# Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model[1–4]

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**ABSTRACT**

**Background:** A better understanding of the associations of early infant nutrition and growth with adult health requires accurate assessment of body composition in infancy.

**Objective:** This study evaluated the performance of an infant-sized air-displacement plethysmograph (PEA POD Infant Body Composition System) for the measurement of body composition in infants.

**Design:** Healthy infants ($n = 49$; age: 1.7–23.0 wk; weight: 2.7–7.1 kg) were examined with the PEA POD system. Reference values for percentage body fat (%BF) were obtained from a 4-compartment (4-C) body-composition model, which was based on measurements of total body water, bone mineral content, and total body potassium.

**Results:** Mean (±SD) reproducibility of %BF values obtained with the PEA POD system was 0.4 ± 1.3%. Mean %BF obtained with the PEA POD system (16.9 ± 6.5%) did not differ significantly from that obtained with the 4-C model (16.3 ± 7.2%), and the regression between %BF for the 4-C model and that for the PEA POD system ($R^2 = 0.73$, SEE = 3.7%BF) did not deviate significantly from the line of identity ($y = x$).

**Conclusions:** The PEA POD system provided a reliable, accurate, and immediate assessment of %BF in infants. Because of its ease of use, good precision, minimum safety concerns, and bedside accessibility, the PEA POD system is highly suitable for monitoring changes in body composition during infant growth in both the research and clinical settings. *Am J Clin Nutr* 2007;85:90–5.

**KEY WORDS** Body composition, infants, air-displacement plethysmography, 4-compartment reference model, dual-energy X-ray absorptiometry

## INTRODUCTION

There is a renewed interest in the importance of early nutrition during infancy and its relation to potential long-term effects on growth and body composition; poor nutrition in early life is related to increased health risks in later life (1–3). Epidemiologic studies indicate that poor growth during fetal and early postnatal life is associated with an increased risk of obesity, hypertension, coronary artery disease, impaired glucose tolerance, and stroke (1–3). In contrast, overnutrition during infancy has also been associated with a risk of similar adverse adult health outcomes, including early onset of diabetes and some cancers (4–11). Taken together, these studies suggest that there may be an optimal range for healthy growth, and deviations from this pattern during early infancy can have a significant effect on health outcomes later in life.

Body weight, which is relatively easy to measure, has been the most frequently used anthropometric-based index of infant growth. It does not, however, provide any assessment of the relative contributions of body fat, lean tissues, and bone, all of which are key indicators of the adequacy of an infant's nutrition. The paucity of information on the clinical relevance of body composition in infancy, especially for longitudinal monitoring of preterm infants, is due, in part, to the absence of safe, reliable, and accurate techniques that can be routinely used in the clinical setting. Instruments developed for body-composition assessment in humans have been designed mainly for adults. Modifications are often needed if used with children, and, in most instances, are not practical for the measurement of infants for both technical and theoretical reasons. Techniques such as stable isotope dilution, dual-energy X-ray absorptiometry (DXA), and magnetic resonance imaging have been used in research studies, but none of them is particularly suitable for general clinical pediatric use, especially for the neonate (12).

Air-displacement plethysmography has proved successful for body-composition measurements in adults and children (13–18). An infant-sized air-displacement plethysmography instrument (PEA POD; Life Measurement Inc, Concord, CA) has recently been developed (19–21). The first human infant study performed with the PEA POD system compared percentage body fat (%BF) with estimates obtained by using deuterium dilution for body water as the reference method (22).

In the present study, a reference 4-compartment (4-C) model of body composition, which directly assesses variations in the body water, protein, and mineral components of fat-free mass

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(FFM), has been used. Many researchers consider this 4-C model to be the best criterion or reference method for the measurement of body fatness in pediatric populations (23–26).

## SUBJECTS AND METHODS

### Subjects

Forty-nine full-term healthy infants (25 boys, 24 girls) participated in the study. The subjects were recruited through advertisement in local communities and via a community-based referral system for pregnant women. The study protocol was reviewed and approved by the Institutional Review Board for Human Research at Baylor College of Medicine, and informed written consent was obtained from a parent of each infant before participation. Measurements of body weight (±2 g) and body length (±0.5 cm) were obtained, along with a brief medical history to confirm a normal health status for each infant.

