text{body5}})/\text{age}] + [1.53 \times (\Phi_{\text{body5}})] - [0.126 \times (X_{\text{body50}}/h)]$ . Fat mass predicted with dual energy X-ray absorptiometry was  $28.11 \pm 9.30$  kg. BIA-predicted FM was  $28.12 \pm 9.11$  kg ( $R = 0.9794$ , adjusted  $R^2 = 0.9561$ , standard error of estimate = 1.95 kg, total error = 1.87 kg). In conclusion, the new developed BIA equation was valid for prediction of FM in healthy subjects aged 23 to 81 years with body mass indices between 15.62 and 39.98  $\text{kg}\cdot\text{m}^{-2}$ . Inclusion of reactance in the  $\text{kg}\cdot\text{m}^{-2}$  single prediction equation appeared to be essential for use of BIA equation in adults with large variations in body mass and age.

**Keywords:** Bioelectrical impedance analysis, Body fat mass, Body parameters, Dual energy X-ray absorptiometry.

**How to cite this article:** Patil BR, Patkar DP, Mandlik SA, Kapse CD, Jindal GD. Estimation of Body Fat Mass from Bioelectrical Impedance Analysis in Indian Adults Aged 23 to 81 Years: A Comparison with Dual Energy X-ray Absorptiometry. *MGM J Med Sci* 2015;2(2):57-65.

<sup>1</sup>Principal, <sup>2,4</sup>Head, <sup>3</sup>Scientific Officer, <sup>5</sup>Professor and Head

<sup>1</sup>Vishwaniketan's Institute of Management Entrepreneurship and Engineering Technology, Raigad, Maharashtra, India

<sup>2</sup>MRI Centre, Balabhai Nanavati Hospital, Mumbai, Maharashtra India

<sup>3</sup>Biomedical Section, Electronics Division, Modular Laboratories Bhabha Atomic Research Centre, Mumbai, Maharashtra, India

<sup>4</sup>Department of Instrumentation Engineering, Government Polytechnic, Mumbai, Maharashtra, India

<sup>5</sup>Biomedical Engineering Department, MGM's College of Engineering and Technology, Kamothe, Navi Mumbai, Maharashtra, India

**Corresponding Author:** GD Jindal, Professor and Head, Biomedical Engineering Department, MGM's College of Engineering and Technology, Kamothe, Navi Mumbai, Maharashtra, India Phone: +91-2265138119, e-mail: gd.jindal@gmail.com

**Source of support:** Nil

**Conflict of interest:** None

## INTRODUCTION

Body fat percentage is the amount of body fat tissue as a percentage of total body weight. It consists of essential body fat and storage body fat. Essential body fat is necessary to maintain life and reproductive functions. Essential fat is 3 to 5% in men, and 8 to 12% in women. Storage body fat consists of fat accumulation in adipose tissue, part of which protects internal organs in the chest and abdomen. Levels of body fat are epidemiologically dependent on gender and age.<sup>1</sup> Different authorities have prescribed different recommendations for ideal body fat percentages. The average acceptable body fat percentages are 18 to 24% for men and 25 to 31% for women. Higher percentage of fat above average levels leads to higher health risk for weight-related illness. Assessment of fat mass (FM) in patients optimizes nutrition support to avoid or minimize muscle wasting or obesity. Therefore, nutrition assessment should include objective body-composition measurements.

A living person's exact body fat percentage generally cannot be determined, but there are several techniques which can be used to estimate it to a good degree of accuracy. Dual energy X-ray absorptiometry (DXA formerly DEXA), is a newer method for estimating body fat percentage and is a method of choice for determining body composition. There are several more complicated procedures that more accurately determine body fat percentage. Some, referred to as multicompartments models, can include DXA measurement of bone, plus independent measures of body water (using the dilution principle with isotopically labeled water) and body volume (either by water displacement or air plethysmography). Various other components may be independently measured, such as total body potassium. *In vivo* neutron activation can quantify all the elements of the body and use mathematical relations among the measured elements in the different components of the body (fat, water, protein, etc.) to develop simultaneous equations to estimate total body composition, including body fat.<sup>2</sup> There exist various anthropometric methods for estimating

body fat. The term anthropometric refers to measurements made of various parameters of the human body, such as circumferences of various body parts or thicknesses of skinfolds. Most of these methods are based on statistical modeling.

The bioelectrical impedance analysis (BIA) method is simple, more affordable and noninvasive way to estimate body fat percentage. In BIA, the body bioelectrical parameters (impedance, reactance, etc.) provide a measure of body fat, since the impedance to sinusoidal current varies between adipose, muscular and skeletal tissue. Fat-free mass (muscles) is a good conductor as it contains a large amount of water (approximately 73%) and electrolytes, while fat is anhydrous and a poor conductor of electric current. Many investigators have developed empirical BIA equations for prediction of body fat mass, fat free mass (FFM) and total body water (TBW).<sup>3-13</sup> Some of these equations have been validated in relatively young, healthy adults against several body-composition techniques.<sup>6</sup> Studies have shown that BIA formulae developed for healthy, normal-weight subjects are not suitable for obese subjects<sup>14,15</sup> and are not valid in elderly subjects.<sup>16</sup> In longitudinal studies, the use of different BIA formulae in the same subject who becomes overweight or older introduces a bias into body-composition studies and raises one question whether the differences in body composition are due to changes in the BIA formula or to changes in body composition. Thus, it would be advantageous to use a single formula that is applicable in young as well as elderly subjects including overweight subjects. Roubenoff et al<sup>16</sup> and others concluded that BIA equations are subject to errors that cannot be determined a priori unless they are validated in the specific population in which they are to be used.<sup>17,18</sup> Thus BIA equations must be validated in a representative population sample against a reference method before its accuracy is accepted. Bioelectrical impedance analysis can be validated against DXA, hydrodensitometry, and total body potassium. Dual energy X-ray absorptiometry is one reference method<sup>5</sup> that has been validated against independent methods, such as *in vivo* neutron activation,<sup>4,8</sup> total body potassium, and hydro densitometry.<sup>9</sup> It is commonly cited as the current standard for body composition testing.

