New body composition reference charts for preterm infants 1,2

Ellen W Demerath,3* William Johnson,5 Bridget A Davern,4 Christina G Anderson,6 Jeffrey S Shenberger,2 Sonya Misra,6 and Sara E Ramel4

3Division of Epidemiology and Community Health, School of Public Health, and 4Division of Neonatology, School of Medicine, University of Minnesota, Minneapolis, MN; 5Medical Research Council Human Nutrition Research, Cambridge, United Kingdom; 6Division of Neonatology, Santa Clara Valley Medical Center, San Jose, CA; and 7Division of Newborn Medicine, Baystate Medical Center, Springfield, MA

ABSTRACT

Background: The American Academy of Pediatrics (AAP) has recommended that nutritional management of the preterm infant should aim to achieve body composition that replicates the in utero fetus, but intrauterine body composition reference charts for preterm infants are lacking.

Objective: Our objective was to create body composition reference curves for preterm infants that approximate the body composition of the in utero fetus from 30 to 36 wk of gestation.

Design: A total of 223 ethnically diverse infants born at 30 + 0 to 36 + 6 wk of gestation were enrolled. Inclusion and exclusion criteria were specified so that the sample would represent healthy appropriately growing fetuses (e.g., singleton, birth weight appropriate for their gestational age, and medically stable). Cross-sectional reference values were generated for fat mass (FM), fat-free mass (FFM), and percentage body fat (PBF) by gestational age (GA), with the use of air-displacement plethysmography (ADP) and the lambda-mu-sigma method for percentile estimation.

Results: GA-specific percentile values and a percentile and z score calculator for FFM, FM, and PBF are presented. These values aligned closely with ADP centile values published for term infants from 36 to 38 wk of gestation. The medians were also similar to the mean values for the reference fetus derived from chemical analysis previously.

Conclusions: To our knowledge, these are the first body composition reference charts for total FM and FFM at birth in preterm infants to assist in following AAP guidelines. Future work will test the clinical utility of body composition monitoring for improving nutritional management in this population. This trial was registered at clinicaltrials.gov as NCT02855814. Am J Clin Nutr 2017;105:70–7.

Keywords: body composition, preterm, infant, fat-free mass, fat mass, adiposity, growth, normative

INTRODUCTION

Because greater proportions of early-preterm infants are surviving without major complications (1), the focus of both research and clinical care has shifted from improving survival to reducing long-term morbidities. This shift has prompted the consideration of the long-term health consequences of achieving rapid early weight gain (2, 3). Early, intensive nutritional management is associated with increased weight gain velocity from birth to discharge and improved neurodevelopmental outcomes (4, 5), and is the standard of care in the neonatal intensive care unit (NICU)8. Yet, despite advances over the past decade, growth restriction continues to affect the majority of very–low birth-weight preterm infants (6). The finding that this growth failure is accompanied by altered body composition, with deficits in lean mass accrual and relatively high fat mass (FM) (7, 8), raises the question of whether, as in term infants with low birth weight, rapid catch-up weight growth in preterm and very–low birth-weight infants confers increased risk of obesity and cardiometabolic disease in adult life [reviewed by Lafeber et al. (9), Lapillonne and Griffin (10) and Pfister and Ramel (11)]. A recurring theme in these reviews is that although the American Academy of Pediatrics has recommended since 1985 (12) that preterm infants grow similarly to the fetus in all markers, including body composition, whether or not this goal is achievable or desirable with respect to long-term outcomes remains largely unknown (11, 13, 14).

Intrauterine growth reference curves exist for assessing the adequacy of growth in size (body weight, length, and head circumference) with the use of measures collected at birth as a proxy for intrauterine growth (15–17); however, to our knowledge, there are no corresponding preterm body composition curves for FM, fat-free mass (FFM), or percentage body fat (PBF) to aid clinical researchers and practitioners in gauging the quality of weight being lost and gained during hospitalization. Descriptions of the aggregate chemical composition of stillborn infants at varied gestational ages (GAs) have been published (18, 19). Plots of body fatness compared with weight with the use of dual-energy.
X-ray absorptiometry (DXA) (20) also have provided useful comparative information across preterm GAs. However, these studies do not provide GA-specific percentile values to allow for the comparison of a given infant against an appropriate population of infants of the same GA. Furthermore, chemical composition studies have used information exclusively from infants who died in utero or shortly after birth, and thus may not represent the normal body composition of the in utero fetus.