### Study design

Infants, with an accompanying parent, were admitted to the Children's Nutrition Research Center at Baylor College of Medicine in Houston, TX between 0900 and 1000. To complete all body-composition measurements needed for the reference model, the infants typically stayed for 3–4 h. Baseline blood samples, urine samples, or both were obtained before the oral administration of a small volume of deuterium-enriched water. Three hours after the dose administration, a second fluid sample was collected. The PEA POD, whole-body counting, and DXA measurements were performed during the 3-h period between the collection of the baseline and 3-h postdose body fluid samples. The order in which these measurements were performed was dependent on the infant's behavior. For the DXA measurement, the infant was usually asleep so as not to introduce movement artifacts during the 3-min scan. For the whole-body counting and PEA POD procedures, the infant's behavior had no significant effect on the measurements. The total measurement times needed for the PEA POD, whole-body counting, and DXA procedures were about 3 min, 15 min, and 5 min, respectively. Note, however, it can often take ≥1 h for the infant to fall asleep, which was needed to obtain a reliable DXA scan.

To determine the precision of the PEA POD measurements, duplicate tests were obtained within 15 min of each other in a subgroup of 31 infants. For accuracy, the PEA POD results for %BF were compared with %BF estimates obtained from a 4-C body- composition model based on the measurements of total body water (TBW) by deuterium dilution, protein calculated from total body potassium (TBK) by whole-body counting, and body mineral based on bone mineral content (BMC) obtained by using DXA (27, 28).

### PEA POD system

Detailed descriptions of the physical design, operating principles, and measurement procedures for the PEA POD system are provided elsewhere (19–22). Each PEA POD test takes ≈3 min to complete and could be repeated as needed without risk. Body length was measured by using an infant board (Holtain Limited, Crymych, United Kingdom). A few drops of baby oil were used to flatten hair closer to the head.

The PEA POD measurement was used to measure body volume, which, coupled with body weight, can be used to calculate body density. If the body is assumed to consist of 2 components, FM and all other tissues (FFM), and each has a known density, then the percentage of body fatness can be calculated if body density is known. For the present study, the density values for FFM reported by Butte et al (27) during early infancy were used.

### Four-compartment reference model

Reference values for %BF were calculated by using a multicompartment model of body composition (27, 28). For this model, the FFM is assumed to consist mainly of body water, protein, and minerals. Three separate measurement techniques were needed to measure the mass of each of these compartments.

The deuterium dilution technique (29) was used to measure TBW. A heel stick (Quickheel Lancet; Becton Dickinson and Company, Franklin Lakes, NJ) was used to obtain blood samples (≥0.25 mL) at baseline and 3 h after the oral tracer dose. Urine samples (>2 mL) were also collected at the same times by using cotton balls placed in the infant's diaper. Collection of urine samples was started after a number of parents would not give consent for the second blood draw. We assayed both blood and urine when available ($n = 35$) and found no significant differences in the TBW estimates [$\bar{x}$ (±SD) difference: −0.02 ± 0.07 kg; $P = 0.421$).

Protein masses were based on the measurement of TBK, which was obtained with a whole-body counter (30). The $^{40}$K counts for an infant were compared with those for an infant-sized phantom with known potassium content. For a counting time of 15 min, the precision for TBK was ≈2.5–3.5%; depending on the infant's body size (30). The infant's behavior, such as excessive movement or crying, did not affect the TBK measurement.

The mineral component of FFM was based on the measurement of BMC obtained by using a DXA scanner (Delphi-A; Hologic Inc, Waltham, MA; software version 11.2). Once the infant was positioned on the bed, the whole-body DXA scans took ≈3 min to complete. To prevent movement during the scan, the infants were swaddled in a light cotton sheet and were usually asleep. DXA precision has been reported at 1.5–4.1% for BMC at body sizes comparable to those of our infants (31).

The reference model was based on the assumption that FFM consists mainly of body water, proteins, and minerals, with a small contribution from glycogen. Body protein was calculated by using the assumption of a constant nitrogen-to-potassium ratio of 0.461 g/mEq during growth, and protein contains 16% nitrogen (27, 28). Body mineral was calculated as BMC plus the mineral content of nonosseous tissues, which could be assumed to be relatively constant at 9.2 g minerals/kg water. Body glycogen stores were assumed to be 0.5% of total FFM. This is the same multicompartment reference model used by Butte et al (27) and Fomon et al (28) to describe changes in body composition during infant growth. Body fat was defined as body weight minus FFM. %BF was defined as 100 × [(body weight – FFM)/body weight].

