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Context: Cross-sectional associations for lean mass (LM) and fat mass (FM) with bone may not reflect longitudinal associations.

Objective: Cross-sectional and longitudinal associations of LM and FM with radial bone measurements in children were compared.

Design and Participants: We conducted a longitudinal study on 370 (232 females) children, 8–18 yr of age.

Main Outcome Measures: LM and FM were measured by dual-energy absorptiometry. Peripheral quantitative computed tomography at the 4% radius (4R) and 20% radius (20R) measured bone mineral content (BMC), volumetric bone mineral density (vBMD), area, and strength [polar stress strain index (pSSI)].

Results: Males at 20R had negative FM cross-sectional and longitudinal associations with cortical area and BMC and pSSI ($P < 0.02$); negative cross-sectional association with total area ($P < 0.001$); and negative longitudinal association with cortical thickness ($P < 0.001$). Females at 20R had FM cross-sectional association with total area, cortical BMC, and pSSI and longitudinal associations with cortical BMC and area, vBMD, and pSSI that went from positive to negative with age and, in some cases, varied with menarche. Both sexes at 4R had a negative FM cross-sectional association with BMC and area ($P < 0.001$) but negative longitudinal association with vBMD ($P < 0.05$). LM associations with bone outcomes were generally positive, except for negative longitudinal associations with cortical BMC and vBMD in young females ($P < 0.01$). LM associations were greater magnitude than FM associations and often depended on age.

Conclusions: For males and older females, cross-sectional associations indicated a reduced bone size with higher FM, whereas longitudinal associations showed a decrease in cortical area without changes in bone size. LM was positively associated with BMC and area. (J Clin Endocrinol Metab 96: 106–114, 2011)

Peak bone mass attained during late adolescence/early adulthood is thought to influence adult fracture risk and osteoporosis risk (1, 2). Despite heredity accounting for a large proportion of variation in bone mineral content (BMC) and bone mineral density (BMD) measurements (3, 4), lifestyle factors that influence bone mass change during childhood and adolescent growth can exert a significant influence on peak bone mass. Because of the potential adverse effect of obesity on health outcomes and the high obesity prevalence (5, 6), numerous studies have addressed the role of body fat on pediatric bone health with conflicting results (7–14).

Cross-sectional studies using dual-energy x-ray absorptiometry (DXA) to measure bone outcomes and lean mass...
(LM) and fat mass (FM) in children and adolescents have resulted in both positive (7, 11, 15) and negative (12, 13, 16, 17) associations between FM and bone. DXA bone measures are based on a two-dimensional image that precludes a direct measure of volumetric BMD (vBMD) and bone circumference. In contrast, peripheral quantitative computed tomography (pQCT) is a three-dimensional imaging method and allows for measurement of vBMD, bone geometry, and estimates of bone strength. Two cross-sectional pQCT studies in young adults (9, 18) reported negative associations of FM with cortical area at both the radius and tibia, but positive cross-sectional associations at the tibia also have been reported (10, 19). Cross-sectional studies in adolescents have consistently shown LM has a positive association with bone outcomes that is greater in magnitude than FM associations (8, 10, 11, 14).

Changes in a bone measure over time, inferred from a cross-sectional association across individuals of different ages, may not accurately reflect what occurs over time in an individual. Indeed, Clark et al. (7) found positive cross-sectional, but negative longitudinal, associations between FM and DXA-measured total body BMC and bone area for young girls. Other longitudinal DXA studies of bone accrual during growth suggest that weight gain in the form of FM has negative effects on hip geometry (20) and height-adjusted BMD (21). To our knowledge, there are no reports addressing the role of FM on pQCT-measured bone parameters in adolescents using a longitudinal design.

We recently reported on the cross-sectional association between FM and DXA bone measures in Hutterite children (16). The Hutterites are a culturally and genetically homogeneous people that live communally on colonies. Studies in this population have the advantage that the potential confounding influence of race and socioeconomic status-related factors are minimized (7, 22, 23). The objective of this study was to investigate longitudinal associations between FM and pQCT bone measurements in Hutterite children and adolescents.

