A 4-compartment model based validation of air displacement plethysmography, dual energy X-ray absorptiometry, skinfold technique & bio-electrical impedance for measuring body fat in Indian adults

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**Background & objectives:** Many methods are available for measuring body fat of an individual, each having its own advantages and limitations. The primary objective of the present study was to validate body fat estimates from individual methods using the 4-compartment (4C) model as reference. The second objective was to obtain estimates of hydration of fat free mass (FFM) using the 4C model.

**Methods:** The body fat of 39 adults (19 men and 20 women) aged 20-40 yr was estimated using air displacement plethysmography (ADP), dual energy X-ray absorptiometry (DEXA), 4-skinfold technique and bio-electrical impedance (BIA). Total body water was estimated using isotope dilution method.

**Results:** All the methods underestimated body fat when compared to 4C model, except for DEXA and the mean difference from the reference was lowest for DEXA and ADP. The precision of the fat mass estimated from 4C model using the propagation of error was 0.25 kg, while the mean hydration factor obtained by the 4C model was found to be 0.74 ± 0.02 in the whole group of men and women.

**Interpretations & conclusion:** The results of the present study suggest that DEXA and ADP methods can provide reasonably accurate estimates of body fat, while skinfold and bio-electrical impedance methods require the use of population specific equations.

**Key words** Air displacement plethysmography - bio-electrical impedance - body composition - 4C model - DEXA - skinfold technique - total body water

The assessment of body fat content is an important aspect of measured outcomes in clinical nutrition. The commonly used ‘simple’ or field techniques for the measurement of body fat are skinfold thickness and bio-impedance, while the more expensive laboratory methods, requiring competent technical expertise, are densitometry, dual energy X-ray absorptiometry (DEXA) and hydrometry.

Hydrometry involves the measurement of total body water (TBW) by the dilution of an isotopic tracer using stable isotopes such as deuterium or ¹⁸O. Although the analysis of body water samples requires a good laboratory, the method itself can be performed in the field. The hydro-densitometry method involves the measurement of the body weight after the body is immersed underwater, from which the volume
and density of the body, and hence body fat, can be calculated. An alternative to hydro-densitometry for the measurement of body volume is air displacement plethysmography (ADP), which is safe, quick, comfortable, non-invasive, and can be used for children, obese and the elderly. The DEXA is also increasingly being used to measure body fat because of its ease in use, availability and low radiation exposure.

Although some measurement methods are more accurate and precise than others, there is no true reference standard for the measurement of body fat. All methods incorporate some assumptions; the best model is, therefore, derived with a combination of measurements, where the use of assumptions is minimized. The 2-compartment model (2C), which divides the body weight into fat mass (FM) and fat free mass (FFM), ignores inter-individual variability in the composition of FFM. The errors associated with the 2C model lie in the validity of its assumption of the density of the FM and FFM, which are based on analyses of 3 cadavers. A 3-compartment (3C) model was proposed by Siri, which is based on measurements of body density and TBW, but still assumes a constant mineral to protein ratio in the dry FFM; this model, therefore, makes no assumptions about the hydration of the FFM. The 4-compartmental (4C) model is more robust with respect to inter-individual variability in the composition of FFM. This model divides the FFM into measured water and mineral mass, and only assumes the density of the remaining components of the FFM (protein, DNA, glycogen, etc), and is, therefore, the best reference method, given the primary measurements available. However, since the 4C model may not be practical in many settings, there is a need to validate the commonly used methods. Previous studies have validated methods such as ADP, DEXA, skinfold and BIA in western populations using the 4C model as reference.

The relationship between body mass index (BMI) and body fat varies in different populations and Indians or people of Indian origin are more adipose for a given BMI, and there are limited good quality data on the body fat of Indians using appropriate methods. To judge the different data sets that are available on body fat measurements by different methods, a reference is needed. Additionally, only one study has measured the hydration factor of FFM in Indians using isotopic methods.

Thus the objectives of the present study were to validate body fat estimates obtained from the ADP, DEXA, skinfold and BIA methods, with the 4C model as a reference method, in Indian men and women aged 20-40 yr, and obtain estimates of the hydration factor of the FFM using the 4C model. Given the importance of this constant in many simple measurements of body fat, it was important to obtain measured values for these constants in Indian adults.

