Comparison of dual-energy X-ray absorptiometry, air displacement plethysmography and bioelectrical impedance analysis for the assessment of body composition in severely obese Caucasian children and adolescents

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The objectives of the present study were to compare body composition assessed by dual-energy X-ray absorptiometry (DXA), air displacement plethysmography (ADP) and bioelectrical impedance analysis (BIA) in severely obese Caucasian children and adolescents and to develop and validate new equations for predicting body composition from BIA using DXA as the reference method. Body composition was assessed in fifty-eight obese children and adolescents (BMI 34·4 (SD 4·9) kg/m²) aged 10–17 years by DXA, ADP and BIA. ADP body fat content was estimated from body density using equations devised by Siri (ADPSiri) and Lohman (ADPLohman). In the whole sample, the Bland–Altman test showed that ADPSiri and ADPLohman underestimated percentage fat mass (%FM) by 2·1 (SD 3·4) and by 3·8 (SD 3·3) percent units (P<0·001), respectively, compared to DXA. In addition, compared to DXA, BIA underestimated %FM by 5·8 (SD 4·6) percent units in the whole group (P<0·001). A new prediction equation (FFM (kg) = 0·87 (stature²/body impedance) + 3·1) was developed on the pooled sample and cross-validated on an external group of sixty-one obese children and adolescents. The difference between predicted and measured FFM in the external group was 2·1·6 (SD 2·9) kg (P<0·001) and FFM was predicted accurately (error < 5 %) in 75 % of subjects.

In conclusion, DXA, ADP and the BIA are not interchangeable for the assessment of %FM in severely obese children and adolescents. The new prediction equation offers an alternative approach to DXA for the estimation of body composition in severely obese children and adolescents.

Dual-energy X-ray absorptiometry: Air displacement plethysmography: Bioelectrical impedance analysis: Body fat: Fat-free mass: Obesity

Because of the increasing prevalence of obesity in industrialised and developing countries(1), assessment of body composition and its variation has to be accurate to manage obesity better and its physiological, clinical and social consequences.

One of the main objectives of obesity management is to reduce fat mass (FM) and to preserve fat-free mass (FFM) during weight loss in order to maintain the physical capacities and energy expenditure of obese subjects(2).

Several studies have pointed out the potential of dual-energy X-ray absorptiometry (DXA) for the assessment of total and regional body composition, because of the relatively quick scan time, the minimal radiation dose and the good reproducibility of measurements(3,4). DXA has been validated against the four-compartment model(5,6), underwater weighing(7) and direct chemical analysis(4,8). The CV of repeated measurements of FFM with DXA is 2 % (3,9). For these reasons, DXA is often used as a reference method for body composition assessment in obese children and adolescents in clinical and research settings(10,11). Underwater weighing has long been considered the gold-standard for body composition assessment(12) and, until recently, was considered the only reliable and valid technique for the measurement of body density in human subjects(13). However, estimation of body density using underwater weighing requires a lengthy and sometimes uncomfortable test, involving the measurement of residual volume in the lungs, as well as complete submersion in water. The development of air displacement plethysmography (ADP) provides another

Abbreviations: ADP, air displacement plethysmography; ADP_siri and ADP_lohman, body-fat-content equations devised by Siri and Lohman respectively; BIA, bioelectrical impedance analysis; BMC, bone mineral content; BMI-SDS, standard deviation score for BMI; BW, body weight; DXA, dual-energy X-ray absorptiometry; FM, fat mass; %FM, percentage fat mass; FFM, fat-free mass; RMSE, root mean squared error of the estimate; Z, whole-body impedance; ZI, impedance index.

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method to measure body density in research and clinical settings and ADP has been proposed as an alternative to underwater weighing\(^{(13)}\). In addition, ADP has been validated in overweight and obese children\(^{(10,11)}\) and adults\(^{(14)}\).

