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Weight Gain Composition in Preterm Infants with Dual Energy X-Ray Absorptiometry

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ABSTRACT

Whole body composition was investigated using dual energy x-ray absorptiometry in 54 healthy preterm infants, birth weight < 1750 g, who were fed fortified human milk ($n = 20$) and preterm formula ($n = 34$) when full enteral feeding was attained and then again 3 wk later at around the time of discharge. Weight gain composition was calculated from the difference between the earlier and later measurement. The minimal detectable changes in whole body composition over time according to the variance of the population (within groups of 20 infants) and the minimal detectable changes according to the dietary intervention (between two groups of 20 infants) were determined at 5% significance and 80% power. Whole body composition was similar in the two groups at the initial measurement, but all the measured variables differed at the time of the second measurement. Formula-fed infants showed a greater weight gain (19.9 ± 3.2 *versus* 15.9 ± 2.2 g·kg^{-1}·d^{-1}, $p < 0.05$), fat mass deposition (5.1 ± 1.9 *versus* 3.3 ± 1.3 g·kg^{-1}·d^{-1}, $p < 0.05$), bone mineral content gain (289 ± 99 *versus* 214 ± 64 mg·kg^{-1}·d^{-1}, $p < 0.05$), and increase in bone area (1.6 ± 0.4 *versus* 1.3 ± 0.3 cm^2·kg^{-1}·d^{-1}, $p < 0.05$) compared with the fortified human milk group. From these data, a minimal increase from the first measurement of 111 g lean body mass, 68 g fat mass, and 3.1 g bone mineral content is needed to be detectable in a longitudinal study that includes 20 infants. For significance between two groups of 20 infants around the time of discharge, dietary intervention needs to achieve minimal differences of 160 g lean body mass, 86 g fat mass, and 4.1 g bone mineral content. With respect to weight gain composition, the minimal differences required to reach significance are 2.1 g·kg^{-1}·d^{-1} for gain in lean body mass, 1.2 g·kg^{-1}·d^{-1} for gain in fat mass, and 76 mg·kg^{-1}·d^{-1} for gain in bone mineral content. We conclude that dual energy x-ray absorptiometry allows evaluation of the effects of dietary intervention on whole body and weight gain composition in preterm infants during the first weeks of life. **(*Pediatr Res* 49: 120–124, 2001)**

Abbreviations
BMDI, bone mineral density index
DXA, dual energy x-ray absorptiometry
FHM, fortified human milk
PTF, preterm formula

In recent decades, with the progressive increase in the survival of preterm infants, there has been increased interest in their nutritional evaluation in the light of knowledge that adequate feeding in the early weeks of life influences short- and long-term development (1, 2). Measurement of body composition is of fundamental importance in the nutritional care for preterm infants, and many techniques have been developed (3–7). Metabolic balances associated with indirect calorimetry allowed the composition of weight gain in preterm infants to be defined (8, 9). The complexity of such techniques and the fact that weight gain composition could only be obtained over a short period of time resulted in the need for a new and reliable method of study. DXA has emerged as an accurate, precise, and reproducible technique for measuring whole body composition *in vivo* in humans. Determination of lean body mass, fat mass, bone area, and bone mineral content can all be done using DXA (10–14). Reference values of body composition in preterm and term infants at birth have been reported (11, 12). Our aim in the present study was to measure body composition and weight gain composition by DXA in preterm infants fed exclusively on either FHM or PTF and to evaluate the sensitivity of the study design by using DXA to measure changes in whole body and weight gain composition in preterm infants during the first weeks of life.

METHODS

Healthy preterm infants without any clinical problems were studied longitudinally. An initial study was performed after full enteral feeding had been achieved and the infant's clinical

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condition allowed us to undertake the DXA examination. A second DXA measurement was done at the time of discharge. Additional inclusion criteria for the present study were a body weight at the first evaluation below 1750 g and a weight gain of more than 500 g between the two evaluations.