### Statistics

All statistical analyses were performed by using SPSS (WINDOWS version 10.0; SPSS Inc, Chicago, IL) and SAS (WINDOWS version 8e; SAS Institute Inc, Cary, NC). Values presented in tables are means ± SDs. Reliability of %BF for PEA POD was determined by calculating the SD, CV, and technical error. The technical error was defined as $\sqrt{\Sigma d^2/2n}$, where $d$ is the

**TABLE 1**
Anthropometric characteristics of the participants

| Number | All infants ($n = 49$) | PEA POD subgroup[1] ($n = 31$) |
|---|---|---|
| Sex (M/F) | 25/24 | 13/18 |
| Ethnicity ($n$) | | |
| European American | 22 | 11 |
| African American | 11 | 10 |
| Hispanic American | 16 | 10 |
| Age (wk) | 8.0 ± 5.4 (1.7–23.0)[1] | 6.0 ± 3.3 (2.0–17.4) |
| Body mass (kg) | 4.7 ± 1.1 (2.7–7.1) | 4.1 ± 0.9 (2.3–6.1) |
| Body length (cm) | 54.9 ± 4.1 (46.0–64.6) | 52.6 ± 3.6 (46.0–60.4) |

[1] $\bar{x}$ ± SD; range in parentheses (all such values).

**TABLE 2**
Body composition measurements[1]

| | Value |
|---|---|
| TBW (kg) | 3.2 ± 0.6 (2.2–4.9) |
| BMC (g) | 91.0 ± 27.0 (29.2–143.7) |
| TBK (g) | 7.8 ± 1.5 (4.0–12.7) |
| $FFM_{4\text{-C Model}}$ (kg) | 3.9 ± 0.7 (2.5–6.1) |
| Percentage TBW (%)[2] | 81.7 ± 1.7 (77.6–85.8) |
| Percentage BMC (%)[2] | 2.3 ± 0.4 (1.2–3.1) |
| Percentage protein (%)[2] | 14.8 ± 1.6 (10.7–18.2) |
| $\%BF_{4\text{-C Model}}$ | 16.3 ± 7.2 (4.0–33.6) |
| $\%BF_{PEA\ POD}$ | 16.9 ± 6.5 (6.3–33.0) |

[1] All values are $\bar{x}$ ± SD; range in parentheses. $n = 49$. TBW, total body water; BMC, bone mineral content; TBK, total body potassium; $FFM_{4\text{-C Model}}$, fat-free mass estimated with the 4-compartment model; %BF, percentage body fat.

[2] Percentage FFM obtained with the 4-C model (for example, percentage TBW = 100 × TBW/$FFM_{4\text{-C Model}}$).

difference between 2 repeated tests for the paired observations ($n = 31$). The nonparametric Kruskal-Wallis test was performed to examine whether the reliability of the PEA POD system was influenced by sex or ethnicity. Linear regression and Bland-Altman analyses (32) were performed to assess individual agreement between %BF estimates from repeated PEA POD tests.

A paired-sample $t$ test was used to detect a significant difference between the %BF values obtained by the PEA POD system and 4-C model. Linear regression analysis for %BF obtained with the 4-C model compared with the PEA POD system was used to determine whether the slope and intercept differed significantly from the line of identity ($y = x$). The coefficient of determination ($R^2$) and the SE of estimate (SEE) from the linear regression were further calculated. Bland-Altman analysis (32) was also used to determine the limits of agreement between the 2 methods as well as potential bias. Multiple regression was used to examine potential effects related to sex, age, ethnicity, body length, and body weight. Stepwise regression analysis was performed to examine whether variability in hydration, protein, or mineral fractions of FFM could account for the differences between the %BF obtained with the PEA POD system and that obtained with the 4-C model.

## RESULTS

The anthropometric characteristics of the total population ($n = 49$) and the subgroup ($n = 31$) that participated in repeated PEA POD tests are given in **Table 1**. For the infants with repeated PEA POD tests, the mean within-subject SD and CV were 0.7%BF and 7.9%, respectively, with a technical error of 0.9%BF. No significant ethnic or sex effects related to these differences were observed ($P > 0.90$). The results of the Bland-Altman analysis for repeated %BF tests are presented in **Figure 1**. Good agreement was observed between the tests, with a mean bias of 0.4%BF and 95% limits of agreement at −2.3 and 3.1%BF. The results of the linear regression analysis confirmed the reproducibility of the PEA POD tests, with slope and intercept values not different from 1.0 and 0.0, respectively (SEE = 1.4%BF; $R^2 = 0.95$; $P < 0.001$).