To date, there has been no specific investigation about the factors that affects the accuracy and performance of BIA equation for prediction of FM in Indian adults. While using BIA method, factors that need to clarify are single *vs* multi-frequency; non-phase *vs* phase sensitive measurements; whole body *vs* segmental approaches; choice of predictor variables and subject factors. The purpose of this study was to validate a single BIA equation for prediction of body FM against DXA in healthy Indian adults with large variations in body mass index and age.

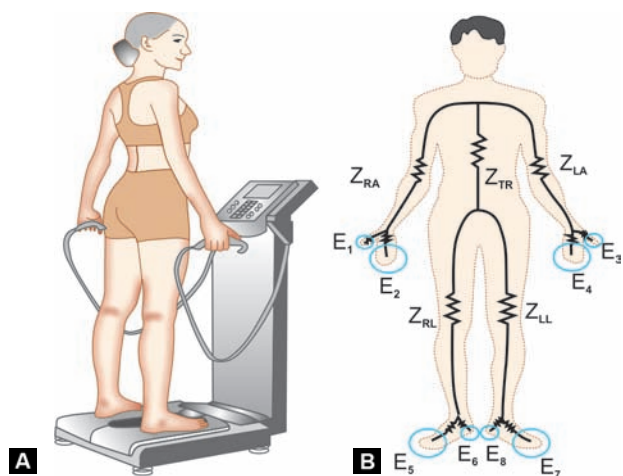
## SUBJECTS AND METHODS

### Subjects

The study group consisted of 113 subjects (28 males and 85 females) in the age group of 23 to 81 years. All subjects were born in India and resided in Mumbai. The subjects were apparently healthy and none was under medication during the last 1 week. The subjects were kept fasting for nearly 2 hours before the measurements. The standing height of each subject was measured to the nearest 0.5 cm. The purpose of the study was explained to all the subjects and their oral consent was taken. Each subject was measured by two methods: FM was measured by dual energy X-ray absorptiometry (Lunar Prodigy, DPX-IQ, General Electric Healthcare, Belgium, Europe) and the segmental bioelectrical parameters (impedance and reactance) at various frequencies were measured by a commercial segmental multi-frequency BIA instrument InBody720 (Biospace Co. Ltd. Seoul, Korea). The total body parameters were derived from segmental parameters.

### Calculation of Total Body Bioelectrical Parameters

InBody 720 provides the segmental bioelectrical parameters (impedance and reactance) at various frequencies using eight-polar tactile-electrode. This commercial product is approved by FDA (Food and Drug Administration, United States, May 2003). It is based on the segmental impedance measurement approach and operated at frequencies of 1, 5, 50, 250, 500 and 1000 kHz, which are pre-set by the manufacturer and introduced into the body in the ascending order of frequency. This device uses contact electrodes located in the handgrips and the footpads. Subjects were asked to stand with the ball and heel of each foot on two metallic electrodes on the floor scale and hold handrails with metallic grip electrodes in contact with the palm and thumb. Laboratory temperature was maintained at  $25 \pm 2^\circ\text{C}$ , in order to avoid any variation in electrical impedance due to temperatures. They were instructed to keep their arms fully extended and abducted approximately  $20^\circ$  laterally (Figs 1A and B). The sequence of measurements were controlled by a microprocessor, proceeds as follows. For 1 kHz programed frequency, an alternating current of 100  $\mu\text{A}$  and for other programed frequencies, an alternating current of 500  $\mu\text{A}$  of intensity (I) is applied between E1 and E5. The recorded voltage difference (V) between E2 and E4 is divided by I to obtain the impedance of the right arm ( $Z_{\text{RA}}$ ). The same operation is performed with V recorded between E4 and E8 to obtain the trunk impedance ( $Z_{\text{TR}}$ ) and with V recorded between E6 and E8 to obtain the impedance of the right leg ( $Z_{\text{RL}}$ ). The alternating current is then applied between E3 and E7



**Figs 1A and B:** Subject position on InBody 720 (A) for measurement of segmental bioelectrical parameters. The eight electrode positions (B) on the body

and the value of  $V$  measured between E2 and E4 is used to calculate the impedance of the left arm ( $Z_{LA}$ ). Lastly, the value of  $V$  measured between E6 and E8 is used to calculate the impedance of the left leg ( $Z_{LL}$ ). No precaution was taken to standardize the subject's posture before BIA, as suggested by the manufacturer. The instrument gave the impedance values of five segments (e.g. arms, trunk and legs) from the measurements at 6 frequencies (1, 5, 50, 250, 500, 1000 kHz) and also the reactance values at three frequencies (5, 50, 250 kHz).

From segmental impedance and reactance values, the respective values of segmental resistance and phase angle were calculated at three frequencies (5, 50, 250 kHz) using the following equations:

Resistance of a segment at 'f' kHz frequency,

$$R_{sf} = \sqrt{(Z_{sf})^2 - (X_{sf})^2} \quad (1)$$

Phase angle of a segment at 'f' kHz frequency,

$$\phi_{sf} = \tan^{-1} \left( \frac{X_{sf}}{R_{sf}} \right) \quad (2)$$

Where,  $[(R)_{sf}]$  is the resistance of the body segment,  $(Z_{sf})$  is the impedance of the body segment,  $[(X)_{sf}]$  is the reactance of the body segment and  $[(\phi)_{sf}]$  is the phase angle of the body segment at 'f' kHz frequency.