At the time of evaluation, all infants were weighed naked on an electronic baby scale (SECA, model 727, Hamburg, Germany) to the nearest 5 g. Crown-heel length (on an infant length board to the next succeeding 0.1 cm) and head circumference (using a paper insertion tape to the next succeeding millimeter) were also determined. All measurements were performed by one of us (C.P.).

The present study was approved by the Human Ethics Committee of the University of Liege. Informed parental consent was obtained.

Total and partial parenteral nutrition was used from admission until an adequate volume of oral feeding had been achieved. Enteral nutrition with human milk was initiated as soon as possible between the first and fifth day of life according to the infant's clinical condition. Supplemental parenteral nutrition was withdrawn after an oral intake of 100–120 $mL \cdot kg^{-1} \cdot d^{-1}$ had been reached. When full oral diet had been attained, the infants were either continued on FHM or were weaned to PTF according to the mother's choice. Mother's own human milk was supplemented with banked human milk as available and by up to 3 to 4% with two similar fortifiers: Eoprotin (Milupa) or BMF (Nutricia). The formula-fed infants received PTF Nenatal (Nutricia), Prematil (Milupa), or Premie (Wyeth). A record of feed intake was kept during the entire study to estimate supply of nutrients.

Body composition was measured with the QDR 2000 bone densitometer (Hologic Inc, Waltham, MA, U.S.A.). Scans were analyzed using infant whole body software V5.65P supplied by the manufacturer (11, 15). The principle of the DXA procedure is described elsewhere (10–13, 16). Quality control scans were performed daily on an anthropometric spine phantom supplied by the manufacturer. The mean coefficients of variation for bone mineral content, bone area, and bone mineral density measurements over a period of 3 y ($n = 486$) were less than 0.5%. For whole body measurements, we used an infant table pad that filters the lower energy beam; this improves system linearity in small subjects and reduces the radiation dose (11, 12, 15). Whole body composition data analysis provides measurements of bone mineral density (g), bone area (cm^2), fat mass (g), and lean body mass (g), whereas the software calculated body weight and bone mineral content (15). In this study, DXA fat mass and lean body mass were recalculated on the basis of our validation study in piglets; these values have already been used to obtain reference values in preterm and term infants (12).

Bone mineral density, calculated as bone mineral content per unit area of bone, is highly dependent on anthropometric variables. Therefore, we prefer to use bone mineral content adjusted for bone area (17) or BMDI [bone mineral content (mg)/(bone area $(cm^2))^{1.7}$], which enables us to obtain a density index independently of anthropometric variables. We have reported measurements made in preterm and term infants at birth (18, 19).

During the DXA measurement, infants were placed in the supine position on an infant table pad. They were scanned only once. No sedation was given. To avoid movement artifacts, defective scans–that is, those showing discontinuity of the lateral edges of skeletal bones on the video monitor–were discarded according to Koo *et al.* (11).

From the difference in body composition values between the second and the first examinations, we determined body weight gain and weight gain composition. Increases in lean body mass, fat mass, and bone mineral content were expressed per kg/d and compared in infants fed FHM and PTF.

The data were analyzed using PC statistical software (Statistica, version for Window 5.0, 1995, Statsoft, Tulsa, OK, U.S.A.). Anthropometric variables parameters in the two groups and longitudinal data in each group were compared using unpaired and paired *t* test. Data of body and weight gain composition in the FHM and PTF groups were compared using ANOVA, taking groups as the independent variable and sex, birth weight, gestational age, and postnatal age as covariates. Differences were considered significant at $p < 0.05$. Values are expressed as mean with SD (M ± 1 SD). Anthropometric and DXA values were also compared with intrauterine reference values (12, 20). For those comparisons, *Z* scores were determined as $(X-M)/S$, where X = individual anthropometric and DXA values, M = mean value of the reference according to gestational age (20) and body weight (12), and S = SD of the reference.

