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Original Article

Bioimpedance analysis with a novel predictive equation - A reliable technique to estimate fat free mass in birth weight based cohorts of Asian Indian males

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ABSTRACT

Aim: To validate bioimpedance based predictive equations for fat free mass (FFM) against DEXA and to derive a novel birth weight based predictive equation for FFM in a birth weight based cohort of healthy Asian Indian men.

Methodology: Whole body composition was done using DEXA and bioimpedance in 117 young Asian Indian men, born of normal birth weight (n = 59, birth weight ≥2.5 kg) or low birth weight (n = 58, birth weight < 2.5 kg). Predictive accuracy of 11 different bioimpedance based equations for FFM was evaluated using Pearson's correlation analysis and the root of mean squared prediction error (RMSE) analysis.

Results: The mean FFM (on DEXA) and total lean mass & impedance index (on bioimpedance) were significantly higher in the low birth weight cohort. Significantly higher body fat percentage was noted on bioimpedance, for the normal birth weight cohort, but not on DEXA. In addition, the mean values of predicted FFM were significantly higher in the low birth weight cohort for 9 different predictive equations. Specifically, the mean FFM values obtained using the predictive equations of Schaefer et al., Hoot cooper et al. and Hughes et al. were in close agreement with the actual FFM values on DEXA. A novel predictive equation (CMC equation) for FFM based on birth weight was derived. FFM = 32.637 + (−0.222*age) + (−32.51*waist-to-hip ratio) + (0.33*body mass index) + (1.58 * 1 or 2 (1 = normal birth weight, 2 = low birth weight) + (0.510*waist circumference).

Conclusions: Our study findings substantiate the validity of Bio-impedance analysis (BIA) as a reliable and noninvasive tool for estimating body composition measures in birth-weight based cohorts of Asian Indian males. Further, we have devised a novel BIA-based predictive equation that can be useful in larger epidemiological studies to look at alterations in body fat in this cohort.

1. Introduction

Asian Indians feature a distinct phenotypic pattern characterized by higher body fat, excess truncal fat and lower lean body mass, resulting in higher predisposition to insulin resistance, metabolic syndrome and development of type 2 diabetes, when compared to Caucasians [1]. Importantly, insulin resistance and body composition are closely related to low birth weight (birth weight < 2.5 kg) in Asian Indians [2] and in other ethnic groups [3].

Measures of body composition namely fat mass, fat free mass, fat percentage, total lean mass and truncal fat mass have gained much importance in studies exploring the mechanisms of adiposity in metabolic disorders such as diabetes, cardiovascular disease and liver cirrhosis. Anthropometric measures such as body mass index (BMI), waist circumference, waist-hip ratio and skin fold thickness have been used extensively to estimate body composition in clinical settings and epidemiological studies. However, these techniques are error prone and show considerable variation across age, gender and ethnicity [4]. Robust techniques for body composition assessment such as underwater weighing (hydrodensitometry), dual energy

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x-ray absorptiometry (DEXA), and magnetic resonance imaging (MRI) are expensive, technically demanding and not feasible for large scale epidemiological studies. Bioimpedance analysis (BIA), by contrast, is relatively simple, inexpensive, faster, non-invasive and yields reliable measurements of body composition with a fair intra-observer and inter-observer variability [5]. In comparison to DEXA, BIA is inexpensive and portable, making it an ideal alternative to assess body composition in epidemiological studies [6]. Furthermore, the results are easily available and reproducible with acceptable error rates of less than 1% on repeated measurements [7]. BIA can also be used in infants, children and gestational mothers in whom DEXA imaging is not feasible or is contraindicated. However, the accuracy of BIA used in a specific population depends on its calibration and validation across different ethnic groups or subgroups of a specific population. In this birth weight cohort based study, we aimed to validate body composition profiles obtained using BIA with those obtained using DEXA and to derive fat free mass (FFM) using previously published bioimpedance based predictive equations. Furthermore, we aimed to derive a novel BIA based equation to predict FFM in the study cohort and validate it using DEXA and previously published predictive equations.

