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Longitudinal effects of fat and lean mass on bone accrual in infants☆

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A B S T R A C T

There are conflicting reports on the influence of lean and fat mass on bone accrual during childhood. No infant's studies have been reported that describe the influence of changes in body composition with changes in bone accrual during the first year of life. The objective of this research was to test the hypothesis that greater gains in lean mass will have a positive effect on bone mineral content (BMC) accrual, while greater gains in fat mass will have a negative effect on BMC accrual in infants. Longitudinal data from 3 previous infant studies were used. Linear mixed models, adjusting for age, sex, dietary calcium, and length were used to investigate longitudinal and cross-sectional associations between total body BMC and lean and fat mass in the individual studies and in a combined analysis. In both individual and combined analyses, we found that lean and fat mass were positively associated with whole body BMC accrual (all, P<0.001). The cross-sectional association of BMC and dietary calcium was negative in one study (P<0.05). No differences in BMC change between sexes were observed in three studies. Our results showed positive cross-sectional and longitudinal associations between total body BMC and lean mass in infants. In contradiction to our hypothesis for fat mass, we found a positive cross-sectional and longitudinal association between total body BMC and fat mass in infants.

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Introduction

Bone mineral accrual in infants depends on several factors, including growth and mineral intake. Body weight is known to influence BMC in adults, children and infants, and numerous studies find the lean mass component of body weight to be positively associated with bone accrual [1–4], but the relationship between fat mass and bone accrual is less clear. In particular, there are several cross-sectional studies that report a positive association between total body fat mass and BMC [1–3,5,6] measured by dual energy X-ray absorptiometry (DXA) while other studies report negative associations between fat and BMC [7–9].

Longitudinal studies in young children and adolescents investigating the relationship between changes in body composition and bone accrual during growth are inconsistent. In a recent report based on children aged 8 to 12 years at baseline, changes in lean mass were positively related to changes in BMC, whereas changes in fat mass were inversely related to BMC change measured by DXA [4,10]. Similar results relating baseline fat mass to bone accrual from 3 to 7 years of age have been reported [11]. Using pQCT to measure radial bone accrual, changes in fat mass were negatively associated with BMC in males; the results for females were mixed because of menarcheal hormone changes [12]. In contrast, Clark et al. [10] found a strong positive association between fat mass and total body less head BMC in both boys and girls aged 9.9 years. No studies have reported on the association of longitudinal changes in total body lean and fat mass with bone accrual during the first year of life.

We previously conducted three different randomized trials that investigated the influence of different mineral intake (Study #1), timing of the introduction of solid foods (Study #2), and gross motor activity (Study #3) on bone and body composition changes during the first year of life. The purpose of the current research was to model the influence of changes in fat mass and lean mass on changes in total body BMC over the first year of life among infants who participated in these three studies. Our hypothesis was that greater gains in lean mass will have a positive effect on BMC accrual, while greater gains in fat mass will have a negative effect on BMC accrual, similar to what we [12] and others [4,12] found in older children.

Subjects and methods

All three of the studies were completed at Cincinnati Children’s Hospital Medical Center using similar procedures. Infant weight was measured with a digital scale, and crown-to-heel length was measured with a length board. Three-day diet records on the infants...
were obtained during the week preceding each visit, and nutrient content was computed using the Minnesota Nutrition Data System (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). Total body BMC, lean mass, and fat mass were measured using dual-energy X-ray, absorptiometry (QDR1000W; Hologic, Inc, Waltham, MA). The Hologic pediatric software (version 5.56) was used for the analysis of the scan results. The coefficient of variation, determined from duplicate scans on 17 infants 3 to 12 months of age was 4.5% for total body BMC.

