Title

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Comparison of multiple methods to measure maternal fat mass in late gestation[1,2]

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ABSTRACT

Background: Measurements of maternal fat mass (FM) are important for studies of maternal and fetal health. Common methods of estimating FM have not been previously compared in pregnancy with measurements using more complete body composition models.
Objectives: The goal of this pilot study was to compare multiple methods that estimate FM, including 2-, 3- and 4-compartment models in pregnant women at term, and to determine how these measures compare with FM by dual-energy X-ray absorptiometry (DXA) 2 wk postpartum.
Design: Forty-one healthy pregnant women with prepregnancy body mass index (in kg/m^2) 19 to 46 underwent skinfold thickness (SFT), bioelectrical impedance analysis (BIA), body density (D_b) via air displacement plethysmography (ADP), and deuterium dilution of total body water (TBW) with and without adjustments for gestational age using van Raaij (VRJ) equations at 37–38 wk of gestation and 2 wk postpartum to derive 8 estimates of maternal FM. Deming regression analysis and Bland-Altman plots were used to compare methods of FM assessment.
Results: Systematic differences in FM estimates were found. Methods for FM estimates from lowest to highest were 4-compartment, DXA, TBW$_{(VRJ)}$, 3-compartment, $D_{b(VRJ)}$, BIA, air displacement plethysmography body density, and SFT ranging from a mean $\pm$ SD of 29.5 $\pm$ 13.2 kg via 4-compartment to 39.1 $\pm$ 11.7 kg via SFT. Compared with postpartum DXA values, Deming regressions revealed no substantial departures from trend lines in maternal FM in late pregnancy for any of the methods. The 4-compartment method showed substantial negative (underestimating) constant bias, and the air displacement plethysmography body density and SFT methods showed positive (overestimating) constant bias. ADP via $D_{b(VRJ)}$ and 3-compartment methods had the highest precision; BIA had the lowest.
Conclusions: ADP that uses gestational age-specific equations may provide a reasonable and practical measurement of maternal FM across a spectrum of body weights in late pregnancy. SFT would be acceptable for use in larger studies. This trial was registered at clinicaltrials.gov as NCT02586714. *Am J Clin Nutr* 2016;103:1055–63.

Keywords: air displacement plethysmography, dual-energy X-ray absorptiometry, maternal body composition, maternal fat mass, maternal obesity, pregnancy, skinfold thickness, total body water

INTRODUCTION

Obesity rates in the United States have risen dramatically over the past several decades (1), including among reproductive aged women. In 2009, it was estimated that 1 in 4 pregnant women were obese before pregnancy with a BMI (in kg/m^2) $\geq$30 (2). Obese women are more likely to have babies who experience excessive fetal growth (3) and to have pregnancies complicated by preeclampsia, gestational diabetes, and the need for cesarean delivery (4, 5). Understanding the physical and metabolic abnormalities experienced by obese women and their babies, especially as they relate to increased maternal body fatness, is a high priority in nutrition research.

The BMI classification of obesity has been useful for epidemiologic studies, for public health evaluations, and in clinical practice because it is simple to calculate by using readily available information (6–8). However, using BMI as a proxy for fat mass (FM)[6] does not account for potential confounding effects of high or low lean (or nonfat) mass on health outcomes. In pregnant women, BMI has been shown to significantly correlate with percent body fat, although this relation weakens in late gestation (9, 10). Thus, BMI is a poor indicator of variations in maternal fat and lean mass later in pregnancy that may regulate fetal and neonatal growth.

Body composition assessment during pregnancy is challenging because of the addition of the uterine contents as well as marked increases in FM and total body water (TBW), which may systematically alter results of common techniques for body composition measurement such as skinfold thickness (SFT) and bioelectrical impedance analysis (BIA) (8). Various studies have used 3-compartment and 4-compartment modeling to quantify whole body fat, water, and fat-free mass (FFM) (consisting primarily of proteins and minerals) and overcome challenges associated with these measurements in pregnancy (11–16). In those studies, body density (D_b) was typically determined with underwater weighing and TBW by isotope dilution (11–13, 17–19). The expense, time, and specialty equipment required to perform these techniques have prohibited their use in large-scale clinical studies. A simpler technique to estimate D_b, air displacement plethysmography (ADP), has become more widely available for research use (20). However, this technique has not been compared with other methods of body composition measurement in pregnancy.

We sought to answer a question that frequently arises among scientists who study body composition changes during pregnancy: how do the aforementioned methods of measuring maternal FM compare, and which is the most appropriate for specific clinical and research applications? We also set out to determine the validity of BMI categories of lean, overweight, and obese, which are commonly used clinically and in research as surrogates for FM during pregnancy.

METHODS

Study design

This was a planned pilot feasibility study in support of a larger project to prospectively study the effects of body composition in pregnancy on placental function. Forty-one healthy pregnant women in their third trimester with a singleton gestation of varying prepregnancy BMIs ($n = 11$ normal weight, BMI 18.5–24.9; $n = 15$ overweight, BMI 25–29.9; and $n = 15$ obese, BMI ≥30) were recruited from Oregon Health & Science University obstetric clinics from July 2012 to August 2013. Exclusion criteria included active maternal infection, documented fetal congenital anomalies, substance abuse, chronic illness requiring regular medication use, maternal diabetes, chorioamnionitis, significant medical conditions (active cancers or cardiac, renal, hepatic, or pulmonary diseases), or an abnormal 2-h 75-g glucose tolerance test. The Oregon Health & Science University institutional review board approved the study protocol, and each subject provided signed informed consent before enrollment.

