Body Composition Analysis by Air Displacement Plethysmography in Normal Weight to Extremely Obese Adults

Kazanna C. Hames1,2, Steven J. Anthony2, John C. Thornton3, Dympna Gallagher3 and Bret H. Goodpaster2

Objective: To compare body composition parameters estimated by air displacement plethysmography (ADP) to dual X-ray absorptiometry (DXA) in body mass index (BMI) classifications that include extremely obese (BMI ≥ 40.0 kg/m2), and to examine if differences between analyses were influenced by BMI.

Methods: Fat-free mass (FFM, kg), fat mass (FM, kg), and body fat (BF, %) were analyzed with both technologies.

Results: All outcome measures of ADP and DXA were highly correlated (r ≥ 0.95, P < 0.001 for FFM, FM, and BF), but Bland–Altman analyses revealed significant bias (P < 0.01 for all). ADP estimated greater FFM and lower FM and BF (P < 0.01 for all). BMI explained 27% of the variance in differences between FFM measurements (P < 0.001), and 37 and 33% of the variances in differences between FM and BF measurements, respectively (P < 0.001 for both). Within normal weight and overweight classifications, ADP estimated greater FFM and lower FM and BF (P < 0.001 for all), but the opposite occurred within the extremely obese classification; ADP estimated lower FFM and greater FM and BF (P < 0.05 for all).

Conclusions: Body composition analyses by the two technologies were strongly congruent, but systematically different and influenced by BMI. Caution should be taken when utilizing ADP to estimate body composition parameters over a wide range of BMI classifications that include extremely obese.

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Introduction

The dramatic increase in extreme obesity over the past decade (1, 2) has elevated the need to determine which body composition methods are valid and reliable in this population. Body composition analysis provides clinical and scientific information relevant to health risks associated with obesity that is more advantageous than body mass index (BMI) alone (3, 4). In addition, body composition analysis can determine the effectiveness of interventions used to induce changes in body composition compartments such as fat and muscle mass.

The gold standard of body composition analysis employs the four compartment model in which fat, mineral, water, and protein are measured (5). However, the body composition technique commonly employed in research studies investigating extremely obese adults is dual X-ray absorptiometry (DXA) (6-12). The popularity of this method can be attributed to the ease of use and widespread availability of DXA, as well as good reliability and reproducibility; the coefficient of variation (CV) is about 2% across several studies with heterogeneous populations (13). Errors in estimation of body composition parameters are due to factors that impact assumed attenuation coefficients for fat and lean tissue, including differentiating bone from lean tissue, hydration status, tissue thickness, and fat distribution (13). These are all issues that become more predominant as BMI increases to the extremely obese classification (BMI ≥ 40 kg/m2). For example, greater tissue thickness lends to a greater estimation of fat tissue (13, 14) so that fat mass (FM) and percentage of total body weight that is body fat (BF) are overestimated in obese adults. Further limitations of using DXA to measure body composition in the obese population include the scanner’s field of view and the table’s maximum weight capacity. Despite these particular disadvantages of using DXA for body composition analysis in extremely obese adults, as well as the method’s lack of establishment as a gold standard, DXA continues to be a predominant method utilized in the literature.

1 Department of Health and Physical Activity, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. Correspondence: Kazanna C. Hames (hames.kazanna@mayo.edu) 2 School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA 3 The New York Obesity Nutrition Research Center, St. Luke’s-Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University, New York, New York, USA

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One of the more promising technological advancements in body composition analysis that has potential utility for extremely obese adults is air displacement plethysmography (ADP). The BOD POD (an ADP unit by COSMED, California, USA) not only accommodates a wider range of body sizes than DXA, but is also non-invasive and relatively rapid and easy to perform (5). The within subject CV for repeated measures of body composition parameters with BOD POD ranges between 2.0 and 3.3% for adults tested on the same day (15) or across several days (16, 17). ADP is a method based on the two compartment model and assumes a constant density of FM and fat-free mass (FFM). The assumption of a constant FFM density may be violated in the extremely obese population and lend to error in estimations of body composition parameters using this method. In the literature thus far, ADP has been compared to other methods of body composition analysis for use in the higher classification of obesity by a limited amount of studies; one used the reference method hydrostatic weighing (18) while the other used a three compartment model (19). Of those studies that compared ADP to DXA, three previous studies included the higher classifications of obesity, with the maximum BMI only reaching 40 kg/m² (15, 17, 20).

