Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery

Sai Krupa Das, Susan B. Roberts, Joseph J. Kehayias, Jack Wang, L. K. George Hsu, Scott A. Shikora, Edward Saltzman, and Megan A McCrory. Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery. Am J Physiol Endocrinol Metab 284: E1080–E1088, 2003. First published February 25, 2003; 10.1152/ajpendo.00185.2002.—Body composition methods were examined in 20 women [body mass index (BMI) 48.7 ± 8.8 kg/m²] before and after weight loss [−44.8 ± 14.6 (SD) kg] after gastric bypass (GBP) surgery. The reference method, a three-compartment (3C) model utilizing body density by air displacement plethysmography and total body water (TBW) by H218O dilution (3C-H218O), showed a decrease in percent body fat (%BF) from 51.4 to 34.6%. Fat-free mass hydration was significantly higher than the reference value (0.738) in extreme obesity (0.756; P < 0.05) and 3C models using 2H2O or bioelectrical impedance analysis (BIA) to determine TBW improved mean %BF estimates over most other methods at both time points. BIA results varied with the equation used, but BIA better predicted %BF than did BMI at both time points. All methods except BIA using the Segal equation were comparable to the reference method for determining changes over time. A simple 3C model utilizing air displacement plethysmography and BIA is useful for clinical evaluation in this population.

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The prevalence of extreme obesity [defined as body mass index (BMI) ≥40 kg/m²] has increased threefold in the US in the last four decades, and 3% of adults are classified as extremely obese (18). Recent reports additionally confirm a rise in the prevalence rates in this population (17). One consequence of this demographic shift is that there is now a need for evaluating the body composition of extremely obese individuals, both in clinical practice and as part of research to evaluate the efficacy of different treatment programs. However, there is very little published research on what body composition methods can be used with confidence in this now prevalent population, either for groups of subjects or for individuals (2, 3, 19). Probably in part due to this lack of methodological examination, recent reports on the composition of weight loss in extremely obese subjects use a wide range of methods, and the reported composition of weight loss varies substantially (66–80% fat) (1, 37, 43).

Several traditional reference methods for measuring body composition in nonobese and moderately obese individuals appear to be inaccurate for use in extremely obese individuals or are inappropriate for practical reasons. For example, dual-energy X-ray absorptiometry and in vivo neutron activation are used as reference techniques (22) in nonobese and moderately obese subjects, but extremely obese subjects frequently exceed the tested weight limits of the instruments and in some cases cannot physically fit into the measurement compartment. Widely accepted two-compartment body composition models, such as 2H2O dilution and densitometry, which rely on standard assumptions of fat-free mass (FFM) hydration (FFM hydration) [0.738, (8)] and density [1.1 g/ml (34)], may be inaccurate in this population, because extremely obese individuals (38) and even postobese individuals (14–16, 20, 24, 26) have excess extracellular water that invalidates these assumptions (8, 34, 35). Three-compartment models that include measurement of total body water (TBW) are theoretically more accurate than the two-compartment models for measuring body composition of extremely obese individuals, since they take into account individual variability in hydration (34), but they are not widely used because they require sophisticated equipment only available in a few research laboratories worldwide.

We therefore conducted a study to characterize the body composition of extremely obese subjects before and after gastric bypass surgery (GBP) and to measure the composition of their weight loss by use of sophisticated research techniques. Furthermore, we evaluated several clinical and research body composition techniques for their ability to measure body composition and the composition of weight loss in these subjects. As...
part of this study, we explored the potential for combining simple body composition methods to develop alternative methods that are simple to use and comparable to the accuracy achieved with research techniques. This general approach has been reported for nonobese and moderately obese individuals (14, 20) but has not been examined in extremely obese persons.