Subjects presented for their study visits in the morning after an overnight fast. During the first visit, at 37–38 wk of gestation, they underwent 4 measures—1) ADP, 2) TBW volume by deuterium dilution (^2H_2O), 3) SFT, and 4) BIA—to generate 8 estimates of fat mass (see below and **Table 1**). To determine maternal FM without the presence of the fetus or placenta and to avoid radiation exposure to the fetus, subjects returned for a second study visit 2 wk postpartum for repeat measurements by the above methods plus a dual-energy X-ray absorptiometry scan (DXA) scan.

Impact of pregnancy on techniques for measuring body composition

Many of the specific assumptions that underlie methods of body composition assessment, particularly hydration and density of FFM, are affected by pregnancy, as reviewed by Widen and Gallagher (25) and Lederman (26). The classic body composition model predicts FFM from TBW by the equation FFM = TBW/0.732, on the assumption that water accounts for 73.2% of FFM. However, FFM hydration increases throughout pregnancy in an amount that varies between individual women (26). Thus, changes in FFM hydration will affect FM calculated from TBW by ^2H_2O dilution and as assessed by ADP. Using the estimate of 73% FFM hydration in ADP calculations will result in an underestimation of TBW and hence an overestimation of FM.

FFM density is essential for the determination of overall D_b used to calculate FM by SFT, 3-compartment, 4-compartment, and ADP methods. Although assumed to be 1.10 g/cm^3 in nonpregnant women, it is thought to decrease during pregnancy (25). Use of the standard FFM density would therefore result in an underestimation of FM. In an effort to correct for these changes in FFM hydration and density during pregnancy, van Raaij (VRJ) et al. (16) generated gestational age-specific equations for D_b and TBW, which we have used in our calculations [$D_{b(VRJ)}$ and $TBW_{(VRJ)}$].

SFT measurements have been shown to significantly change shortly after delivery despite suspected minimal changes in edema or FM (25). This suggests a potential alteration in the distribution of body fat and water during pregnancy rather than a quantitative change (26).

TABLE 1
Equations used for maternal fat mass calculations[1]

	Anatomic region	Equation	Reference
Anthropometric equations (SFT)	D_b	1.1581 − 0.0720 (log of the sum of TSF + BSF + SSSF + SISF)	(21)
	Fat, %	$[(4.95/D_b) − 4.5] \times 100$	(22)
	Fat mass, kg	Fat (%) × wt	
BIA	Fat mass, kg	Fat (%) × wt	
$D_{b(VRJ)}$ for 37 wk of gestation	Fat mass, kg	$(Wt/100) \times [519/D_b) − 476]$	(16)
$TBW_{(VRJ)}$ for 37 wk of gestation	Fat mass, kg	Wt − (TBW/0.747)	(16)
3-C model	Fat mass, kg	$Wt (2.118/D_b) − 0.78 (TBW/wt) − 1.354$	(23)
4-C model	Fat mass, kg	2.747 Bvol − 0.71 TBW + 1.46 BMC − 2.05 wt	(24)

[1]BIA, bioelectrical impedance analysis; BMC, bone mineral content (kg); BSF, biceps skinfold (mm); Bvol, body volume (m^3); D_b, body density (kg/m^3); $D_{b(VRJ)}$, van Raaij body density; SFT, skinfold thickness (mm); SISF, suprailiac skinfold (mm); SSSF, subscapular skinfold (mm); TBW, total body water (kg); $TBW_{(VRJ)}$, van Raaij total body water; TSF, triceps skinfold (mm); wt, weight (kg); 3-C, 3-compartment; 4-C, 4-compartment.

Because of low-level radiation use, DXA is contraindicated during pregnancy and was only used postpartum in the present study. DXA determines soft tissue composition, including FM, by analyzing the proportion of lean and fat in areas lacking bone as determined by differential photon attenuation coefficients in each pixel (27, 28). As such, DXA is limited in its ability to assess lean and fat in soft tissue areas that overlie bone and may also be affected by tissue depth, especially in morbidly obese individuals (29). Although DXA is less reliant than other methods on assumptions regarding constant values for density and hydration of FFM (28), concern remains that overhydration may result in small but systematic errors in FM estimation with DXA as well (30).

Body composition measurements

ADP

Whole-body composition was measured by ADP by using a BodPod (Life Measurement Inc.). The subject changed into a bathing suit or spandex clothing, including a swimming cap, and body weight and height were measured. The subject sat inside the BodPod while the air displaced by the body was measured. Results included percent body fat, FM, lean mass, body volume, and D_b [$D_{b(BodPod)}$]. Residual lung volume was directly measured in 35 of 41 subjects. Six subjects were unable to successfully perform direct lung volume measurement, and therefore the standardized volume was substituted, which has been shown in pregnancy to have a very slight overestimation on body fat assessment (31).

TBW by ^2H_2O

After an overnight fast, a baseline saliva sample (at least 1 mL) was obtained with Sarstedt salivettes. After collection, the salivette was spun at $1500 \times g$ at room temperature for 10 min. The liquid was transferred to a 1.2-mL internally threaded cryogenic vial tightly capped and sealed to avoid dead space and frozen at $-80°C$. The 99.8% enriched ^2H_2O (Cambridge Isotope Laboratories) was diluted with deionized water and divided into aliquots of $\sim$5-g individual doses, which the subjects were then given to drink. The bottles were weighed to the nearest 0.1 mg before and after each dose administration to determine the exact amount of ^2H_2O ingested. Subjects were instructed not to eat or drink anything after the dose. It has been suggested that full isotopic equilibration with the TBW pool may require up to 4 h or more in late gestation (32). However, in practical terms, a prolonged overnight fast in late gestation is quite difficult, and thus the postdose sample was collected 3 h after ^2H_2O ingestion as per Lederman et al. (17). If isotopic equilibration was not reached by 3 h, our results would overestimate TBW and underestimate FM. Saliva samples were frozen for later batch analysis. The samples were processed in duplicate for measurement of deuterium enrichment by wavelength-scanned cavity ringdown spectroscopy (Isotopic Water Analyzer, L2120-I; Picarro), and TBW was calculated as previously described (33).