**Study #1: varying mineral intake**

A total of 101 (55 females) white infants were enrolled. The overall purpose was to determine the influence of varying mineral intake during the first year of life on BMC accretion [13]. The study was a randomized trial conducted in two phases. In phase I, 67 subjects were randomized, after stratifying by sex, into one of two formula groups; a low mineral content formula or a moderate mineral content formula. In this phase, an additional 34 subjects whose mothers elected to breastfeed for approximately six months were included. The subjects were enrolled shortly after the birth and formula-feeding was begun preferably at discharge, but no later than 2 weeks of age. In phase II, all subjects were re-randomized to the type of feeding between 6 and 12 months of age: a cow-milk feeding group, formula feeding with a moderate mineral content formula, or a high mineral containing formula. Due to concerns regarding cow-milk feeding in infants less than 12 months of age, mothers or their pediatricians who were concerned about randomization to this group were assigned to the high mineral containing formula. Briefly, we found that during the first 6 months, significant differences among the feeding groups were observed in changes between 1 and 6 months of age in weight, length, and BMC. The moderate mineral content group had greater increases in weight and BMC compared to the human milk-fed group, and the low mineral feeding group was intermediate between the moderate mineral and breast-fed groups. During the second 6 months no significant differences were observed in growth parameters or BMC gain between the high and moderate mineral feeding groups; infants who received human milk during the first six months had greater BMC accretion between 6 and 12 months of age than the low or moderate-mineral formula groups. By 12 months of age there was no influence of early feeding on total body BMC. Total body BMC and body composition, weight, length, head circumference and dietary calcium intake were measured at 1, 3, 6, 9 and 12 months of age. Because we did not have dietary calcium intake for the breast-fed infants in this study, they were omitted from the current analysis.

**Study #2: introduction to solid foods**

A total of 174 (80 females) infants were enrolled in this study, which was designed to determine whether early versus late introduction of solid foods and commercially prepared versus parent's choice of solid foods affects growth or body composition in the first year of life [14]. This randomized factorial designed study was conducted on healthy infants from the Greater Cincinnati area. This study consists of 161 white infants, 8 black infants, 2 infants of other race, and 3 infants with no information on race. Infants were recruited before 3 months and were randomized to one of the four groups: commercially prepared solid foods from 3 to 12 months, commercially prepared solid foods from 6 to 12 months, parent's choice solid foods from 3 to 12 or parent's choice solid foods from 6 to 12 months. Infants were permitted to consume human milk prior to randomization at 3 months of age, but all infants received proprietary formula between 3 and 12 months of age. Total body BMC and body composition were measured at 3, 6 and 12 months of age. Infant weight, length, head circumference, and dietary calcium intake were measured at 3, 6, 9 and 12 months of age. Briefly, there were no significant differences in anthropometric or body composition measurements found between early versus late introduction group or commercial versus parent's choice group over the first year of life.

**Study #3: gross motor activity**

A total of 87 (45 females) infants were enrolled in a study designed to determine whether infants randomized to a 1-year gross motor activity program had a greater BMC accrual than infants randomized to a fine motor activity program [15]. This study included 72 white and 15 black infants. Infants were randomized at 6 months of age to either a gross motor or a fine motor activity program. Total body BMC and body composition, anthropometric measurements, 3-day diet records, and 48-h activity levels using miniature motion sensors were obtained at 6, 9, 12, 15 and 18 months of age. All infants were supplied with the same proprietary formula between 6 and 12 months of age. Briefly, a greater bone mass accretion was observed in infants with higher calcium intake, but there was no beneficial effect of gross motor activity on bone mass accretion. Calcium intake appeared to modify the effect of activity on bone accrual; there did not appear to be an effect of activity on bone accrual in infants consuming a moderately high calcium intake, whereas gross motor activity appeared to lead to a reduced bone accrual in infants consuming a moderately low calcium intake.

**Statistical analysis**

Data through 12 months of age from the three individual studies were analyzed using linear mixed effect models for longitudinal data [16]. We examined the association between changes in lean mass and fat mass with changes in BMC after controlling for other covariates (i.e., length, sex, age, dietary calcium intake). All the studies had baseline measurements; although, these occurred at different ages.