SUBJECTS

The subjects were 20 women aged 39 ± 10 yr with a BMI of 37.5–76.4 kg/m². They were patients who underwent GBP surgery for weight reduction at the Tufts-New England Medical Center Hospital and had measurements made of body composition before surgery (extreme obesity) and after weight loss and weight restabilization at 14 ± 2 mo (weight-reduced state). Exclusions for the study included diabetes, cancer, coronary heart disease, endocrine disorders or other acute or chronic diseases, and use of medications known to influence body composition (such as diuretics and corticosteroids). Measurements were conducted in the Clinical Study Unit of Tufts-New England Medical Center Hospital and at the Jean Mayer USDA Human Nutrition Research Center at Tufts University. The study was approved by the Human Investigation Review Committee of Tufts University and the Tufts-New England Medical Center Hospital. All subjects gave written, informed consent before participating.

METHODS

Study protocol. Percent body fat (%BF) and FFM were determined by densitometry, isotope dilution, bioelectrical impedance analysis (BIA), and three-compartment modeling during a period of weight stability in the extremely obese state before GBP surgery and at a weight-reduced follow-up 14 ± 2 mo later. To ensure weight stabilization at each time period, subjects weighed themselves at home or at the clinical facility three times a week in the fasting state in the month before scheduled testing. Subjects who were not weight stable (defined as within 2.3 kg of starting weight) during this period were monitored further and were scheduled when the defined weight stability was achieved. All body composition measurements were conducted after a 12-h overnight fast and by the same scientist (S. K. Das) at each time point.

Subjects were admitted to the Clinical Study Unit on the evening before the start of the study. On the following morning, TBW was measured by H218O and 2H2O dilutions, and evening before the start of the study. On the following morning, TBW was measured by H218O and 2H2O dilutions, and on cross-calibrated scales each test day, and differences in body weight between the 2 days were assumed to be due to differences in body water, as we will describe.

Densitometry. Air displacement plethysmography (BOD POD, Life Measurement, Concord, CA) was used to measure body density, as described elsewhere (28). The principles of this method have been detailed previously (13). Measurements were conducted with the subject in a Lycra-style swim cap and minimal skin-tight shorts or underwear, dry, and in the resting state according to the manufacturer’s instructions. Body weight was measured to the nearest 1 g on the instrument’s electronic scale, which was calibrated daily. After the standard calibration of the plethysmograph’s chamber, subjects entered the chamber for measurements of raw body volume and thoracic gas volume (Vr,T). Vr,T measurements were repeated until a figure of merit value <1.0 (signifying compliance) was obtained for all subjects. The obtained Vr,T and the average of two raw body volume measurements that agreed within 0.2% were used in subsequent calculations. Body density was calculated as body weight per body volume, where body volume was corrected for Vr,T and a surface area artifact, as described previously (13). Body weight and the corrected body volume were used to calculate body density, and %BF was derived by using the two-compartment Siri formula (35). Calculations were performed by the BOD POD’s software (version 1.69).

Body density was also determined by hydrostatic weighing in a subset of the study group (n = 11) in the extremely obese state only, and %BF was calculated using the Siri formula (35). Reasons for excluding other subjects from the hydrostatic weighing procedure included a history of discomfort and/or apprehension about being tested in the water tank, inability to perform the maneuvers required for satisfactory testing, and other such physical constraints. In subjects who performed the procedure, weight under water during maximal exhalation to residual lung volume (VR) was determined until three body fat estimates agreed within 1% for this preliminary agreement, a predicted VR (10) was temporarily used. VR was measured at the pulmonary laboratory at Tufts-New England Medical Center Hospital before hydrostatic weighing by nitrogen washout (12) with a Sensor Medics Vmax 229 (Yorba Linda, CA) with software version 4.8. Measurements were made until three values were within 10%, and the best values (criteria for “best” as defined by the Sensor Medics operation manual section 9.23, p. 0296C) were used in the calculation of %BF. No correction was made for gastrointestinal gas volume. The three %BF values were recalculated using measured VR, and the mean was used in data analysis.