Material & Methods

The subjects for this cross-sectional study were selected at the Nutrition and Lifestyle Management Clinic, St. John’s Medical College Hospital, Bangalore, Karnataka, India, between January to May 2011. Thirty nine healthy individuals (19 men and 20 women) were recruited, aged 20 – 40 yr, all of whom reported weight stability in the preceding one year. They were normal healthy individuals from urban areas of Bangalore. Some of them were staff of the St. John’s National Academy of Health Sciences, while others were normal individuals accompanying their family members/friends, who had come for consultation to the nutrition clinic. The subjects were mainly students, doctors, laboratory technicians, drivers, and administrative assistants, and were classified as “healthy”, based on medical history and available records. They were not put on any nutritional treatment. The sample size was calculated, to observe a correlation coefficient of 0.9 between the individual method and 4C method, with 80 per cent power and 5 per cent level of significance. This sample size was also sufficient to estimate a average difference of 1.3 kg between the reference method and the individual methods with 25 per cent precision and 95 per cent confidence interval. The exclusion criteria were any chronic illness, hormonal abnormalities and pregnancy. The study was approved by the Institutional Ethics Review Board of St. John’s Medical College and Hospital. The objectives of the study were clearly explained to the subject and a written informed consent was obtained.

Measurements: The subjects reported at 0700 h to the Nutrition Clinic. General information regarding age and weight history was recorded. The following measurements were carried out:

Anthropometry and skinfolds - The anthropometric measurements were performed by trained staff. Anthropometric measurements of body weight, height, waist circumference and skinfold thickness were measured by utilizing standard methodology. The body weight was measured to the nearest 0.1 kg using an electronic scale (Essae Teraoka Limited, India). The weighing scale was calibrated regularly using standard
weights. The height was measured with the subjects standing erect, without shoes, looking straight on a level surface with heels together and toes apart and measurement was performed to nearest 0.1 cm. Waist circumference was measured with a non-stretchable tape by trained nutritionists at the midpoint of the lowest rib cage and the iliac crest to the nearest 0.1 cm\textsuperscript{14}. Skinfold thickness measurements were performed at the sites of biceps, triceps, subscapular and suprailiac\textsuperscript{9} using a Holtain Caliper (Holtain Ltd, Crymych, UK). The skinfold measurements were used in age and gender equations to determine body density\textsuperscript{15} and body fat was derived using Siri’s equation\textsuperscript{4}.

Total body water (TBW) - A basal saliva (2 aliquots of 1 ml from each) sample was collected. An oral dose (0.2 g/kg body weight) of \^18O was used to measure total body water using \(^18\)O dilution. The individuals were provided with 50 ml of water after the dose and further saliva samples were collected at 3 and 4 h post dose. An aliquot of the dose was stored for future analysis of the isotope, which was incorporated in the calculation sheet for determining the pool size of each subject. The study individual was provided with three biscuits after the dose and then was not allowed to eat or drink till the completion of the study. Saliva samples were analyzed in triplicate using an isotope ratio mass spectrometer (IRMS, Thermo Scientific, Germany), with internal and external precision of <0.2 and 0.5 ppm (SD), respectively, using the equilibration method. The TBW was calculated from the \(^18\)O dilution using the plateau enrichment at 3 and 4 h post-dose\textsuperscript{16}. Since the TBW values can be overestimated, due to the exchange of the \(^18\)O isotope with non-aqueous exchangeable oxygen, conventionally the \(^18\)O dilution space is converted to TBW by dividing by a factor of 1.00717.

Dual energy X-ray absorptiometry (DEXA) - Bone mineral content (BMC), bone mineral density (BMD), FFM and FM were determined using the Lunar Prodigy Advanced PA+301969 (GE Medical Systems, USA) whole body scanner, with software version 12.30. Scans were performed with the subject wearing light clothing and no metal objects. Values for FM, FFM (lean tissue + BMC) were obtained directly from the results. The DEXA machine was calibrated daily. The BMC reported in the DEXA represents ashed bone\textsuperscript{18}, which is the total bone mineral mass minus the volatile components lost in ashing. Bone mineral mass (BMM) was estimated from the bone mineral content from DEXA (ash) by multiplying by 1.0436, assuming that 4 per cent of the bone mineral is lost during ashing\textsuperscript{19}. The internal coefficient of variation (CV) in our laboratory for ash content was 2.1 per cent.

Bio-electrical impedance (BIA) - The Quadscan 4000 (Bodystat, UK) was used to measure body composition. The subject was made to lie in a supine position on a non-conducting surface, with the arms slightly abducted from the trunk and the legs slightly separated. Four surface electrodes were placed on the right side of the body on the dorsal surface of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively and also medially between the distal prominences of the radius and ulna and between the medial and lateral malleoli at the ankle\textsuperscript{20}. The body fat values obtained from the instrument, based on the proprietary equation used by the manufacturer, were used for statistical analysis.