Values of resistance, reactance, impedance and phase angle of total body at three frequencies (5, 50 and 250 kHz) were calculated as follows:

Body resistance at 'f' kHz frequency,

$$R_{bodyf} = (R_{RAf}) + (R_{LAf}) + (R_{TRf}) + (R_{RLf}) + (R_{LLf}) \quad (3)$$

Body reactance at 'f' kHz frequency,

$$X_{bodyf} = (X_{RAf}) + (X_{LAf}) + (X_{TRf}) + (X_{RLf}) + (X_{LLf}) \quad (4)$$

Body impedance at 'f' kHz frequency,

$$[Z_{bodyf}] = \sqrt{[R_{bodyf}]^2 + [X_{bodyf}]^2} \quad (5)$$

Phase angle of body at 'f' kHz frequency,

$$[\phi_{bodyf}] = \tan^{-1} \frac{[X_{bodyf}]}{[R_{bodyf}]} \quad (6)$$

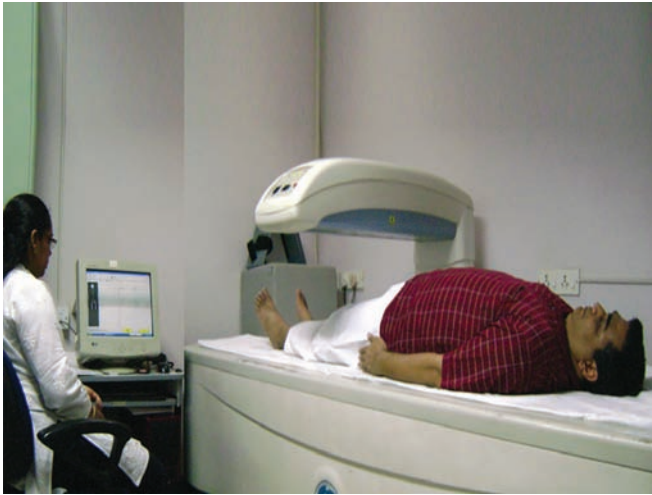
where  $[R_{bodyf}]$  is the resistance of the body,  $[Z_{bodyf}]$  is the impedance of the body,  $[X_{bodyf}]$  is the reactance of the body and  $[\phi_{bodyf}]$  is the phase angle of the body at 'f' kHz frequency. The body segments right arm, left arm, trunk, right leg and left leg are represented by RA, LA, TR, RL and LL respectively.

### Dual Energy X-ray Absorptiometry

Fat mass of each subject was measured by DXA. The DXA scanning technique measures the differential attenuation of two different levels of X-ray energy as they pass through the body, thereby allowing determination of bone mineral content and soft-tissue mass on a pixel-by-pixel basis. Dual energy X-ray absorptiometry allows the separation of total and segmental body mass (BM) into fat mass (FM), lean tissue mass (LTM) and bone mineral content (BMC). The sum of LTM and BMC gives fat-free mass (FFM). The X-ray source (fan beam) mounted beneath the patient generates a narrow, tightly collimated beam of X-ray that pass through the patient at rapidly changing energies. The transmitted intensity of each energy level is measured by a radiation detector mounted on a movable arm directly above the X-ray source. Simultaneous with the measurement of the skeleton, the percentage of fat is determined from the attenuation ratio of lower energy to higher energy detected by the beam. This ratio is calculated from all non-skeleton pixels scanned and extrapolated of the skeleton-containing pixels. In the literature,<sup>28</sup> the effective total body-radiation dose was reported as 5.4  $\mu$ Sv. All measurements were performed with a Lunar Prodigy instrument DPX-IQ, General Electric Healthcare, Belgium, Europe (Fig. 2).

### Statistics

The total body bioelectrical parameters like  $(Z_{bodyf})$ ,  $(X_{bodyf})$ ,  $(\Phi_{bodyf})$  and  $(R_{bodyf})$  are derived from segmental parameters at 5, 50 and 250 kHz frequencies. The physical parameters comprise of weight ( $w$ ), stature ( $h$ ), gender and age. By combining physical and bioelectrical parameters, the new parameters like  $(h^2/Z_{bodyf})$ ,  $(Z_{bodyf}/w)$ ,  $(Z_{bodyf}/h)$ ,  $(R_{bodyf}/h)$ ,  $(X_{bodyf}/h)$ ,  $(w \times h^2/Z_{bodyf})$ , etc. are derived at 5, 50 and 250 kHz frequencies. Descriptive statistics for all parameters were calculated and expressed as mean



**Fig. 2:** Measurement of fat mass by dual-energy X-ray absorptiometry (lunar prodigy, DPX-IQ and general electric healthcare)

± standard deviation (SD). Simple regressions were calculated to test correlations between FM obtained from DXA and BIA parameters. FM measured by DXA was used as the criterion measurement.

With the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The predictor variables entered into the BIA model in the order of highest correlation coefficient and smallest standard error of estimation (SEE) were ( $R_{\text{body250}}$ ), (sex),  $w$ , ( $h^2/Z_{\text{body50}}$ ),  $h$ , ( $X_{\text{body250}} - X_{\text{body5}}$ )/age, ( $\Phi_{\text{body5}}$ ), ( $X_{\text{body50}}/h$ ). In addition to correlation and regression techniques, error analysis was performed. Standard error of estimation was calculated and used as error of prediction for DXA derived FM and predicted FM by new BIA equation. The total error (TE) was calculated as:

$$TE = \sqrt{\frac{\sum_{i=1}^n [(FM1)_i - (FM2)_i]^2}{n}} \quad (7)$$

Where (FM1) is the observed value of FM by DXA and (FM2) is the predicted value of FM by BIA equation. The total error of measurement estimates the magnitude of the error for a given measurement and is defined as the difference between measurements for the individual ( $i$ ),  $i = 1 \dots n$ , where  $n$  is the number of individuals.<sup>20</sup> The total error was compared with the SEE. A small difference between total error and SEE indicates high accuracy of the prediction. To assess the agreement between the two clinical measurements, the difference between the values was plotted against their means, because the mean was the best available estimate of the true value. This analysis allows for the calculation of bias (estimated by the mean differences), the 95% confidence interval for the bias and the limits of agreement (two SDs of the difference).<sup>19</sup> Statistical significance was set at  $p \leq 0.05$

for all tests. In order to verify the different factors which may affect the performance of the BIA method, the different sets of parameters were selected and for each set, with the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The change in % adjusted  $R^2$  and SEE were recorded for each set of variables.