Total body BMC was the outcome variable and predictors included as fixed effects were fat mass (kg), length (m), dietary calcium intake (g/day), sex, lean mass (kg). Within each study, an indicator variable for the specific group assignment was included. Random intercept and slope for age in months were included in the model to account for within subject random variability. The PROC MIXED procedure in SAS® software (Statistical Analyses System software, version 9.2; SAS Institute, Cary, NC) was used to fit the model. For analysis, we converted the dependent variable BMC (g), lean and fat mass (g) to kilograms, age to months, length of the infant (cm) to meters and dietary calcium intake (mg/day) to grams/day. The bone outcome variable total body BMC is equated to the linear sum of baseline covariates to model cross-sectional effects, change covariates to model longitudinal effects, and random effects to model among and within random error. The coefficient for fat mass change is interpreted as the within-individual change in total body BMC in kilograms per 1 kg within-individual change in fat mass with adjustment for the follow-up time and other change covariates in the model.

We conducted analyses using either fat mass or percent fat as standardized variables, along with the other covariates. We found the standardized coefficient estimates to be similar, indicating that the strength of the effect of fat on bone does not depend on whether it is expressed in absolute terms as mass or relative terms as percent of body weight. Multicollinearity, a problem that occurs when two or more predictor variables in a statistical model are highly correlated and leads in possible errors in coefficient estimates, was checked by calculating the variance inflation factors (VIF) using repeated measures within the REG procedure in SAS. The highest VIF in all three studies was 2.4, indicating that multicollinearity among the predictor variables was not a problem.
The only significant treatment effect was in Study #1 where the formula group the infant was assigned to in the first 6 months had a significant effect on changes in total body BMC. Inclusion of treatment effect did not change the relevance of lean and fat mass to total body BMC accretion. For ease of presentation, we have omitted treatment groups from the presentation of final models given in Table 2.

Results

Descriptive statistics for the three studies by age are given in Table 1. Descriptive characteristics of the study populations by age. Data are mean±SD.

<table>
<thead>
<tr>
<th>Study</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>N=67</td>
<td>N=65</td>
<td>N=59</td>
<td>N=60</td>
<td>N=58</td>
</tr>
<tr>
<td>Age (months)</td>
<td>1.1±0.1</td>
<td>3.1±0.2</td>
<td>6.0±0.7</td>
<td>9.1±0.5</td>
<td>12.2±0.5</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>54.3±2.4</td>
<td>59.8±3.0</td>
<td>66.5±2.6</td>
<td>71.0±2.6</td>
<td>75.1±2.8</td>
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<tr>
<td>Lean mass (kg)</td>
<td>3.47±0.44</td>
<td>4.28±0.49</td>
<td>5.31±0.57</td>
<td>6.25±0.63</td>
<td>7.00±0.85</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>1.11±0.29</td>
<td>2.04±0.57</td>
<td>2.67±0.70</td>
<td>2.82±0.75</td>
<td>3.02±0.92</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>330±63</td>
<td>394±850</td>
<td>466±106</td>
<td>745±240</td>
<td>743±281</td>
</tr>
<tr>
<td>Study 2</td>
<td>N=173</td>
<td>N=163</td>
<td>N=156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>1.2±0.2</td>
<td>6.0±0.2</td>
<td>12.1±0.3</td>
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<td></td>
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<tr>
<td>Length (cm)</td>
<td>61.2±2.2</td>
<td>67.2±2.4</td>
<td>75.5±2.6</td>
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</tr>
<tr>
<td>Lean mass (kg)</td>
<td>4.29±0.40</td>
<td>5.18±0.55</td>
<td>6.96±0.76</td>
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<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>2.13±0.59</td>
<td>2.79±0.73</td>
<td>3.10±0.70</td>
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<tr>
<td>Calcium intake (mg/day)</td>
<td>472±114</td>
<td>569±136</td>
<td>789±308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 3</td>
<td>N=86</td>
<td>N=81</td>
<td>N=77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>6.1±0.3</td>
<td>9.1±0.2</td>
<td>12.3±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>67.0±2.4</td>
<td>71.5±2.6</td>
<td>75.3±2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>5.34±0.64</td>
<td>6.25±0.73</td>
<td>7.13±0.90</td>
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<tr>
<td>Fat mass (kg)</td>
<td>2.73±0.71</td>
<td>2.94±0.80</td>
<td>3.18±0.73</td>
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<tr>
<td>Calcium intake (mg/day)</td>
<td>524±111</td>
<td>508±143</td>
<td>669±234</td>
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</table>