**Subjects and Methods**

**Participants**

A convenience sample of Hutterite children aged 8–18 yr were recruited from 16 colonies in eastern South Dakota starting in 2001. Measurements at the 4% radius (4R) and 20% radius (20R) were available on 380 subjects (237 females) and 373 subjects (234 females), respectively. Individuals with a history of a medical condition or medication known to affect bone metabolism were excluded (two males with type 1 diabetes, two females who were lactating, and one female with amenorrhea). Bone measurements were obtained at 18-month intervals for up to 90 months in this ongoing observational study; this report is for the first 3 yr of follow-up. In some cases, DXA or pQCT measurements were missing at baseline, but data for later visits were available; then the timing for the DXA or pQCT measures and covariate data were shifted so that 18-month measurements became baseline, the 36-month became the 18-month measurements, and if available, the 54-month became the 36-month measurements. This allowed maximum use of data but resulted in slightly different datasets for 4R and 20R outcomes. We also required that complete covariate data (LM, FM, height, and physical activity) be available for a visit. Accordingly, the analysis included 370 subjects (233 females) and 363 subjects (230 females) for the 4R and 20R.

Questionnaires were completed by each child with the help of the mother and study personnel and included medical history, medication use, and menstrual status. Verbal consent to participate was obtained from the minister of each colony, written consent from each participant or their parent, and assent was obtained from all children. The protocol was approved by the SDSU Human Subjects Committee.

**Bone, body composition, and anthropometric measurements**

Height without shoes was measured to the nearest 0.5 cm, and weight in light clothing was measured to the nearest 0.1 kg. Total body LM and FM were measured using DXA (Hologic QDR Discovery, software version 12.3; Hologic Inc., Waltham, MA). The coefficients of variation were 0.6% for LM and 1.8% for FM based on 15 subjects aged 7–17 yr. pQCT measurements of the left 4R and 20R were obtained using a Norland-Stratec XCT2000 densitometer (Pforzheim, Germany) using previously described methods (24). Cortical vBMD at the 4R site was not determined due to partial volume effects (25). The indicator of bending strength, polar stress strain index (pSSI), was estimated at the 20R using previously described methods (24). Coefficients of variation from duplicate scans obtained on nine adults after repositioning with a scout view were 0.5, 1.2, and 0.5% for cortical vBMD, cortical thickness, and total area, respectively, at the 20R site and 2.6 and 2.4% for total area and vBMD, respectively, at the 4R site.

**Physical activity measurement**

Physical activity was estimated from a 7-d physical activity recall and was obtained quarterly (26). The 7-d physical activity recall estimates the amount of time spent sleeping, sitting, or participating in moderate or vigorous activity. The remaining time was classified as light activity. Activity patterns for both weekdays and weekend days were included. Baseline average percentage of time in moderate plus vigorous activity per day was based on the mean of measurements at 0, 3, and 6 months; 18-month estimates were based on the mean of measurements at 0, 3, and 6 months; and 36-month estimates were based on the mean of measurements at 0, 3, and 6 months.

**Statistical analysis**

Analyses were performed separately for males and females. The independence of categorical variables was evaluated using a χ² test, and continuous variables were evaluated using t test or ANOVA. The association of LM or FM with change in bone outcomes was evaluated using a linear mixed-effects model approach that allows simultaneous evaluation of cross-sectional and longitudinal effects (27, 28). Analyses were performed using variables transformed to SD units; analyses using nonstandardized variables yielded identical results. This approach models random error among and within individuals and thus accounts
for correlated repeated measures within an individual. Each bone outcome of interest 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 change covariates are calculated as the difference of a follow-up measurement for a predictor from the corresponding baseline measurement. The standardized coefficient for FM change is interpreted as the within-individual change in BMC in SD units per one SD within-individual change in FM with adjustment for the follow-up time and other change covariates in a model. Note that change in a bone measure (dependent variable) is estimated using a value for the baseline bone measure based on a linear fit to the baseline predictors. This allows simultaneous evaluation of cross-sectional and longitudinal effects as well as adjustment for baseline covariates.