From the mean and SD of the differences between the values of body weight, lean body mass, fat mass, bone mineral content, and bone area at the two determinations, the minimal significant longitudinal changes were calculated for a sample size of 20 infants. The minimal significant difference according to dietary intervention for body composition and weight gain composition variables at the end of the study was also evaluated for a sample size of 20 infants in each group. The calculations were carried out for a 5% level of significance (α risk = 0.05) and 80% power (β risk = 0.20) (21).

RESULTS

Fifty-four healthy preterm infants (29 female and 25 male) were included in the study. Forty-five were appropriately grown for gestational age (20). Twenty infants were fed FHM (FHM group) and thirty-four received a PTF (PTF group). In the two groups, anthropometric variables (Table 1) and whole body composition (Table 2) were similar at birth and at the start of the nutrition study period, performed at a mean age of 21 d (group FHM) and 22 d (group PTF). Over the whole period of study, milk intake was significantly higher in the FHM group than in the PTF group (165 ± 11 *versus* 147 ± 11 $mL \cdot kg^{-1} \cdot d^{-1}$, $p < 0.0001$).

At the time of the second measurement, body weight was significantly lower in preterm infants fed FHM than in those fed PTF, whereas body length and head circumference were similar in the two groups (Table 1). Lean body mass, fat mass, bone mineral content, and bone area were significantly lower in preterm infants fed FHM than in those fed PTF (Table 2). Lean body mass and fat mass were, respectively, 89.2 and 9.5% of

Table 1. *Anthropometric characteristics of FHM- and PTF-fed infants*

	FHM (n = 20)	PTF (n = 34)
At birth		
Sex	7 M/13 F	18 M/16 F
IUG status	5 SGA/15 AGA	4 SGA/30 AGA
Weight (g)	1298 ± 317	1269 ± 261
Length (cm)	38.7 ± 3.4	38.6 ± 2.8
Head circumference (cm)	27.8 ± 2.1	27.5 ± 2.0
Gestational age (wk)	31 ± 2	30 ± 2
At 1° examination		
Age (d)	21 ± 11	22 ± 14
Weight (g)	1482 ± 164	1448 ± 185
Length (cm)	40.4 ± 2.1	40.3 ± 1.9
Head circumference (cm)	29.4 ± 1.4	29.2 ± 1.3
At 2° examination		
Age (d)	44 ± 12	47 ± 16
Weight (g)	2112 ± 166	2362 ± 231*
Length (cm)	43.6 ± 1.5	44.1 ± 1.5
Head circumference (cm)	32.3 ± 1.4	33.0 ± 0.9

* $p < 0.05$ *vs* FHM-fed infants.

IUG indicates intrauterine growth; SGA, small for gestational age; AGA, appropriate for gestational age.

body weight in the FHM group and 85.5 and 13.2% in the PTF group, whereas bone mineral content accounted for 1.3% in both groups. BMDI was similar but decreased significantly from the first to the second evaluation in the two groups ($p < 0.001$). At the end of the study, lean body mass, fat mass, and bone area were in the range of the reference values related to body weight and determined in preterm and term infants at birth (12). By contrast, bone mineral content related to body weight or to bone area, as well as BMDI, were similar in the two groups, although significantly lower than the reference value (Fig. 1).

During the study, weight gain was lower in infants fed FHM (15.9 ± 2.2 g·kg^{-1}·d^{-1}) than in those fed PTF (19.9 ± 3.2 g·kg^{-1}·d^{-1}) ($p < 0.001$), whereas length gain and head circumference were not significantly different ($p = 0.45$ and $p = 0.07$, respectively). The gains in lean body mass, fat mass, bone mineral content, and bone area were significantly lower in infants fed FHM than in those fed PTF (Table 2). The contribution of fat mass to weight gain was significantly less in the FHM group (20.9 ± 7.3 *versus* 25.8 ± 7.3%, $p < 0.05$).