The primary aim of this study was to compare the absolute and proportionate amounts of FM and FFM estimated by ADP to the reference method, DXA, in a group that included extremely obese adults. We hypothesized that body composition parameters estimated using ADP would not significantly differ from DXA across the spectrum of BMI classifications ranging from normal weight (BMI 18-24.9 kg/m²) to extremely obese.

Methods

Men and women greater than 18 years of age were recruited from 2007 to 2010. An additional 23 subjects from an ancillary study examining body composition and resting energy expenditure responses to bariatric surgeries also had pre-operative data included in the following analysis. All participants provided written informed consent approved by the Institutional Review Board of the University of Pittsburgh, and completed testing at the Endocrinology and Metabolism Research Center at the University of Pittsburgh.

Body Composition Assessment

Participants were asked not to exercise the day of their appointment, and not to drink or eat anything other than water for three to four hours prior to their appointment. Upon arrival, subjects were asked to void, and women completed a urine pregnancy test to exclude those who were pregnant. Subjects removed all excess clothing and accessories, except for swimsuits, compression shorts, or other close fitting undergarments. Height (cm) and total body weight (BW, kg) were measured on a calibrated, digital scale (Tanita Corporation of America, Inc., Illinois, USA) to calculate BMI (kg/m²). No participants were excluded due to exceeding the maximum weight capacity or field of view of the DXA. BW (kg), FFM (kg), FM (kg), and BF (%) were determined by both methods. Each participant completed a test with the ADP unit and a total body DXA scan on the same day and within the same hour. The testing sequence was random.

Air Displacement Plethysmography

The BOD POD was calibrated prior to each test. Subjects were provided a swim cap to wear over their head with their hair tucked in. BW was determined with the equipment’s scale. After completing three tests, the software (version 2.14) predicted the following variables in all subjects: thoracic gas volume, FFM, FM, and BF.

Dual X-ray Absorptiometry

All measurements were made with Lunar Densitometry model (General Electric Medical Systems Lunar, Wisconsin, USA) and analyzed with the accompanying software (enCORE 2003, version 8.50). The equipment was calibrated at the beginning of each test day, and also calibrated quarterly with phantom spine scans. In addition to the clothing worn for the assessments, subjects were offered a hospital gown. All subjects had a total body scan completed while lying motionless in the supine position. BW was calculated as the sum of bone mineral content, lean tissue and FM. Although DXA provides estimates of three compartments of body composition, the current study focused on the estimates of two compartments (fat and fat-free content) to compare to ADP analyses. FFM was calculated as bone mineral content plus lean tissue. BF was calculated by dividing FM by BW and multiplying by 100.

Statistical Analysis

The Statistical Package for Social Science (SPSS 17.0, SPSS Inc., Illinois, USA) was used for the following analyses with significance level set at P < 0.05. According to the Shapiro–Wilk test, the assumption of normality was not met by any of the body composition parameters among the total sample. For descriptive purposes, frequencies (n) and percentages (%) of categorical data and medians with 25th and 75th percentiles of continuous variables were presented. Body composition parameters measured by ADP were compared to those estimated by DXA with Wilcoxon-signed rank tests in the total sample and among each BMI classification group. Spearman’s rank correlation coefficients were used to evaluate the strength of the relationships between the methods’ estimations of FFM, FM, and BF. Bland–Altman plots (21) were also used to determine agreement between each methods’ outcome measures.