2. Methodology

Ethical approval for this cross sectional study was obtained from the institutional research board and ethics committee of Christian Medical College, Vellore, India (Research Committee Minute Number: 5879, 2006 and Administrative Committee Minute Number: 50-y: 6–2006) of Christian Medical College, Vellore (India). Data was obtained from a previous published cohort study from our group [2]. Briefly, a cohort of 117 unrelated men, aged between 18 and 22 years, born of normal birth weight ($n = 59$, birth weight ≥ 2.5 kg) or of low birth weight ($n = 58$, birth weight < 2.5 kg) were recruited with informed mutual consent from a rural region of Tamil Nadu, South India. The study was conducted in accordance to the guidelines mentioned in declaration of Helsinki [8]. Fasting blood samples were analysed for lipids and glycaemic profile. Body composition analysis through bioimpedance analysis was performed using a non −invasive, bioimpedance analyser (BodySTAT 1500). The bioimpedance analyser was calibrated every day and all measurements were recorded with the subject in the supine position. Two self-adhesive electrodes were placed on the right foot; one electrode was placed behind the second toe and the other on the ankle between the medial and lateral malleoli. Further, two electrodes were placed on the right hand; one electrode was placed behind the knuckle of the middle finger and the other on the wrist next to the ulnar head. Values for fat mass, lean mass, fat percent, bioimpedance and average energy requirement were recorded in accordance to the standard instructions of the National Institutes of Health Research (NIHR), Southampton, USA. In addition, whole body composition analysis was performed in all subjects using Dual Energy X ray absorptiometry (DEXA) with a DEXA scanner (Hologic DEXA Discovery QDR 4500). The scanner was calibrated daily using an aluminum phantom. Variables of body composition such as fat mass and lean mass in hands, legs and abdomen, were imaged using the same. Bilateral sections and whole body composition data were obtained by analysis of the regions of interest (ROI) using APEX software [9]. Fat free mass was calculated from bioimpedance analysis using eleven different predictive equations shown in Table 1and validated it with FFM obtained using DEXA. In addition, we also derived a BIA based novel predictive equation for FFM specific to the birth weight based cohorts of the present study and validated the novel equation with the FFM derived from DEXA and the other eleven bioimpedance based predictive equations.

3. Data analysis

Data were checked for normative distribution and summarised as Mean & SD/median using independent samples *t*-test. Data on body composition obtained by bioimpedance was compared with that of DEXA using Pearson's correlation coefficient analysis. Further, the root of mean squared prediction error (RMSE) analysis was used to indicate the accuracy of the predictive equation with respect to the study cohorts. The RMSE value was calculated based on the difference between the BIA predicted value and the DEXA reference value with all individual differences squared. The mean of the squared differences and the root of the mean value were calculated. Statistical analysis was performed using SPSS package (version 16) and R software was used for RMSE analysis.

4. Results

The low birth weight and normal birth weight cohorts were matched for age and BMI. The biochemical profile showed no significant differences for measures of glycaemia and the lipid profile (Table 2). Body composition analysis showed a significantly higher body fat percentage on bioimpedance, for the normal birth weight cohort but not on DEXA.Specifically, the low birth weight cohort had significantly higher mean values of fat free mass (on DEXA), total lean mass and impedance index (on bioimpedance) as compared to the normal birth weight cohort (Table 3).

In addition, the mean values of equation derived FFM were significantly higher in the low birth weight cohort for nine out of the eleven different predictive equations. Importantly, the mean FFM values obtained using the predictive equations of Schaefer et al. [11], Hout cooper et al. [12] and Hughes et al. [18] were in close agreement with the actual FFM values obtained using DEXA (Table 4). We performed agreement analysis for all predictive equations for FFM in the low birth weight and normal birth weight cohorts and observed that only for the normal weight cohort, the equation by Houtcooper et al. [12] was in close agreement with the actual values of FFM obtained using DEXA. Furthermore, in the normal weight cohort, the accurate predictive value of 43.1 was highest for the equation by Houtcooper et al. [12], with a minimum bias rate of 0.69 as compared to other groups (Table 5). In the low birth weight cohort, the equation by Schaefer et al. [11] showed the highest accurate predictive value of 41.3 and minimum RMSE value of 0.64F and bias rate: −5.37) as compared to other predictive equations. Moreover, the mean value of FFM derived using this equation was 42.6 ± 4.2 kg as compared to FFM derived from DEXA (45.3 ± 5.3), showing the highest degree of agreement as compared to other equations. The predictive equations by Bhat et al. [10], Lohman et al. [13], Segal et al. [5], and Tyrell et al. [16] performed poorly with higher degrees of under predictions in the normal birth weight and low birth weight cohorts of Asian Indian men (Table 6).