Air-displacement plethysmography (ADP) provides a precise and valid measure of infant body composition (21–23) and has been used to generate body composition reference curves in term infants (24–26). ADP is a good candidate for use in the NICU for routine nutritional status monitoring because the measurement is rapid, does not expose the infant to radiation, is well tolerated by small infants (27), and is able to detect changes in body composition after changes in diet (28).

The objective of our study (NCT02855814) was to create ADP-based body composition reference curves for preterm infants that approximate, as close as is feasible in live-born infants near birth, the body composition of the in utero fetus from 30 to 36 wk of gestation. This curve would provide a tool that could assist in the clinical management of individual preterm infants and advance the capacity for nutritional research.

METHODS

Subjects

The general study design was a cross-sectional assessment of body composition at birth, with a goal of ~30 infants/gestational week (15 male and 15 female) so that the curves were equally stable across the range of GAs. Infants were eligible for the study if they were born between 30 + 0 and 36 + 6 wk of gestation, had an appropriate-for-gestational-age (AGA) birth weight [between the 10th and 90th weight percentile (16)], were from a singleton pregnancy, and were medically stable, allowing for their measurement to occur within the first 72 h after birth. This last inclusion criterion minimized the influence of postnatal changes and growth restriction that typically follow preterm birth. Exclusion criteria included congenital conditions affecting fetal growth, birth weight <1 kg, and the inability to tolerate room air for 5 min without desaturation or bradycardia. The latter 2 exclusions stem from design constraints of infant ADP. The study was conducted between 2012 and 2015 at 3 tertiary NICUs in the United States: the University of Minnesota Masonic Children’s Hospital in Minneapolis, Minnesota; Santa Clara Valley Medical Center in San Jose, California; and Baystate Medical Center in Springfield, Massachusetts. The majority of the infants were enrolled at the Minnesota and Santa Clara centers, with the third center (Massachusetts) added in 2014 to enhance recruitment of infants born at earlier GAs. Approval was obtained from the institutional review board at each facility, and written consent was obtained from the parent or parents of each participant.

Data collection procedures

Growth and demographic information

Infant sex, birth weight, GA (and its method of determination; either ultrasound before 12 wk or last menstrual period), and exact time of birth were obtained from medical records. Maternal and paternal race/ethnicity were determined by parental self-report. Weight, recumbent length, and occipitofrontal (head) circumference (OFC) were measured at the time of body composition analysis by trained study personnel. Weight was measured (without clothing) on an electronic scale accurate to the nearest 0.1 g, recumbent length by length board to the nearest 0.1 cm, and OFC with the use of a flexible cloth measuring tape to the nearest 0.1 cm. SD z scores for these anthropometric measurements were calculated with the use of the Fenton 2003 growth reference data for preterm infants (16).

Body composition measurement

Body composition was assessed at all centers with the use of infant ADP (PEA POD; COSMED) with identical measurement protocols. A detailed description of the PEA POD’s physical design and measurement procedures are provided elsewhere (22, 29, 30). Infant ADP was first validated with the use of bovine sample phantoms, which showed high technical validity (29), followed by validation in live piglets, which showed high accuracy ($R^2 = 0.83$) compared with chemical composition at 2 d of life (31). Piglets at that age are 1.5 kg with 3% body fat, which is quite similar to the total composition of the preterm infants in our study. ADP also was validated in 10 preterm infants with a mean GA at measurement of 32.5 wk against isotope dilution. Validity was very good ($R^2 = 0.63$, $r = 0.77$, $P < 0.001$) (23).