SFT

Maternal anthropometric measurements were performed by the same trained examiner during both visits by using a method validated in nonpregnant populations (34). Midarm circumference was measured with the right arm hanging relaxed at the patient's side, at the midpoint between the acromion and olecranon process. Measurements were performed with a calibrated Lange caliper that applied 10 g/mm^2 pressure at 4 sites: *1*) triceps skinfold—a vertical fold at the same level as the midarm circumference measurement, on the posterior aspect of the arm, with the right arm held vertically; *2*) biceps skinfold—a vertical fold at the same level as the midarm circumference, over the short head of the biceps, with the arm placed supinated on the patient's thigh; *3*) subscapular skinfold—just below the tip of the inferior angle of the scapula, at an angle of 45° to the vertical; and *4*) suprailiac skinfold—immediately above the iliac crest, in the midaxillary line.

BIA

Whole-body composition was measured with the Body Composition Analyzer TBF-410 (Tanita Corporation of America Inc.). The electrical impedance was measured when a low-level electric current (500 μA) was applied between the feet, placed on pressure contact stainless steel food pads. Weight and bioelectrical impedance were measured and, with input of age and height, a report generated for percent body fat. To account for the weight of clothing worn, 0.8 kg was subtracted from all weights.

DXA

Whole-body composition was assessed by using DXA scans at 2 wk postpartum only. Bone mineral content (BMC) and FM were attained with a QDR Discovery A Densitometer (Hologic Inc.).

Calculations of FM

Most of our FM estimates were based on 2-compartment models. Equations for the calculation of FM by SFT and BIA are shown in Table 1. FM by DXA was generated by the equipment software. FM from ADP was measured in 2 ways. FM $D_{b(BodPod)}$ was generated by the equipment software. FM $D_{b(VRJ)}$ was calculated from $D_{b(BodPod)}$ by using an equation to account for alterations in FFM hydration and density at 37 wk of gestational age as described by van Raaij et al. (16) (Table 1). Similarly, calculation of FM from TBW measured with ^2H_2O dilution was done by using equations modified for pregnancy (16) (Table 1).

The 3-compartment body composition model, as described by Forbes (23) and explained in Hopkinson et al. (12), was used to calculate FM with the D_b determined from ADP and TBW from ^2H_2O (Table 1). A 4-compartment model used BMC obtained by DXA in addition to D_b from ADP and TBW from ^2H_2O to calculate FM (24) (Table 1).

Statistical analysis

Statistical analysis was performed by using the R package Method Comparison Regression, version 1.2.1 (https://cran.r-project.org/web/packages/mcr/index.html). The postpartum DXA FM was elected as the comparison method to show the variation between methods because it is an independent assessment technique that, unlike the 4-compartment model, does not depend on multiple other methods (TBW, D_b via ADP, and BMC via DXA), each of which is subject to its own limitations in pregnancy that may result in propagation of error and the potential bias of including the same numeric result in a comparison

calculation [e.g., 4-compartment method includes the same D_b value as $D_{b(BodPod)}$, $D_{b(VRJ)}$, and the 3-compartment method] (28). Estimates of mean FM were compared by using ANOVA. The bias and 95% ± SD limits of agreement for comparison of the estimates of body FM were established as per Fuller et al. (24). The line of calibration for FM as estimated by $D_{b(VRJ)}$, $D_{b(BodPod)}$, $TBW_{(VRJ)}$, 3-compartment model [$D_{b(BodPod)}$ and ^2H_2O], 4-compartment model [$D_{b(BodPod)}$, ^2H_2O, and BMC via DXA), SFT, BIA, and DXA was determined by Deming regression analysis (35) rather than simple linear regression to account for expected errors within each set of measurements. The precision of the Deming regression model was assessed by bootstrap resampling to construct a 95% CI around the coefficient for the slope. If the slope is far from 1, this suggests the alternative measure differs from the comparison according to a systematic trend (i.e., more than just a constant). Bland-Altman plots (36) were subsequently created to analyze the agreement between FM estimates obtained with each method compared with each of the 7 other methods.

RESULTS

There were no differences in gestational age at delivery, infant birth weight, or cesarean delivery between the normal-weight, overweight, and obese groups of women (**Table 2**). Obese women were more likely to be multiparous (66%) compared with overweight (33.3%) and normal-weight (9.1%) women.

All 41 subjects presented for the first study visit at 37–38 wk of gestation and have complete data for SFT, BIA, $D_{b(BodPod)}$, and $D_{b(VRJ)}$. The second visit took place a mean ± SD of 13 ± 4.4 d postpartum. The duration between the visits was a mean ± SD of 4.1 ± 1.4 wk. One subject delivered at an outside hospital and did not present for the second study visit. Two other subjects did

not present for the second study visit and were lost to follow-up; therefore, DXA results are available for 38 women. During the first study visit, 7 women appeared to have drunk fluids between ^2H_2O administration and saliva collection, resulting in very dilute saliva enrichments, which led to calculated TBW greater than total body weight. Thus, ^2H_2O data were excluded for these 7 women and $TBW_{(VRJ)}$ and the 3-compartment method were not calculated, leaving data from 34 subjects available for assessment by these methods. Four-compartment modeling relies on both TBW via ^2H_2O and BMC via DXA, and hence 4-compartment data were determined for 33 women. There are complete data for both visits for 33 women.