## RESULTS

A total of 113 healthy adults aged 23 to 81 years and weighting 39 to 97.7 kg were used as subject. Table 1 shows the anthropometric and BIA characteristics of male and female subjects grouped by age. The prediction equation developed from all subjects is shown in Table 2. The order of entry of predictor variables was ( $R_{\text{body250}}$ ), (sex),  $w$ , ( $h^2/Z_{\text{body50}}$ ),  $h$ , ( $(X_{\text{body250}} - X_{\text{body5}})/\text{age}$ ), ( $\Phi_{\text{body5}}$ ), ( $X_{\text{body50}}/h$ ). For the BIA model (Table 3) weight ( $w$ ) alone accounted for 64.26% of the variability (SEE = 5.56 kg) of the equation whereas ( $X_{\text{body50}}/h$ ) accounted for 13.75% of the variability (SEE = 8.63 kg). Inclusion of  $w$ , (age),  $h$  and (sex) without BIA parameters accounted for 92.73% of the variability with a SEE of 2.51 kg. Thus inclusion of BIA parameters clearly improved the prediction power (95.61%) and decreased the SEE (1.95 kg) compared with anthropometric parameters only. The significant variables (predictors) for estimating the value of FM are listed in Table 4. The beta (standardized regression coefficient) value is the measure of how strongly each predictor variable influences the criterion variable. Higher the absolute beta value, greater is the impact of the predictor variable on criterion variable. The  $t$ -value and statistical significance ( $p$ ) value give the rough indication of the impact of each predictor variable: a big absolute  $t$ -value and small  $p$ -value suggests that a predictor variable has a large impact on the criterion variable. The tolerance values are a measure of the correlation between the predictor variables and can vary between 0 and 1. The closer to zero the tolerance value is for a variable, the stronger the relationship between this and the other predictor variables. We should worry about the variables that have a very low tolerance. Variance inflation factor is an alternative measure of collinearity (in fact it is the reciprocal of tolerance) in which a large value indicates a strange relationship between the predictor variables. Graph 1A shows a comparison of FM measured by BIA equation (a new non-invasive technique) and DXA (established method). Graph 1B shows the correlation and mean difference, according to Bland and Altman,<sup>19</sup> using the prediction equation in all subjects. Here, the mean difference was 0.018 kg; the 95% confidence interval was from -3.737 to

**Table 1:** Anthropometric and bioelectrical impedance analysis characteristics of healthy subjects grouped by age

	Age (years)			
	Below 40	41–50	51–60	Above 60
<b>Male (n = 28)</b>				
n	7	7	9	5
h (m)	1.67 ± 0.076	1.71 ± 0.101	1.70 ± 0.056	1.70 ± 0.033
w (kg)	70.56 ± 14.15	76.36 ± 3.48	73.51 ± 12.30	71.30 ± 6.01
BMI (kgm <sup>-2</sup> )	25.01 ± 3.56	26.38 ± 2.13	25.37 ± 3.71	24.64 ± 1.93
(Z <sub>body5</sub> ) (Ω)	1333.28 ± 161.34	1272.47 ± 84.30	1288.99 ± 165.05	1323.97 ± 121.87
(Z <sub>body50</sub> ) (Ω)	1167.17 ± 137.35	1112.27 ± 86.41	1153.75 ± 153.52	1210.24 ± 151.46
(Z <sub>body250</sub> ) (Ω)	1055.77 ± 119.43	1007.59 ± 79.17	1049.99 ± 140.68	1086.27 ± 113.85
(R <sub>body5</sub> ) (Ω)	1331.52 ± 161.21	1270.96 ± 84.46	1287.75 ± 165.06	1322.62 ± 121.88
(R <sub>body50</sub> ) (Ω)	1161.14 ± 136.21	1116.56 ± 86.42	1149.16 ± 153.18	1205.78 ± 151.48
(R <sub>body250</sub> ) (Ω)	1052.19 ± 118.84	1003.82 ± 78.66	1046.81 ± 140.20	1083.13 ± 113.66
(X <sub>body5</sub> ) (Ω)	68.43 ± 7.77	61.13 ± 9.84	56.00 ± 8.53	59.20 ± 7.95
(X <sub>body50</sub> ) (Ω)	118 ± 20.91	112.44 ± 12.69	102.53 ± 12.84	103.26 ± 11.12
(X <sub>body250</sub> ) (Ω)	85.96 ± 13.40	86.15 ± 15.94	81.59 ± 12.92	82.48 ± 8.59
(Φ <sub>body5</sub> ) (degree)	2.948 ± 0.185	2.767 ± 0.499	2.508 ± 0.389	2.573 ± 0.357
(Φ <sub>body50</sub> ) (degree)	5.779 ± 0.570	5.766 ± 0.639	5.116 ± 0.394	4.928 ± 0.556
(Φ <sub>body250</sub> ) (degree)	4.658 ± 0.343	4.898 ± 0.731	4.452 ± 0.311	4.364 ± 0.287
(h <sup>2</sup> /Z <sub>body5</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00214 ± 0.00037	0.00229 ± 0.00020	0.00228 ± 0.00033	0.00220 ± 0.00025
(h <sup>2</sup> /Z <sub>body50</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00224 ± 0.00041	0.00259 ± 0.00022	0.00255 ± 0.00039	0.00243 ± 0.00035
(h <sup>2</sup> /Z <sub>body250</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00269 ± 0.00044	0.00289 ± 0.00023	0.00280 ± 0.00043	0.00269 ± 0.00036
<b>Female (n = 85)</b>				
n	11	22	22	30
h (m)	1.57 ± 0.028	1.58 ± 0.058	1.57 ± 0.049	1.55 ± 0.052
w (kg)	64.96 ± 17.71	63.87 ± 12.09	68.24 ± 11.28	64.46 ± 15.32
BMI (kgm <sup>-2</sup> )	26.29 ± 7.28	25.55 ± 5.24	27.74 ± 4.24	26.88 ± 5.79
(Z <sub>body5</sub> ) (Ω)	1584.09 ± 258.92	1600.89 ± 205.91	1522.76 ± 190.49	1563.15 ± 232.68
(Z <sub>body50</sub> ) (Ω)	1419.39 ± 224.65	1449.84 ± 195.48	1380.75 ± 172.56	1433.10 ± 217.48
(Z <sub>body250</sub> ) (Ω)	1288.10 ± 199.54	1321.71 ± 189.54	1266.63 ± 161.01	1320.25 ± 203.69
(R <sub>body5</sub> ) (Ω)	1582.59 ± 258.58	1599.60 ± 205.87	1521.55 ± 190.32	1562.12 ± 232.54
(R <sub>body50</sub> ) (Ω)	1413.58 ± 223.41	1444.95 ± 195.31	1376.31 ± 172.15	1429.11 ± 217.01
(R <sub>body250</sub> ) (Ω)	1283.91 ± 198.46	1317.90 ± 189.14	1263.09 ± 160.55	1316.77 ± 203.18
(X <sub>body5</sub> ) (Ω)	68.53 ± 15.45	63.56 ± 10.90	60.07 ± 12.20	55.65 ± 14.69
(X <sub>body50</sub> ) (Ω)	127.95 ± 25.51	118.55 ± 13.04	109.90 ± 16.92	105.89 ± 20.46
(X <sub>body250</sub> ) (Ω)	103.63 ± 22.01	100.14 ± 13.83	94.36 ± 14.45	95.38 ± 17.218
(Φ <sub>body5</sub> ) (degree)	2.473 ± 0.275	2.291 ± 0.371	2.259 ± 0.349	2.027 ± 0.499
(Φ <sub>body50</sub> ) (degree)	5.159 ± 0.387	4.722 ± 0.419	4.576 ± 0.503	4.248 ± 0.589
(Φ <sub>body250</sub> ) (degree)	4.585 ± 0.319	4.358 ± 0.269	4.275 ± 0.364	4.146 ± 0.414
(h <sup>2</sup> /Z <sub>body5</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00159 ± 0.00026	0.00159 ± 0.00020	0.00164 ± 0.00023	0.00156 ± 0.00028
(h <sup>2</sup> /Z <sub>body50</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00178 ± 0.00029	0.00176 ± 0.00024	0.00181 ± 0.00025	0.00171 ± 0.00031
(h <sup>2</sup> /Z <sub>body250</sub> ) (m <sup>2</sup> Ω <sup>-1</sup> )	0.00196 ± 0.00031	0.00194 ± 0.00028	0.00197 ± 0.00028	0.00186 ± 0.00034