Body composition was measured without clothing, and the weight and volume of any articles unable to be detached from the infant (e.g., nasogastric tubes, umbilical clips, and pulse oximetry) were tared before testing. All infants wore a tight-fitting cap to minimize volume measurement artifact from hair. Infant heart rate and oxygen saturation were monitored continuously via wireless techniques throughout the study visit (Nonin Medical), and the protocol was halted if heart rate was <100 or oxygen saturation was <80%.

Statistical analysis

Descriptive statistics

Means, SDs, and ranges were calculated for all continuous variables, whereas frequencies and percentages were calculated for categorical variables. Significant differences in pregnancy and infant characteristics by method of assessing pregnancy dates and by center were evaluated with the use of a chi-square test to establish whether the frequencies in these factors systematically varied.

Lambda-mu-sigma method

The lambda-mu-sigma (LMS) method was used to produce percentile curves for each outcome (FM, FFM, and PBF). Briefly, the LMS method models variation in size across age (here, GA at birth) as a function of 3 curves: 1) the $\lambda$ curve describes the Box-Cox power needed to remove skewness, 2) the $\mu$ curve describes the median, and 3) the $\sigma$ curve describes the CV (32). With these 3 curves, it is possible to compute any centile; we produced reference charts that included the 95th, 90th, 75th, 50th, 25th, 10th, and 5th centiles.

Model choice was guided by the Bayesian Information Criterion (33) and visual inspection of the centiles against the observed
data. We further assessed the fit of the curves to the data by comparing expected and observed percentages of participants with values above each standard centile (e.g., 5% of subjects should have values above the 95th percentile, whereas 25% of subjects should have values above the 75th percentile). PBF percentile values at 36 wk then were compared with published ADP body composition percentiles in late-preterm and early-term infants born at 36–37 wk of gestation to judge alignment of our curves with existing term reference data (26). Finally, to gauge the consistency of ADP-generated values to those of the reference fetus, the mean FM, FFM, and PBF values from our data set were compared with the estimated lipid, nonlipid, and PBF values for the reference fetus for each completed week from 30 to 36 wk (18).

RESULTS

Sample description

There were a total of 605 infants admitted to the NICU during the study period who were AGA and within the correct GA range. Of these, 343 (56.6%) were medically stable within 72 h of birth (i.e., off respiratory support and without central arterial lines). Parents could be contacted and provided consent for 231 (67%) of

FIGURE 1 Flowchart of participant inclusion and exclusion for the present study. AGA, appropriate for gestational age; GA, gestational age.
these. A complete, valid body composition assessment was obtained for 223 (96.5%) of the consented infants (Figure 1). The growth and demographic characteristics of the 223 infants included in the analysis are described in Table 1. The sample included approximately equal numbers of male and female infants. Mean birth weight, mean test weight, and mean OFC were all near sex- and GA-specific medians. Mean recumbent length was slightly lower than the population median (z = −0.14, P = 0.002).

There were no significant differences in GA, infant size, or body composition by method of dating or site of enrollment (P > 0.5). The Minnesota infants were measured somewhat later (10 h later, on average; 53 h after birth) than the Santa Clara and Massachusetts infants (44 h after birth) (P < 0.05). Scatter plots of FM and FFM by study center (Supplemental Figure 1) showed that body composition values across the 3 centers did not systematically differ from one another (P > 0.05 for all).

Body composition percentile charts

Percentile curves for FFM, FM, and PBF are provided in Figures 2–4. As expected, given the known relation of lean and adipose tissue accrual during infant development, FFM increased fairly linearly across the GA range, whereas FM and PBF demonstrated a more curvilinear relation, with faster increases in FM and PBF at later GAs. To demonstrate the fit of the curves to the data, Table 2 shows that the proportion of individuals observed to fall above selected centiles corresponded to the proportion that would be expected. A data set containing LMS values for each day of life between 30 + 0 and 36 + 6 wk is provided in Supplemental Table 1. Stata statistical code is provided in Supplemental File 1, and the LMS values in Stata data set format from which to calculate z scores and percentiles for batched body composition data with the code are provided in Supplemental File 2. An Excel spreadsheet calculator to convert individual FM, FFM, and PBF measurements into percentiles and z scores can be found in Supplemental File 3.