5. Predictive equation

In this study, we derived a BIA based predictive equation for prediction of fat free mass (FFM) specifically for the study cohort and validated it with FFM derived from DEXA. The equation named as Christian Medical college (CMC) equation is mentioned below:

FFM = 32.637 + (−0.222 × age) + (−32.51 waist-to-hip ratio) + (0.33 × body mass index) + (1.58 × 1 or 2 (normal birth weight coded as 1, Low birth weight coded as 2) + (0.510 × waist circumference). The mean value of 95% confidence intervals for the normal birth weight cohort and the low birth weight cohort were in range [NBW cohort: 1.45; lower limit: −7.4 & upper limit: 10.3) and LBW cohort: 1.45; lower limit: −5.4, upper limit: 8.4]. On applying the CMC equation, we observed that the mean value of FFM was in

Table 1
Bioimpedance based predictive equations for fat free mass.

Authors	Predictive equation for fat free mass (FFM)
Bhatt et al., 2005 [10]	FFM = 0.287 Height (cms) squared/Impedance + 0.3064 * Weight (kg) +12.297
Schaefer et al., 1994 [11]	FFM = 0.65 * Height (cm^2)/Impedance + (0.68 × age (years) + 0.15
Houtcooper et al., 1992 [12]	FFM = 0.61 * Height (cm^2)/impedance + (0.25 × weight (kg) + 1.31
Lohman et al., 1992 [13]	FFM = 0.475 * Height (cms) squared/Impedance + 0.295 *weight (kg) + 5.49
Duerenberg et al., 1991 [14]	FFM = 0.64 * Height (cms) squared/impedance + 4.83
Lukaski et al., 1986 [15]	FFM = 0.838 * Height (cms) squared/impedance squared + 4.179
Tyrell et al., 2001 [16]	FFM = 0.31 * Impedance index + 0.17 * height (cms) +0.11 * weight (kgs) + 0.942 * (Gender = 1 for females; 2 for males) −1496
Pietrobelli et al., 2002 [17]	FFM = 0.6375 (Impedance index) + 5.9913
Hughes et al., 2015 [18]	FFM = 0.432 * Impedance index-0.086 * age + 0.269 * Height squared + 16.42
Lorenzo et al., 1998 [19]	FFM = 0.6375 * Impedance index + 5.99
Segal et al., 1988 [5]	FFM = 0.00132 * Height (cms) squared + 0.3052 * weight (kgs) squared − 0.1676 * age (yrs) squared - 0.0439 * impedance + 22.66

Table 2
Baseline characteristics of the study cohort.

Variables	Normal birth weight cohort (n = 59)	Low birth weight cohort (n = 58)	P value
Age (years)	19.5 ± 1.0	19.7 ± 0.7	0.06
Birth weight (kg)	3.1 ± 0.2	2.1 ± 0.2	**0.00**
Body mass index (kg/m^2)	19.5 ± 2.6	19 ± 2.1	0.39
Waist circumference (cms)	70.9 ± 7.0	69.4 ± 7.5	0.25
Waist -to- hip ratio	0.82 ± 0.05	0.83 ± 0.03	0.48
Fasting plasma glucose (mg/dl)	87.2 ± 6.0	88.2 ± 6.6	0.38
Fasting serum insulin (μU/ml)	4.7 ± 4.9 3.5[a] (0.5, 29.6)[b]	5.5 ± 5.7 4.1[a] (0.2, 31.4)[b]	0.23
Fasting C - peptide (ng/ml)	1.5 ± 1.18 1.1[a] (0.1, 5)[b]	2.2 ± 4.5 1.4[a] (0.1, 34)[b]	0.25
Total cholesterol (mg/dl)	129.8 ± 27.3	133.7 ± 32.5	0.47
Serum triglycerides (mg/dl)	78.8 ± 32.4 74[a] (32, 158)[b]	83.7 ± 47.6 70[a] (20, 281.4)[b]	0.50
High density lipoprotein cholesterol (mg/dl)	31.0 ± 5.6	31.6 ± 8.2	0.65
Low density lipoprotein cholesterol (mg/dl)	80.0 ± 22.8	82 .0 ± 26.2	0.65

Values are presented as mean ± standard deviation. *P* value < 0.05: Statistically significant.
[a] Indicates median values & numbers in parentheses.
[b] Indicate minimum and maximum median values respectively.