Isotope dilution. On day 1, subjects were given a mixed dose of 2H2O and H218O containing 0.1 g of 18O and 0.07 g of 2H2 per kg body weight for determination of TBW. The dose was administered orally after a collection of baseline urine early in the morning. Subjects remained in the Clinical Study Unit while urine specimens were collected every hour for 5 h after dose administration. All samples were aliquoted into airtight storage tubes (CryoGene cryogenic vials, Vanguard International, Neptune, NJ) immediately after collection and were stored at −20°C until analysis.

Baseline and 5-h postdose urine samples were used for determination of TBW. Urinary isotope enrichments were determined by isotope ratio mass spectrometry (Hydra, PDZ Europa, Cheshire, UK) by use of the method of Prosser and Scrimgeour (30) for sample preparation. The coefficient of variation (CV) for day-to-day repeated measurements of 18O and 2H abundances in standards averaged 0.08% for 18O and 0.03% for 2H, respectively. Isotope dilution spaces were calculated with the assumption of no loss of isotope between dosing and 5 h (11). To correct for the known isotopic exchange with nonaqueous organic compounds, TBW was calculated as the H218O dilution space at 5 h postdose divided by 1.01, and as the 2H2O dilution space at 5 h postdose divided by 1.04 (31). TBW was calculated as a volume measurement and converted to kilograms by using the conversion factor of 0.99336 (density of water at normal body temperature). FFM was calculated from TBW by assuming a FFM hydration...
coefficient of 0.738 (8), and %BF was computed as [(body wt – FFM)/body wt·100]. FFM was also calculated by using the mean measured hydration coefficients in extreme obesity and in the weight-reduced state, with FFM hydration calculated as we will describe.

ECW. The intravenous NaBr method (36) was used to determine ECW volume. An intravenous dose of a 3% solution of NaBr was administered at 1 ml/kg body wt. Blood samples were collected before the dose was administered and 2, 3, and 4 h after dose administration. The bromide concentration in plasma samples was analyzed by ion chromatography (44). Since there were no significant differences between the measurements at the three time points, indicating that bromide had equilibrated in the extracellular space by 2 h, the mean of 2-, 3-, and 4-h measurements was used for the calculation of dilution space. ECW was calculated from the increase in bromide concentration between baseline and mean postdose blood samples, the amount of bromide injected (44), an assumed factor of 0.90 for the nonextracellular distribution of bromide (9), and the Donnan equilibrium factor of 0.95 (9). ECW was calculated as a volume measured converted to kilograms by using the conversion factor of 0.99336 (density of water at normal body temperature). Intracellular water (ICW) was calculated as TBW – ECW. To further examine the metabolically active component of FFM, cellular mass (CM) was calculated as FFM – ECW, where FFM was calculated by the Siri 3C-H₂¹⁸O method described below. Solids were not measured in this study but were estimated as 100 – ECW + ICW + fat mass, each expressed as a percentage of body weight. The NaBr protocol was ready for use only after the first four subjects completed all of their presurgery measurements, and therefore ECW measurements could not be obtained in these four subjects. The "n" is therefore equal to 16 in analyses involving ECW data.

BIA. BIA measurements were made using a four-terminal bioelectrical impedance analyzer (model 103B, RJL Systems, Mt. Clemens, MI) in thermoneutral ambient conditions. Subjects removed their shoes, socks, and all accessories containing metal. They then rested supine for 15 min before the start of the measurement. During the measurement, the subject rested supine on a nonconducting surface and without limbs touching each other or any other part of the body. A small electrical current of 800 μA at 50 kHz was introduced through two of the terminals, which were affixed to the skin on the dorsal surfaces of the left hand and foot with self-adhesive electrode pads. The two detection terminals, again attached to the skin of the left hand and foot with self-adhesive electrode pads, were placed between the distal prominences of the radius and ulna and between the lateral and medial malleoli. Three values for resistance and reactance were recorded at each measurement, and the means were used in subsequent calculations. FFM was calculated using the equations of RJL (the manufacturer), Lukaski et al. (25), and the obese-specific equations of Segal et al. (33). TBW by BIA was also calculated using the equations of Kushner and Schoeller (23).