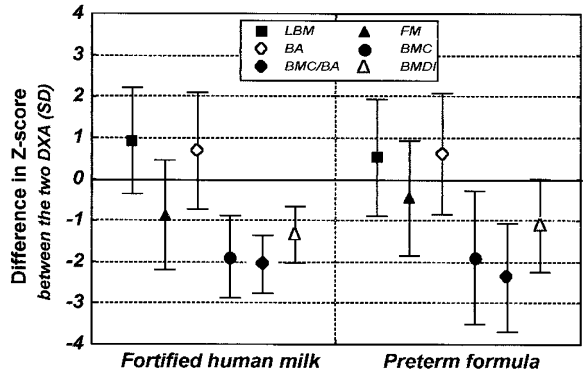

Figure 1. Body composition change in Z score between the two DXA. Z scores were related to our reference values determined in preterm and term infants at birth (12, 19).

Table 2. *Body composition and weight gain composition in preterm infants fed FHM or PTF*

	FHM (n = 20)	PTF (n = 34)
At 1° examination		
LBM (g)	1391 ± 147	1355 ± 147
FM (g)	71 ± 35	75.4 ± 55.5
FM (%)	4.7 ± 2.3	4.9 ± 2.5
BMC (g)	19.5 ± 3.1	18.0 ± 3.5
BA (cm^2)	157 ± 17	153 ± 18
BMDI	3.4 ± 0.3	3.3 ± 0.3
At 2° examination		
LBM (g)	1883 ± 161*	2015 ± 190*,†
FM (g)	200 ± 54*	315 ± 122*,†
FM (%)	9.5 ± 2.6	13.2 ± 4.5*,†
BMC (g)	28.0 ± 3.5*	31.3 ± 5.4*,†
BA (cm^2)	210 ± 15*	225 ± 22*,†
BMDI	3.0 ± 0.2*	3.0 ± 0.3*
Between examinations		
Weight gain (g/kg/d)	15.9 ± 2.2	19.9 ± 3.2†
Length (cm/wk)	1.0 ± 0.3	1.1 ± 0.3
Head circumference (cm/wk)	0.9 ± 0.2	1.1 ± 0.3
LBM (g/kg/d)	12.4 ± 1.9	14.5 ± 3.5†
FM (g/kg/d)	3.3 ± 1.3	5.1 ± 1.9†
FM gain (%)	4.8 ± 2.3	8.3 ± 2.5†
BA (cm^2/kg/d)	1.3 ± 0.3	1.6 ± 0.4†
BMC (mg/kg/d)	214 ± 64	289 ± 99†

BA, bone area; BMC, bone mineral content; BMDI = BMC(mg)/(BA(cm^2))$^{1.71}$; FM, fat mass; LBM, lean body mass.

* $p < 0.05$ *vs* 1° examination.

† $p < 0.05$ *vs* FHM-fed infants.

At the 5% level of significance and 80% power, the minimal detectable changes over time within a group of 20 infants weighing approximately 1500 g represented 154 g (10.5%) for body weight, 111 g (8.1%) for lean body mass, 13.9 cm^2 (9.0%) for bone area, 3.1 g (16.7%) for bone mineral content, and 68 g for fat mass (91.9% of the extremely low fat mass content at the first evaluation in Table 3). As fat mass represented on average 20.9 and 25.8% of the weight gain, respectively, in the FHM and PTF groups, the minimal total body weight gain necessary to obtain a significant difference from the first examination in all the whole body composition variables in a cohort of 20 preterm infants can be estimated as 325 g (68 g/0.209) in the FHM group and 264 g (68 g/0.258) in the PTF group, corresponding to an interval of 12 and 8 d in a longitudinal study.