Bioelectrical impedance analysis (BIA), a safe, non-invasive and portable method, is also widely used for estimating body composition\(^{(15,16)}\). However, several factors limit its application to obese subjects: body geometry and body water distribution are in fact different from those of normal-weight subjects\(^{(17)}\). The prediction equations developed in normal-weight subjects generally overestimate FFM in obese adults\(^{(18)}\) and children\(^{(16)}\). However, DXA and ADP are expensive methods for assessing body composition in the clinical setting so that a cheap, quick and reliable method is needed to assess body composition of severely obese subjects.

The objectives of the present study were (1) to compare body composition measured by DXA, ADP and BIA in severely obese Caucasian children and adolescents and (2) to develop and cross-validate prediction equations of body composition in obese children and adolescents from BIA measurements using DXA as the reference method.

**Subjects and methods**

**Study protocol**

The study group comprised fifty-eight obese subjects (twenty-seven boys and thirty-one girls) aged 10–17 years with a BMI above the ninety-seventh percentile for sex and age\(^{(19)}\). The subjects were recruited from the Division of Auxology, Italian Institute for Auxology, IRCCS, Piancavallo (Verbania, Italy). Subjects who had overt metabolic and/or endocrine diseases or who were taking medications that would alter body water content were excluded from the study. The study protocol was approved by the Ethical Committee of the Italian Institute for Auxology, Milan (Italy). The purpose of the study was carefully explained to each subject and his or her parents, who gave their written informed consent. Anthropometry, DXA, ADP and BIA were performed on the same day by the same investigators.

An external group of sixty-one obese children and adolescents recruited at the Paediatrics Department of the Clermont-Ferrand Hospital (France), who had participated in previous studies\(^{(20,21)}\), was used to validate the new body-composition equation developed in the present study.

**Anthropometry and pubertal assessment**

Body weight (BW) was measured to the nearest 0.1 kg with a mechanical weighing scale (Seca 709; Seca Ltd, Germany). Stature was measured to the nearest 0.1 cm using a floor-standing stadiometer (Model 220; Seca Ltd, UK). BMI was calculated as weight (kg) divided by height (m)\(^2\)\(^{(22)}\). The standard deviation score of BMI (BMI-SDS) was determined using the least-mean-square method with Italian reference values\(^{(19)}\). Circumferences at the waist and hip were measured in triplicate to the nearest 0.1 cm using an inelastic tape following the Anthropometric Standardization Reference Manual\(^{(23)}\).

Pubertal stage was evaluated according to Tanner\(^{(24)}\) on a scale from 1 to 5, with stage 1 being prepubertal, and stage 5 being adult. The same paediatrician assessed Tanner staging during the study.

**Dual-energy X-ray absorptiometry**

Body composition was assessed using a GE/Lunar Prodigy densitometer (GE Lunar Medical Systems, Milwaukee, WI, USA)\(^{(25)}\). The scanner was calibrated daily against the standard calibration block supplied by the manufacturer in order to control for possible baseline drift. The subjects lay supine on the bed and were scanned from head to toe. The scanner utilises a narrow fan beam (4.5°) parallel to the longitudinal axis of the body. Scans were analysed using Paediatric software version 1.5. Manufacturer’s algorithms provide a three-compartment analysis consisting of non-bone lean tissue mass, FM, and bone mineral content (BMC) ash. BMC was calculated as (BMC ash × 1.0436)\(^{(26)}\). FFM was defined as the sum of lean tissue mass and BMC.

For ethical reasons, repeated measurements were not performed on the study children. In our laboratory, the within-day CV for measurement of percentage fat-mass (%FM) in eight obese adults measured twice (with repositioning) is 2.3 % (data not shown).

**Air displacement plethysmography**

Body density was assessed using BOD-POD (Life Measurement Incorporated, Concord, CA, USA) coupled with software version 1.69, according to the manufacturer’s directions and procedures\(^{(12)}\). Before subject evaluation, a two-point chamber calibration was performed using the empty chamber and a 50.218 litre calibration cylinder. Subjects were clothed in a tight-fitting bathing suit and acrylic bathing cap and were weighed to the nearest 0.1 kg using the ADP system’s electronic scale. The scale was calibrated daily using a 20 kg weight.

