**Title page**

**Title**

Evaluation of bioelectrical impedance analysis in measuring body fat in 6-to-12-year-old boys compared to air displacement plethysmography

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**Abbreviations**

Percentage fat mass (%FM), dual-energy x-ray absorptiometry (DXA), three-compartment model (3C), four-compartment model (4C), air displacement plethysmography (ADP), limits of agreement (LoA), bioelectrical impedance analysis (BIA), percentage fat mass measured by air displacement plethysmography (%FMADP), percentage fat mass measured by bioelectrical impedance analysis (%FMBIA), intraclass correlation coefficients (ICCs), Technical error of the measurement (TEM), body volumes (Vb), thoracic gas volumes (TGV), skin surface area (SSA), Effect size (ES), 95% confidence intervals (95%CI).

**Running Title**

Body fat by BIA v ADP in 6-12 year olds

**Keywords**

Body composition, obesity, pediatric, concurrent validity, reliability

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**Introduction**

Childhood obesity is associated with significant morbidity and mortality.1,2 Comorbidities associated with childhood obesity affect almost every body system, including, but not limited to, endocrine, cardiovascular, cardiometabolic, and musculoskeletal systems.3 Worldwide prevalence of childhood overweight and obesity increased from 12.8% in 2000 to 14.2% in 2013 and is expected to reach 15.8% in 2025.4 Growth trajectories for childhood obesity into adulthood indicate that 57.3% of today’s children and 75% of children currently with obesity will be obese at the age of 35.5 Monitoring and tracking of obesity in childhood appears critical to determine when preventative or management interventions should be taken.

Obesity is defined as excess fat accumulation that may impair health.6 However, obesity is commonly measured by Body Mass Index (BMI) which, in children, is transformed into BMI z-scores to define age- and gender-specific cut-offs for overweight and obesity.7 Body Mass Index is useful for tracking changes in obesity prevalence in populations, however, the relationship between BMI and adiposity is not consistent across populations and assumes a linear increase in body mass and fat mass through childhood.8,9 Measures of adiposity (i.e. fat mass relative to body mass [%FM]), rather than weight relative to height, provide accurate assessment of obesity status and may provide better indication of the effectiveness of weight loss programmes.10,11

Reference methods of measuring adiposity include computerised tomography, magnetic resonance imaging, dual-energy x-ray absorptiometry (DXA), isotope dilution, and combinations of methods to construct three (3C) and four (4C) compartment models. Reference methods are accurate assessments of %FM (compared to ‘gold-standard’ cadaver analysis),12 because measurements of hydration status and mineral content are included in the %FM calculation.9 However, in comparison to two compartment (2C) models of body composition, that partition the body into fat mass and fat-free mass (e.g. air displacement plethysmography and bioelectrical impedance analysis), reference methods are costly, time consuming, invasive, and may not be suitable for children.13 Although 2C models of body composition are subject to error arising from variation in fat-free mass composition,9 they are more accessible to clinicians and researchers and less burdensome on participants.

Air displacement plethysmography (ADP) is an indirect method to determine body volume, using a volumetric chamber into which a participant is introduced, by recording pressure changes under isothermal and adiabatic conditions.14 Equations that include assumed densities of fat and lean tissues are used to calculate %FM. Bioelectrical impedance analysis (BIA) is an indirect measure of total body water from which an empirical relationship with fat-free mass can be derived using subject-specific regression equations. Previous studies generally indicate that measures of %FM by ADP (%FMADP)11,15, 16, rather than BIA (%FMBIA)9,17, have better agreement with reference measures in paediatric populations. However, age, gender, BMI, and BIA device all impact the estimation of %FM, and should therefore be considered in %FM prediction equations.11,18

Few studies have compared measures of %FM derived from ADP and BIA in paediatric populations,19 generally finding that %FMADP was greater than %FMBIA 20,21. Whilst these studies benefit from large sample sizes, comparisons between methods were not distinguished based on weight status which can impact estimates of body composition.22 One study which did compare %FMADP and %FMBIA in both participants with and without obesity23 , measured %FMBIA using a foot-to-foot device (measuring only part of the body) and %FMADP using general,24 rather than child-specific regression equations.11,25 Comparisons between %FM methods should be made using age-specific equations, controlling for gender and weight status.16,26,27