Discussion

In this paper we examined the cross-sectional and longitudinal associations of BMC accrual in infants during the first year of life in relation to changes in fat and lean mass. We are unaware of any study to date that has investigated longitudinal relationships between BMC accrual and lean or fat mass in infants. Our results showed positive associations between total body BMC and lean mass in infants from both cross-sectional and longitudinal perspective, which supports our hypothesis. We also observed a positive cross-sectional and longitudinal association between total body BMC and fat mass in infants, which contradicts our hypothesis.

Our findings of a relationship between BMC change and lean mass are consistent with previous cross-sectional DXA studies [1,5,17]. Crabtree et al., in a cross-sectional study, found that lean mass was the strongest predictor of total body and spine BMC in chronic disease children aged 5 to 18 years [2]. On the other hand, the association between fat mass and BMC is not uniform among different cross-sectional studies [1,5,6,8,9,17]. Frost’s mecanostat theory provides a theoretical mechanism for a lean mass effect on bone that includes...
It is possible that higher body fat in studies of older children is a reflection of inactivity, and inactivity and decreased bone loading have been shown to be associated with lower BMC accrual [20–24]. In infants, a higher body fat may not necessarily translate into a decrease in bone loading and thus, an inverse relationship between BMC accrual and fat mass would not be expected.

Burrows et al. investigated bone accrual across 7 years of growth and reported a positive relationship between BMC accrual and lean mass, and a negative relationship between BMC accrual and fat mass of children aged from 8 to 12 years [4]. Previous studies among children and adults have found a stronger association of bone with lean mass than with fat mass [6,12,17,25,26]. However, we did not find major differences in the magnitude of lean and fat mass cross-sectional and longitudinal associations with total body BMC in infants (Table 2). There are a number of mechanisms by which fat mass may influence bone, including subsequent bone loading due to increased body weight, or through indirect systemic pathways that involve growth hormone, insulin-like growth factor (IGF-1), or leptin. These hormones have a significant influence on growth and body composition and both cord concentrations of IGF-1 and leptin have been shown to be associated with birth length and weight [27]. Studies in piglets have shown that leptin

### Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Longitudinal effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>0.004 ± 0.001*</td>
<td>0.005 ± 0.001*</td>
<td>0.004 ± 0.001*</td>
</tr>
<tr>
<td>Length (m)</td>
<td>−0.007 ± 0.044</td>
<td>0.018 ± 0.050</td>
<td>−0.119 ± 0.067</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>0.012 ± 0.006*</td>
<td>0.004 ± 0.005</td>
<td>0.004 ± 0.005</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.015 ± 0.002*</td>
<td>0.018 ± 0.002*</td>
<td>0.011 ± 0.005*</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.018 ± 0.003*</td>
<td>0.018 ± 0.002*</td>
<td>0.021 ± 0.003*</td>
</tr>
<tr>
<td><strong>Cross-sectional effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>−0.016 ± 0.010</td>
<td>0.001 ± 0.003</td>
<td>−0.007 ± 0.005</td>
</tr>
<tr>
<td>Length (m)</td>
<td>0.010 ± 0.058</td>
<td>0.035 ± 0.045</td>
<td>0.026 ± 0.082</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>0.055 ± 0.022*</td>
<td>−0.029 ± 0.006*</td>
<td>−0.011 ± 0.015</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.029 ± 0.005*</td>
<td>0.025 ± 0.001*</td>
<td>0.025 ± 0.002*</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.020 ± 0.003*</td>
<td>0.017 ± 0.003*</td>
<td>0.017 ± 0.003*</td>
</tr>
<tr>
<td>Sex (F)</td>
<td>−0.003 ± 0.002</td>
<td>0.003 ± 0.002</td>
<td>0.003 ± 0.003</td>
</tr>
</tbody>
</table>

Model estimates are for predicting total body BMC in kg and are adjusted for age, length, calcium intake, fat mass, lean mass and sex.