Anthropometry. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer, and weight was measured to the nearest 1 g by use of a calibrated scale (Detecto-Cardinal Scale Manufacturing model CN-20, Webb City, MO). One week later, body weight was measured to the nearest 1 g on a different calibrated electronic scale (BOD POD, Life Measurement). There was no significant difference in the accuracy of the two scales as determined by calibration weights, and any differences in body weight between the two test days were assumed to be water. Waist circumference was measured at the level of the umbilicus, and hip circumference was measured with a quilting tape as the maximal circumference at the level of the buttocks.

3C models. The 3C model of Siri (35) modified by Modlesky et al. (29), which incorporates body density (d) and TBW as a fraction of body weight (w), was used to calculate %BF as 2.1176/d – 0.78w – 1.351, and FFM was calculated as the difference between body weight and fat mass. The modification by Modlesky et al. incorporated the assumption that the mineral-to-protein ratio in the fat-free body is a constant (6.8/19.4), on the basis of work by Brozek et al. (8), whereas the original equation by Siri (34, 35) assumes that the mineral-to-protein ratio is 5/12.

The reference technique for %BF in this study was the 3C model that used TBW determined by H₂¹⁸O and body density determined by air displacement plethysmography (3C-H₂¹⁸O). Other 3C estimates of %BF were also calculated, using body density by air displacement plethysmography and TBW by either ²H₂O dilution (3C-²H₂O) or BIA [TBW prediction equations are those of Kushner and Schoeller (23)] (3C-BIA).

FFM hydration coefficient. The FFM hydration coefficient was calculated in standard fashion as TBW/FFM (4), by use of TBW determined from ²H₂O and FFM determined by 3C-H₂¹⁸O. By using different isotope dilutions for TBW (²H₂O) and FFM (H₂¹⁸O) in the calculation, the denominator remained independent of the numerator, and error propagation was minimized.

"The Reference Female." For the purposes of this paper, the Reference Female values used were obtained as follows. The value for the %BF compartment was obtained from the Reference Female model work done by Katch and colleagues (21, 27) with the theoretical model of Behnke and Wilmore (5, 6). For body water compartments, i.e., ECW and ICW, the values for the Reference Man in the review by Wang et al. (42) were used. Solids were derived as 100% minus the sum of %BF and %TBW.

STATISTICS

Statistical analyses were performed by using SPSS 10.0.7 and SYSTAT 9.0.1 (SPPS, Chicago, IL) and SAS (version 8, SAS Institute, Cary, NC). Values are expressed as means ± SD unless otherwise specified. Student's t-tests for paired data were used to examine whether there were significant changes over time in body compartments after weight loss, and one-sample t-tests were used to compare body composition in the extremely obese and weight-reduced states with Reference Female values. Bonferroni corrections were made to adjust for multiple comparisons. Linear regression analysis was used to determine whether FFM hydration varied systematically with %BF.

The agreement between the reference method and the test method was assessed by using ANOVA and Bland-Altman analysis (7). The mean ± SD difference (bias) for %BF between the reference method (3C-H₂¹⁸O) and the alternative methods was calculated by subtracting each alternative method from the reference method. Bland-Altman analysis was performed, and limits of agreement were calculated to determine the range of agreement between methods for individual subjects. ANOVA with Dunnett's post hoc test was used as a test of whether %BF by the alternative methods differed significantly from the reference method.

Regression analysis was used to determine the relationships between BMI and %BF and between FFM and fat mass. Pearson correlation coefficients were calculated to determine the associations between the mean difference of each
method and the reference method and physiological characteristics, such as ECW, ICW, ratio of ECW to ICW, ECW as % of TBW, FFM hydration, BMI, and waist-to-hip ratio to determine whether these characteristics affected the accuracy of the methods.

For all tests, statistical significance was accepted at $P < 0.05$.