Reliability of %FM measurements in children have been conducted, showing intraclass correlation coefficients (ICCs) of >0.90 from BIA,12 and >0.93 from air displacement plethysmography.28 Vicente-Rodriguez et al29 reported intra-day reliability of %FMADP and %FMBIA in 84 adolescents (13-to-17-years-old). Technical error of the measurement (TEM) was 1.07% and 0.74% for ADP and BIA respectively, with correlation coefficients of 0.989 and 0.993 for ADP and BIA respectively. However, there is a paucity of research that has assessed the reliability of ADP and BIA methods in one cohort, with no studies investigating this in a cohort of children < 12 years.

A recent systematic review suggests that ADP has similar validity to DXA and isotopic dilution methods to assess %FM in children with obesity.30 ADP has been considered as a ‘standard’ method of body composition assessment23 to which BIA methods can be compared for validity and reliability.20,21 Measures of body fat by ADP offers greater agreement with reference measures, but BIA offers faster, more convenient and inexpensive field-based measures of body fat. Therefore, the aim of this study was to measure concurrent validity and reliability of %FMADP and %FMBIA in 6-to-12-year-old children with and without obesity. We hypothesise that %FMBIA will be underestimated compared to %FMADP, and that in boys with obesity, %FMBIA will be underestimated to a greater extent compared to boys without obesity. Compared to studies that have not used age-specific equations for body composition, we expect to find less difference between %FMADP and %FMBIA. Finally, we hypothesise that both %FMADP and %FMBIA methods will be reliable, in keeping with literature involving older children. The findings will help practitioners determine whether %FMADP and %FMBIA can be used interchangeably and reliably in children.

**Method**

*Participants*

Seventy-one boys underwent assessment of body composition by BIA and ADP (age: 10.1 ± 1.70 years, height: 1.43 ± 0.11 m, mass: 39.4 ± 11.2 kg). Ten boys took part in the intra-day reliability analysis of BIA and ADP (age: 10.0 ± 2.63 years, height 1.39 ± 0.17 m, mass 33.8 ± 10.8 kg). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the host institution (Ref No. ETH/13/11). Written and verbal informed consent were obtained from parents and children (verbal consent was witnessed and formally recorded). Parents completed a health medical questionnaire prior to data collection; all participants were reportedly healthy at the time of the study. Obesity was defined as an %FM >25%.23

*Procedure*

Participants were tested in pairs and a randomised, cross over design was used whereby pairs were randomly assigned to be tested by either ADP or BIA, after which they completed the other test procedure immediately after the first. Each participant wore tight fitting swimming shorts with no shoes or socks throughout both testing procedures. Participants were instructed not to eat, drink, or exercise two hours before the measurement and to void their bladder 30 minutes before testing. Estimates of %FM from ADP (%FMADP) and BIA (%FMBIA) were measured within the same day by the lead author. For assessment of reliability, %FMADP, %FMBIA, body volume, and resistance measurements were repeated within 10 minutes of the first test in order to avoid biological variation in hydration and temperature.

*ADP*

Air displacement plethysmography (ADP) was measured using the Bodpod device following manufacturer’s protocols.14 Each participant wore a swim cap to cover and compress head hair. The Bodpod weighing scale was calibrated before each testing session with known 20kg weights; all calibrations were within ±0.01kg. The chamber was calibrated against a known volume cylinder (50.024l) before each testing session. Five repeated measures of cylinder volume were made during the calibration procedure. The average estimated volume was 50.047 ± 0.007l, within the accuracy and variability range of repeated measures previously reported for volumetric measures by the Bodpod.14

The ADP procedure involved three successive measurements of raw body volume, the total procedure time was less than one minute. If body volume differed by more than 0.015L between the measures the procedure was repeated. The mean of the three raw body volumes (Vb) was corrected for isothermal conditions of air in the lungs and around the skin surface. Raw Vb was corrected for thoracic gas volumes (TGV) (and skin surface area [SSA]) using child specific equations detailed in Table 1. Body density was calculated by dividing the corrected body volume by body mass and converted to %FM using gender- and age specific equations published by Lohman25 (Table 1).