After the calibration was completed, the subject entered the ADP. Body volume was corrected for lung air volume, with thoracic gas volume measured during tidal breathing and during exhalation against a mechanical obstruction. Subjects were excluded from the study if thoracic gas volume could not be measured. Body density was calculated as the ADP-measured body mass divided by (total body volume + 0.40 × thoracic gas volume − surface area artefact). The surface area artefact was calculated by the ADP software to account for the changes in air temperature close to the subject’s skin. Body fatness was then calculated by using the general equation of Siri (ADP\(_{siri}\)\(^{(27)}\)) and the age- and sex-specific equations of Lohman (ADP\(_{lohman}\)\(^{(28)}\)). In our laboratory, the CV for measurement of %FM in ten obese adolescents measured twice on the same day was 3.4 % (data not shown).

**Bioelectrical impedance analysis**

Whole-body impedance (Z) was measured using a multifrequency (from 5 to 250 kHz) impedance-meter (Human IM Plus II; DS Medica, Milan, Italy). Measurements were performed according to the method of Lukaski\(^{(29)}\) after 25 min resting in a supine position with arms and legs relaxed and abducted at an angle of 45°. The impedance index (ZI) was calculated as (stature (cm)\(^2\) divided by Z at
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50 kHz (Ω). Estimates of body composition were obtained from the equipped software (unknown equations). FM was obtained by subtracting the equipment’s body mass (unknown formula), and FM was obtained by subtracting the equipment’s body mass (unknown formula) from the body weight (unknown formula) of the individual. The within-day CV for three repeated measurements of FM in ten obese adolescents (with repositioning) was 2.2% (data not shown).

Statistical analysis

Statistical analysis was performed using STATA 9.2 (StataCorp, College Station, TX, USA). Statistical significance was set to a two-tailed P value of <0.05. All continuous variables were normally distributed (Shapiro–Wilk test) and are shown as means and standard deviations (SD). The Bland–Altman (31) method was used to calculate the limits of agreement (±2 SD) between ADP and BIA vs. DXA for the assessment of %FM and Pitman’s test was used to evaluate proportional bias (32). Bias was defined as the difference between %FM estimated by BIA or measured by ADP and %FM measured by DXA.

For the development of new BIA equations, selection of variables was performed by stepwise bootstrapped linear regression on 1000 random samples of the fifty-eight subjects (32). Candidate predictors were sex, age, body mass, ZI and phase angle and Tanner staging. For internal cross-validation, the standard error and 95% CI of the final prediction model were calculated by bootstrap analysis on 1000 random samples of the fifty-eight subjects (33). Values of the adjusted coefficient of determination and of the root mean squared error of the estimate (RMSE) with bootstrapped 95% CI were used to assess the accuracy of predictors.

An external cross-validation of the prediction equation was performed on an independent group of sixty-one obese children and adolescents enrolled in a previous study (20,21). Differences between FM measured by DXA and FM estimated from BIA were evaluated using the Bland–Altman method (30). In addition, we calculated the percentage of subjects whose FM was predicted within 5% of measured FM. This limit was chosen as being consistent with technical measurement errors of 5% or less.

Results

Subject characteristics

The physical characteristics of the study group are shown in Table 1. All subjects were obese, with BMI-SDS ranging from 2.1 to 4.1 (19). Age, BW, waist circumference and waist:hip ratio were not significantly different between boys and girls. Stature was significantly higher in boys than in girls (P<0.001). However, pubertal stage, BMI, BMI-SDS and hip-circumferences were significantly lower in boys than in girls (P<0.005).

FM obtained by DXA was significantly higher in boys than in girls (P<0.005) while FM and %FM were significantly lower in boys than in girls (P<0.005). BMC, Z and phase angle were not significantly different between boys and girls.