Body weight ranged from 64.2–134.5 kg at 37 wk of gestation to 55.2–128.1 kg at 2 wk postpartum. The mean ± SD change in body weight between visits was 7.6 ± 2.3 kg. At the 37-wk study visit, there was more than a 10-kg difference between estimates of FM, with the highest estimate seen with SFT (mean ± SD: 39.1 ± 11.7 kg) and the lowest estimate with the 4-compartment method (mean ± SD: 29.5 ± 13.2 kg) (**Table 3**). The mean ± SD TBW by ^2H_2O was 42.0 ± 6.4 kg, and the mean ± SD hydration constant for FFM was 0.767 ± 0.08 with FM as determined by $D_{b(VRJ)}$. At the 2-wk postpartum visit, the estimated mean ± SD body fat ranged from 28.9 ± 13.6 kg via the 4-compartment method to 35.4 ± 11.7 kg via SFT. The mean ± SD TBW by ^2H_2O was 35.7 ± 5.4 kg, and the mean ± SD hydration constant for FFM was 0.739 ± 0.05 with FM as determined by $D_{b(BodPod)}$. At 2 wk postpartum, there was a reduction in FM with all measurements. The greatest reduction (3.7 kg) was seen with the SFT method, whereas the smallest change was seen with the 3-compartment and 4-compartment methods (0.8 kg and 0.6 kg, respectively). If one compares what in theory are the best ADP-based methods before delivery and postpartum, $D_{b(VRJ)}$ at 37 wk and $D_{b(BodPod)}$ 2 wk postpartum,

TABLE 2
Maternal demographic characteristics

	Normal weight (*n* = 11)	Overweight (*n* = 15)	Obese (*n* = 15)
Age, y	31.5 ± 4.9[1]	31.6 ± 6.5	27.2 ± 10.0
Parity, *n* (%)			
0	10 (90.9)	10 (66.7)	5 (33.3)
1	1 (9.1)	5 (33.3)	8 (53.3)
2	0	0	2 (13.3)
Race, *n* (%)			
American Indian/Alaskan native	0	0	1 (6.7)
Asian American	1 (9.1)	1 (6.7)	1 (6.7)
Black/African American	0	1 (6.7)	1 (6.7)
Pacific Islander	0	1 (6.7)	0
White/Caucasian	10 (90.9)	11 (73.3)	10 (66.7)
Unknown	0	1 (6.7)	2 (13.3)
Ethnicity, *n* (%)			
Hispanic	0	1 (6.7)	4 (26.7)
Non-Hispanic	11 (100)	14 (93.3)	10 (66.7)
Unknown	0	0	1 (6.7)
Height, cm	167.4 ± 11.3	164.4 ± 6.8	165.7 ± 6.9
Prepregnancy BMI, kg/m²	22.2 ± 2.0	27.4 ± 1.5	37.5 ± 5.1
BMI at study enrollment, kg/m²	26.3 ± 2.8	32.4 ± 2.6	37.3 ± 11.2
Gestational age at visit 1, wk	37.9 ± 0.7	37.7 ± 0.5	37.6 ± 0.6
Gestational age at delivery, wk	40.2 ± 1.1	40.0 ± 1.1	40.1 ± 1.1
Infant birth weight, kg	3.5 ± 0.5	3.5 ± 0.3	3.6 ± 0.5
Cesarean deliveries, *n* (%)	3 (27.3)	4 (26.7)	4 (28.6)

[1] Mean ± SD (all such values).

TABLE 3
Fat mass estimate by method for all subjects with complete data ($n = 33$)[1]

Model	37 wk of gestation fat mass, kg	2 wk postpartum fat mass, kg	Change in fat mass, kg
SFT[2]	39.1 ± 11.7	35.4 ± 11.7	−3.7 ± 16.6
BIA[2]	36.2 ± 14.5	32.8 ± 14.4	−3.4 ± 20.4
Postpartum DXA[3,5]	—	33.6 ± 13.1	—
$D_{b(BodPod)}$[2]	37.9 ± 13.2	35.0 ± 14.0	−2.9 ± 19.2
$D_{b(VRJ)}$[3,4]	35.9 ± 13.0	—	—
$TBW_{(VRJ)}$[3,4]	34.9 ± 13.5	—	—
3-C[2]	35.5 ± 13.3	34.7 ± 13.3	−0.8 ± 18.8
4-C[2,5]	29.5 ± 13.2	28.9 ± 13.6	−0.6 ± 19.1

[1]All values are means ± SDs. BIA, bioelectrical impedance analysis; $D_{b(BodPod)}$, air displacement plethysmography body density; $D_{b(VRJ)}$, van Raaij body density; DXA, dual-energy X-ray absorptiometry; SFT, skinfold thickness; $TBW_{(VRJ)}$, van Raaij total body water; 3-C, 3-compartment; 4-C, 4-compartment.
[2]Maternal-fetal fat mass.
[3]Maternal fat mass.
[4]Calculated during pregnancy only.
[5]DXA performed postpartum.

the difference by those methods was similar to the 3-compartment and 4-compartment models at 0.9 kg.

Complete values for all Deming regression analyses for comparison of FM between methods at 37 wk of gestation are shown in **Table 4**. Comparisons of FM measurements by Deming regressions revealed no major departures from DXA trend lines in maternal FM at 37 wk of gestation for any of the methods (**Figure 1**). As FM increased, BIA showed increased bias (overestimation) and SFT showed decreased bias (overestimation at lower fat mass) away from DXA compared with the other methods. Substantial constant bias was seen in the 4-compartment, $D_{b(BodPod)}$, and SFT methods, with the 4-compartment method showing negative (underestimating) and

$D_{b(BodPod)}$ and SFT showing positive (overestimating) constant bias. The tightest precision was obtained via use of ADP determination of D_b by both $D_{b(VRJ)}$ and $D_{b(BodPod)}$ with Pearson's $r = 0.983$ and 0.984, respectively, followed by the 4-compartment method with Pearson's $r = 0.982$.