For each bone outcome, the mixed model included fixed terms for age, height, LM, FM, and physical activity as both a baseline covariate and as a change covariate; the model also included a random term for the intercept. For all but a few bone outcomes, the inclusion of other random terms for time-varying covariates was deemed unnecessary based on comparison of nested models using a likelihood ratio test (28). For consistency, the intercept was the only random effect included in models. Interactions with age for both genders and menarche for females were evaluated and retained only random effect included in models. Interactions with age for both genders, there was no difference among measurement times in the distribution of sample size across five baseline age categories shown in Tables 1 and 2 ($\chi^2$ analysis, $P > 0.05$; data not shown). In addition, there was no difference in mean gender- and age-specific FM at baseline for subjects with complete data compared with subjects with missing data at 18 or 36 months (ANOVA, $P > 0.05$; data not shown).

### Results

#### Population

Descriptive statistics at baseline for body composition, physical activity, and pQCT bone measures are given in Tables 1 (females) and 2 (males). As reported previously (16), this Hutterite population is lighter and shorter and has a lower mean body mass index than other South Dakota children residing in the same counties and lower prevalence of obesity (data not shown) than U.S. non-Hispanic White children (29). Follow-up at the 20R was 73% at 18 months and 60% at 36 months, and follow-up at the 4R was 76% at 18 months and 63% at 36 months. For both genders, there was no difference among measurement times in the distribution of sample size across five baseline age categories shown in Tables 1 and 2 ($\chi^2$ analysis, $P > 0.05$; data not shown). In addition, there was no difference in mean gender- and age-specific FM at baseline for subjects with complete data compared with subjects with missing data at 18 or 36 months (ANOVA, $P > 0.05$; data not shown).

#### Cross-sectional associations of bone measures with LM and FM

In males at the 20R (Fig. 1), LM had a positive association with total and cortical area, thickness, BMC, and pSSI (all $P < 0.001$) but not cortical vBMD. For cortical BMC, an interaction of LM with age ($P < 0.01$) resulted in a greater effect at older ages. In contrast, FM in males was negatively associated with total and cortical area, BMC, and pSSI (all $P < 0.001$) but not cortical thickness and vBMD. There was an interaction of FM with age on cortical BMC ($P < 0.05$) such that a negative effect was evident only at older ages (13–17 yr; Fig. 1). At the 4R, there was a positive association of LM with total BMC, vBMD (both $P < 0.001$), and area, but for area, the association was attenuated at older ages ($P < 0.05$ for interaction). FM was negatively associated with 4R total

### TABLE 1. Baseline characteristics by age for females

<table>
<thead>
<tr>
<th>Age Group</th>
<th>n</th>
<th>Postmenarcheal (%)</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>LM (kg)</th>
<th>FM (kg)</th>
<th>BMI (kg/m²)</th>
<th>Physical activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8–10 yr</td>
<td>55</td>
<td>0</td>
<td>8.9 ± 0.5</td>
<td>133.4 ± 6.0</td>
<td>22.2 ± 3.3</td>
<td>7.2 ± 3.3</td>
<td>16.9 ± 2.5</td>
<td>17.7 ± 11.2</td>
</tr>
<tr>
<td>10–12 yr</td>
<td>39</td>
<td>3</td>
<td>11.0 ± 0.6</td>
<td>147.0 ± 8.8</td>
<td>30.0 ± 5.5</td>
<td>10.5 ± 5.5</td>
<td>18.9 ± 3.2</td>
<td>13.4 ± 7.3</td>
</tr>
<tr>
<td>12–14 yr</td>
<td>28</td>
<td>5</td>
<td>12.8 ± 0.6</td>
<td>156.2 ± 7.1</td>
<td>35.7 ± 5.4</td>
<td>11.1 ± 3.7</td>
<td>19.6 ± 2.6</td>
<td>13.8 ± 6.2</td>
</tr>
<tr>
<td>14–16 yr</td>
<td>36</td>
<td>67</td>
<td>15.0 ± 0.6</td>
<td>160.8 ± 4.2</td>
<td>39.1 ± 3.9</td>
<td>15.1 ± 5.2</td>
<td>21.4 ± 3.1</td>
<td>17.0 ± 7.3</td>
</tr>
<tr>
<td>≥16 yr</td>
<td>76</td>
<td>100</td>
<td>17.4 ± 1.0</td>
<td>161.9 ± 4.9</td>
<td>41.2 ± 4.3</td>
<td>16.4 ± 6.2</td>
<td>22.5 ± 3.3</td>
<td>20.4 ± 7.2</td>
</tr>
</tbody>
</table>

