Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults\textsuperscript{1–3}

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ABSTRACT

Background: Over the past decade, considerable attention has been paid to accurately measuring body composition in diverse populations. Recently, the use of air-displacement plethysmography (AP) was proposed as an accurate, comfortable, and accessible method of body-composition analysis.

Objective: The purpose of this study was to compare measurements of percentage body fat (%BF) by AP and 2 other established techniques, hydrostatic weighing (HW) and bioelectrical impedance analysis (BIA), in adults.

Design: The sample consisted of healthy men (n = 23) and women (n = 24). %BF was measured by AP, HW, and BIA.

Results: In the total group, %BF\textsubscript{AP} (25.0 ± 8.9%) was not significantly different from %BF\textsubscript{HW} (25.1 ± 7.7%) or %BF\textsubscript{BIA} (23.9 ± 7.7%), and %BF\textsubscript{AP} was significantly correlated with %BF\textsubscript{HW} (r = 0.944, P < 0.001) and with %BF\textsubscript{BIA} (r = 0.859, P < 0.01). Compared with HW, AP underestimated %BF in men (by 1.24 ± 3.12%) but overestimated %BF in women (by 1.02 ± 2.48%), indicating a significant sex effect (P < 0.05). The differences in estimation between AP and BIA and between BIA and HW were not significantly different between the sexes.

Conclusion: AP is an accurate method for assessing body composition in healthy adults. Future studies should assess further the cause of the individual variations with this new method.

KEY WORDS  Adiposity, body fat, body density, humans, validity, reliability, body composition, hydrostatic weighing, bioelectrical impedance analysis, air-displacement plethysmography

INTRODUCTION

The assessment of body fat and fat-free mass provides valuable information about the physical and metabolic statuses of humans. In addition, the ability to accurately measure body fat is important because of the established association between high amounts of body fat and a variety of disease processes such as hypertension, diabetes mellitus, coronary artery disease, and hyperlipidemia (1). Various methods are available for indirectly measuring body fat, including anthropometry, hydrodensitometry, bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry, measurement of total-body potassium, in vivo neutron activation analysis, and sophisticated imaging techniques.

Hydrodensitometry, or hydrostatic weighing (HW), is considered to be the gold standard of the densitometric methods (2). HW uses the Archimedes principle to determine total body volume by measuring the difference between a subject’s weight in water and that in air and thus determining whole-body density (3). This technique typically requires the subject to be completely submerged underwater while exhaling maximally (yielding residual lung volume) to minimize the effect of buoyancy from lung air (3). The limitations associated with this method include time, labor intensity, subject discomfort, and inaccessibility for many special populations such as the elderly, disabled, and chronically ill. The validity and reliability of HW has been well established in the literature (4–6).

An alternative method for measuring body fat is BIA (4, 7, 8), which estimates body resistance, or impedance, from a voltage drop initiated from a small current passed between electrodes (9). The level of impedance, an indication of the water and electrolyte composition of the body, is used to estimate lean tissue content and body water volume from developed regression equations. Assuming a hydration fraction of lean tissue, additional regression equations are used to estimate lean body mass and fat mass (8, 9). The reliability and validity of BIA have also been described previously (1, 7, 9).

Researchers have also experimented with a variety of plethysmographic alternatives to HW. Air-displacement plethysmography (AP) determines body volume by measuring the reduction in chamber volume caused by introduction of a subject into a chamber with

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a fixed air volume (2, 10). Similar to HW, whole-body density is therefore determined and body composition is calculated by using previously validated prediction equations. The purpose of the present study was to compare measurements of body fat by AP with those by 2 established techniques, HW and BIA, in a heterogeneous sample of healthy men and women.

SUBJECTS AND METHODS

Subjects

After obtaining approval from the Committee for the Protection of Human Subjects, a sample comprising healthy men (n = 23) and women (n = 24) aged 19–48 y was recruited for the study from university health promotion facilities and the surrounding community. Subjects were screened for good health and were excluded if they 1) had a history of heart disease, including coronary artery disease, congestive heart failure, or symptomatic dysrhythmia; 2) had a history of chronic obstructive pulmonary disease; 3) were pregnant; or 4) were afraid of submersion in water.