The external and the internal study group had similar values of age, stature and FM. The external group had lower values of BW, BMI, BMI-SDS, FM, %FM and BMC (P<0.001) (Table 1).

Table 1. Physical and biological characteristics of subjects. Fat-free mass (FFM), fat-mass (FM) and bone mineral content (BMC) were obtained from dual-energy X-ray absorptiometry; impedance and angle phase were obtained from bioelectrical impedance analysis†

<table>
<thead>
<tr>
<th></th>
<th>Study group§</th>
<th>External group‖</th>
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<tbody>
<tr>
<td></td>
<td>All (n 58)</td>
<td>Boys (n 27)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age (year)</td>
<td>14.2 ± 1.9</td>
<td>14.1 ± 2.0</td>
</tr>
<tr>
<td>Pubertal stage‡</td>
<td>4.0 ± 1.1</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.64 ± 0.10</td>
<td>1.69 ± 0.11</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>92.5 ± 14.5</td>
<td>91.8 ± 13.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.3 ± 4.9</td>
<td>32.2 ± 2.8</td>
</tr>
<tr>
<td>BMI-SDS</td>
<td>2.8 ± 0.6</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Waist circumference (m)</td>
<td>1.11 ± 0.12</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>Hip circumference (m)</td>
<td>1.17 ± 0.10</td>
<td>1.13 ± 0.07</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.95 ± 0.09</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>47.8 ± 9.1</td>
<td>51.0 ± 10.2</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>44.7 ± 9.9</td>
<td>40.8 ± 6.7</td>
</tr>
<tr>
<td>FM (%)</td>
<td>48.2 ± 6.3</td>
<td>44.7 ± 5.8</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>2.33 ± 0.46</td>
<td>2.56 ± 0.57</td>
</tr>
<tr>
<td>Impedance (Ω)</td>
<td>553.7 ± 61.0</td>
<td>526.0 ± 66.3</td>
</tr>
<tr>
<td>Angle phase (°)</td>
<td>6.5 ± 0.7</td>
<td>6.4 ± 0.9</td>
</tr>
</tbody>
</table>

BMI-SDS, standard deviation score for BMI.

Mean values were significantly different from those of the boys by ANOVA of the main effects of sex: *P<0.05; **P<0.001.

Mean values were significantly different between groups (Study group v. External group; ANOVA): †P<0.001.

† For details of procedures, see Subjects and methods.

§ Study group subjects were recruited from the Division of Auxology, Italian Institute for Auxology, IRCCS, Piancavallo (VB), Italy.

‖ External group subjects were recruited from the Paediatrics Department of the Clermont-Ferrand Hospital, France (20,21).

‡ For details of procedures, see Subjects and methods.

¶ Study group subjects were recruited from the Division of Auxology, Italian Institute for Auxology, IRCCS, Piancavallo (VB), Italy.

The external group had lower values of BW, BMI, BMI-SDS, FM, %FM and BMC (P<0.001) (Table 1).
Comparison of dual-energy X-ray absorptiometry and other methods

The mean difference between BW measured by DXA and BW measured by air-displacement plethysmography (ADP) was −0.2 (SD 0.8) kg (P=0.025) and the corresponding value for ADP was −0.2 (SD 0.6) kg (P=0.005). Even if the mean differences were significantly different from 0, the bias values are low and of no practical relevance.

The Bland–Altman method revealed wide limits of agreement between DXA and ADP or BIA for the assessment of %FM so that the methods could not be considered interchangeable (Table 2 and Fig. 1). As compared to DXA, the ADP SIRI method underestimated %FM by 21 (SD 3.4) percent units in the whole group (P<0.001, Fig. 1 (a)). In boys, the difference was not significant (−1.2 (SD 3.8) percent units, P=0.097), but in girls a significant underestimation of −2.9 (SD 2.9) percent units (P<0.001) was found. In addition, the ADP LOHMAN method underestimated %FM by 3.8 (SD 3.3) percent units in the whole group (P<0.001, Fig. 1 (b)). In boys and in girls %FM was significantly underestimated by −2.8 (SD 3.6) and −4.7 (SD 2.7) percent units, respectively, (P<0.001). Moreover, %FM estimated by the ADP SIRI method was significantly higher than %FM estimated by the ADP LOHMAN method (+1.7 (SD 1.4) percent units, P<0.001) (Table 2).