**RESULTS**

Table 1 shows the body composition of the subjects in the extremely obese and weight-reduced states by use of the reference 3C-H$_2^{18}$O method for body composition. Weight loss was substantial, with mean BMI decreasing from 47.8 to 30.4 kg/m$^2$. Also, as shown in Table 1, weight loss was primarily as fat mass loss (79 ± 11% of weight loss was fat, and 21 ± 8% was FFM). In both the extremely obese and weight-reduced states, the subjects had relatively high FFM hydration compared with the reference value of 0.738 (8), but the difference was significant only in the extremely obese state ($P < 0.001$). No significant difference in FFM hydration was observed between the two physiological states. ECW/ICW ratios also did not differ significantly between extreme obesity and weight-reduced states and were significantly higher than reference values (0.76) (42). Figure 1 shows the body compartments of the subjects expressed as a percentage of body weight compared with values in our Reference Female. Both ECW and ICW expressed as percentages of body weight were significantly lower ($P < 0.05$) in the extremely obese state compared with the Reference Female values. In the weight-reduced state only, ICW expressed as a percentage of body weight was significantly lower ($P < 0.05$) than the Reference Female values. As shown in Fig. 2, FFM hydration did not increase significantly with increasing %BF, either in extreme obesity ($r = 0.06$; $P < 0.81$) or after weight reduction ($r = 0.28$; $P < 0.22$). However, FFM hydration was more variable after weight reduction, with values ranging from 0.733 to 0.798 in extreme obesity and from 0.663 to 0.786 after weight reduction. In addition, the ratio of ECW to ICW increased with increasing %BF both in extreme obesity ($r = 0.54$; $P < 0.05$) and in the weight-reduced state ($r = 0.61$; $P < 0.05$) (data not shown).

Table 2 shows a comparison of %BF determined by the different methods in the extremely obese and weight-reduced states, with the 3C-H$_2^{18}$O model used as the reference method. In general, mean differences

<table>
<thead>
<tr>
<th>Detection of %BF and Body Composition</th>
<th>Extremely Obese</th>
<th>Weight Reduced</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m$^2$</td>
<td>47.8 ± 8.8</td>
<td>30.5 ± 7.0$^*$</td>
<td>-17.3 ± 5.6</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>124.3 ± 24.1</td>
<td>79.6 ± 19.1$^+$</td>
<td>-44.7 ± 14.6</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>64.4 ± 16.0</td>
<td>28.7 ± 14.7$^+$</td>
<td>-35.7 ± 12.2</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>60.0 ± 9.5</td>
<td>50.9 ± 7.7$^+$</td>
<td>-9.1 ± 4.1</td>
</tr>
<tr>
<td>Body fat, %body wt</td>
<td>51.4 ± 3.6</td>
<td>34.6 ± 9.3$^+$</td>
<td>-16.8 ± 8.5</td>
</tr>
<tr>
<td>Cell mass, kg</td>
<td>33.1 ± 6.0</td>
<td>28.0 ± 5.0$^+$</td>
<td>-5.1 ± 3.4</td>
</tr>
<tr>
<td>TBW, kg</td>
<td>45.5 ± 7.3</td>
<td>38.2 ± 5.8$^+$</td>
<td>-7.3 ± 3.2</td>
</tr>
<tr>
<td>ECW, kg</td>
<td>26.0 ± 5.6</td>
<td>21.8 ± 3.9$^+$</td>
<td>-4.2 ± 4.8</td>
</tr>
<tr>
<td>ICW, kg</td>
<td>18.8 ± 4.7</td>
<td>15.6 ± 3.3$^+$</td>
<td>-3.2 ± 3.6</td>
</tr>
<tr>
<td>ECW/ICW</td>
<td>1.48 ± 0.57</td>
<td>1.44 ± 0.30</td>
<td>-0.04 ± 0.64</td>
</tr>
<tr>
<td>TBW/FFM</td>
<td>0.756 ± 0.01</td>
<td>0.747 ± 0.03</td>
<td>-0.009 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD by use of the reference 3-compartmental (3C-H$_2^{18}$O method (%body fat, fat mass, and FFM). BMI, body mass index; FFM, fat-free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Δ, Extremely obese-weight reduced. There are 20 subjects for all values, except for ECW, ICW, ECW/ICW, and cell mass, for which $n=16$. *Measured by H$_2^{18}$O dilution. Significantly different from baseline: $^+$P < 0.001; $^*$P < 0.01 by Student’s paired t-test.