Before any measurements were made, volunteers were provided with written instructions about the testing protocol. The protocol criteria included the following: 1) a 12-h fast, 2) no consumption of alcohol for 12 h before measurements, 3) no intensive exercise within 12 h of the measurements, 4) adequate hydration, and 5) avoidance of excessive use of moisturizing lotions. After protocol adherence was verified, the purpose and possible risks of the study were explained to all subjects and their voluntary, written consent was obtained.

Equipment

The air-displacement plethysmograph used in this study was the BOD POD body-composition system (model 2000A; Life Measurement Instruments, Concord, CA). The device consists of a computer-integrated, dual-chambered air plethysmograph; digital weight scale; and BOD POD software version 1.68. The system is located within the General Clinical Research Center of the Vanderbilt University Medical Center.

The equipment used for HW included a hydrostatic tank (≈2 m³, or 74.25 ft³), 3 electronic force sensors (Omega Engineering Inc, Stamford, CT), a nitrogen analyzer (model 5000; Consulting Western Services, Lakewood, CA), a calibrating syringe (model 454; Hans Rudolph, Inc, Kansas City, MO), a personal computer (Tandy 1000 RLX with BIOS ROM version 2.0 capability software; Exertech Fitness Products, Dresbach, MN), and a chart data recorder (Servogor 120; Norma Goerz Instruments, Elk Grove, IL). Additional supportive devices included a weighing scale (Healthmeter 5000; Consulting Western Services) and a barometer (model 453; Primeeco, South Hampton, MA). BIA was measured by using a body-composition analyzer (model 310; Biodynamics, Seattle).

Experimental procedures

All measurements were performed by 2 experienced testers according to the manufacturers’ requirements and suggestion; the procedures followed were consistent with standard procedures reported in the literature. Measurements in each subject were first made by AP, followed immediately by BIA. HW was performed within 3 d of the original AP and BIA measurements.

Air-displacement plethysmography

For the AP measurement, subjects were asked to change into a tight-fitting swimsuit and to remove all jewelry. Subjects also wore a tight-fitting swim cap during this segment of the study to minimize the effect of the hair on body volume assessment (2, 10). Body mass was first measured to the nearest 0.02 kg on a calibrated electronic scale. Each subject was then asked to sit in the air-displacement plethysmograph for body volume measurement. Subjects were instructed to sit quietly with an erect posture and normal respiration, with their hands folded in their laps and their feet placed on the floor of the device. A minimum of two 50-s tests were conducted to ensure reliability of measures. The body volume measurement was repeated if the 2 measures were not within 150 mL of each other (2). After these initial measurements, subjects were connected to a breathing circuit internal to the system for measurement of thoracic gas volume. The subjects were instructed to apply a nose clip and to continue normal breathing for ≈2–3 full breaths to allow the system to record a real-time breathing record on the computer screen. The investigator observed this breathing pattern and signaled the subjects just before airway occlusion. Subjects then alternately contracted and relaxed the diaphragm while airway and chamber pressures were recorded simultaneously. The thoracic gas volume measurement was repeated if either gas leaks or puffing too hard by the subjects was detected by the automated program (2). Once these measurements were completed (after ≈3–5 min), body density (Dₜ) was calculated by the system’s computer with the following equation:

\[ Dₜ(\text{AP}) = \frac{\text{mass}}{Vₜ(\text{AP})} \]  

where \( Vₜ(\text{AP}) \) is body volume determined by AP.

Percentage body fat by AP (%BFₜ) was derived by using Shutte et al’s (11) formula for African American subjects and Siri’s (5) formula for all other volunteers:

\[ \text{Shutte: } %\text{BF}_\text{AP} = (4.374/Dₜ) - 3.928 \]  
\[ \text{Siri: } %\text{BF}_\text{AP} = (4.95/Dₜ) - 4.50 \]

Bioelectrical impedance analysis

Two signaling electrodes were placed on the dorsal surface of the right foot at the space between the metatarso-phalangeal joints of the great and second toes, as well as on the dorsal right hand at the space between the metacarpophalangeal joints of the second and third digits. The 2 detecting electrodes were placed between the styloid processes of the right radius and ulna and between the medial and lateral malleoli of the right ankle. During the measurement, subjects remained still in a supine position with their hands and feet slightly abducted from the midline to ensure that no contact was made between the extremities and the torso. Body fat content was calculated by using an unpublished regression equation of the impedance measurements and %BF.