Table 1.

*BIA*

A multi-frequency BIA device (Quantum II, RJL systems, Inc. Clinton Township, Michigan, USA) was used to measure body impedance in the participants. The BIA device was calibrated before each testing session using known resistance and reactance. The device recorded mean resistance figures of 384 ± 0.34Ω and reactance of 44.9 ± 1.22Ω which were within the manufacturer’s guidelines.

The participants were instructed to lay supine on a portable couch for five minutes prior to testing as per the manufacturer’s instructions to allow extracellular water to level out across the body. Electrodes were placed on the ipsilateral bony prominences of the wrist and ankle (metacarpal and metatarsal lines) ensuring the electrodes were 5 cm apart.

Reactance (*X*) and resistance (R) were outputted for each participant for calculation of %FM based on gender- and age-specific equations. The equation of Horlick et al33 was chosen to estimate FFM (Table 1) based on regression analysis of impedance measures from the same manufacturer (RJL) used in the current study and has shown to be valid in paediatric populations.19 FFM was then converted to %FM (Table 1).

*Statistical analysis*

*Concurrent validity*

With obesity and without obesity group differences for age, height, body mass, raw body volume (m3), resistance (Ω), %FMADP, and %FMBIA were assessed by independent t tests. Comparisons between %FMADP, and %FMBIA were made for the full sample, and within the with obesity and without obesity groups. Differences between %FMADP and %FMBIA were assessed by paired samples t tests. Effect sizes (ES) were calculated based on Cohen’s d and defined as <0.2 weak, 0.2 to 0.49 small, 0.5 to 0.79 medium, and >0.79 large.34 Pearson correlation coefficients were performed to measure the strength of association between %FMADP and %FMBIA, with 95% confidence intervals (95%CI). Correlation coefficients <0.29 were defined as weak, between 0.3 and 0.49 moderate, and >0.5 strong.34 Agreement between %FMADP and %FMBIA were analysed using Bland-Altman analysis.35 This involved the calculation of the mean difference between two methods together with LoA, based on 95% confidence intervals (95%CI), calculated from the SD of the mean difference for each participant (multiplied by 1.96). Proportional bias, error affected by the magnitude of measurement, were determined by Pearson’s correlation coefficient r>0.5.36 Predicting %FMADP is considered the ‘standard’ method for this study, to which %FMBIA was compared. To address clinical acceptability, a minimal acceptable standard for estimating %FM of ± 3.5% (group-level difference) from the reference measure was employed.37 The sample size of 71 was calculated based on the minimal acceptable standard,37 standard error of measurement for BIA,12 with 80% power and two-sided significance of 0.05.

*Reliability*

For comparison with previous literature on the reliability of %FM measures, three reliability statistics were calculated; technical error of the measurement (TEM and TEM%), coefficient of reliability (*rxx*), and ICC as detailed in Table 2.

Table 2.

**Results**

Table 3 presents data for all participants, and for the with obesity and without obesity groups. No significant differences were found between groups for age (t(69) = 1.85, p = 0.069), height (t(69) = 1.09, p = 0.212), and resistance (t(69) = 0.32, p = 0.748). The with obesity group were significantly heavier (t(69) = 2.36, p = 0.021), had a higher BMI (t(69) = 4.97, p <0.001), greater raw body volume (t(69) = 0.75, p = 0.004), and a higher %FMADP (t(69) = 14.15, p <0.001), and %FMBIA (t(69) = 8.80, p <0.001).

Table 3.