The results for the measured parameters (TBW, BMC, and TBK) are presented in **Table 2**, along with the calculated values of FFM obtained with the 4-C reference model. The percentage contributions of water, bone mineral, and protein to total FFM are also included in the table, along with the %BF values obtained with the PEA POD system and 4-C reference model. The PEA POD estimates for %BF were not significantly different from the values obtained with the 4-C reference model (mean difference: 0.6 ± 3.7%BF; $P = 0.62$).

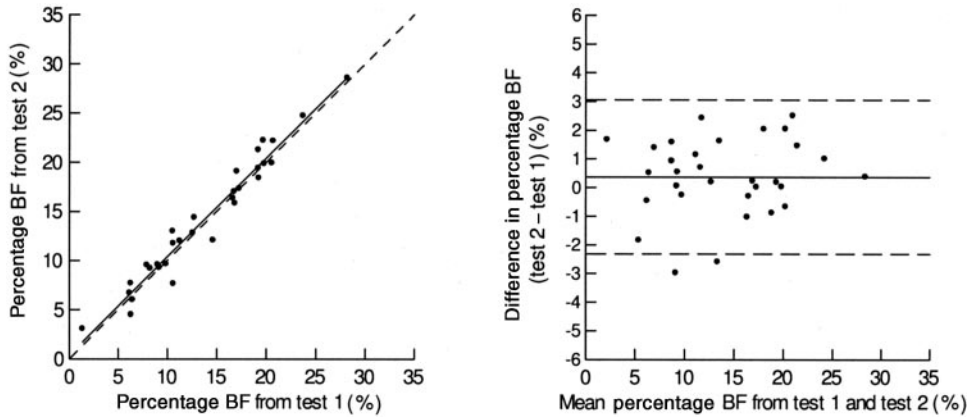


**FIGURE 1.** Left: Comparison of the air-displacement plethysmography (PEA POD) test 1 and test 2 results for percentage body fat (%BF). The regression line ($R^2 = 0.95$; SEE = 1.4%BF; $P < 0.001$) is not significantly different from the line of identity (dashed line; $y = x$). Right: Bland-Altman scatter plot for the 2 PEA POD tests. The solid line represents the mean difference (bias = 0.4%BF), and the 2 dashed lines are the 95% limits of agreement (±2 SD from the mean difference).

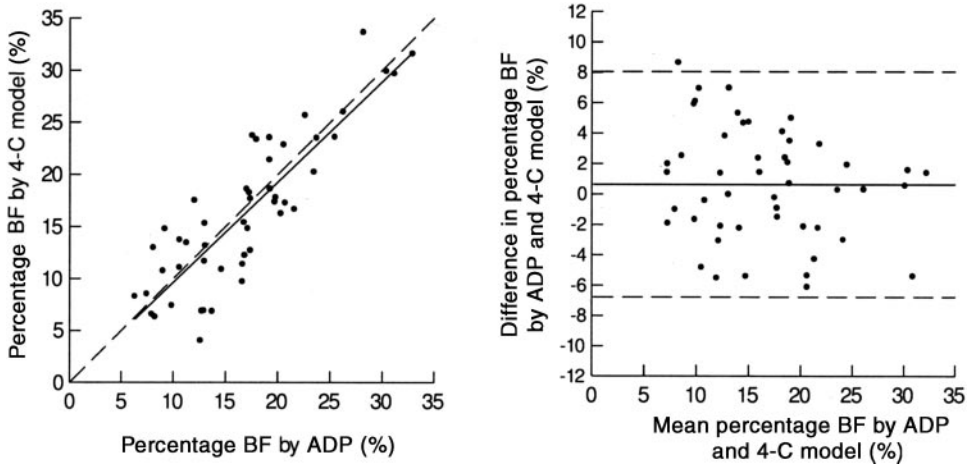


**FIGURE 2.** Left: Relation of percentage body fat (%BF) obtained with the air-displacement plethysmography (ADP; PEA POD system) and 4-compartment (4-C) reference model ($R^2 = 0.73$; SEE = 3.7%BF; $P < 0.001$). The dashed line represents the line of identity ($y = x$). Right: Bland-Altman scatter plot for the 2 methods. The solid line represents the mean differences between the 2 methods (bias = 0.6%BF), and the dashed lines are the 95% limits of agreement (±2 SD from the mean difference). No significant correlation was observed between the difference and the mean values of the 2 methods ($r = -0.22$, $P = 0.13$).