Hydrostatic weighing

The following measurements were obtained before HW: weight in air (in kg), height (in cm), air temperature (in °C), and barometric pressure (in mm Hg). The nitrogen analyzer was calibrated according to the manufacturer’s specifications. After subjects entered the tank and removed any air trapped in their swimsuits, in their hair (by running their hands through their hair while submerged in water), or on their skin (by rubbing the surface of their skin to get rid of air bubbles), they...
were instructed to immerse themselves in the water to chin level and to bend at the knees to simulate a sitting position during the measurement of residual lung volume by the nitrogen dilution method. This procedure was repeated until 2 values within ±100 mL of each other were obtained; the mean of these 2 values was used to calculate $D_{n\text{HW}}$.

Subjects were then instructed to sit in a chair that was submerged in the water tank and attached to multiple electronic loading sensors. After the subjects were completely submerged, they exhaled maximally. A computer-integrated scale readout was obtained after the subject signaled for the end of maximal exhalation and all air bubbles from the exhalation disappeared. This procedure was repeated until 2 measures of weight agreed to ±50 g.

Body volume was calculated according to the Archimedes principle, with correction for residual lung volume. This volume value was used to compute whole-body density (body mass/volume) and the density value was entered into the equation of Siri (5) or Shutte et al (11) for estimation of %BF.

### Statistical analysis

Body-composition data are presented as means ± SDs. All statistical analyses were performed with SPSS (SPSS Inc., Chicago). The correlation analyses were conducted by using Pearson’s correlation coefficients ($r$). Two-factor analysis of variance was used to examine differences between methods and between sexes. Analyses performed on the differences between measurements included SEE (calculated as the SD of the difference between 2 measurements); scatter plots; Bland-Altman plots (12), which express the difference between the 2 measurements with respect to the mean of the 2 measurements; and multiple linear regression analysis (stepwise), to examine the mean and limits of agreement and the contribution of disagreements from %BF, weight, height, and age. P values <0.05 were considered to be significant.

### RESULTS

The characteristics of the study sample, including age, weight, height, and %BF measured by the 3 methods, are shown in Table 1. %BF AP was not significantly different from %BF HW or %BF BIA and was significantly correlated with both %BF HW ($r = 0.944$, $P < 0.001$; Figure 1) and %BF BIA ($r = 0.859$, $P < 0.01$; Figure 2). %BF BIA and %BF HW were also significantly correlated ($r = 0.857$, $P < 0.01$; Figure 3). Overall, there was no method-by-sex effect of the 3 methods of body-composition measurement.

The measurement bias, expressed as the mean of the difference in %BF measured by AP and that measured by HW (AP – HW) was small (−0.09% of %BF); the limits of agreement, defined as the mean ± 2 SDs, were from −6.10% to 5.93% (Figure 1). Moreover, there existed a significant ($P < 0.01$) and positive correlation between the difference and the mean of the 2 %BF measurements by AP and HW (Figure 1). AP underestimated %BF in men (by −1.24 ± 3.12%) and overestimated %BF in women (by 1.02 ± 2.48%); this sex effect was significant ($P < 0.05$). Other characteristics such as age, weight, and height, however, did not significantly contribute to the differences in %BF measurements by stepwise multiple regression analysis.

When AP was compared with BIA, the measurement bias was 1.12% and the limits of agreement were from −8.00% to 10.22% (Figure 2). There was a positive but nonsignificant correlation between the difference and the mean of the 2 measures. There was no significant contribution to the difference between the 2 measures from sex, age, weight, or height by stepwise multiple regression analysis.

When BIA was compared with HW, the measurement bias was −1.20% and the limits of agreement were from −9.48% to 7.07% (Figure 3). There was a slight positive but nonsignificant correlation between the difference and the mean of the 2 measures. There was no significant contribution to the difference between the 2 measures from sex, age, weight, or height by stepwise multiple regression analysis.