Comparison with other estimates

To assess how well our curves match with existing body composition reference curves with the use of ADP in early term infants, we compared centile values for PBF at 36 + 6 wk of gestation in the present study to published centile values for PBF at 36–40 wk of gestation for term infants also measured with the use of ADP at or near birth (26) (Supplemental Table 2). This shows close correspondence in mean PBF in our sample at 36 wk to published values for early term infants at 36–38 wk of gestation. We also show close concordance of mean FFM, FM, and PBF values in the present study to the estimated values by week of gestation for the reference fetus in Ziegler et al. (18) (Supplemental Table 3).

DISCUSSION

One key barrier in determining the optimal balance between supporting neurodevelopment and avoiding later metabolic disease in preterm infants is the lack of body composition reference data, which hinders the interpretation of individual measurements (34). To address this barrier, the present study developed LMS growth charts with the use of cross-sectional body composition data from an ethnically and racially diverse sample of healthy preterm infants, assessed as close as possible to the time of birth, as a way of representing the pattern of fetal body composition variation. The centile values we provided at 36 wk were close to the corresponding centile values for early term infants from 36 to 38 wk of gestation, suggesting that the preterm curves presented here dovetail with those of the healthy fetus, at least near term. The mean values of FFM, FM, and PBF were close to the lipid, nonlipid, and PBF values for the reference fetus, respectively, at each completed week of gestation (18), suggesting that the percentile values obtained on preterm infants with the use of ADP did not give a picture of fat and fat-free tissue amounts different from that suggested by the classic reference data.
Recently, BMI-for-GA reference curves were developed for preterm infants in order to assess proportionality of weight and length growth (35). Although these reference data are likely to be highly useful for that purpose, BMI is not a good proxy for relative fatness or leanness in neonates (36). In term infants measured at birth, BMI explained only 43% of the variation in FM and had high estimation errors (37). In preliminary analyses of preterm infants born at 30–36 wk of gestation, we found that BMI explained only 27% of the variation in PBF at birth (38).

The only body composition study in live preterm infants aimed at developing reference information was that of Rigo et al. (20), who used DXA to develop FFM-for-length and FM-for-length...
regression models. That study included 53 preterm infants measured between 2 h and 2 wk after birth (20); percentiles and \( z \) scores were not provided. There are 2 sources of postmortem body composition information on preterm infants. Elemental content of the whole body was estimated with the use of 40K counting and total body neutron activation analysis in 23 infants of varying GAs who survived from a few hours to 192 d after varying postpartum illnesses (19). Reference values for total body chemical composition also were culled from a variety of studies for 22 infants born at 23–42 wk of gestation and who died in utero or shortly thereafter (18). The data reported in the latter study are widely cited and used in clinical practice, and they may represent the best available data to date. However, the data were the combined results of multiple studies, the methods of data collection and analysis of which differed.

With the accompanying statistical code, a given ADP measurement of FM, FFM, or PBF obtained from an infant measured at (but not necessarily born at) 30–36 postconceptual weeks can be converted easily to a \( z \) score or percentile value that represents their comparison to a group of healthy AGA singleton preterm infants before extrauterine growth retardation has begun. This tool can help determine whether achieving fat and FFM growth that deviates from intrauterine body composition is, in fact, associated with poorer outcomes, the assumption underlying the American Academy of Pediatrics recommendation (12). These body composition reference data could provide a useful common yardstick for assessing the impact of different nutritional interventions on preterm infant growth and body composition, as part of the larger project of improving future neurobehavioral and metabolic outcomes (39–41).