Air displacement plethysmography (ADP): The body fat of subjects was measured in light clothing using ADP (BOD POD, Life Measurement Inc, USA), according to the instructions of the manufacturer. The subject was made to empty their bladder, weighed on an accurate digital scale and made to sit in the chamber of the instrument. Body volume was measured thrice, for 40 seconds each. Predicted lung volume was used for the calculation of body volume using equations\textsuperscript{6}. Appropriate corrections for thoracic gas volume and skin surface area artefact in adults were applied to the raw measurement to obtain actual BV\textsuperscript{21}. Body density was obtained by using the values of body weight and body volume. The per cent fat and fat mass was obtained from body density using Siri’s equation\textsuperscript{4}. The internal CV of body fat by this method was found to be 2.3 per cent.

Four compartment model: This model divides the body into fat, water, protein and mineral, and avoids the assumptions that the hydration fraction of the FFM, as well as the ratio between mineral and protein in FFM, are constant. The 4C model is based on the assumption that the density is 0.9007 kg/l for body fat, 0.99371 kg/l for water at 36\(^\circ\)C, 2.982 kg/l for mineral mass and 1.5157 kg/l for protein plus mineral. The minor components such as glycogen and DNA are ignored. Body volume was obtained from ADP, body water from TBW measurement and bone mineral mass from DEXA, as stated above. The 4C model uses the primary measurement values and densities (water, mineral and protein) to derive a value for the density of FFM\textsuperscript{8}. This derived value of the density of FFM and value for density of fat (above) were used in the densities...
and proportions equation of the 2C model (FM and FFM) to compute the body fat content. Rearranging these equations and collecting terms yields a single equation:

\[ \text{Fat (kg)} = 2.747 \times \text{BV} - 0.710 \times \text{TBW} + 1.460 \times A - 2.050 \times Wt \]

Where BV is body volume, TBW - total body water, A- ash and Wt - body weight.

The hydration factor of FFM was calculated as the ratio of TBW from \(^{18}\text{O} \) dilution and the FFM derived from 4C model.

**Statistical analysis:** The 4C model was used as the reference method, and the resulting fat mass was compared with the other individual methods. Bias between methods was calculated as the difference in body fat by an individual method, from that obtained from 4C model. The mean bias for each method is reported with the corresponding 95 per cent confidence interval (95 % CI). Bland Altman plots of bias against the fat mass obtained from 4C model were examined, since it has been demonstrated that the correlation in the bias is lower across ranges of errors of the test measure when the criterion method is used in the x axis, instead of the mean

Estimates of the overall measurement precision of fat mass and hydration factor from the 4C model were calculated using the propagation of error method (http://burro.case.edu/Academics/Astr306/Stats/Appendix_V_Error_Prop.pdf). The reason for calculating this error was that each primary measurement’s precision propagated into the final 4 compartment model estimate. The precision estimates were based on a 60 kg person with 60 per cent TBW. The values for error in the basic measurements were as follows: body volume 0.09 litres (obtained from 10 consecutive measurements on an individual using the ADP method; TBW 0.036 litres (obtained from 10 consecutive measurements), total body mineral ash 0.028 kg (obtained from 10 consecutive DEXA measurements on an individual), and body weight 0.01 kg. The precision of estimated amount of fat using the 4C model, obtained from the propagation of error method for a 60 kg person was ± 0.25 kg. It is clear from the equation that since the coefficients for body volume and body weight are higher in the equation used to derive body fat; greater precision in these measurements needs to be maintained. The precision associated with the measurement of the hydration factor was ± 0.008.

**Results**

The mean age of the study subjects was 26.3 ± 3.6 yr, while the mean body weight and BMI were 57.3 ± 11.8 kg and 21.8 ± 2.8 kg/m\(^2\), respectively (Table). The sample size of the study was not adequate for male-female comparison.

The mean estimate of body fat by the reference 4C model was 17.6 ± 5.6 kg (16.1 ± 6.2 in men and 18.1 ± 6.2 kg in women). The mean estimates of body fat using the various methods, for the whole group and based on gender are presented in the Table. The mean hydration factor was found to be 0.74, 95 per cent CI : 0.729, 0.745 in the whole group (0.73, 95% CI: 0.717, 0.745 in men and 0.74, 95% CI: 0.7345, 0.750 in women).