For each bone outcome, the mixed model included fixed terms for age, height, LM, FM, and physical activity as both a baseline covariate and as a change covariate; the model also included a random term for the intercept. For all but a few bone outcomes, the inclusion of other random terms for time-varying covariates was deemed unnecessary based on comparison of nested models using a likelihood ratio test (28). For consistency, the intercept was the only random effect included in models. Interactions with age for both genders and menarche for females were evaluated and retained in models when significant ($P < 0.05$). Analyses were performed using SAS software (SAS Institute, Inc., Cary, NC).

Values are mean ± sd.
area and BMC (both $P < 0.001$), but there was no association with vBMD. In females, associations of LM with bone measures at the 4R and 20R were similar to males, except there was no association with total vBMD at the 4R and a greater positive effect on pSSI after menarche than before menarche (Fig. 1). The associations of FM with bone measures at the 4R and 20R also were similar to males, except total area and pSSI (20R), where higher FM at older ages exerted a negative influence, and total area (20R), where FM postmenarcheal exerted a positive influence (all $P < 0.05$ for interactions). In general, the magnitudes of the associations with LM were greater than those for FM.

**Longitudinal associations of bone measures with LM and FM**

Age-specific mean changes in LM, FM, and bone measures were estimated from the difference of the 18-month and baseline visits (Table 3) and were not different (all $P > 0.05$) than mean 18-month change estimated using 36-month data, except female 4R total area and LM for 10- to 12-yr-olds (data not shown). In these two cases the second 18-month means were lower. Most changes are greater than zero in growing subjects, but a notable exception is a negative change in 4R total area ($P < 0.05$) for older adolescents ($\geq 16$ yr). For longitudinal associations at the 20R in males, LM was positively associated with total area ($P < 0.001$) and cortical thickness ($P < 0.05$) but not cortical vBMD (Fig. 2). The associa-

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**FIG. 1.** Cross-sectional associations of LM and FM with pQCT bone measurements. Standardized coefficient estimates for all ages (horizontal spike is 95% confidence limit) are given for females (upper panel) and males (lower panel). For a significant interaction ($P < 0.05$) with baseline age, estimates are given for ages 9 ( ), 13.5 ( ), and 17 ( ) years. For a significant interaction with menarche, estimates are given for before ( ) and after ( ) menarche; when there is significant interaction ($P < 0.05$) with both age and menarche, the estimate for 9 yr is before menarche, the estimate for 17 yr is after menarche, and estimates for 13.5 yr are for both before ( ) and after ( ) menarche. A separate model is fit for each bone outcome; including baseline age and height, physical activity, and LM and FM (and menarcheal status for females). A standardized coefficient greater than zero indicates a positive association between a bone outcome and covariate; a coefficient less than zero indicates a negative association. Thk, Thickness.
TABLE 3. The 18-month change from baseline for body composition and bone outcomes