The linear regression and Bland-Altman analysis for %BF values obtained with the PEA POD system and 4-C model are presented in **Figure 2**. The slope and intercept values were 0.96 (95% CI: 0.80, 1.13) and −0.005 (95% CI: −3.05, 3.04), respectively, with $R^2 = 0.73$ and SEE = 3.7%BF. Sex ($P = 0.10$), age ($P = 0.21$), ethnicity ($P = 0.57$), body length ($P = 0.22$), and body weight ($P = 0.90$) had no significant effect on this relation. For the Bland-Altman analysis, the differences between the values obtained with the PEA POD system and the 4-C model were not a function of the mean values for the 2 methods ($r = -0.22$, $P = 0.13$), indicating there was no systematic bias as body fatness increased. The 95% limits of agreement were at −6.8 and 8.1%BF.

The relation of the differences between the %BF obtained with the PEA POD system and that obtained with the 4-C reference model with variation in hydration, protein, and bone mineral fractions of the FFM was examined by using stepwise multiple regression analysis. The results are presented in **Table 3**. The mineral fraction explained about 16% of the variation ($P = 0.004$), and the hydration and protein fractions explained only an additional 0.1% ($P = 0.80$) and 0.2% ($P = 0.79$), respectively. The best-fit model derived from stepwise regression analysis indicated that the mineral fraction of the FFM was the only significant contributing factor to the individual differences between the %BF values obtained with the PEA POD system and those obtained with the 4-C model.

**TABLE 3**
Stepwise multiple regression analysis of the effects of hydration and mineral and protein fractions of fat-free mass on the differences in percentage body fat between the PEA POD technique and the 4-compartment reference model

| Variables | β Coefficient | SE | Partial $R^2$ | $P$ |
|---|---|---|---|---|
| Best-fit model | | | | |
| Mineral fraction | −3.82 | 1.27 | 0.16 | 0.004 |
| Excluded variables | | | | |
| Hydration fraction | — | — | 0.002 | 0.79 |
| Protein fraction | — | — | 0.001 | 0.80 |

## DISCUSSION

The 4-C reference model we have used in the present study is considered the best choice when assessing body composition in humans (12, 25, 26, 33, 34). For infants, ours is the first study that has compared %BF obtained by using the PEA POD instrument with the values obtained with this reference model. Good agreement was observed between the 2 methods, with a mean difference of 0.6%BF, which was not significant. This was confirmed by the regression results where the values did not deviate significantly from the line of identity and there were no effects due to sex, ethnicity, age, body length, and body weight. Furthermore, the reliability of the %BF measurement obtained with the PEA POD system was exceptional, as indicated by the mean difference of 0.4%BF for repeated tests. This finding agrees with previous reports of the reliability of PEA POD measurements in infants (21, 22). We conclude that the PEA POD measurement provided an accurate and reliable assessment of %BF for healthy infants in the weight range we examined.

To further assess the individual differences between the %BF values obtained with the PEA POD system and those obtained with the 4-C reference model, we examined the effects of variation in the hydration, protein, and mineral fractions of FFM. We found that the hydration of FFM was not a substantial contributing factor, which indicates that the PEA POD measurement is relatively independent of minor changes in FFM hydration. Likewise, the protein fraction was also not a significant factor. Further testing with younger infants is needed to verify these finding at these ages.

If an estimate of %BF is the focus, the PEA POD measurement clearly has technical advantages over the multiple techniques that are required when using the 4-C model. Although the stable-isotope dilution technique is well established for the measurement of TBW (35), the practicality of the method, at least from a clinical perspective, is rather limited. One could argue the same for the TBK measurement, because this technology is not commonly found in most clinical centers, much less in a pediatric setting. Whole-body magnetic resonance imaging techniques have been used in research to examine body composition in

infants (36–38), but routine use of these instruments for this purpose remains highly unlikely because movement of the infant during the scan can significantly reduce the reliability of the results. DXA instruments, similar to the one we used in the present study to assess bone mineral content, are relatively common in clinical settings, but most do not have the ability to scan infants. Also, there are concerns about the accuracy of DXA for soft tissue measurements in infants (39–41).

In contrast, the PEA POD measurement is easy to perform. It takes only a few minutes to complete, and infant movement during the measurement is not a significant factor. Also, the measurement can be repeated as frequently as needed, and the results are immediately available. The calibration of the PEA POD instrument we used for the present study was based, in part, on the density values for FFM for healthy full-term infants starting at 2 wk of age (27). Further studies, therefore, may be needed to assess the performance of the PEA POD instrument with preterm infants and during the period immediately after birth when there can be considerable fluctuation in the hydration of the FFM.

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