Complete values for all Bland-Altman analyses for comparison of FM between methods at 37 wk of gestation are shown in **Table 5**. After accounting for agreement between DXA and each method via Bland-Altman analysis (**Figure 2**), the most precise measurement with the tightest 95% CI for the difference was $D_{b(VRJ)}$ with a mean ± SD FM difference of −2.31 ± 2.37 kg, followed by the 3-compartment method with a mean ± SD FM difference of −1.35 ± 2.83 kg. Careful

TABLE 4
Pairwise comparison of Deming regression estimates between methods at 37 wk of gestation for maternal fat mass (kg)[1]

Method	BIA	PP DXA	$D_{b(BodPod)}$	$D_{b(VRJ)}$	$TBW_{(VRJ)}$	3-C	4-C
SFT ($n = 41$)							
Intercept	9.2 (5.6, 12.5)	9.2 (5.8, 12.4)	6.2 (3.6, 8.5)	7.6 (5.3, 10.1)	10.6 (4.3, 16.1)	7.3 (1.9, 11.2)	13.2 (9.4, 16.3)
Slope	0.8 (0.8, 0.9)	0.9 (0.8, 1.0)	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.8 (0.7, 1.0)	0.9 (0.8, 1.1)	0.9 (0.8, 1.0)
BIA ($n = 41$)							
Intercept		−0.03 (−6.2, 3.9)	−3.6 (−9.2, 0.8)	−1.9 (−7.4, 2.7)	−1.0 (−12.4, 5.4)	−4.1 (−14, 1.8)	3.8 (−3.1, 8.4)
Slope		1.1 (1.0, 1.3)	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	1.1 (0.9, 1.5)	1.2 (1.0, 1.4)	1.1 (1.0, 1.3)
PP DXA ($n = 38$)							
Intercept			−3.6 (−6.7, −0.8)	−2.0 (−5.0, 0.8)	−0.32 (−5.2, 4.5)	−2.0 (−5.2, 1.2)	4.3 (2.0, 6.5)
Slope			1.0 (0.1, 1.1)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
$D_{b(BodPod)}$ ($n = 41$)							
Intercept				4.9 (−1.0, 11.0)	4.9 (−1.0, 11.0)	1.7 (−3.5, 5.0)	8.5 (5.0, 11.2)
Slope				1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (1.0, 1.2)	1.0 (0.9, 1.1)
$D_{b(VRJ)}$ ($n = 41$)							
Intercept					3.5 (−3.7, 9.3)	0.2 (−5.3, 3.7)	6.9 (3.8, 9.6)
Slope					1.0 (0.8, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.1)
$TBW_{(VRJ)}$ ($n = 34$)							
Intercept						−3.2 (−7.7, −1.6)	4.7 (2.0, 7.0)
Slope						1.1 (1.0, 1.2)	1.0 (1.0, 1.1)
3-C ($n = 34$)							
Intercept							6.2 (5.0, 7.0)
Slope							1.0 (1.0, 1.0)

[1]All values are coefficients; 95% CIs in parentheses. BIA, bioelectrical impedance analysis; $D_{b(BodPod)}$, air displacement plethysmography body density; $D_{b(VRJ)}$, van Raaij body density; DXA, dual-energy X-ray absorptiometry; PP, postpartum; SFT, skinfold thickness; $TBW_{(VRJ)}$, van Raaij total body water; 3-C, 3-compartment; 4-C, 4-compartment.
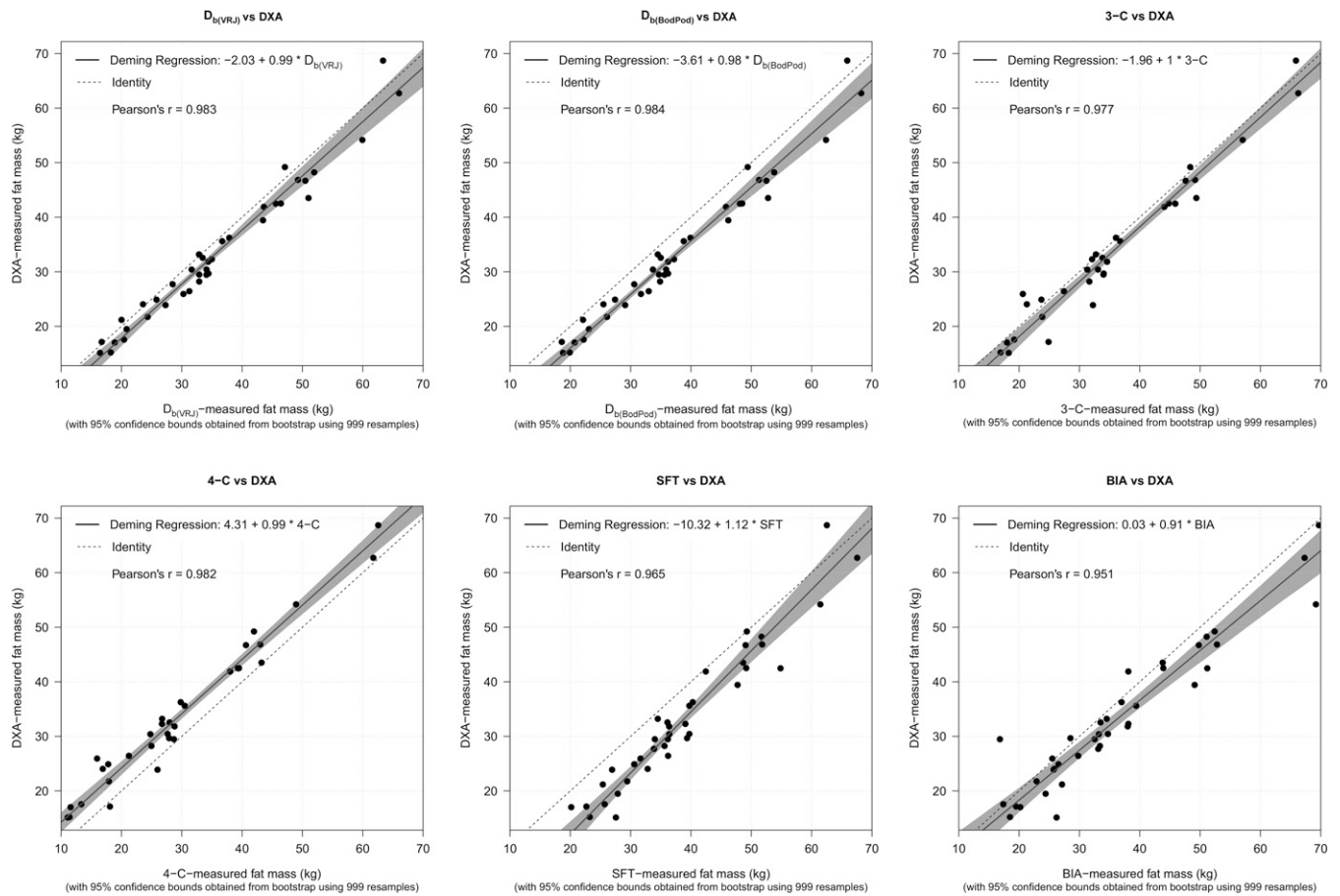
FIGURE 1 Deming regressions showing relation between fat mass (kg) estimated with DXA scan ($n = 38$) compared with $D_{b(VRJ)}$ ($n = 41$), $D_{b(BodPod)}$ ($n = 41$), 3-C method ($n = 34$), 4-C method ($n = 33$), SFT ($n = 41$), and BIA ($n = 41$). BIA, bioelectrical impedance analysis; $D_{b(BodPod)}$, air displacement plethysmography body density; $D_{b(VRJ)}$, van Raaij body density; DXA, dual-energy X-ray absorptiometry; SFT, skinfold thickness; 3-C, 3-compartment; 4-C, 4-compartment.