Fig. 1. Body composition in extremely obese and weight-reduced states compared with Reference Female values (21, 27, 42). All body compartments (except solids) are significantly different in the extremely obese state compared with the weight-reduced state ($P < 0.05$), and body compartments in extreme obesity (except solids) and weight-reduced state (except solids and extracellular water [ECW]), are significantly different from Reference Female values ($P < 0.05$). ICW, intracellular water.

Fig. 2. Association between percent body fat (%BF), determined with the reference 3-compartmental (3C-H$_2^{18}$O method, and hydration of fat-free mass in extremely obese and weight-reduced states.

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in %BF (reference method – alternative method) for each method did not differ significantly from the reference method in extreme obesity or in the weight-reduced state, with the exception of H$_2^{18}$O and H$_2$O dilutions (with assumed hydration values), air displacement plethysmography in the extremely obese state, and BIA at both time points (which varied with the particular prediction equation used; $P < 0.05$). However, for each method, interindividual variability was greater in the weight-reduced state than in extreme obesity, as indicated by the larger SEs and Bland-Altman limits of agreement. In both the extremely obese and weight-reduced states, the methods that gave mean values closest to the reference method were the 3C-H$_2$O and 3C-BIA models and $^{2}$H$_2$O dilution calculated with the mean measured FFM hydration coefficient. %BF determined by H$_2^{18}$O dilution and $^{2}$H$_2$O dilution using the standard hydration coefficient of 0.738 had narrower limits of agreement than 3C-BIA, but the mean %BF values were 1.0–1.3%BF higher than the reference method (significant for H$_2^{18}$O and $^{2}$H$_2$O dilution with the assumed hydration coefficient in extreme obesity, $P < 0.05$). %BF measured by air displacement plethysmography averaged 1.8%BF higher than the reference method (significant in extreme obesity, $P < 0.05$), and the limits of agreement were similar to or slightly larger than for dilutions. Hydrostatic weighing also slightly overestimated %BF, by 0.6%BF in extreme obesity, but limits of agreement were somewhat wider than those for air displacement plethysmography. There was a strong agreement between body volume measured by hydrostatic weighing and that by air displacement plethysmography (adjusted $R^2 = 0.9995$; $P < 0.0001$). For %BF determined by BIA (Table 2), each of the three equations tested resulted in %BF that differed significantly from the reference method. We found that the equations by Lukaski et al. (25) provided mean %BF values that were closest to the %BF measured by the reference method at both time points. Although mean %BF values by the Segal equation (33) were 1.5%BF lower than the reference method in extreme obesity, the mean difference was large (4.8%BF) and significantly different from the reference method ($P < 0.01$) in the weight-reduced state. Estimates of %BF using the (RJL) manufacturers’ equation were also significantly and largely different from the reference method both in extreme obesity and after weight reduction ($P < 0.05$). For all BIA equations tested, the limits of agreement were much wider than for the two-compartment (dilution, densitometry) and 3C models.