### Table 3

<table>
<thead>
<tr>
<th>Variables</th>
<th>BMC</th>
<th>Bone area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Longitudinal effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>0.005 ± 0.001*</td>
<td>0.004 ± 0.001*</td>
</tr>
<tr>
<td>Length (m)</td>
<td>−0.034 ± 0.028</td>
<td>0.261 ± 0.056*</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>0.006 ± 0.003*</td>
<td>0.001 ± 0.005</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.018 ± 0.001*</td>
<td>0.039 ± 0.002*</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.018 ± 0.001*</td>
<td>0.056 ± 0.003*</td>
</tr>
<tr>
<td>Study (1)</td>
<td>−0.014 ± 0.014</td>
<td></td>
</tr>
<tr>
<td>Study (2)</td>
<td>−0.012 ± 0.008</td>
<td>−0.019 ± 0.017</td>
</tr>
</tbody>
</table>

Model estimates are for predicting total body BMC in kg and bone area (in cm²) and are adjusted for age, length, calcium intake, fat mass, lean mass, study and sex.

### Table 4

<table>
<thead>
<tr>
<th>Variables</th>
<th>BMC</th>
<th>Bone area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cross-sectional effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>−0.003 ± 0.003</td>
<td>0.001 ± 0.006</td>
</tr>
<tr>
<td>Length (m)</td>
<td>0.039 ± 0.035</td>
<td>0.304 ± 0.069*</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>−0.012 ± 0.006*</td>
<td>−0.013 ± 0.012</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.024 ± 0.001*</td>
<td>0.042 ± 0.002*</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.018 ± 0.002*</td>
<td>0.054 ± 0.004*</td>
</tr>
<tr>
<td>Sex (F)</td>
<td>−0.001 ± 0.001</td>
<td>0.005 ± 0.003</td>
</tr>
</tbody>
</table>

Model estimates are for predicting total body BMC in kg and bone area (in cm²) and are adjusted for age, length, calcium intake, fat mass, lean mass, study and sex.

### Fig. 1

a: Spaghetti plot of total body BMC (kg) versus age (months) for infants in all the three studies. b: Spaghetti plot of fat mass (kg) versus age in months for infants in all the three studies. c: Spaghetti plot of lean mass (kg) versus age in months for infants in all the three studies.
concentrations influence both fat and bone mass and are elevated early in life [28].

Although other investigators have found a greater contribution of lean mass on bone than fat mass, it is possible that these previous observations are a result of long-term muscle contractions on bone and loading that result when in an upright position. Muscle contractions in infants are thought to be associated with BMC accrual [15], and some individuals have proposed that fetal movements are associated with early BMC [29].

Calcium intake was associated with increased BMC accrual both as cross-sectional and longitudinal effects in the one study that included a wide range of calcium intake by design (Study #1). This is not surprising since the purpose of this study was to determine the effect of formulas with varying mineral content on total body bone mass accretion, whereas in the other two studies the infants were provided with the same proprietary formulas in order to minimize differences in calcium intake. Therefore, the range in calcium intakes was larger in Study #1 than it was in the other two studies. One of the other studies found a negative cross-sectional association between calcium intake and BMC accrual. The relationship between calcium intake and BMC accrual can be confounded by body size since larger infants typically have greater calcium intake and the inclusion of body size parameters (length, mass) into the statistical model may over control for these effects, resulting in what appears to be a negative relationship. This in fact was the case with Study #2, since removing length and mass from the statistical model lead to a non-significant effect of calcium intake on BMC accrual. The combined model found a positive longitudinal relationship between dietary calcium and BMC accrual, although this effect is relatively small compared to the influence on lean and fat mass.

In summary, we found positive cross-sectional and longitudinal associations between total body BMC and lean and fat mass in infants. The positive effect of fat mass on bone accrual in infants is not consistent with the effects at older ages. In particular, the role of fat mass, which has been found to be detrimental to bone accrual later in childhood, does not adversely influence bone accrual during infancy.

Acknowledgments

We would like to acknowledge the families who participated in this research.

References