BIA underestimated %FM by 5.8 (SD 4.6) percent units (P<0.001, Fig. 1 (c)). In boys and girls, %FM was underestimated to a similar degree (6.1 (SD 4.2) and 5.6 (SD 4.2) percent units, respectively, P<0.001).

The ADP–DXA bias tended however to increase for increasing levels of %FM (Pitman’s t 0.401, P=0.002 for Siri and r 0.315, P<0.001 for Lohman) so that the agreement between APD and DXA was influenced by the underlying level of adiposity. This was not apparent however for the BIA–DXA relationship (Pitman’s r 0.103, P<0.441).

Development and validation of bioelectrical impedance analysis equations

To identify predictors of FFM for BIA algorithms, we performed a bootstrapped stepwise regression using FFM assessed by DXA as the outcome variable and sex, age, Tanner stage, weight, ZI and phase angle as predictors. ZI was a predictor in 1000 out of 1000 bootstrap samples, weight in 868, sex in 584, Tanner staging in 414, age in 115 and phase angle in 265. Thus, we focused on ZI and weight as predictors for the BIA algorithm.

Because weight explained only 50% of the variance of FFM assessed by DXA (RMSE = 6.4 kg, P<0.001) as compared to a value of 91% for ZI (RMSE = 2.7 kg, P<0.001) and the addition of weight to ZI did not improve the prediction (adjusted coefficient of determination = 0.92, RMSE = 2.6 kg, P<0.001), we considered only ZI as predictor, with the following algorithm:

\[ \text{FFM (kg)} = 0.87 \times ZI (\text{cm}^2/\Omega) + 3.4 \] (adjusted coefficient of determination = 0.91; RMSE = 2.7 kg, P<0.001).

The 95% bootstrapped CI of the regression coefficients are 0.81 to 0.92 (P<0.0001) for ZI and 0.29 to 5.87 (P=0.03) for intercept.

In the external cross-validation group, the mean estimated FFM was significantly lower than assessed FFM (48.9 (SD 8.7) v. 50.4 (SD 9.9) kg, P<0.001), with limits of agreement between +4.1 and −7.2 kg. FFM was predicted within 5% of the true value in 75% of subjects, overestimated in 3% and underestimated in 22% (Fig. 2).

Discussion

The results of the present study show that the methods used (DXA, ADP and BIA) are not interchangeable for the assessment of body composition of obese children and adolescents. The suitable alternative approach to DXA to assess body composition, in these subjects, was BIA using a new prediction equation based on the ZI parameter.

The results of validation tests against the four-compartment model, hydrodensitometry, and chemical analysis show that DXA is an accurate and precise method for measuring soft-tissue body composition in cross-sectional and longitudinal studies in human subjects.

The ADP method was well correlated with DXA in athletes and in adults and children. However, the results of our study suggest that ADP SIRI and ADP LOHMAN underestimate %FM in obese individuals, compared to DXA and this

Table 2. Comparison between measured percentage fat-mass (%FM) by dual-energy X-ray absorptiometry (DXA) v. air-displacement plethysmography (ADP SIRI and ADP LOHMAN) and bioelectrical impedance analysis (BIA) using the Bland–Altman method. ADP body fatness was estimated from body density using the general equation of Siri (ADP SIRI) and the age- and sex-specific equations of Lohman (ADP LOHMAN).