review of the outliers identified on the Bland-Altman analyses shows no common trends, including BMI category, between individuals that could explain the variations in measurement with DXA values. The complete Deming regression and Bland-Altman analyses for comparison of FM between methods at the 2-wk postpartum visit are available in **Supplemental Tables 1** and **2**.

Based on the $D_{b(VRJ)}$ estimation, mean maternal FM and percent body fat (**Figure 3**) increased with increasing BMI category. Although normal-weight women consistently had

lower FM and percent body fat than did overweight and obese women, there was substantial overlap in values between overweight and obese women, including 3 obese women with FM <75th percentile for overweight women.

DISCUSSION

The present report was undertaken to compare multiple methods of maternal FM assessment in late pregnancy in women with a wide range of BMIs. Assessment of maternal FM in pregnancy is

TABLE 5

Bland-Altman mean difference in maternal fat mass (kg) between methods at 37 wk of gestation[1]

	BIA	PP DXA	$D_{b(BodPod)}$	$D_{b(VRJ)}$	$TBW_{(VRJ)}$	3-C	4-C
SFT ($n = 41$)	2.40 ± 4.45	-5.64 ± 3.48	-1.40 ± 3.11	-3.40 ± 3.12	-5.14 ± 8.40	-4.27 ± 5.54	-9.70 ± 4.15
BIA ($n = 41$)		-3.09 ± 4.37	1.0 ± 4.62	-1.0 ± 4.78	-2.49 ± 9.52	-1.65 ± 6.98	-6.83 ± 5.49
PP DXA ($n = 38$)			-4.29 ± 2.31	-2.31 ± 2.37	-1.35 ± 4.38	-1.79 ± 2.83	4.16 ± 2.55
$D_{b(BodPod)}$ ($n = 41$)				-2.0 ± 0.30	4.02 ± 7.98	3.13 ± 4.76	8.44 ± 2.89
$D_{b(VRJ)}$ ($n = 41$)					2.04 ± 7.97	1.14 ± 4.74	6.48 ± 2.95
$TBW_{(VRJ)}$ ($n = 34$)						-0.93 ± 3.35	5.44 ± 2.31
3-C ($n = 34$)							5.94 ± 0.89