Concurrent validity

Table 4 presents the mean difference and LoA of %FMADP and %FMBIA for all participants, the with obesity group, and the without obesity group. Compared to %FMBIA, %FMADP was significantly higher in all participants (t(70) = 5.11, p < 0.001, ES = 0.42) and in the without obesity group (t(45) = 2.98, p = 0.005, ES 0.52; Table 3); although mean differences observed were clinically acceptable (< 3.5%), LoA were 22.3% and 21.8% in all participants and those without obesity, respectively . In the with obesity group, %FMADP was significantly higher compared to %FMBIA (t(24) = 4.76, p < 0.001, ES = 0.90; Table 3), with the mean difference (-5.20 ± 5.46%) exceeding the clinically acceptable threshold of 3.5%, and LoA of 21.8%. A strong, significant positive correlation was found between %FMADP and %FMBIA when examining all participants (r = 0.80, p < 0.001, 95%CI 0.64 to 0.95). and participants with obesity (r = 0.60, p = 0.001, 95%CI 0.11 to 1). In the without obesity group, a moderate, significant positive correlation was found (r = 0.44, p = 0.003, 95%CI 0.26 to 1). Figure 1 presents Bland-Altman plots of %FMADP and %FMBIA for all participants, those with obesity, and those without obesity. No proportional bias was detected (r = 0.001) meaning agreement between measures was not affected by the magnitude of %FM.

Table 4.

Figure 1.

Reliability

Reliability analysis revealed that ADP resulted in lower error of %FM measures compared to BIA, with TEMs of 0.55% and 0.65%, respectively. Coefficient of reliability and ICCs were also higher in %FMADP measures (0.92 and 0.95, for rxx and ICC respectively) compared to %FMBIA measures (0.89 and 0.93; Table 5).

Table 5.

**Discussion**

The aim of this study was to compare validity of %FMBIA to the ‘standard’ %FMADP and assess intra-day reliability of both methods in the same cohort. Compared to ADP, BIA underestimated %FM in the study population, but there was no bias in differences between methods relating to obesity status (i.e. magnitude of %FM). Despite the significant correlation, there was a significant difference and large limits of agreement between measures of %FMBIA and %FMADP. The reliability findings reported in this study reveal that %FMADP is a more reliable measure compared %FMBIA, but both methods were highly reliable in the cohort.

Concurrent Validity

Underestimation of %FMBIA compared %FMADP in the current study is in general agreement with previous studies.20,21,23,40 Previous studies have shown %FMBIA to be underestimated by 0.5 – 5.6% in children and adolescents compared to %FMADP; although some %FMBIA prediction equations have resulted in an overestimation.21 The mean underestimation of 3.4% found in the present study is within the range previously reported. The differences between %FMBIA and %FMADP within the with- and without obesity groups also agree with Azcona et al23 who reported mean underestimation of %FMBIA compared to %FMADP among the full sample (3.39%), without obese (2.49%) and with obesity groups (5.01%). Despite different BIA devices and %FM equations used between the current study and Azcona et al,23 the mean differences between %FMBIA and %FMADP are similar.

Compared to the clinically acceptable differences reported by Heyward and Wagner,37 %FM differences in the without obesity group were within the ± 3.5% clinically acceptable threshold, but in the with obesity group differences would be deemed clinically unacceptable (> 3.5%). Despite no significant bias in differences between devices detected across levels of body fat, it does appear that BIA underestimates %FM to a greater extent. Furthermore, the LoA found in the current study are in general agreement with values of 15.3-20.6% reported in previous studies.20,21,23 Whilst no consensus has been reached on what level of LoA is clinically acceptable (a range of 2 to 20% has been reported in the literature),30,41,42 the large LoA in the current study indicates that BIA and ADP cannot be used interchangeably to measure an individual’s %FM. Assessment of body composition must be accurate on an individual basis to correctly identify overweight and obesity.43

Reliability

The findings from the current study suggest that repeated measurements of %FM from ADP and BIA are highly reliable in young children. These findings are comparable to other studies examining the intra-day reliability of %FMADP and %FMBIA in older children. Vicente-Rodriguez et al29 measured intra-day reliability in 84 adolescents (13-17 years old), finding %FMADP TEM of 1.07% FM and rxx = 0.99, and %FMBIA TEM of 0.74% and rxx = 0.99. Resistance and body volume reliability in the current study also compare well with values of Vicente-Rodriguez et al29; resistance TEM of 10.2Ω and rxx = 0.99, and body volume TEM of 0.58m3 and rxx = 0.99. Comparable reliability in the current younger cohort to adolescents reveals that children were able to adhere to the BIA and ADP procedures and follow instructions.