n: number of subjects; h: height; w: weight; BMI: body mass index; Z<sub>body5</sub>: body impedance at 5 kHz; Z<sub>body50</sub>: body impedance at 50 kHz; Z<sub>body250</sub>: body impedance at 250 kHz; R<sub>body5</sub>: body resistance at 5 kHz; R<sub>body50</sub>: body resistance at 50 kHz; R<sub>body250</sub>: body resistance at 250 kHz; X<sub>body5</sub>: body reactance at 5 kHz; X<sub>body50</sub>: body reactance at 50 kHz; X<sub>body250</sub>: body reactance at 250 kHz; Φ<sub>body5</sub>: body phase angle at 5 kHz; Φ<sub>body50</sub>: body phase angle at 50 kHz; Φ<sub>body250</sub>: body phase angle at 250 kHz

3.773 kg. The mean difference is closer to the zero, i.e. the bias is negligible. Thus, BIA equation tends to give a closer reading having the limits of agreement (-3.737 to 3.773 kg) are small enough for us to be confident that BIA equation can be used for assessment of FM.

The elderly subjects were analyzed separately to determine whether greater error occurred with the BIA equation in aged subjects (Table 5). Fat mass measured for

35 subjects with age above 60 years from DXA was 28.9222 ± 10.1149 kg. Fat mass predicted by equation was 28.6267 ± 9.8814 kg. The mean difference was 0.2955 ± 1.5354 kg (p > 0.05, paired t-test; R = 0.9885, total error = 1.7481 kg). Subjects with BMIs above 27 kg.m<sup>-2</sup> were also analyzed separately to determine whether greater error occurred with the BIA equation in obese subjects (Table 6). Fat mass measured for 44 subjects with BMIs above 27 kgm<sup>-2</sup>

**Table 2:** Bioelectrical impedance analysis prediction equation for fat mass using all subjects (n = 113)

$FM = 15.45 + (0.0074(R_{\text{body}250})) - (3.89 \times (\text{sex}); \text{men} = 1, \text{women} = 0) + (0.844 w) - (6938 \times (h^2/Z_{\text{body}50})) - (22.22 \times h) + (3 \times ((X_{\text{body}250} - X_{\text{body}5})/\text{age})) + (1.53 \times (\Phi_{\text{body}5})) - (0.126 \times (X_{\text{body}50}/h))$
FM predicted with DXA = 28.11 ± 9.30 kg
FM predicted with BIA equation = 28.12 ± 9.11 kg (R = 0.9794, adjusted R <sup>2</sup> = 0.9561, SEE = 1.95 kg, TE = 1.87 kg)
n: number of subjects; FM: fat mass; DXA: dual energy X-ray absorptiometry; BIA: bioelectrical impedance analysis; w: weight; h: height; R <sub>body250</sub> : body resistance at 250 kHz; Z <sub>body50</sub> : body impedance at 50 kHz; X <sub>body5</sub> : body reactance at 5 kHz; X <sub>body50</sub> : body reactance at 50 kHz; X <sub>body250</sub> : body reactance at 250 kHz; Φ <sub>body5</sub> : Phase angle of the body at 5 kHz; R: validity coefficient; SEE: standard error of the estimate; TE: total error