At the time of discharge, the minimal detectable differences in body composition between groups when comparing the dietary intervention (Table 3) represented around 8% for body weight (182 g), lean body mass (160 g), and bone area (17.1 cm^2), 13.6% for bone mineral content (4.1 g), and 31.6% for fat mass (86 g). For weight gain composition calculated between the two DXA, the minimal detectable difference was, respectively, 2.3 g·kg^{-1}·d^{-1} for body weight, 2.1 g·kg^{-1}·d^{-1} for lean body mass, 1.2 g·kg^{-1}·d^{-1} for fat mass, 76 mg·kg^{-1}·d^{-1} for bone mineral content, and 0.32 cm^2·kg^{-1}·d^{-1} for bone area.

DISCUSSION

These data represent some of the first published values on early weight gain composition obtained with DXA in preterm infants in relation to the type of feeding regimen (13, 18, 22,

Table 3. *Minimal significant difference of whole body composition and weight gain composition detectable with DXA in preterm infants during the first weeks of life*

Gain	Within groups $n = 20$	% (*)	Between groups $n = 20$/group	% (†)	Between groups $n = 20$/group	% (‡)
Body weight	154 g	10.5	182 g	8.0	2.3 g/kg/d	12.5
LBM	111 g	8.1	160 g	8.1	2.1 g/kg/d	15.3
FM	68 g	91.9	86 g	31.6	1.2 g/kg/d	27.0
BMC	3.1 g	16.7	4.1 g	13.6	76 mg/kg/d	29.1
BA	13.9 cm^2	9.0	17.1 cm^2	7.8	0.32 cm^2/kg/d	21.9

* % of the body composition at first DXA.
† % of the body composition at second DXA.
‡ % of the mean weight gain composition values.

23). We designed our present study on the basis of our previous results obtained with metabolic balances and indirect calorimetry, which showed significant differences in weight gain and weight gain composition in preterm infants fed FHM and PTF. It was not a randomized study as the feeding regimen was determined by the mother's choice, but it was performed with the aim of evaluating the sensitivity of nutritional studies by use of DXA for the detection of significant differences related to nutritional intervention. Weight gains of 15.9 and 19.9 $g \cdot kg^{-1} \cdot d^{-1}$, respectively, in the FHM and PTF groups are comparable to data reported in similar groups of preterm infants (8, 9, 24, 25). The overall weight gain during the period of study was 630 and 913 g in the two groups, an increase of 42 and 63% over initial body weight. During the course of a longitudinal study, these values represent twice the minimal estimated weight gain to obtain a significant difference in all the whole body variables in a cohort of 20 infants fed FHM and more than three times the minimal weight gain in a similar cohort of infants fed PTF.

Growth and weight gain composition of the preterm infant during the first weeks of life differ from that of the fetus *in utero*. From birth to the time of discharge, body weight and body-length growth deviate from intrauterine reference values (20) in the FHM group by −1.04 and −1.30 SD and in the PTF group by −0.45 and −1.33 SD**,** respectively. Such a difference has often been reported in very-low-birth-weight infants (26, 27) and may lead to a long-term reduction in linear growth (28, 29).

We recently reported data on whole body composition obtained using DXA in preterm and term infants soon after birth and showed that the values were similar to intrauterine reference values reported previously using carcass analysis of deceased fetuses and neonates. Compared with those data and relative to body weight, the whole body lean mass and fat mass contents determined in our two groups at the end of the study fell within the reference range (Fig. 1), although the fat mass was significantly higher in infants fed PTF than in those fed FHM.

After birth, the use of the gastrointestinal tract instead of the umbilical cord to provide all nutrients for growth does not change the energy and protein supply necessary for bone growth significantly. However, it causes a large reduction in calcium availability for mineralization and, thus, leads to relative osteopenia characterized by a disproportionate reduction in bone mineral to the extent that the relative amount of unmineralized matrix (osteoid) is increased above normal levels (18, 19, 30). Our data obtained at the end of the study seem to confirm such an interpretation. Indeed, whereas bone area was in the range of the reference values according to body weight, bone mineral content, both absolute and related to bone area, was significantly decreased. In addition, from the first to the second examination and compared with the reference, there was a significant change in bone mineral content (−1.9 SD in both groups) and bone mineral content related to bone area (−2.1 SD) in the FHM group and −2.4 SD in the PTF group, confirming the insufficient postnatal mineral supply and retention in orally fed preterm infants.