Multiple linear regression was used to examine if BMI contributed to the differences between each method’s determination of body composition variables (FFM, FM, and BF). Differences were calculated by subtracting the estimates of body composition parameters of the reference method from the ADP method. Demographic data (age, sex, race) were used to adjust for potential confounding in regression analyses, as was the difference in BW determined by ADP from DXA. Two participants’ data were identified as points with high residual and leverage; removing their data was believed to substantially change the estimate of coefficients and therefore their data were eliminated from the dataset. The participants were a 25-year-old Hispanic female with a BMI of 19.3 kg/m² and a 23-year-old Caucasian female with a BMI of 17.7 kg/m². All other assumptions of the multiple linear regression models were not violated.

Results

Subjects

Subject characteristics are presented in Table 1 for the total sample and by BMI classification. Of the 109 participants who completed the study, 98 were non-Hispanic whites, 6 were non-Hispanic blacks, 2 were Hispanic, and 3 were of other racial background. The BMI range for the normal weight group was 17.8-24.9 kg/m², 25.0-
29.8 kg/m² for the overweight group, 30.3-39.2 kg/m² for the obese group, and 41.1-51.5 kg/m² for the extremely obese group.

**Comparison of FFM Estimations by ADP and DXA**

There was strong agreement between the two methods’ estimates of FFM ($r = 0.95$, $P < 0.001$; Figure 1A) with ADP estimating higher FFM compared to DXA ($P < 0.001$; Table 2). The Bland–Altman analysis detected significant bias ($P = 0.002$; Figure 2A). When stratified by BMI groups, ADP continued to estimate significantly higher FFM than DXA in only the normal weight and overweight participants ($P < 0.001$ for both groups), but significantly lower FFM than DXA in extreme obese participants ($P < 0.05$; Table 3). There was no difference between the methods’ estimates of FFM in the obese group ($P = 0.84$; Table 3).

The difference in the estimation of FFM by ADP compared to DXA had a large, negative correlation with BMI ($r = -0.52$, $P < 0.001$; Figure 3A). BMI explained 37% of the proportion of variability in the difference of FFM estimated by both methods. For every 5 kg/m² increase in BMI, there was a 2.59 kg decrease in the difference between FFM estimated by ADP and DXA ($P < 0.001$). This suggests that as BMI increases, ADP underestimates FFM compared to DXA. In addition, Figure 3A indicates that the estimate of FFM by ADP in normal weight subjects is greater than the FFM estimate by DXA, but as BMI increases to the obese classifications, ADP estimates a lower FFM compared to DXA. When examining for possible covariates in the regression model, the difference between FFM estimated by ADP compared to DXA ($P < 0.001$) and the male gender ($P < 0.01$) were significant predictors of the difference in estimation of FFM, whereas age ($P = 0.48$) and race ($P = 0.51$) were not. BMI remained significantly associated with the difference between estimates of FFM when difference in BW and sex were held constant ($P < 0.001$).

**Comparisons of Fat Content Estimations by ADP and DXA**

There was strong agreement between the two methods’ estimations of fat content regardless of the unit of expression (kg for FM or % for BF) (FM: $r = 0.99$, $P < 0.001$, Figure 1B; BF: $r = 0.98$, $P < 0.001$, Figure 1C), although ADP provided significantly lower estimates of fat content ($P < 0.001$ for both FM and BF; Table 2). Bland–Altman analysis detected a significant bias in both FM ($P < 0.001$, Figure 2B) and BF ($P < 0.0001$, Figure 2C) estimated by ADP compared to DXA. When stratified by BMI groups, ADP continued to estimate significantly lower fat content (expressed as either FM or BF) than DXA in normal weight and overweight participants ($P < 0.001$ for both groups; Table 3), but estimated higher fat content in the extreme obese group ($P < 0.01$ for both FM and BF; Table 3). There were no significant differences in fat content estimated by the two methods in the obese group of class 1 and 2 participants (FM: $P = 0.47$; BF: $P = 0.80$; Table 3).