(Values are means with standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>%FM</th>
<th>Difference (percent unit)</th>
<th>Limits of agreement (percent unit)</th>
<th>P†</th>
<th>r-Pitman‡</th>
<th>P-Pitman†</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA</td>
<td>48.21 6.32</td>
<td>−2.11 3.43</td>
<td>−8.82 4.61</td>
<td>0.001</td>
<td>0.401</td>
<td>0.002</td>
</tr>
<tr>
<td>ADPSIRI</td>
<td>46.10 7.66</td>
<td>−3.80 3.30</td>
<td>−10.27 2.67</td>
<td>0.001</td>
<td>0.315</td>
<td>0.001</td>
</tr>
<tr>
<td>ADPLohman</td>
<td>44.41 7.33</td>
<td>−5.84 4.62</td>
<td>−14.90 3.22</td>
<td>0.001</td>
<td>0.103</td>
<td>0.441</td>
</tr>
</tbody>
</table>

*For details of procedures, see Subjects and methods.
†Test between measured %FM by DXA v. air-displacement plethysmography (ADP SIRI and ADP LOHMAN) and BIA.
‡r-Pitman, Pearson’s correlation coefficient for the Pitman’s test; P-Pitman, P value corresponding to r value for the Pitman’s test.
is in agreement with previous studies\(^{38,39}\). The major disadvantage of techniques based on the two-compartment model (FFM and FM) is the assumption concerning density of FFM\(^{40}\). In children and adolescents, FFM is sensitive to variability in hydration status resulting in an unstable density of FFM in relation to growth\(^{17}\). Further uncertainty arises in obese subjects as the result of excess fat\(^{41}\). In addition, there was a trend towards the underestimation of %FM by ADP relative to DXA in obese children and adolescents therefore remain to be elucidated.

Compared to DXA, BIA underestimated %FM in a similar way to the results of previous studies\(^{16,44}\). The differences in %FM between BIA and DXA might result both from overestimation of FFM hydration in obese subjects\(^{17,41}\), and from the peculiar distribution of FM in the body\(^{18}\). The assumption that 73.2% lean body mass consists of total body water is possibly not correct. In healthy persons the water content of FFM is constant only after age 20 years\(^{45}\). In the obese, however, FFM hydration may be up to 75%\(^{17,41}\). This finding could cause an overestimation of the FFM which in turn could underestimate the FM\(^{18}\).

The second objective of this study was to develop and validate new prediction equations of body composition in obese adolescents from BIA using DXA as the reference method. The present study focused on the prediction of FFM rather than FM, because of the necessity of preserving FFM in weight-reduction programmes, and the ability of BIA to predict FFM from its high water and electrolyte content\(^{46}\). BIA is a popular, simple, rapid, and non-invasive method to estimate total body water and FFM in healthy people. This technique has been cross-validated in children against methods using measurements of total body water by deuterium dilution\(^{47}\) and total body K\(^{48}\). However, several factors limit its application to obese subjects: assumption of constancy in the hydration factor of FFM, and body water distribution and body geometry. Therefore, the use of the prediction equations must be limited to the type of population in which they have been validated\(^{15,18}\). Thus, when prediction equations developed in non-obese subjects are used for obese subjects, FFM is overestimated and consequently FM is underestimated\(^{16,49–51}\). The prediction equation developed in the present study allowed a satisfactory estimation of body composition in obese adolescents from measurements of stature and body impedance. In addition, the high accuracy observed in an external validation group (75% of subjects with predicted values within 5% of true values), shows that the new equation may be
useful for health care professionals, who have access to BIA equipment, for the estimation of FFM in severely obese children and adolescents.

Strengths of this study include the use of DXA, a robust and well-accepted measure as the criterion method and the use of Bland–Altman comparisons in the interpretation of results. Limitations include the relatively small sample size and the fact that the new equation is only applicable for overweight and obese Caucasian children and adolescents. In addition, the results obtained from the comparison between methods may not be applicable to other BIA and ADP devices or software.

In conclusion, the results of the present study show that DXA, ADP and BIA are not interchangeable for the assessment of body composition in obese children and adolescents and that a population-specific BIA equation may be a suitable approach for assessing body composition in these children and adolescents.

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