**Table 3:** Contribution and order of entry of variables to the bioelectrical impedance analysis model and anthropometric model for fat mass (n = 113 subjects)

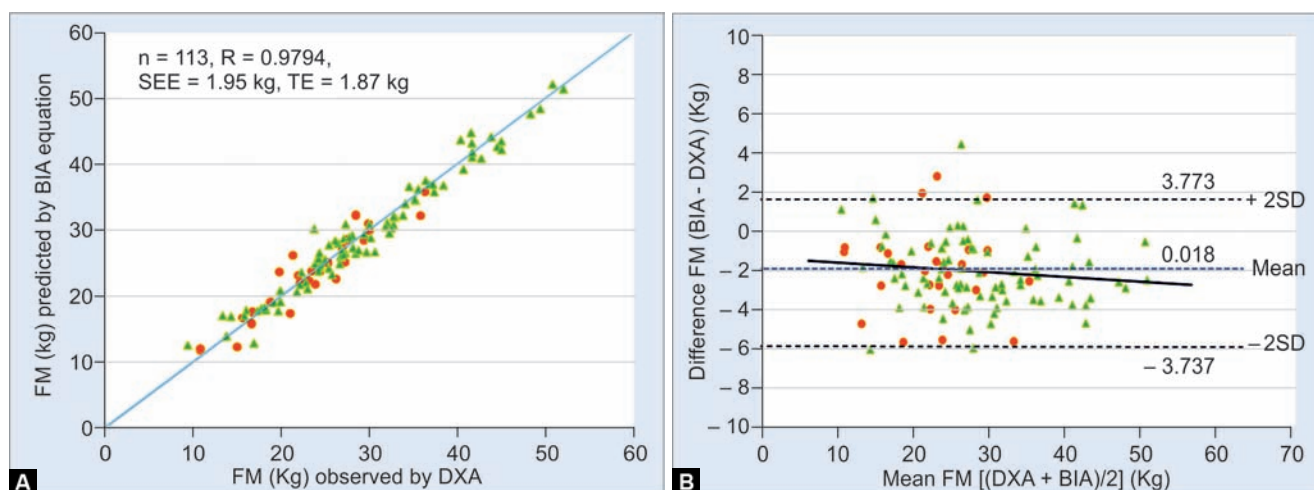
Model and variables	Cumulative variables used in model			Variables		
	% Ad.R <sup>2</sup>	SEE	p	% Ad.R <sup>2</sup>	SEE	p
<b>BIA</b>						
(R <sub>body250</sub> )	13.65	8.64	0.000	13.65	8.64	0.000
+ (sex)	49.55	6.60	0.000	08.08	8.91	0.001
+ w	91.97	2.68	0.000	64.26	5.56	0.000
+ (h <sup>2</sup> /Z <sub>body50</sub> )	94.78	2.12	0.000	02.43	9.18	0.054
+ h	95.00	2.08	0.020	00.14	9.29	0.248
+ [(X <sub>body250</sub> - X <sub>body5</sub> )/age]	95.06	2.07	0.133	06.67	8.98	0.003
+ (Φ <sub>body5</sub> )	95.09	2.06	0.198	00.00	9.33	0.689
+ (X <sub>body50</sub> /h)	95.61	1.95	0.000	13.75	8.63	0.000
<b>BMI</b>						
w				64.26	5.56	0.000
w + (age)				65.19	5.48	0.049
w + (age) + h				84.21	3.69	0.000
w + (age) + h + (sex)				92.73	2.51	0.000

n: number of subjects; BIA: bioelectrical impedance analysis; BMI: body mass index; h: height; w: weight; R<sub>body250</sub>: body resistance at 250 kHz; Z<sub>body50</sub>: body impedance at 50 kHz; X<sub>body5</sub>: body reactance at 5 kHz; X<sub>body50</sub>: body reactance at 50 kHz; X<sub>body250</sub>: body reactance at 250 kHz; Φ<sub>body5</sub>: phase angle of the body at 5 kHz; R: validity coefficient; SEE: standard error of the estimate; TE: total error; p: significance of contribution of each additional individual parameter to the stepwise multiple regression model; %Ad.R<sup>2</sup>: percentage adjusted squared value of the validity coefficient

**Table 4:** Significant variables in bioelectrical impedance analysis model

	Standardized coefficient (beta)	t-value	p	Collinearity statistics	
				Tolerance	VIF
(R <sub>body250</sub> )	0.163	1.887	0.062	0.053	18.940
(sex)	-0.181	-4.657	0.000	0.258	3.871
w	1.212	32.615	0.000	0.284	3.522
(h <sup>2</sup> /Z <sub>body50</sub> )	-0.327	-2.805	0.006	0.029	34.587
h	-0.191	-3.028	0.003	0.098	10.190
[(X <sub>body250</sub> - X <sub>body5</sub> )/age]	0.106	3.194	0.002	0.356	2.808
(Φ <sub>body5</sub> )	0.077	2.018	0.046	0.271	3.696
(X <sub>body50</sub> /h)	-0.169	-3.656	0.000	0.184	5.429

h: height; w: weight; R<sub>body250</sub>: body resistance at 250 kHz; Z<sub>body50</sub>: body impedance at 50 kHz; X<sub>body5</sub>: body reactance at 5 kHz; X<sub>body50</sub>: body reactance at 50 kHz; X<sub>body250</sub>: body reactance at 250 kHz; Φ<sub>body5</sub>: phase angle of the body at 5 kHz; p: significance of contribution of each additional individual parameter to the stepwise multiple regression model



**Graphs 1A and B:** (A) Correlations of fat mass (FM) in all subjects measured by DXA and predicted by BIA equation, (B) differences of FM in all subjects determined by using BIA and DXA. Solid line represents the linear relation between mean differences in FM (FM by BIA-FM by DXA) and average FM [(FM by DXA + FM by BIA)/2]; dark abbreviated line represents the mean difference and light abbreviated lines represent the 95% confidence interval. BIA, bioelectrical impedance analysis; DXA, dual energy X-ray absorptiometry; n: number of subject; R: validity coefficient; SEE: standard error of estimation; TE: total error; circles indicate men; triangles indicate women