[1]All values are means $\pm$ SDs. BIA, bioelectrical impedance analysis; $D_{b(BodPod)}$, air displacement plethysmography body density; $D_{b(VRJ)}$, van Raaij body density; DXA, dual-energy X-ray absorptiometry; PP, postpartum; SFT, skinfold thickness; $TBW_{(VRJ)}$, van Raaij total body water; 3-C, 3-compartment; 4-C, 4-compartment.
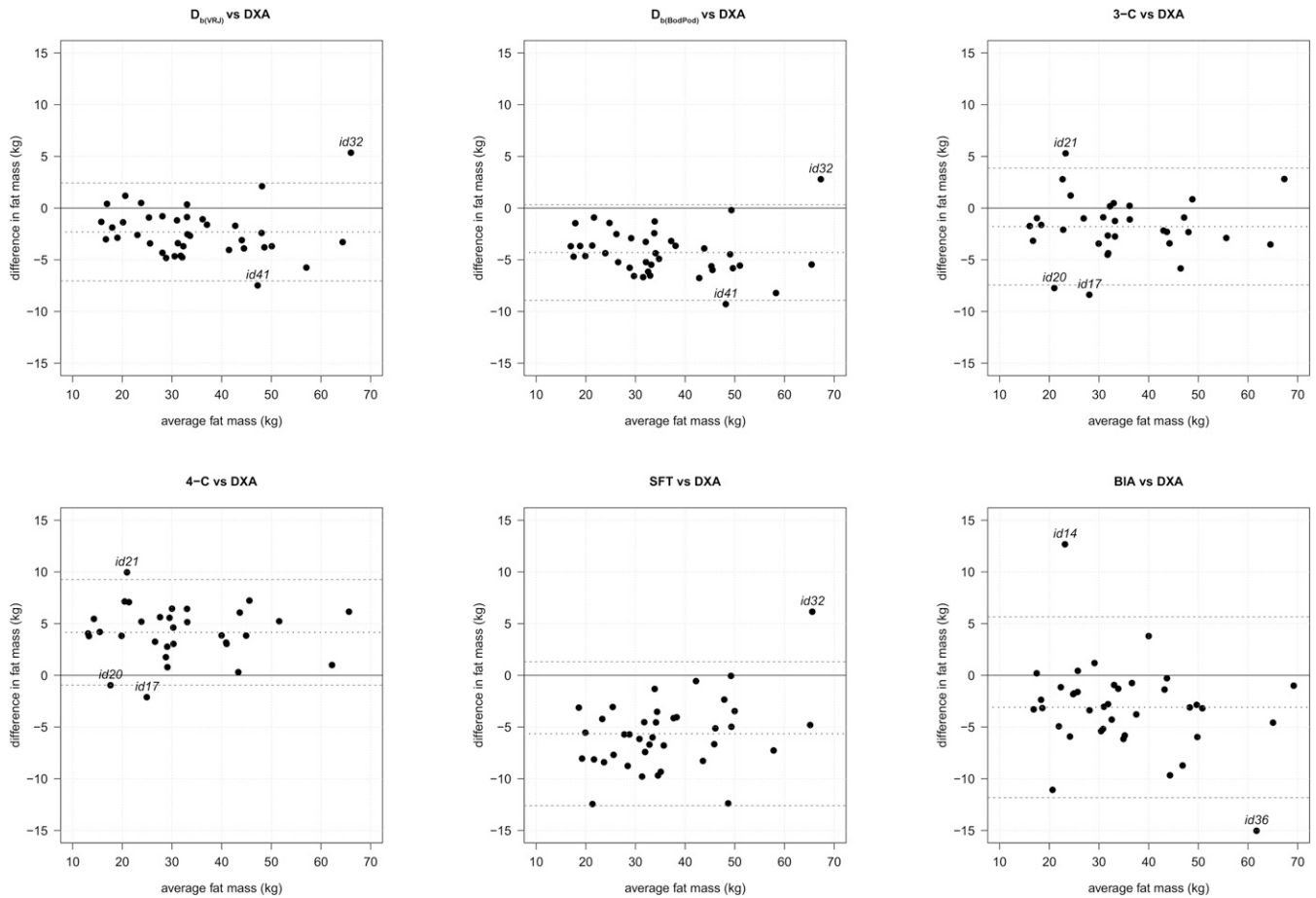
FIGURE 2 Bland-Altman plots of fat mass estimated from DXA scan ($n = 38$) compared with $D_{b(VRJ)}$ ($n = 41$), $D_{b(BodPod)}$ ($n = 41$), 3-C method ($n = 34$), 4-C method ($n = 33$), SFT ($n = 41$), and BIA ($n = 41$). Middle dotted line indicates mean difference. Dashed lines indicate ± 2 SD. Outliers identified by study identification number. BIA, bioelectrical impedance analysis; $D_{b(BodPod)}$, air displacement plethysmography body density; $D_{b(VRJ)}$, van Raaij body density; DXA, dual-energy X-ray absorptiometry; SFT, skinfold thickness; 3-C, 3-compartment; 4-C, 4-compartment.

particularly challenging due to the inability to separate the uterine contents, including fetus, placenta, and amniotic fluid, from the maternal tissues. Some authors have attempted to correct for this by using gestational age–specific equations (16), but these methods, including SFT, BIA, $D_{b(BodPod)}$, and 3-compartment and 4-compartment methods, still cannot disentangle the maternal-fetal unit.

Choosing a reference or "gold-standard" method in this population is challenging. Imaging methods, such MRI and

DXA scans, are currently considered the most accurate to quantify fat and lean mass. However, MRIs are labor intensive to analyze and cost-prohibitive for use in larger scale studies. DXA scans are less expensive and simpler to analyze but are contraindicated during pregnancy due to radiation exposure. Some studies use a 4-compartment model as the reference comparison (12). However, this 4-compartment model relies on the same numerical values that are individually subject to their own limitations in pregnancy (TBW, D_b via ADP) as previously
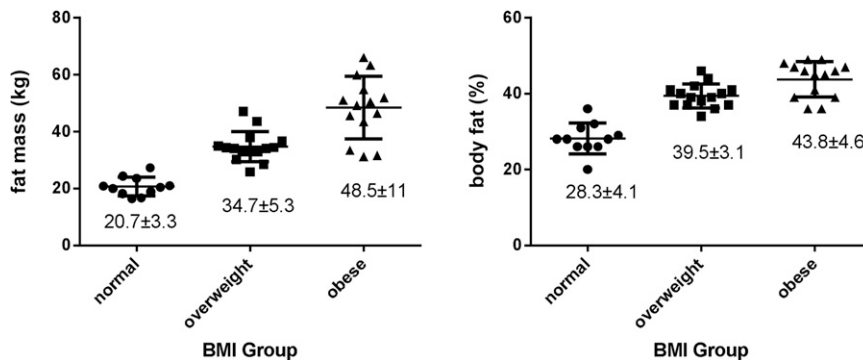
FIGURE 3 Maternal fat mass (left graph) and percent fat (right graph) by BMI group ($n = 41$). Values are given as means $\pm$ SDs.

described and BMC via DXA, which can only be measured in the postpartum state. Thus, the 4-compartment model will result in the propagation of any error generated by those other methods. Ultimately, we reasoned that a DXA scan 2 wk after delivery would reflect FM in the late third trimester, without the confounding factors contributed by the fetus, amniotic fluid, placenta, and increased TBW. However, DXA may be affected by changes in tissue depth and soft tissue hydration after delivery (29, 30); thus, although DXA is validated for FM assessment in other populations (27), including at 12 wk postpartum (28), it has not been validated at the 2-wk post-partum time point used in this study.