Table 3
Body composition profile quantified by DEXA and bioimpedance in the study cohort.

Measures of body composition	Normal birth weight cohort (n = 59)	Low birth weight cohort (n = 58)	P value
Total fat mass (kgs) (on DEXA)	7.8 ± 5.0	8.2 ± 4.0	0.60
Total fat free mass (kgs) (on DEXA)	43.1 ± 5.8	45.2 ± 5.3	**0.03**
Total body fat percentage (kgs) (on DEXA)	14.4 ± 6.0	14.4 ± 4.9	0.94
Total body fat percentage (kgs) (on BIA)	18.1 ± 4.2	15.9 ± 4.0	**0.005**
Total lean mass (kgs) (on BIA)	11.0 ± 5	13.1 ± 4.2	**0.01**
Bioimpedance (Ohms)	678 .9 ± 73.0	667.1 ± 76.0	0.39
Impedance index (on BIA)	41.7 ± 5.1	44.7 ± 6.2	**0.00**

Values are presented as mean ± standard deviation. *P* value < 0.05: Statistically significant.

Table 4
Comparison of FFM obtained using predictive equations with FFM obtained using DEXA.

Predictive equations for fat free mass	Normal birth weight cohort (n = 59)	Low birth weight cohort (n = 58)	P value
Predicted FFM mass (kg) (Segal et al.) [5]	6.3 ± 5.8	8.0 ± 6.6	0.41
Predicted FFM mass (kg) (Bhat et al.) [10]	28.8 ± 2.8	29.9 ± 2.5	**0.03**
Predicted FFM mass (kg) (Schaefer et al.) [11]	**40.8 ± 3.4**	**42.5 ± 4.3**	**0.01**
Predicted FFM mass (kg) (Houtcooper et al.) [12]	**40.1 ± 4.7**	**42.8 ± 5.5**	**0.00**
Predicted FFM mass (kg) (Lohman et al.)	21.3 ± 2.7	22.3 ± 2.4	**0.03**
Predicted FFM mass (kg) (Deurenberg et al.)	31.5 ± 3.3	33.4 + 4.0	0.00
Predicted FFM mass (kg) (Lukaski et al.)	37.7 ± 5.4	40.2 ± 5.9	0.01
Predicted FFM mass (kg) (Tyrell et al.)	33.9 ± 3.4	36.0 ± 3.7	0.00
Predicted FFM mass (kg) (Pietrobelli et al.)	32.1 ± 4.7	34.0 ± 5.4	0.04
Predicted FFM mass (kg) (Hughes et al.)	**46.8 ± 4.7**	**49.1 ± 5.0**	**0.01**
Predicted FFM mass (kg) (Lorenzo et al.)	37.7 ± 5.4	40.2 ± 5.9	0.01
Predicted FFM mass (kg) (CMC equation)	44.4 ± 4.1	46.9 ± 3.6	0.00
Actual FFM (kg) (On DEXA)	**43.1 ± 5.8**	**45.2 ± 5.3**	**0.03**

Values are presented as mean ± standard deviation. *P* value < 0.05: Statistically significant.

Table 5
Comparison of bioimpedance based predictive equations for FFM with the novel equation for normal birth weight cohort.

Normal birth weight cohort (n = 59)	FFM (kg)	Accurate Predictions	Under Predictions	Over Predictions	RMSE
CMC Equation	44.4 ± 4.2	34.48	18.97	46.55	0.61
Lohman et al.	21.3 ± 2.7	0.00	100.00	0.00	2.92
Bhat et al.	28.8 ± 2.8	0.00	100.00	0.00	1.95
Segal et al.	5.9 ± 4.6	0.00	100.00	0.00	4.92
Schaefer et al.	40.8 ± 3.4	34.48	46.55	18.97	0.77
Houtcooper et al.	40.1 ± 7.7	43.10	50.00	6.90	0.69
Deurenberg et al.,	31.5 ± 3.2	1.72	98.28	0.00	1.67
Lorenzo et al.	38.1 ± 4.3	15.52	81.03	3.45	0.87
Pietrobelli et al.,	32.5 ± 3.3	1.72	96.55	1.72	1.54
Hughes et al.,	47.1 ± 4.0	12.07	8.62	79.31	0.75
Tyrell et al.,	34.1 ± 2.9	0.00	100.00	0.00	1.31
Lusaki et al.	39.1 ± 4.3	24.14	63.79	12.07	0.88

Values of FFM are presented as Mean ± SD.
Equations with 100% under predictions or 0% accurate predictions are considered inferior.