**Table 5:** Paired t-test for FM by DXA Vs BIA predicted FM for subjects with age above 60 years (n = 35)

	Mean ± SD
FM (kg) by DXA	28.9222 ± 10.1149
BIA predicted FM (Kg)	28.6267 ± 9.8814
Difference (FM by DXA-BIA predicted FM) (Kg)	0.2955 ± 1.5354
95% confidence interval for mean difference: (-4.48, 5.07)	
t-test of mean difference = 0 (Vs not = 0); t-value = 0.12;	
p-value = 0.902; DF = 67	

n: number of subjects; FM: fat mass; BIA: bioelectrical impedance analysis; DXA: dual energy X-ray absorptiometry; SD: standard deviation; p: level of significance; DF: degree of freedom

from DXA was  $36.5356 \pm 7.6410$  kg. Fat mass predicted by equation was  $36.4505 \pm 7.5671$  kg. The mean difference was  $0.0851 \pm 1.9586$  kg ( $p > 0.05$ , paired t-test;  $R = 0.9669$ , total error = 0.5643 kg). Thus, it is possible to estimate FM with the same equation for elderly and obese subjects.

## DISCUSSION

### Validity of BIA

It is generally agreed that the accuracy of BIA depends on the variables included in the prediction equation and on using a specific prediction equation validated for a specific population.<sup>17</sup> Our equation for BIA-predicted FM was used, in order of entry, ( $R_{\text{body250}}$ ), (sex), w, ( $h^2/Z_{\text{body50}}$ ), h, [ $(X_{\text{body250}} - X_{\text{body50}})/\text{age}$ ], ( $\Phi_{\text{body5}}$ ), ( $X_{\text{body50}}/h$ ). Although weight (w) alone accounted for 64.26% of the variability (Table 3), all other variables entered added significantly to the BIA-predicted FM. The correlation obtained with the new developed equation is observed to be 97.94% against DXA. The cross validation of BIA equation is important to test for its accuracy. Slightly

**Table 6:** Paired t-test for FM by DXA Vs BIA predicted FM for subjects with BMI above  $27 \text{ kgm}^{-2}$  (n = 44)

	Mean ± SD
FM (kg) by DXA	36.5356 ± 7.6410
BIA predicted FM (Kg)	36.4505 ± 7.5671
Difference (FM by DXA-BIA predicted FM) (Kg)	0.0851 ± 1.9586
95% confidence interval for mean difference: (-3.14, 3.31)	
t-test of mean difference = 0 (Vs not = 0); t-value = 0.05; p-value = 0.958; DF = 85	

n: number of subjects; FM: fat mass; BIA: bioelectrical impedance analysis; DXA: dual energy X-ray absorptiometry; SD: standard deviation; p: level of significance; DF: degree of freedom

lower accuracy could be expected in subjects older than 81 years. Validity of the BIA equation in subjects older than 81 years is unknown and requires further validation; it is also necessary in subjects with BMIs below  $15.62 \text{ kgm}^{-2}$ . In addition, validation studies must be conducted in subjects with nutritional disorders that affect body water. Further validation of BIA is necessary to understand the mechanisms for the changes in acute illness, fat/lean mass ratios, extreme heights, and body shape abnormalities.

### Variations in BIA Parameters

Measurement of body parameters by segmental multiple frequency bioelectrical impedance analysis (MF-BIA) technique with tetra-polar electrode gives high degree of accuracy and precision. Bedogni et al<sup>21</sup> found that the precision of eight-polar segmental MF-BIA technique was quite good (coefficient of variation, CV < 3.0% for between days; CV < 2% for within days measurements) as compared to four-polar total body BIA performed at 50 kHz.

Several factors are known to influence the bioelectrical parameters, such as age, height, weight, gender and ethnic origin.<sup>22,23</sup> Several investigators have found a positive relation between age and impedance<sup>24,25</sup> a negative relation between age and reactance,<sup>24-26</sup> and a negative relation between age and phase angle.<sup>25-27</sup> The present study (Table 1) too, validated that a negative relation between age and phase angle in both genders. Impedance and resistance increased with age in both sexes after 60 years. Reactance decreased with age progressively in men and women until 60 years. Bioelectrical impedance analysis parameters are frequency dependent. For any individual, body impedance and resistance decrease as frequency increases. However, the body reactance and the phase angle initially increase with frequency but after certain frequency they decrease with the increase in frequency (Table 1).

Bioelectrical impedance analysis values are affected by numerous variables including body position, hydration status, consumption of food and beverages, ambient air, skin temperature, recent physical activity, and conductance of the examining table. Reliable BIA requires standardization and control of these variables. A specific and well-defined procedure for performing routine BIA measurements is required.

#### *Factors affecting the Performance of BIA Equation*

In order to verify the different factors which may affect the performance of the BIA equation, the different sets of parameters were selected and for each set, with the help of SPSS package version 17, stepwise multiple regression analysis was carried out to derive BIA prediction equation for FM. The change in '% adjusted R<sup>2</sup>' and SEE were recorded for different set of variables. Through this exercise, it was found that simple measurement at 50 kHz was not sufficient. By adding frequencies help in the improvement of prediction and reducing the error. The single frequency measurement results at 50 kHz are superior to the results at 5 kHz or 250 kHz. It was also found that, inclusion of reactance/phase angle improves the prediction and reduces the error.

#### **CONCLUSION**

The results of this study show that the newly developed single prediction BIA equation validated against DXA can be used to predict FM in subjects aged 23 to 81 years and with BMIs ranging from 15.62 to 39.98 kgm<sup>-2</sup>. The BIA equation developed can be used in populations with large variations in age and body mass.