The mean bias for the DEXA, ADP, BIA and skinfold methods were -0.5 ± 1.6 (95% CI:-1.04, 0.01), 0.6 ± 1.5 (95% CI: 0.15, 1.10), 3.4 ± 2.7 (95% CI: 2.48, 4.24) and 4.6 ± 2.4 (95% CI: 3.74, 5.29) kg, respectively. All the methods underestimated body fat when compared to the 4C model, except for DEXA method. Bland and Altman plots (Fig.) were used to examine the pattern of bias between the reference method (4C) and individual methods. ADP was the only method where the bias was random across the range of fat mass and the mean bias was relatively small (0.6). There was a systematic bias observed for DEXA, skinfold method and BIA. For DEXA, although the mean bias was the smallest, there was overestimation at lower values and underestimation at higher values of fat mass.

**Discussion**

This study examined the accuracy, precision and bias of fat mass as assessed by DEXA, ADP, skinfold and BIA methods relative to a reference 4C method in Indian adults. The study also provided data on the hydration of FFM in Indian adults.

The DEXA method provided estimates of body fat with the lowest bias, when compared to 4C method, with slight overestimation of body fat. However, the negative association of bias in DEXA body fat with the same measurement from the reference method suggests that the magnitude of error is not constant across the magnitude of measurement. Studies on DEXA for measuring body composition have shown mixed results. An earlier study showed that the DEXA, regardless of gender, underestimated the per cent body fat of lean individuals when compared to a 4C model\(^2\). Some of the reasons suggested for the difference between the DEXA and 4C method are biological variations in FFM
hydration, variations in surrogates of anterior-posterior tissue thickness and age dependent differences in body composition estimations\textsuperscript{25}. Nevertheless, the DEXA offers a quick, non-invasive and easy body composition assessment and has also been shown to be superior to many other methods in assessing body fat\textsuperscript{26}. Further, the use of DEXA to evaluate body composition uniquely provides whole body and regional measurements. The results of the present study supports the existing literature that suggests that DEXA provides accurate group mean estimates of body fat, but that there are small errors in individual estimates; therefore, an understanding of the limitations is important while interpreting results. Additionally, since variations exist between the manufacturers and software version, it is important to provide details of the instrument including the manufacturer and software version. Since the regression line deviated from the line of identity for DEXA in this study, it overestimated fat mass for individuals with low fat and underestimated for those with higher fat. These findings could have implications, for the estimation of fat mass using DEXA in the Indian populations who have been reported to have greater adiposity for a given BMI\textsuperscript{10}. However, more studies with larger sample size are needed to confirm these findings.

There was underestimation of body fat mass by the ADP method in comparison with the 4C model (about 1.1\%) and the magnitude of the difference was constant across different magnitudes of fat measurement. Previous studies, comparing the ADP to the 4C model have demonstrated that it underestimated per cent body fat by about 2-3 per cent\textsuperscript{27,28} and these differences have been partly explained by limitations in the assumptions of 2C model, rather than by limitations of the ADP technique\textsuperscript{27}. The findings of our study suggest that the ADP method can be used in Indian subjects, who are shown to be more adipose for a given BMI\textsuperscript{10} and can provide accurate estimates of body fat within 5 per cent of the reference measurement. The ADP is a safe, comfortable, non-invasive, radiation free method to get population estimates of body fat and can be used in children, obese and elderly. However, since the ADP method makes assumptions about the lung volume, it needs to be validated. Additionally, it is important to follow the right testing protocol; in particular, the use

<table>
<thead>
<tr>
<th>Variable</th>
<th>Combined (n= 39)</th>
<th>Women (n= 20)</th>
<th>Men (n= 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.3 ± 3.6</td>
<td>25.9 ± 2.9</td>
<td>26.8 ± 4.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>57.3 ± 11.8</td>
<td>52.0 ± 8.5</td>
<td>62.8 ± 12.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.61 ± 0.1</td>
<td>1.60 ± 0.1</td>
<td>1.68 ± 0.1</td>
</tr>
<tr>
<td>Body mass index, BMI (kg/m\textsuperscript{2})</td>
<td>21.8 ± 2.8</td>
<td>21.6 ± 2.4</td>
<td>22.4 ± 3.1</td>
</tr>
<tr>
<td>Total body water, TBW by \textsuperscript{18}O dilution (kg)</td>
<td>29.3 ± 6.8</td>
<td>24.6 ± 3.8</td>
<td>34.3 ± 5.3</td>
</tr>
<tr>
<td>Body density (g/ml)</td>
<td>1.032 ± 0.017</td>
<td>1.021 ± 0.010</td>
<td>1.045 ± 0.010</td>
</tr>
<tr>
<td>Bone mineral content (kg)\textsuperscript{4}</td>
<td>2.3 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Fat mass (4 C-kg)</td>
<td>17.6 ± 5.6</td>
<td>18.1 ± 6.2</td>
<td>16.1 ± 6.2</td>
</tr>
<tr>
<td>Fat mass (DEXA-kg)</td>
<td>18.1 ± 6.8</td>
<td>20.1 ± 5.5</td>
<td>15.9 ± 7.4</td>
</tr>
<tr>
<td>Fat mass (ADP-kg)</td>
<td>16.9 ± 5.8</td>
<td>18.4 ± 4.9</td>
<td>15.4 ± 6.3</td>
</tr>
<tr>
<td>Fat mass (BIA-kg)</td>
<td>14.2 ± 4.3</td>
<td>16.0 ± 4.0</td>
<td>12.4 ± 3.7</td>
</tr>
<tr>
<td>Fat mass (SKF-kg)</td>
<td>13.0 ± 4.2</td>
<td>14.3 ± 3.8</td>
<td>11.6 ± 4.4</td>
</tr>
</tbody>
</table>