For measuring changes over time with weight reduction (also shown in Table 2), nearly all methods gave mean results that were similar to those of the reference technique. The 3C and two-compartment models all provided group mean estimates of the change in %BF that were nearly identical to the reference method. BIA estimates of mean %BF change were also good when the RJL or Lukaski et al. (25) equations were used ($-0.5$, $-0.4$%BF, respectively), but not when the Segal equation (33) was used. For estimating %BF change in individual subjects, the 3C-BIA model and $^{2}$H$_2$O dilution (with either the standard or the mean measured FFM hydration coefficients) resulted in wider limits of agreement than the 3C-$^{2}$H$_2$O model, H$_2^{18}$O dilution, or air displacement plethysmography. Therefore, using BIA with body density in a three-compartment model (3C-BIA) did not offer an advantage over air displac-
ment plethysmography alone for measuring changes over time in this population. In addition, the limits of agreement were much wider for all BIA equations than for the other techniques.

The relationship between %BF by the reference method and BMI was also examined at baseline and follow-up and is summarized in Fig. 3. BMI was significantly associated with %BF in both extremely obese and weight-reduced states, but the correlation was stronger in the weight-reduced state than in the extremely obese state. Partial correlations of BMI with fat mass (controlled for FFM) and FFM (controlled for fat mass) showed that, both in extreme obesity and after weight reduction, BMI was significantly associated with fat mass independent of FFM but not with FFM itself.

Selected subject characteristics related to body size and hydration, including FFM hydration and BMI, were examined as predictors of method differences in %BF (reference method minus alternative method) to help determine potential factors associated with method differences. In extreme obesity, %BF was increasingly underestimated with increasing FFM hydration by use of dilution techniques ($r = 0.911–0.943; P < 0.0001$) and by BIA with the RJL equation ($r = 0.49; P = 0.051$), but it was increasingly overestimated with increasing FFM hydration when air displacement plethysmography ($r = -0.937; P < 0.0001$) was used. In contrast, in the weight-reduced state, only the difference in %BF for $^2$H$_2$O dilution (with either the actual or assumed hydration coefficient; $r = 0.99; P < 0.0001$) was increasingly underestimated with increasing FFM hydration.

**DISCUSSION**

Extreme obesity poses challenges to the measurement of body composition because of altered body hydration, large individual variations in the hydration state, other potential alterations in the composition of FFM (26, 38, 39), and physical size limitations. However, our results show that %BF in the extremely obese and the weight-reduced states and the change in body composition with weight loss can be measured in groups and individuals by using a variety of techniques, including three-compartment models combining methods such as air displacement plethysmography and BIA that are commercially available, technically simple to perform, and minimally invasive. Using these methods and also the reference $3C$-$^3$H$_2$O technique, we found that the mean composition of massive weight loss in extremely obese patients undergoing GBP surgery was comparable to reported summaries of the composition of weight loss in overweight individuals losing modest amounts of weight (32), indicating relatively stable body composition changes under widely different weight loss circumstances. As characterized in this study, the compartmental shifts with respect to fat and FFM were large relative to the Reference Female with normal body weight, and despite massive weight loss, some of these variations persisted. The mean FFM hydration remained somewhat elevated after weight reduction induced by GBP compared with the reference value (0.747 vs. 0.738), although not significantly so. However, in contrast to

![Fig. 3. Relationship between body mass index (BMI) and %BF (A) in extremely obese and weight-reduced states; partial plots show relationship between BMI and fat-free mass (FFM, B) and between BMI and fat mass (C) in extreme obesity and weight-reduced states. Extreme obesity, ●, solid line; weight-reduced state, ○, dotted line.](http://ajpendo.physiology.org/)