The difference in FM estimated by ADP compared to DXA had a large, positive correlation with BMI ($r = 0.61$, $P < 0.001$). BMI explained 37% of the difference between estimates of FM provided by ADP and DXA. A 5 kg/m² increase in BMI was associated with a 3.03 kg increase in the difference in FM as estimated by ADP compared to DXA ($P < 0.001$). This suggests that as BMI increases, ADP estimates a greater FM than DXA. At the lower end of BMI, ADP underestimates FM compared to DXA, but, as BMI increases into the obese classification, ADP overestimates FM (Figure 3B). None of the possible covariates were significant predictors in the regression model (difference in BF, $P = 0.34$, age, $P = 0.50$, males, $P = 0.06$ and race, $P = 0.64$).

The results of the regression analysis were similar when examining the influence of BMI on fat content expressed as BF. The difference in the estimates of BF had a large, positive correlation with BMI ($r = 0.57$, $P < 0.001$). For every 5 kg/m² increase in BMI, there was a 2.87 % increase in the difference in estimation of BF by ADP compared to DXA such that a higher BMI resulted in a higher BF estimated by ADP compared to DXA ($R^2 = 0.33; P < 0.001$). At the lower end of BMI, ADP underestimates BF compared to DXA, but, as BMI increases into the obese classification, ADP overestimates BF (Figure 3C). When examining potential covariates in the regression model, the difference in BF and male gender were significant predictors ($P = 0.003$ and $P = 0.046$, respectively). When these covariates were held constant in the regression model, BMI remained a significant predictor of the variance in the difference in BF as estimated by ADP compared to DXA ($P < 0.001$).

**Discussion**

This study compared body composition estimates analyzed by ADP to another independent method of analysis based on the two compartment model approach, DXA. The study population included a heterogeneous population of adults with a range of ages and mixed races, as well as BMI classifications ranging from normal weight to extremely obese. The results confirmed that body composition parameters assessed by ADP and DXA were strongly correlated, but the precise estimates of body composition parameters significantly
The direction of differences between ADP and DXA was not uniform across the BMI spectrum, and the differences were influenced by BMI. Overall, ADP overestimated FFM and underestimated fat content compared to DXA. This was in agreement with prior studies demonstrating ADP underestimated fat content compared to DXA analysis of adults with BMI values ranging from 19 to 36 kg/m² (15, 17, 22, 23). The current study extended these findings to a population with an even broader range of BMI values that included extremely obese adults with a maximum BMI of 52 kg/m², but were limited to those that fit the requirements of the equipment (e.g., the weight limits of ADP and DXA and the field of view of the DXA scanner). Additional analyses were carried out to assess if differences in estimates of body composition parameters by ADP and DXA were influenced by BMI. Within the normal weight, overweight, and extremely obese groups, significant differences were found in FFM, FM, and BF estimated by these two laboratory techniques (Table 3). Previous research suggested that BMI might explain the outcome measurements of body composition analysis. Among participants with a BMI range of 17-42 kg/m², the greatest difference between estimation of BF by ADP and DXA was among those participants with higher adiposity (15). More specifically, ADP tended to overestimate BF in those with higher adiposity, but underestimate BF in those with lower adiposity. This was shown with comparisons between ADP and DXA in children (16), ADP and hydrostatic weighing in adults (17), and ADP and a three compartment model using body density by H\textsuperscript{18}O dilution in adults with extreme obesity (24). This trend was not significant when comparing analysis by ADP to DXA in adults with a maximum BMI of 40 kg/m² (4, 20). However, the current study found that higher BMI values were associated with greater overestimation of fat content (expressed as either mass or percentage of total BW) and underestimation of FFM by ADP compared to DXA. At the lower end of BMI values, the opposite was true; ADP underestimated fat content (expressed as either mass or percentage of total BW) and overestimated FFM compared to DXA. BMI contributed significantly to differences in body composition outcome variables between ADP and DXA, and whether ADP under or overestimated a given outcome variable of body composition analysis in adults was dependent on which BMI classification group was of interest. In other words, the direction of the differences in the two methods’ estimations of body composition parameters varied across BMI classification groups. These results may contrast those of Levenhagen et al. (17) and Nunez et al. (16) due to the current study’s larger sample size and the inclusion of extremely obese adults that extended the range of body fatness levels. In addition, differences between studies may be due to the estimations of body composition parameters estimated by different versions of the Lunar DXA software utilized by each study.
In addition to BMI, the male gender was also a significant confounding variable in the differences in FFM and BF (but not FM) measured by ADP and DXA. Differences between sexes may be more of a factor of body size and composition, but with males tendency to present with greater BW and FFM and lower BF (17).
extracting how sex influences the estimation of body composition parameters by different analyses is difficult. Such analysis was not a part of the current study’s design, and may not be adequately powered to examine this relationship. However, the significant influence of body size (expressed as BMI in the current study) on differences in body composition parameters by ADP and DXA, suggests that this may have more of an effect on body composition analysis compared to a sex effect.