Previous studies have typically evaluated body FM measurements in pregnancy by using one or 2 methods, most commonly SFT and underwater weighing (13, 17–19, 37–40); some have used their data to generate new anthropometric equations specifically for pregnant women (11, 16, 18). SFT is likely to vary throughout pregnancy, especially later in gestation due to increased water retention (41, 42), and may not accurately reflect unique changes in maternal fat deposition and distribution that occur as gestation progresses (26). Our study confirmed prior investigations showing a bias toward higher FM values by SFT compared with other methods (18), suggesting that the increase in TBW in late gestation may contribute to greater SFT values, which, in turn, decrease D_b calculations and overestimate total FM, especially in normal-weight women. Despite these potential limitations, we found that the Deming regression for FM between DXA and 37-wk SFT was reasonably close (slope of 1.1; 95% CI: 0.99, 1.3), suggesting that SFT may be acceptable for use in field studies including large numbers of pregnant women. On the other hand, the wide range of values of percent body fat obtained with BIA, despite its ease of use, raises significant concerns regarding the usefulness of this method in late pregnancy.

We found that use of ADP for determination of D_b was well tolerated in the third trimester of pregnancy and, with the use of the VRJ equation [$D_{b(VRJ)}$] based on gestational age (16), provided the most precise estimate of maternal FM compared with postpartum DXA. Using the pregnancy-specific calculation [$D_{b(VRJ)}$] resulted in a tighter correlation with DXA values for FM and also a narrower 95% CI for the difference in the Bland-Altman plots compared with the standard $D_{b(BodPod)}$ calculation. Our 3-compartment data showing a mean ± SD difference in FM of −1.79 ± 2.83 kg from DXA supports the use of this model for late-gestation maternal FM evaluation as well. However, we found obtaining reliable TBW measures for use in the 3-compartment model to be challenging in this population (see below), and given the slightly tighter precision of the $D_{b(VRJ)}$ method, this latter technique was judged to be a superior tool for FM measurement.

Our data can be used to highlight the variations in body fat (kg) and percent body fat in pregnant women within current BMI categories for overweight and obese. For example, 2 women with class 1 obesity (BMI 31 compared with 33) had a 15% difference in percent body fat (24.7% compared with 39.2%), which corresponds to a 22-kg difference in FM (21.2 kg compared with 43.0 kg). Even greater overlap between the overweight and obese groups was found for percent body fat measurements. These discrepancies may prevent the proper categorization of distinct physiologic and metabolic characteristics important for perinatal

complications based on the patients' degree of adiposity and may contribute to the significant variations in results from perinatal epidemiologic studies using BMI as a surrogate for body fatness.

This study has several limitations. We could not obtain DXA scan data at the 37-wk study visit for use in the 4-compartment model and, instead, relied on a 2-wk postpartum measurement. Previous studies have found minimal change in BMC immediately after delivery (43), and 2 wk was believed to allow for resolution of delivery-related fluid shifts before significant changes in FM would be expected to occur. As anticipated, our FM values determined from the 2-wk postpartum DXA scan were lower than the 37-wk fat FM assessments, likely due to the loss of the uterine contents. Although we cannot rule out changes in FM between visits, the precision and minimal bias in FM between DXA and $D_{b(VRJ)}$ are reassuring that the accuracy did not vary based on obesity status. Therefore, we felt it was reasonable to use a postpartum DXA both to measure BMC for use in the 4-compartment calculation and to become our comparison value for maternal FM measured at 37 wk of gestation. We acknowledge that researchers may have other preferences for a comparison standard and have provided our raw data for Deming regression and Bland-Altman analyses between each method to allow for additional comparisons (Tables 4 and 5, Supplemental Tables 1 and 2). In addition, despite strict instructions and close monitoring, 7 subjects appeared to have consumed fluids during the ^2H_2O salivary equilibration period, which can alter the TBW determination. We found that the required overnight fast, followed by an additional 3 h with no water or food intake and minimized activity during the salivary collection period, is challenging for women during the third trimester of pregnancy and likely contributed to variation in the TBW measurements determined by ^2H_2O in this study. These experiences highlight the challenges associated with attempting to accurately measure body composition in pregnancy. This study was completed in a midsized metropolitan area among subjects in late pregnancy with a range of BMIs from normal to obese and included 18% non-Caucasians but may not be generalizable to other populations or other time points in pregnancy.

In summary, this study compared multiple body composition measurement methods for maternal FM used in both clinical and research settings of women in late pregnancy. We specifically examined women of various prepregnancy BMI weight categories to help determine the most effective tool for studies of obesity-related comorbidities. For larger field studies and studies with limited resources, SFT can reasonably estimate maternal FM, whereas BIA measures of FM appear to be less reliable. Where available, the use of ADP with pregnancy-specific formulas is perhaps the preferred method for assessing maternal FM in late pregnancy. It is well tolerated by subjects and less prone to measurement error than obtaining salivary collection samples for ^2H_2O for use in 3-compartment equations.

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