<table>
<thead>
<tr>
<th></th>
<th>8–10 yr</th>
<th>10–12 yr</th>
<th>12–14 yr</th>
<th>14–16 yr</th>
<th>≥16 yr</th>
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</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>42</td>
<td>28</td>
<td>20</td>
<td>29</td>
<td>59</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>5.2 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>FM (kg)</td>
<td>2.2 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>20R</td>
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<td>n</td>
<td>36</td>
<td>28</td>
<td>20</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>Total area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>10.8 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0 ± 7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 7.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.4 ± 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortical area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>6.3 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cortical thickness (mm)</td>
<td>0.14 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cortical BMC (mg/mm)</td>
<td>6.4 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.5 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9 ± 2.6&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cortical vBMD (mg/cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>-1.2 ± 30.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.3 ± 32.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.2 ± 18.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.2 ± 25.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7 ± 20.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>pSSI (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>21.7 ± 9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.2 ± 15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.7 ± 17.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8 ± 12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1 ± 15.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td><strong>Males</strong></td>
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<td>n</td>
<td>21</td>
<td>21</td>
<td>16</td>
<td>20</td>
<td>28</td>
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<tr>
<td>LM (kg)</td>
<td>4.5 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>FM (kg)</td>
<td>1.8 ± 1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.1 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.2 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>pQCT, 20R</td>
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<td>n</td>
<td>20</td>
<td>16</td>
<td>16</td>
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<td>28</td>
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<tr>
<td>Total area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>10.6 ± 9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.3 ± 8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.9 ± 12.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.6 ± 11.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortical area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>4.8 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.9 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Cortical thickness (mm)</td>
<td>0.07 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cortical BMC (mg/mm)</td>
<td>5.5 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.7 ± 7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0 ± 7.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.9 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Cortical vBMD (mg/cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>12.6 ± 34.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.2 ± 24.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.3 ± 26.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.2 ± 29.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.7 ± 23.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>pSSI (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>23.6 ± 12.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.7 ± 17.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.0 ± 35.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.6 ± 31.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.3 ± 24.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>pQCT, 4R</td>
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<td>n</td>
<td>21</td>
<td>21</td>
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<td>27</td>
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<tr>
<td>Total BMC (mg/mm)</td>
<td>9.4 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 16.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.9 ± 12.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3 ± 9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total vBMD (mg/cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>-10.9 ± 26.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-4.7 ± 18.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3 ± 15.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.6 ± 25.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.3 ± 16.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>39.3 ± 31.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.8 ± 49.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.1 ± 38.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 40.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-7.5 ± 16.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01.
<sup>b</sup> P < 0.05. Means that are significantly less than zero are in bold.
<sup>c</sup> Means that are not significantly different from zero (P > 0.05).

...tions of LM with pSSI, cortical BMC, and area were dependent on age with a larger positive effect at older ages (all P = 0.01 for interaction). For FM, a negative association with cortical area, thickness, BMC (all P < 0.001), and pSSI (P < 0.02) was observed. At the 4R, there was a positive association of LM with total area, BMC, and vBMD (all P < 0.01). For FM, a significant negative association with total vBMD (P < 0.05) was observed.

For females at the 20R, there was a positive association between LM and cortical area (P < 0.001), and with total area and pSSI after menarche (P < 0.01). In contrast, a negative association of LM and cortical BMC and vBMD was observed for young females (both P < 0.01) and became positive for older females (P < 0.001 for interaction). The effect of FM on several measures at the 20R for females was modified in opposing directions by age and menarche. Young females had positive associations with cortical BMC (P = 0.07), vBMD (P < 0.05), and pSSI (P < 0.01) that became negative at older ages (cortical BMC at 13 yr, P < 0.01; vBMD at 17 yr, P = 0.05; pSSI at 17 yr, P < 0.08; all P < 0.05 for interaction with age). In contrast to age, menarche had a positive influence on FM associations with cortical area, BMC, and vBMD (all P < 0.01 for interaction). The modifying influences of age (P < 0.05 for interaction) and menarche (P < 0.001) resulted in a negative association of FM with cortical area that was observed only for older premenarcheal females (P < 0.05) and the oldest females (P < 0.05 for 18 yr). For cortical thickness, the association with FM was negative before menarche vs. positive after menarche (P = 0.05 for interaction) but was weak in both cases. At the 4R in females, LM was positively associated with total area and BMC (both P < 0.001). FM was negatively associated with total BMC and vBMD before menarche (both P < 0.01) but not after menarche. As with the cross-sectional associations, the magnitudes of the associations with LM were greater than those for FM.
Influence of physical activity on LM and FM associations

Inclusion of physical activity in models did not affect the significance of main associations of LM or FM, except male 4R total BMC for which change in FM became non-significant ($P < 0.07$). However, several interactions of age with LM or FM became nonsignificant ($P < 0.05$) with activity in the model (male longitudinal: age-by-LM with 20R total area and 4R BMC; female longitudinal: age-by-LM with 20R cortical area and age-by-FM with 4R vBMD; female cross-sectional: age-by-LM with 20R cortical area and menarche-by-LM with 20R cortical area).