Table 6
Agreement analysis of bioimpedance based predictive equations for FFM with the novel equation for the low birth weight cohort.

Low birth weight cohort (n = 58)	Mean FFM (kg)	Accurate Predictions	Under Predictions	Over predictions	RMSE
CMC Equation	46.7 ± 3.5	37.9	13.9	48.28	0.49
Lohman et al.	22.3 ± 2.5	0.00	100.00	0.00	3.06
Bhat et al.	29.9 ± 2.6	0.00	100.00	0.00	2.07
Segal et al.,	7.5 ± 5.3	0.00	100.00	0.00	4.98
Schaefer et al.,	42.6 ± 4.2	41.38	51.72	6.90	0.64
Houtcooper et al.,	42.9 ± 5.5	37.93	56.90	5.17	0.55
Deurenberg et al.,	33.5 ± 4.0	1.72	98.28	0.00	1.63
Lorenzo et al.,	40.7 ± 5.1	17.24	81.03	1.72	0.75
Pietrobelli et al.,	34.6 ± 4.0	1.72	98.28	0.00	1.50
Hughes et al.,	49.4 ± 4.6	12.07	3.45	84.48	0.67
Tyrell et al.,	36.3 ± 3.3	0.00	100.00	0.00	1.28
Lusaki et al.	41.7 ± 5.2	27.59	68.97	3.45	0.72

Values of FFM are presented as Mean ± SD.
Equations with 100% under predictions or 0% accurate predictions are considered inferior.

close agreement with the FFM values quantified by DEXA in the study cohorts. A significantly higher mean value of FFM was observed for the low birth weight cohort as compared to normal weight cohort. Furthermore, in the normal birth weight cohort, the mean FFM values derived by using CMC equation were similar to the mean FFM values derived using the equation of Scheafer et al., [11] with lower degree of under predictions (Table 5). In the low birth weight cohort, the CMC equation derived the best prediction value of FFM close to that of DEXA, with accurate predictions and RMSE values being similar to that of Houtcooper et al. [12]. Contrastingly, lower degrees of accurate predictions were observed for the predictive equations of Duerenberg et al. [14], Pietrobelli et al. [17], and Tyrell et al. [16], as shown in Table 6.

6. Discussion

This is the first birth weight cohort based study to derive a BIA based predictive equation for fat free mass in young, healthy Asian Indian males. In this study on two birth weight based cohorts from Tamil Nadu, South India, we observed significantly higher mean values of fat free mass (on DEXA) and fat free mass derived using three different BIA based predictive equations. These equations had been validated earlier in other ethnic groups but not in Asian Indians. Schaefer et al., 1994 [11] used bioimpedance to estimate body composition and compared to potassium (40 K) spectrometry to derive a predictive equation to derive fat free mass in German adolescents. We validated this equation in comparison to DEXA and found the highest degree of accurate predictions (41.3%) when compared to other equations. The term accurate prediction indicates the degree of precision with which the predictive equation can derive values closer to the actual value obtained using gold standard techniques. On the other hand, the term under prediction indicates the error rate of the equation in deriving the actual value. Schaefer et al., used skinfolds and bioimpedance and derived a predictive equation for comparative analysis with the equations of Houtcooper et al. [12] and Deurenberg et al. [14], and found good agreement with that of Deurenberg et al. [14], which was based on simple variables such as body impedance, sex, age and anthropometric variables in a group of 246 Dutch children and young adults, ranging in age from 7 to 25 years. In the current study, we observed higher degrees of agreement of Schaefer et al. [11] and Houtcooper et al. [12] with DEXA quantified FFM for the normal birth weight and low birth weight cohorts respectively, but not for Deurenberg et al. It may be noted that Houtcooper et al. [12] derived a bioimpedance based predictive equation for FFM using simple variables such as height, weight and impedance in adolescents aged 10–19 years and compared it with FFM determined from body density (underwater weighing) and body water (deuterium dilution) (FFB-DW) as standards for comparison, instead of DEXA. However, in comparison to DEXA, underwater weighing and deuterium dilution methods are cumbersome and no longer in vogue from a clinical perspective due to considerable discomfort to study participants. Lukaski et al. [15], reported a strong correlation between FFM determined by hydro-densitometry and FFM mass derived using a BIA based predictive equation in healthy 114 male and female subjects aged between 18 and 50 years. Compared to hydro-densitometry, the BIA method had a lower predictive error in estimating body composition as compared to anthropometry. However, on applying this equation in the current study, we observed the highest degree of under predictions (63–68%) with a mean difference of 5 kg FFM quantified using DEXA, in the normal birth weight cohort.