The intra-day reliability of body fat mass measures from ADP and BIA are dependent on environmental conditions, instructor competence, and participant adherence to the procedures. Environmental variation includes pressure changes within the laboratory (from opening doors or drafts) during the procedure that can affect ADP reliability and, temperature changes in the ten minutes between repeated measures that can affect BIA reliability. Correct electrode placement on the ipsilateral bony prominences of the wrist and ankle (the metacarpal and metatarsal lines)44 can be subjective. Electrode placement variability can alter impedance readings by 4%,45 and would have reduced reliability in this study. Variability due to procedural adherence includes movement of the participant in the Bodpod chamber or irregular breathing. These can cause pressure changes within the Bodpod influencing raw body volumes.46 For this reason, ADP measures from Bodpod were taken in triplicate and, if the raw body volumes differed by >0.015L, the procedure was started again. In order to maximise intra-day reliability of %FMADP and %FMBIA measures environmental conditions, protocols and participant preparation should be strictly monitored throughout testing procedures.

Limitations of the current study comprise the use of predicted lung volumes in ADP measurements which may impact the accuracy of %FMADP. However, young children struggle with the protocol for lung volume measurement and error in the correction of raw body volume for air in the lungs is relatively small.47 Other age- and gender-specific %FM equations are available for ADP that account for changes in hydration status with age and gender.11 However, the Lohman25 equation has been validated against 4C48 and, in boys, compares well with more recent equations for %FMADP.11 The relatively short duration of food and drink abstention may have affected BIA measurements. However, longer abstention may be unethical and impractical.49 We could not collect pubertal status from our sample and it is acknowledged that pubertal status may have improved the accuracy of both %FMADP and %FMBIA. Particularly for %FMBIA measurements, puberty/maturation status has an impact on total body water (TBW), but the current study used standardised procedures and age-appropriate equations to limit extraneous variation. Indeed, as reported by Horlick et al33 when developing the BIA equation used in the current study, including Tanner stage to the regression model for TBW had little effect on the predictive power above measures of age, height, mass and gender.

**Conclusion**

The results of the intra-day reliability tests revealed that both %FMADP and %FMBIA are highly reliable in boys age 6-to-12-years-old. %FMBIA was significantly correlated with %FMADP in children with and without obesity. However, %FMBIA was significantly underestimated in both groups, but only in the with obesity group was it beyond the minimal acceptable standard of ±3.5% (Heyward & Wagner, 2004). Therefore, BIA may be suitable for determining %FM in boys without obesity age 6-to-12 years-old. Similar to the findings of previous studies that have used different devices (e.g. foot-to-foot BIA), %FM equations (proprietary or adult), sample age (e.g. adolescents), and do not consider obesity status, the large limits of agreement between %FMADP and %FMBIA in the current study indicate that the devices cannot be used interchangeably in boys age 6-to-12-years-old.

**Conflicts of Interest statement**

The authors declare no conflict of interest in relation to this study.

**Author Contributions**

All authors contributed to conceptualisation, study design, data analysis, data interpretation, presentation of tables and figures, writing of the manuscript and manuscript revisions. Ryan Mahaffey collected data.

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**Tables**

Table 1. Equations used in ADP and BIA procedures

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| --- | --- |
| **Equations** | **Reference** |
| **Used in ADP procedure** | |
|  | Fields et al31 |
|  | Haycock et al32 |
|  | Lohman25 |
| **Used in BIA procedure** | |
|  | Horlick et al33 |
|  |  |

*TGV*, thoracic gas volume; *Ht*, height in cm (derived by height in m x 100); *SSA*, skin surface area; *Wt*, body mass in kg; *%FM*, percent fat mass; *k*1 and *k*2, gender and age specific constants; *Db, body density; FFM, fat free mass;* R, resistance; *A, age in years; S*, gender specific constants.

