

Online supplemental material

Bioelectrical Impedance Analysis (BIA)

The Tanita BIA (Model TBF-310, Tanita Corporation of America, Inc, Arlington Heights, IL) consists of a platform with an integrated scale, a display unit, a maximum weight of 270 kg (600 lbs) and has been previously described [1]. While standing on extra-large stainless steel footprints (that contain the electrodes) positioned on the platform with the hind foot positioned on the posterior electrode and the forefoot on the anterior electrode, the participant, wearing a hospital gown, was instructed to stand erect with arms hanging alongside the body but not touching or making contact with the trunk. The within-subject coefficient of variation in our laboratory for two repeated BIA measurements on four adults ages 38 to 63, ranging in weight from 69 to 111 kg, was 0.007 for %fat and 0.003 for TBW.

The BIA current travels through the path of least resistance in body segments; in obese adults where the thighs make contact, the electrical current may not travel the full length of its intended path but rather be short-circuited where the thighs make contact. In our laboratory, we evaluated whether placing a towel between the thighs influenced BIA TBW and %fat estimates. In 16 adults (mean BMI of 33 kg/m²; range 24-47 kg/m²), TBW estimates with or without a towel were not different (p=0.08); but placement of a towel was associated with 0.4% lower fat (p=0.03). While placement of a towel between thighs was not associated with BIA TBW estimates, towel placement was associated with a small decrease (0.4%) in BIA %fat estimates; however, we do not believe this influenced study findings.

Total Body Water (TBW)

TBW was measured using the isotope deuterium oxide (D_2O or 2H_2O) as the reference method. The dose for this study was ~ 0.1 g/kg for all subjects and weighed in a dose cup, up to ± 0.002 gm in accuracy according to the subject body weight. With this dose the D_2O concentration at equilibrium is less than 0.03% of body water. With the subject fasting, a venous blood sample (~ 7 ml) was taken from an antecubital vein after which the subject ingested the dose orally. The dose vial was rinsed three times with 10 ml of regular water and all rinsings were consumed. Three hours following the initial dose administration, a second fasting blood specimen was taken (~ 7 ml). Both baseline (3 ml plasma) and 3 hour (3 ml plasma) specimens were centrifuged in a refrigerated centrifuge at 3000 rpm for 10 minutes, transferred, and stored at $-20^\circ C$ until analyses were performed. After lyophilization, the dose concentration was measured on a single frequency infrared-spectrophotometer (Nicolet 380, Thermo Electron Co.). TBW was calculated from our laboratory D_2O standard calibration based on six working standards concentration ranges of 0.0, 0.15, 0.30, 0.45, 0.60, 0.75 mg/ml from which a standard curve equation was derived: $Y = AX + B$, where $X =$ concentration, mg/ml and $Y =$ (absorbance reading).

The concentration of D_2O in each specimen calculated from the standard equation

$TBW (L) = D_2O (g) / (\text{Post-Pre}) \text{ concentration (g/l)}$. TBW precision in our laboratory is $\pm 1.0\%$ [2].

Air displacement plethysmography

Body density was measured using the BOD POD (Cosmed, Chicago, IL; software version 2.3 confirm) as previously described [3, 4]. Subjects were clothed in a Lycra-style swim cap and tight fitting underwear. Body weight was measured to the nearest 1 g on the Bod Pod electronic weight scale. Following standard calibration procedures, the subject's body volume was

measured with correction made for thoracic gas volume (VTG) estimated using the BOD POD breathing circuit system. The final VTG and the average of two body volume measurements within 0.2% were used to calculate body density [3]. The within-subject day-to-day CV for body density for four adults ages 22 to 33 and weighing from 52 to 95 kg was 0.0062 kg/L [5].

3-Compartment Calculations

The 3-C model was originally derived by Siri (34). Fat-free mass and fat mass were measured using a modified 3-compartment model that was developed in obese subjects in our laboratory that assumes body core temperature is 1-2°C under basal conditions, therefore values of 0.9007 and 0.9937 were utilized for the densities of fat and water at 36°C, respectively [6]: Fat (kg) = $2.122 \cdot (BW/D) - 0.779 \cdot TBW - 1.356 \cdot BW$, where BW is body weight in kg (measured by the Weight Tronix scale), D is body density derived from BodPod, and TBW is total body water in kg. Fat-free mass was calculated from body weight minus FM, and percentage fat was estimated.

Several weights (Bod Pod, Tanita 310, Weight Tronix Scale) were obtained on subjects at each observation. Weight Tronix weights were used in 3C calculations, due to independence from other 3C measures. Wilcoxon signed-rank tests evaluated whether the difference in medians of Weight Tronix weights and other scale weights were zero. Tanita 310 and Weight Tronix weights were not different at T0, T12, or change, (range median differences: 0.0-0.2 kg, $p > 0.05$). BodPod weights were lower than BIA weights at T1 and change (median difference: 0.05 kg, $p = 0.01$; 0.13 kg, $p = 0.05$, respectively), and Weight Tronix at T12 (median difference: 0.28 kg, $p = 0.03$), but not at other times, which may be attributable to the subject removing the hospital gown for the BodPod weight.

Detectable differences for two-sided one-sample t-tests given 80% power and a significance level of 0.05.

T0: With a sample size of 41 we can detect a difference of -4.2 L in TBW from the criterion value (median (IQR):45.8 (11.8) L), and a difference of -3.1% in %fat from the criterion value (median (IQR):51.4(9.6) %).

T12: With a sample size of 41 we can detect a difference of -3.9 L in TBW from the criterion value (median (IQR):40.8(15.2) L), and a difference of -4.8% in %fat from the criterion value (median (IQR):35.0(14.8)%).

Δ : With a sample size of 32 we can detect a difference of -2.5 L in change in TBW from the criterion value (median (IQR):6.4(6.4) L), and a difference of -4.6% in change in %fat from the criterion value (median (IQR):-14.8(13.4) %).