Lohman et al. [13], derived a BIA based predictive equation for FFM using simple variables namely resistance, body weight and

height. In our study, this equation showed the least degree of accurate predictions with mean values of predicted FFM being 20 kg less than the actual FFM obtained using DEXA. A similar trend was observed for the BIA based predictive equation by Bhat et al. [10], derived from a sample of 141 males aged between 29 and 51 years. Though the equation by Bhat et al. [10] was derived in Asian Indians men, the predictive accuracy was minimal in our study cohort. This discordance could be attributed to differences in the age group of subjects in the present study and that of Bhat et al. It may be noted that variables such as age, gender, BMI, body fat percentage are potential confounders resulting in nearly 45% variation in the estimation of FFM by BIA as shown in a large study on 653 healthy subjects aged between 20 and 90 years [20]. In line with this observation, the lowest degree of accurate predictions for FFM was observed for the BIA based equation by Tyrell et al. [16] for young children aged 8.1 ± 1.5 years. A study by Pietrobelli et al. (2003) in healthy Italian subjects aged 7–14 years included triceps skinfold thickness and derived gender specific BIA based equations for FFM and found significant agreement (87%) with FFM measured using DEXA [17]. Contrastingly, this equation showed less predictive accuracy in our cohort, thereby limiting its application for Asian Indian males. An earlier study in urban Asian Indians aged between 45 and 50 years (BMI 16–34 kg/m^2) compared body composition profile using segmental bioimpedance and skinfold measurement with DEXA [6]. Interestingly, the measurement of body fat by the leg-to-leg impedance and the skinfold method had better agreement with DEXA than the hand-held impedance. However, in the study of reference, fat free mass was not estimated using BIA.

7. Cohort specific predictive equation for FFM

It is intriguing to note that despite the agreement of FFM values calculated using these predictive equations with DEXA, the degrees of accurate predictions were highly variable for each equation. This may primarily be due to differences in body composition among ethnic groups and due to bias in measurement techniques between each study. Thus, the need for an ethnicity specific predictive equation is inevitable. In this study on two birth weight based cohorts, we derived a bioimpedance based predictive equation for FFM using simple variables such as age, waist circumference, waist -to-hip ratio, BMI and birth weight. Importantly, the degree of accurate predictions was the higher as compared to other predictive equations for FFM. Besides, this equation can be applied in any bioimpedance analyser as it does not require resistance values which only few BIA devices provide. Furthermore, validation of the predicted FFM has been done with the FFM quantified on DEXA, which is considered the gold standard technique for whole body composition.

In summary, our study observations show that BIA can be used as a fairly reliable and non-invasive method in epidemiological studies to measure body composition, in Asian Indian males of South India. The predictive equation obtained in this study can be applied for BIA based estimation of fat free mass in BIA devices wherein FFM cannot be estimated directly. However, the novel equation must be validated with DEXA prior to its application in large scale epidemiological studies across other populations of Asian Indian ethnicity. It is striking to note that nearly 20% of infants are born in India are underweight (birth weight < 2500 g), predominantly owing to maternal under-nutrition before and during pregnancy [21]. Individuals born with low birth weight have an inherent propensity for decreased lean mass, increased adiposity and early onset of metabolic syndrome, type 2 diabetes and cardiovascular diseases [22,23]. In this scenario, bioimpedance based measures can be effectively used in prospective studies to evaluate the phenotypic changes in body composition of Asian Indians.

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