### DISCUSSION

Since its development, the AP system for body-composition assessment has been evaluated in terms of its reliability and validity (2, 10). The reproducibility of the method was reported to be excellent in a variety of adult volunteers ($n = 68$), yielding an average CV of $1.7 \pm 1.1\%$ in 2 consecutive tests on the same day (10). Furthermore, the CV for test-retest variations in AP measurements was even slightly lower than that for HW ($2.3 \pm 1.9\%$, NS) (10). With our machine, we observed similar reproducibility for the AP system for the same individual under the same testing conditions ($2.0 \pm 2.1\%$, unpublished observations; 1998). In terms of validity, McCrory et al (10) concluded that AP was in “excellent agreement” with the gold standard of HW. The mean difference and correlation coefficient value between %BF assessed by AP and by HW in our study were similar to those reported in a previous validation study (10). However, results in our study showed less agreement between the 2

### TABLE 1

Subject characteristics and percentage body fat (%BF) measured by air-displacement plethysmography (AP), hydrostatic weighing (HW), and bioelectrical impedance analysis (BIA)\(^{1}\)

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 23)</th>
<th>Women (n = 24)</th>
<th>All (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33.3 ± 8.7 (20–48)</td>
<td>30.7 ± 7.2 (19–42)</td>
<td>32.0 ± 8.0 (19–48)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>84.1 ± 13.1 (62.1–113.8)</td>
<td>68.1 ± 17.1 (53.3–135.0)</td>
<td>75.9 ± 17.1 (53.3–135.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.5 ± 5.9 (167.0–188.0)</td>
<td>168.0 ± 7.3 (156.2–184.8)</td>
<td>173.6 ± 8.8 (156.2–188.0)</td>
</tr>
<tr>
<td>%BF AP (%)</td>
<td>20.2 ± 7.5 (6.0–34.0)</td>
<td>20.7 ± 7.9 (15.8–48.9)</td>
<td>25.0 ± 8.9 (6.0–48.9)</td>
</tr>
<tr>
<td>%BF HW (%)</td>
<td>21.5 ± 6.2 (8.5–32.7)</td>
<td>28.6 ± 7.5 (13.7–46.8)</td>
<td>25.1 ± 7.7 (8.5–46.8)</td>
</tr>
<tr>
<td>%BF BIA (%)</td>
<td>20.2 ± 6.3 (11.1–36.4)</td>
<td>27.5 ± 7.4 (13.5–44.1)</td>
<td>23.9 ± 7.7 (11.1–44.1)</td>
</tr>
</tbody>
</table>

\(^{1}\) ± SD; range in parentheses. Subjects included 3 African American men and 4 African American women. There were no significant differences in %BF measured by BP, HW, or BIA in the group as a whole.

\(^{2}\) Significantly different from men, P < 0.01.
methods. This was reflected by the larger SEE (3.1%BF compared with 1.81%BF) and, perhaps more disturbingly, the significant correlation between the difference in measured %BF and the mean of the 2 measures in the present study. Possible causes for these differences may be the smaller size of our subject group (n = 47 compared with 68) and our subjects’ lack of prior experiences with either of the 2 methods.

We found a significant sex difference in %BF measured by AP compared with HW. One explanation for the underestimation of %BF by AP in men compared with the overestimation in women is that body hair may have negatively influenced the body volume measurement (2). In our study, as would be expected, we noticed this effect to be more prominent in men than in women. Although none of our male subjects had beards, the amounts of hair on other areas of the body such as the back, chest, arms, and legs varied greatly between individuals, more so in men than in women. Therefore, it is reasonable to expect a lower measurement of average body volume, which would lead to an underestimation of %BF in men compared with women. The overestimation of %BF in women could have been a compensatory correction for the entire subject group because the group means were calibrated in the initial development of the general estimation equations (2). Further evidence of the effect of body hair can be seen in the larger SEE for the measurement of %BF by AP compared with HW in men (3.12%BF) than in women (2.48%BF). According to the manufacturer and the developer of the AP method, such a difference is secondary and small (2). Small differences in measured body volume, however, can cause significant changes in calculated %BF (2, 10). Thus, this factor should not be overlooked when an accurate measure of body composition is needed for research and clinical studies. Furthermore, the significant group difference in %BF between men and women may also have contributed to the correlation between the difference and the mean of the %BF measurements by AP and HW (Figure 1).

**FIGURE 1.** Percentage body fat (%BF) measured by air-displacement plethysmography (AP) versus %BF by hydrostatic weighing (HW). The dashed line represents the regression equation: %BFHW = 0.82 × %BFAP + 4.59 (R² = 0.891); the regression line was not significantly different from the line of identity. Also shown is the difference in %BF by AP and HW versus the mean %BF by the 2 methods. The horizontal dashed lines represent the mean ± 2 SDs of the group differences. Regression equation: Difference in %BF = 0.14 × mean %BF − 3.71 (R² = 0.155, P < 0.05).