*Fig. 3. Relationship between body mass index (BMI) and %BF (A) in extremely obese and weight-reduced states; partial plots show relationship between BMI and fat-free mass (FFM, B) and between BMI and fat mass (C) in extreme obesity and weight-reduced states. Extreme obesity, ●, solid line; weight-reduced state, ○, dotted line.*
what was speculated previously (40), FFM hydration did not increase systematically with increasing %BF within the range of 20–50%BF. It should be noted that this study may not be adequately powered to conclusively establish the nature of this relationship, and further research in a large number of subjects is needed to validate this finding. Although some of the observed individual variation in FFM hydration may be attributed to biological variations and measurement error, these findings imply that the hydration-related assumptions associated with the two-compartment models may not be the same for all individuals across varying %BF levels in this population (i.e., the assumed hydration of 0.738 may not be applicable through the range of %BF of the subjects in either the extremely obese or weight-reduced states, as demonstrated by the small and nonsignificant mean difference in %BF between the reference method and 2H2O dilution method when the mean measured FFM hydration value was used instead of the assumed hydration value). The findings also help to explain why nearly all of the methods used in this study did not increase systematically with increasing %BF (by ~1.8%BF) in the extremely obese state and in the weight-reduced state (with measured VTOT used at both time points) relative to the reference method; however, our subjects were able to perform these measurements with relative ease compared with hydrostatic weighing, and there were no size constraints imposed by the machine over the wide range of BMI (37–77 kg/m²) tested in our study. The ease and speed with which measurements can be obtained and other considerations (for example, if quantifying fat mass loss is the objective) make air displacement plethysmography an attractive option for measurement of body composition in the extremely obese and weight-reduced states that were in the formerly extremely obese. However, these findings need to be validated in larger studies. The relative accuracy of the dilution techniques for the group of subjects as a whole was improved by using the group mean measured hydration coefficient for FFM in the population; however, in the absence of accepted published values for different population groups, a measurement of actual hydration may be required for each population studied, and this would require technically demanding measurements of TBW by both 2H2O and H218O. Additionally, FFM hydration appeared to be associated with the mean difference in %BF of some of the tested methods compared with the reference method in both the extremely obese and the weight-reduced state, further indicating the need for population-specific values for FFM hydration. The relative accuracy of the other method tested, BIA, was highly dependent on the equations used to calculate %BF.

Concerning the other methods tested in this study, isotope dilution to measure TBW gave slightly higher values for %BF than the three-compartment models when a standard hydration factor was used because of high hydration in the extremely obese state (mean water % in FFM was 75.6% compared with the reference value of 73.8%). It was also noted that the hydration of FFM was higher (although not significantly so) than the reference value after weight loss, suggesting that the standard hydration factor may also be inappropriate for use in a weight-reduced population that was formerly extremely obese. However, these findings need to be validated in larger studies. The relative accuracy of the dilution techniques for the group of subjects as a whole was improved by using the group mean measured hydration coefficient for FFM in the population; however, in the absence of accepted published values for different population groups, a measurement of actual hydration may be required for each population studied, and this would require technically demanding measurements of TBW by both 2H2O and H218O. Additionally, FFM hydration appeared to be associated with the mean difference in %BF of some of the tested methods compared with the reference method in both the extremely obese and the weight-reduced state, further indicating the need for population-specific values for FFM hydration. The relative accuracy of the other method tested, BIA, was highly dependent on the equations used to calculate %BF.

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method more suitable for population groups than for individuals. An additional result from this study was that the relationship between BMI and %BF appeared to change between the extremely obese and weight-reduced states, perhaps indicating a plateau in the relationship between %BF and BMI at higher BMI values. Although both BMI and %BF represent a mathematical combination of fat mass and FFM, we found that BMI was associated with fat mass but not FFM at both time points, in contrast to our previous findings in subjects ranging from 12 to 47%BF (45). These findings indicate that further research is needed to examine the utility of BMI to accurately predict %BF in different subject groups. In the meantime, it should be noted that BIA (using any of the tested equations) was more strongly associated with %BF than BMI and therefore could be more widely used as a tool to measure body fatness in clinical practice and population-based research.

In conclusion, a simple three-compartment model combining measurements of body density by air displacement plethysmography and TBW by BIA can provide measurements of %BF that are comparable to the broad utility of these methods. Most methods gave values for body composition change that were comparable to values obtained by the reference method, including isotope dilution combined with a group-specific factor for the hydration of FFM and air displacement plethysmography alone. Further studies are needed to confirm the broad utility of these assessments of body composition methodology in extremely obese and other populations.

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