\textsuperscript{4}Values obtained from DEXA
4C- 4 compartment model; DEXA, dual energy X-ray absorptiometry; ADP, air displacement plethysmography; BIA, bio-electrical impedance; SKF, skinfold thickness

Values are mean ± SD
of tight fitting clothing during the measurement, since excess clothing causes a significant underestimation of body volume. This is because the air that comes in contact with the cloth will remain isothermal as pressure fluctuates.

The skinfold method underestimated the per cent body fat by 8 per cent. In present study the prediction equation of Durmin and Womersley was used to arrive at estimates of body fat. To our knowledge, only one study has used a 4C model to validate skinfold prediction equations, and the results of the study demonstrated that the Durmin and Womersley equation underestimated per cent body fat by about 7 per cent.

On the other hand, it was observed that in Indian men, there was no systematic difference in the body fat measurement by skinfolds method in relation to D2O measurements, suggesting that skinfold fat measurement by Durmin and Womersley equation might be used in Indians without any change in the prediction equation. This was similar to another study in 100 Indian men and women, in whom hydrodensitometric estimates of body fat showed little bias when compared with the skinfold method. Even so, the random error of this method is high, and its use, while quick, easy, non-invasive and suited for large epidemiological studies, is still best suited to group analyses. It is also important
to use equations which are best suited for a specific population.

The BIA method underestimated per cent body fat by 5.5 per cent when compared to the 4C method. The BIA measurement has been shown to systematically differ in body fat measurement in comparison to the deuterium dilution method in Indian men, underestimating it at lower and overestimating it at higher body fat. The present study also supports these findings and keeping in mind the large random error observed between the BIA and 4C methods, the readings of body fat taken directly from the BIA machine software should be interpreted with caution. There is also a need to devise new robust age and gender based Indian equations. The results of the present study could have significant implications with regard to the wide use and interpretation of results from portable BIA machines in clinical practice and research studies.

The hydration fraction of FFM obtained from the present study was 0.74 ± 0.02 (0.73 ± 0.03 in men and 0.74 ± 0.02 in women). The range of hydration factor was 0.68 to 0.78, which was similar to values reported earlier, but different from an earlier deuterium dilution study on 10 adult men and women, where it was lower (0.70 ± 0.03 in men and 0.72 ± 0.02 in women). In general, it is fair to assume that all ethnic groups have the same constant hydration factor of FFM (0.73). There could be ethnic differences in the hydration factor and climatic conditions could cause a directional bias. The difference between the earlier 3C study and the present study could also be due to methodological differences, which could occur due to the different assumptions used. The hydration factor obtained in the present study was possibly as accurate as could be obtained in the present circumstances, since it was from a 4C model with a minimum number of assumptions, and with a precision of ± 0.008.

The main limitation of the study was the sample size which though adequate, was not large. However, due to the very high costs of the stable isotope, we were not able to use a large sample size. We were also not able to validate the derived regression rule on an external data set due to the cost constraints. Although the range of BMI of the subjects in the present study was from 17-30 kg/m², the mean BMI was 21.8 ± 2.8 kg/m². Additional studies are needed to ascertain whether these results can be extended to obese or thin people. In terms of methodology, it should be remembered that the skinfold techniques and DEXA have limitations with obese individuals.

In conclusion, the 4C model, with its own precision of <5 per cent, has advantages over the 2C model because it largely eliminates the reliance on assumptions. However, the cost and technical expertise needed does not allow its wide implementation. The results of the present study suggest that the DEXA and the ADP method can provide accurate estimates of body fat within 5 per cent of the reference measurement, while the more accessible field methods using the skinfold technique and BIA are less accurate. These latter methods are more suited to group estimates, and may have to make use of population specific equations.

**References**