#### **ACKNOWLEDGMENT**

The authors thank Dr TS Ananthkrishnan, Head, Electronics Division, Bhabha Atomic Research Centre

and Dr SK Narayankhedkar, Principal, MGM's College of Engineering and Technology for their support and help throughout the study. We also thank Ms Aarti Karkera and MM Kuswarkar for helping in data collection.

#### **REFERENCES**

1. Jackson AS, Stanforth PR, Gagnon J, Ranknen T, Leon AS, Rao DC, Skinner JS, Bouchard C, Wilmore JH. The effect of sex, age and race on estimating percentage body fat from body mass index: the heritage family study. *Int J Obesity* 2002;26(6):789-796.
2. Cohn SH, Vaswani AN, Yasumura S, Yuen K, Ellis KJ. Improved models for determination of body fat by in vivo neutron activation. *Am J Clin Nutr* 1984;40(2):255-259.
3. Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nut* 1985;41(4): 810-817.
4. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol* 1986;60(4):1327-1332.
5. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. *Am J Clin Nutr* 1986;44(3): 417-424.
6. Graves JE, Pollack ML, Calvin AB, Van Loan M, Lohman TG. Comparison of different bioelectrical impedance analyzers in the prediction of body composition. *Am J Hum Biol* 1989;1(5):603-611.
7. Segal KR, Van Loan M, Fitzgerald PL, Hodgdon JA, Van Itallie TB. Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. *Am J Clin Nut* 1988;47(1):7-14.
8. Van Loan M, Mayclin P. Bioelectrical impedance analysis. Is it a reliable estimator of lean body mass and total body water? *Hum Biol* 1987;59(2):299-309.
9. Jackson AS, Pollock ML, Graves JE, Mahar MT. Reliability and validity of bioelectrical impedance in determining body composition. *J Appl Physiol* 1988;64(2):529.
10. Lukaski HC, Bolonchuk WW. Estimation of body fluid volumes using tetrapolar bioelectrical measurements. *Aviat Space Environ Med* 1988;59(12):1163-1169.
11. Heitmann BL. Prediction of body water and fat in adult danes from measurement of electrical impedance: a validation. *Int J Obes* 1990;14(9):789-802.
12. Deurenberg P, Van der Kooij K, Evers P, Hulshof T. Assessment of body composition by bioelectrical impedance in a population aged > 60 years. *Am J Clin Nut* 1990;51(1):3-6.
13. Deurenberg P, Vander KK, Leenen R, Westrate JA, Seidell JC. Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross-validation study. *Int J Obes* 1991;15(1):17-25.
14. Stolarczyk LM, Heyward VH, Van Loan MD, Hicks VL, Wilson WL, Reano LM. The fatness-specific bioelectrical impedance analysis equations of Segal et al: are they generalizable and practical? *Am J Clin Nut* 1997;66(1):8-17.
15. Pichard C, Kyle UG, Slosman DO. Fat-free mass in chronic illness: comparison of bioelectrical impedance and dual-energy X-ray absorptiometry in 480 chronically ill and healthy subjects. *Nutrition* 1999;15(9):668-676.



16. Roubenoff R, Baumgartner RN, Harris TB, Dallal GE, Hannan MT, Economos CD, Stauber PM, Wilson PW, Kiel DP. Application of bioelectrical impedance analysis to elderly population. *Journals of Gerontology: Biological Sciences and Medical Sciences* 1997;52(3):M129-M136.
17. Kyle UG, Pichard C, Rochat T, Slosman DO, Fitting JW, Thiebaud D. New bioelectrical impedance formula for patients with respiratory insufficiency: comparison to dual-energy X-ray absorptiometry. *Eur Respir J* 1998;12(4):960-966.
18. Pichard C, Kyle U, Gremion G, Gerbase M, Slosman D. Body composition by X-ray absorptiometry and bioelectrical impedance in elite female runners. *Med Sci Sports Exerc* 1997;29(11):1527-1534.
19. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1(8476):307-310.
20. Wellens R, Chumlea WC, Guo S, Roche AF, Reo NV, Siervogel RM. Body composition in white adults by dual energy X-ray absorptiometry, densitometry, and total body water. *Am J Clin Nutr* 1994;59(3):547-555.
21. Bedogni G, Malavolti M, Severi S, Poli M, Mussi C, Fantuzzi AL, Battistini N. Accuracy of an eight-point tactile-electrode impedance method in the assessment of total body water. *Eur J Clin Nutr* 2002;56(11):1143-1148.
22. Vellas B, Guigoz Y, Garry PJ, Nourashemi F, Bennahum D, Lauque S, Albarede JL. The mini nutritional assessment (MNA) and its use in grading the nutritional state of elderly patients. *Nutrition* 1999;15(2):116-122.
23. Maggiore Q, Nigrelli S, Ciccarelli C, Grimaldi C, Rossi GA, Michelassi C. Nutritional and prognostic correlates of bioimpedance indexes in hemodialysis patients. *Kidney International* 1996;50(6):2103-2108.
24. Kyle UG, Genton L, Hans D, Karsegard VL, Michel JP, Slosman DO, Pichard C. Total body mass, fat mass, fat-free mass and skeletal muscle in older people: cross-sectional differences in 60-year-old persons. *J Am Geriat Soc* 2001;49(12):1633-1640.
25. Kyle UG, Unger P, Mensi N, Genton L, Pichard C. Nutrition status in patients younger and older than 60 years at hospital admission: a controlled population study in 995 subjects. *Nutrition* 2002;18(6):463-469.
26. Kyle UG, Unger P, Dupertuis YM, Karsegard VL, Genton L, Pichard C. Body composition in 995 acutely ill or chronically ill patients at hospital admission: a controlled population study. *J Am Diet Assoc* 2002;102(7):944-955.
27. Piccoli A, Pillon L, Dumler F. Impedance vector distribution by sex, race, body mass index, and age in the United States: standard reference intervals as bivariate Z scores. *Nutrition* 2002;18(2):153-167.
28. Lewis MK, Blake GM, Fogelman I. Patient dose in dual X-ray absorptiometry. *Osteoporosis Int* 1994;4(1):11-15.