Table 2. Equations used to assess reliability of data

|  |  |
| --- | --- |
| **Equation** | **Reference** |
|  |  |
|  |  |
|  | Ulijaszek & Kerr38 |
|  | Shrout & Fleiss39 |

TEM, technical error of measurement; *d,* difference between measurements; *n*, number of individuals measured; *x*, mean percentage fat mass (%FM); *rxx,* reliability coefficient*; SD*, standard deviation; *ICC*, intraclass correlation coefficient; *k,* number of measurements; *BMS,* between subject variance; *EMS*, error (residual) mean square variance.

Table 3. Age and anthropometric variables according to weight status

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **All participants** (n = 71)  Mean SD | | **Without obesity** (n = 46)  Mean SD | | **With obesity** (n = 25)  Mean SD | |
| Age (years) | 10.1 | 1.70 | 10.3 | 1.94 | 9.56 | 0.96 |
| Mass (kg) | 39.4 | 11.2 | 37.1 | 11.0 | 43.5 | 10.6\* |
| Height (m) | 1.43 | 0.11 | 1.42 | 0.12 | 1.43 | 0.07 |
| Body mass index (kg/m2) | 18.7 | 3.70 | 17.3 | 2.76 | 21.2 | 3.83\* |
| Raw body volume (m3) | 36.6 | 10.9 | 34.0 | 10.2 | 41.5 | 10.7\* |
| Resistance (Ω) | 674 | 96.2 | 677 | 101 | 669 | 89.5 |
| %FMADP | 21.6 | 9.00† | 16.1 | 4.03† | 32.2 | 5.49\*† |
| %FMBIA | 18.2 | 8.87 | 13.7 | 6.14 | 27.0 | 6.59\* |

%FMBIA, percentage body fat measured by bioelectrical impedance analysis; %FMADP, percentage body fat measured by air displacement plethysmography

\* denotes significant difference between non-obese and obese groups at 0.05 level

† denotes significant difference within group between ADP and BIA methods at 0.05 level

Table 4. Differences in %FM measured by ADP and BIA (%FMBIA - %FMADP)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All participants** (n = 71)  %FM | **Without obesity** (n = 46)  %FM | **With Obesity** (n = 25)  %FM |
| Mean ± SD | -3.38 ± 5.60 | -2.40 ± 5.45 | -5.20 ± 5.46 |
| 95% CI | -4.30, -2.46 | -3.51, -1.28 | -6.71, -3.68 |
| LoA | -14.5, 7.78 | -13.3, 8.50 | -16.1, 5.73 |

%FM, percentage fat mass. 95% CI; 95% confidence interval; LoA, Limits of Agreement

Table 5. Mean, SD and within-day test re-test for intra-day reliability of %FM measures (n=10)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Session 1**  Mean SD | | **Session 2**  Mean SD | | **TEM** | **TEM%** | **rxx** | **ICC** (95%CI) |
| %FMBIA | 11.4 | 7.92 | 12.5 | 7.86 | 0.65 | - | 0.89 | 0.93 (0.78-0.98) |
| %FMADP | 13.3 | 9.16 | 14.1 | 8.17 | 0.55 | - | 0.92 | 0.95 (0.85-0.98) |
| Resistance (Ω) | 670 | 83.3 | 685 | 71.3 | 5.72 | 1.63 | 0.90 | 0.95 (0.85-0.98) |
| Raw body volume (m3) | 30.7 | 10.3 | 30.8 | 10.2 | 0.11 | 0.34 | 0.92 | 0.99 (0.98-1.00) |

%FMBIA, percentage body fat measured by bioelectrical impedance analysis; %FMADP, percentage body fat measured by air displacement plethysmography. TEM% is not presented for %FM since the units are already a percentage

**Figure Titles**

Figure 1. Bland-Altman plot of percentage fat mass (%FM) from ADP and BIA. Black circles represent the without obese group, and open circles represent the with obesity group. Dashed line is mean difference (bias), solid lines are limits of agreement (± 1.96 SD). Dotted line is the line of best fit (proportional bias).