There are several potential reasons for the disagreement between body composition measurements by ADP and DXA. Inherent errors of methodological assumptions were expected to affect the outcome measurements of body composition analyses between ADP and DXA. An assumption associated with ADP is that density of FFM is a constant (25). A violation to this assumption can occur with differences in hydration status and fluid distribution. Among extremely obese subjects, there is a significant variation in fluid distribution (examined by the ratio of extracellular to intracellular water) compared to reference values (24) and controls with normal weight and overweight BMI classifications (26). Greater hydration of FFM, resulting in lower FFM density and an underestimation of FFM, would explain the lower FFM found by ADP compared to DXA in the extremely obese group (Table 3). In an attempt to limit hydration status as a source of variability, the current study asked participants to not exercise the day of the study and to fast for 3 to 4 h prior to testing. The assumption of a constant density of FFM may also be due to racial differences; it has been suggested that blacks have a higher FFM density than other racial groups, but previous studies have found this variable to be similar between blacks and whites (27). The current study did not find race to be a significant contributor to the difference in body composition parameters estimated by ADP and DXA, but, with the majority of the sample being one race (i.e., Caucasian), the study may not have been powered to detect the effects of race.

The ability of both technologies to accurately estimate body composition parameters is more fraught with error due to differential effects of bone mineral density, which is a potential issue for obese adults who often present with higher bone content. DXA has greater error in estimating soft tissue under- and overlying bone, such that those with a higher bone mineral content lead to overestimation of FM (13). With ADP, higher bone content would lead to an overestimation of FFM. Variations in bone content and their potential effects on body composition analyses by each technology did not seem to influence the outcome measurements in the current study’s population since estimation of FM was lower in DXA compared to ADP, and FFM was lower in ADP compared to DXA in the obese BMI classification groups (Table 3).

Deviations from protocols were another potential source of error. With ADP, the isothermal air trapped near skin, hair and clothing, as well as in the lungs must be accounted for (5). The current study limited this source of error by enforcing the recommendations of BOD POD to use swim caps and close fitting garments. To account for the isothermal conditions in the lungs (5), the lung volume can be measured or predicted with the equipment and software provided with the BOD POD. In the current study, lung volume was predicted instead of measured. Although this introduces a source of error, Collins and McCarthy (28) found no difference between predicted and measured lung volumes with BOD POD, nor did the use of either value of lung volume have a significant influence on the outcome measurements.

In conclusion, estimates of body composition parameters by ADP were strongly associated with DXA across a heterogeneous population with a wide range of BMI values. However, FFM, FM, and BF estimated by ADP were systematically different from DXA and
affected by BMI. The extent of the discrepancy between ADP and DXA was no greater in extremely obese then other BMI classifications, but the direction of the differences was not uniform. In comparison to DXA, ADP estimated a higher absolute and proportional fat content in adults with higher BMI, but estimated lower fat content in those with lower BMI. ADP’s estimation of FFM was lower at higher BMI values, but higher at lower BMI values. These results do not support interchanging these two methodologies when a comparison of body composition is being conducted in only one BMI classification or across classifications.

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