At the time of discharge, the sensitivity of our study design for detecting significant differences in whole body composition between two groups of 20 infants ranged from 8 to 31.6% according to the variable assessed. For fat mass, and considering the relatively low fat-mass content at the time of discharge, the sensitivity appears to be better than could be obtained by other indirect methods (6). Considering that DXA provides an assessment of the whole body in three separate compartments, it appears to be one of the most interesting noninvasive methods presently available for investigating whole body composition in preterm infants.

The weight gain composition determined by DXA was in the range of the values we reported previously using metabolic balance studies and indirect calorimetry in preterm infants fed on similar regimens (25, 31). In the present study, dietary intervention was the main determinant of weight gain and whole body composition in preterm infants. As suggested by Cooke *et al.* (13), sex was an additional significant independent variable, resulting in an increase in fat mass and bone mineral content in female infants (32), but the contribution of this was relatively small compared with the feeding regimen. By use of DXA, the sensitivity for detecting significant differences in weight gain composition appeared to be at least similar to that obtained by more invasive techniques. Between two groups of 20 preterm infants, the sensitivity was approximately 14% for lean body mass and 27% for fat mass. The figures for bone area (18%) and bone mineral content (23%) were intermediate between those values.

Our results relating to bone mineralization need additional comment. Bone growth is related to the protein and energy supply necessary for osteoid matrix synthesis, and growth in bone area in the two groups may reflect the difference in body weight gain. In contrast, bone mineral content has been con-

sidered to be a measure of the hydroxyapatite content and gain in bone mineral content to be a reflection of mineral accretion. In our validation study, we observed a calcium to bone mineral content ratio of 46.5%, and we reported that the whole body calcium content was accurately measured by DXA with an error of estimation of only 4.4%. Using the same type of conversion equation, the whole body calcium content estimated in preterm and term infants at birth was similar to the intrauterine reference values. In our present study, the lower bone mineral accretion observed in the infants fed FHM suggests that calcium retention was lower in those infants than in the PTF-fed infants (99 ± 31 and 133 ± 47 mg of calcium per kg/d, respectively). These values, which are close to the fetal accretion rate, are higher than expected from published calcium intake and metabolic balance studies. Indeed, in preterm infants fed similar human milk fortifier or PTF, a calcium retention of 45 to 60 $mg \cdot kg^{-1} \cdot d^{-1}$ was previously obtained (33, 34), and similar values were determined in 20 of the preterm infants fed fortified PTF enrolled in this study (our unpublished data). By contrast, the similar reduction in our two study groups in BMDI and bone mineral content related to bone area suggests that the bone mineral content to calcium ratio may be modified after birth by disproportionate bone growth in relation to mineral deposition. These data suggest that when using the infant whole body software, which has a low threshold value for assessing the pixel count of bone, the attenuation coefficient of the matrix could be such that it is erroneously counted as bone, leading to a relative overestimation of bone mineral mass. In our study, the significant difference in accretion of bone mineral content between the two groups disappeared when the data were adjusted for the weight gain difference, suggesting that the effect was largely caused by differences in bone matrix growth rate between the two groups.

CONCLUSIONS

Our study suggests that DXA is a useful technique for evaluating whole body and weight gain composition in preterm infants. The sensitivity for detecting significant within-group differences in a longitudinal study and between groups in a parallel study depends on the variance of the population and on the various indices assessed but appears to be relatively high, allowing comparison of various feeding regimens in preterm infants during the first weeks of life. However, owing to the low threshold level for bone detection, the bone mineral content measurement does not represent bone mineral mass exclusively and, therefore, cannot be directly converted to calcium content and accretion.

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