The sample was multiethnic, with 22.9% Hispanic, 18.8% African-American, 7% Asian, 3% Native American, and 48% white non-Hispanic infants, but it was restricted to the subset of infants who were relatively healthy at birth despite their preterm

![Percentage body fat percentile curves for 223 appropriate-for-gestational-age, medically stable preterm infants born at 30–36 wk of gestation, measured within 72 h of birth.](https://academic.oup.com/ajcn/article-abstract/105/1/70/4633928)

### TABLE 2

<table>
<thead>
<tr>
<th>Centile</th>
<th>Expected percentage of values above this centile</th>
<th>Observed percentage of values above this centile (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass</td>
<td>Fat-free mass</td>
<td>Percentage of fat mass</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
<td>5.38</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>8.52</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>25.11</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>55.16</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>73.99</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>87.89</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>95.52</td>
</tr>
</tbody>
</table>

\(^1\)P value for the chi-square test for concordance of expected and observed percentage above selected centiles was >0.99 for all body composition variables.
status, and were born predominantly in Minnesota. Thus, the results may not be generalizable to all US infants. Because the inclusion criteria were purposefully narrow in the present case, many fewer infants could be included than are typically included in reference curves for routine growth data collected from term newborns [e.g., Olsen et al., 2015 (35)]. With a larger sample, more extreme percentiles (3rd, 97th) could be estimated reliably and sex-specific curves also could be generated. This is potentially important because greater FM generally is found in female infants and greater FFM is found in male infants at term (42, 43). Multicomponent reference data showed no difference before 2 wk after birth (44), but a possible next step would be to expand the study to generate sex-specific ADP body composition reference percentiles.

ADP is an appropriate method for infant body composition reference curves because it has been validated for preterm neonates (23, 29, 31), and has high interdevice reliability (23), which has not been shown for DXA (45). Nonetheless, a weakness of densitometric approaches is the necessity of making assumptions regarding FFM density, which varies significantly in the chemically immature young infant (46). A future improvement would be additional 3-compartment model studies that can confirm the assumptions of the FFM density reference values used in ADP at present. Nonetheless, ADP uses the best available FFM density estimations derived from a reference 4-compartment model (44, 47), and a recent update to the proprietary ADP algorithm also adjusts for the greater expected weight loss immediately after birth in preterm compared with term infants.

Strengths and weaknesses in ADP should also be weighed against the strengths and weaknesses of alternative techniques (36). Isotopic dilution for total body water estimation can be highly accurate and well tolerated in infants (48), but it is time-consuming and inappropriate for routine use in the clinical setting, and still relies on density models for estimation of body composition. Radiation exposure for DXA, although minute, nonetheless is an obstacle for routine repeated measurement in infants. Imaging techniques such as magnetic resonance avoid the necessity of density assumptions, and potentially can be performed when infants are asleep, but they require specially trained operators and usually require moving the infant outside the unit. A limitation of infant ADP that is shared with most other whole-body composition methods (other than isotope dilution) is that sick infants on respiratory support and with lines cannot be measured. Unfortunately, this means that infants are unable to be measured at a time when nutrition and growth are often restricted because of instability. However, this reinforces our suggestion that ADP, and these curves, are best used to monitor the nutritional status of preterm infants during their convalescent period between 30 and 36 wk, once they have recovered from their initial illness, and at a time when nutritional adjustments are feasible and more likely to be tolerated.

To our knowledge, this study presents the first body composition reference curves designed to represent, as closely as is feasible, the variation in total body fat and FFM values from 30 to 36 wk of gestation in the healthy fetus. This tool may facilitate comparisons of body composition status between groups of preterm infants under different intervention conditions in the research setting, and ultimately may help to guide personalized nutritional treatment of individual infants in the clinical setting. Future work will test the clinical utility of body composition monitoring in preterm infants with the use of these reference values, to answer the question of whether it is either feasible or desirable for preterm infants to achieve the same growth rate and body composition as the unborn fetus.

The authors’ responsibilities were as follows—EWD and SER: designed the research; EWD, BAD, CGA, JSS, SM, and SER: conducted the research; EWD and WJ: analyzed the data; EWD and SER: wrote the paper and had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study. COSMED, Inc. was involved in the initial design of the study. COSMED, Inc. was not involved in the implementation, data collection, statistical analysis, interpretation of the data, or manuscript preparation and writing.

REFERENCES