**FIGURE 2.** Percentage body fat (%BF) measured by air-displacement plethysmography (AP) versus %BF by bioelectrical impedance analysis (BIA). The dashed line represents the regression equation: %BFBIA = 0.75 × %BFAP + 5.20 (R² = 0.738); the regression line was not significantly different from the line of identity. Also shown is the difference in %BF by AP and BIA versus the mean %BF by the 2 methods. The horizontal dashed lines represent the mean ± 2 SDs of the group differences. Regression equation: Difference in %BF = 0.15 × mean %BF − 2.53 (NS).
The dashed line represents the regression equation: \( \%BF_{\text{BIA}} = 0.86 \times \%BF_{\text{HW}} + 2.36 \) (\( R^2 = 0.734 \)); the regression line was not significantly different from the line of identity. Also shown is the difference in \%BF by HW and BIA versus the mean \%BF by the 2 methods. The horizontal dashed lines represent the mean \pm 2 SDs of the group differences.

It is also possible that inaccuracies in HW contributed to the variation in the difference in \%BF measurements by AP and HW. The reliability and accuracy of the HW technique has been reviewed previously (13–15). Although HW is generally considered to be the gold standard in whole-body densitometry, the method involves multiple measurements such as water temperature, atmospheric temperature and pressure, nitrogen analyzer calibrations, force sensor calibrations, subjects’ residual lung volume, and the underwater weighing itself. Each measurement can introduce inaccuracies that contribute to the overall error in estimating body density and \%BF. Moreover, variation in accuracy and compliance can be an even larger problem in obese, elderly, and diseased populations.

We also compared AP with BIA. Because of its practical nature, BIA is widely used in general health evaluations as well as in research studies, perhaps more so than HW. The reliability and validity of BIA has been proven and reviewed in the literature (1, 16, 17). The average group value for \%BF by AP was slightly higher than that by BIA (NS), with wide limits of agreement between the 2 methods. These wide limits of agreement were likely due to the underestimation of \%BF by BIA and to larger variations in BIA measurements than in AP measurements, as shown by comparisons between BIA and HW. However, multiple linear regression analysis did not reveal any independent contribution to the measured difference in \%BF by AP and BIA from age, sex, body mass, height, or average \%BF.

Compared with HW, BIA accurately estimated \%BF for the entire subject group (\( r = 0.86, 1.2\% \) underestimation, NS), although with a considerably wide variation (SEE: 4.14\%BF) as shown in Figure 3. The individual differences in \%BF as measured by BIA and HW were not correlated with the mean of the 2 measures. Our findings of a difference between BIA and HW are similar to findings in previous validation studies (18–20).

Overall, the findings of this study further indicate the validity of the relatively new AP method of body-composition measurement. Although our results did not show the “excellent” agreement between AP and HW reported in a previous validation study (10), our study represented a more realistic situation, ie, subjects with no prior knowledge of or experience with any of the body-composition methods. We also identified sex as a factor in the difference in the average and variation in \%BF as measured by AP and HW, and body hair may have caused this difference. Nevertheless, AP is accurate for group and individual assessment of \%BF compared with the current gold standard of HW. BIA is also accurate when used for group estimation of \%BF, but the large variations in \%BF by this method could be limiting in terms of accuracy for certain individuals. Although accurate, HW can be time consuming, tedious for subjects and testers, and limiting for special populations. Further attention should be paid to physical and physiologic factors that can influence the accuracy and reproducibility of body-composition measurements by AP, BIA, HW, and other techniques.

FIGURE 3. Percentage body fat (\%BF) measured by hydrostatic weighing (HW) versus \%BF by bioelectrical impedance analysis (BIA). The dashed line represents the regression equation: \( \%BF_{\text{BIA}} = 0.86 \times \%BF_{\text{HW}} + 2.36 \) (\( R^2 = 0.734 \)); the regression line was not significantly different from the line of identity. Also shown is the difference in \%BF by HW and BIA versus the mean \%BF by the 2 methods. The horizontal dashed lines represent the mean \pm 2 SDs of the group differences.

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