Discussion

These data describe similarities and differences in cross-sectional and longitudinal relationships of pQCT-measured bone characteristics with LM and FM in children, ages 8–18 yr. The longitudinal associations reflect relationships within individuals over 18–36 months. Cross-sectional associations are relationships across individuals at a point in time and thus do not account for duration of exposure to LM or FM. If short-term effects represented by longitudinal associations do not change with time (i.e. with age), then cross-sectional associations should be similar to longitudinal associations as effects accumulate. If there are other modifying influences such as puberty or an adaptive response to a high LM or FM environment, then cross-sectional associations may differ from longitudinal associations. For both genders, we found strong positive cross-sectional associations of radial bone outcomes with LM that were similar to longitudinal associations, except for a negative longitudinal association of LM with cortical BMC and vBMD for young premenarcheal females. For FM, the pattern of cross-sectional associations did not match longitudinal associations, and for females, the associations were modified by opposing effects of age and menarche, with increasing age pushing in a negative direction and menarche pushing in a positive direction. In males, FM had a negative association, both longitudinal and cross-sectional, with cortical area, but a negative association with total area only cross-sectionally. This suggests that FM-associated smaller bones occur only after relatively long-term exposures to FM gains. A similar profile of changes in females was found only for older ages and was opposed by menarche.

We know of no other longitudinal studies in adolescents of LM and FM associations with radial bone outcomes. A number of cross-sectional studies have found positive associations between LM or muscle cross-sectional area and radial (30) or tibial (8, 10, 31) cortical bone area for both adolescent males and females. The negative longitudinal association of LM with cortical BMC and cortical vBMD in young females that became positive in older females suggests LM-associated increases in cortical bone size precede increases in cortical BMC. This is consistent with results from a longitudinal study where tibia area was increasing while vBMD was decreasing 3 yr before menarche (32). Also in a cross-sectional study at the 65% radius, muscle area was negatively associated with vBMD in prepubertal girls but not early-puberty girls (8). It may be that among young females of the same age, those with high LM are at a more advanced pubertal stage and have been exposed to high LM longer. Previous cross-sectional studies have found FM to be negatively associ-
lated with cortical area in a population of older (average 18 yr) adolescent females (9) and a population of 25- to 45-yr-old males (18); however, no association was found for a population of young (average 19 yr) adult males (19). Similar to our findings, Taes et al. (18) reported a negative association between FM and periosteal circumference and no association of FM with cortical thickness at the radius. Cross-sectional studies of cortical bone (at the tibia) indicate that FM has a positive association with cortical area (10, 19), but one study found a negative association of FM with cortical area, thickness, and periosteal circumference (18). Because of greater loads at the tibia, these results may not be comparable with the radius. Furthermore, it is possible that response may vary by different regions on the same bone (33).

Our results for cortical bone at the 20R for males indicate that greater positive longitudinal change in FM is associated with a reduction in cortical area, but no association with total cross-sectional bone size. This was accompanied by smaller cortical thickness and no change in vBMD. Older females followed a similar pattern showing no association with total bone size, but a negative association with cortical area was weaker, and there was no association with cortical thickness, possibly because of the influence of estrogen during puberty. In contrast, both males and females with higher FM (cross-sectional association) have smaller bones but similar cortical thickness. This difference in longitudinal vs. cross-sectional results may be explained as an adaptation to maintain bone strength in response to long-term gain in FM and is best illustrated by the result for males. Evidence that high-fat children in a cross-sectional sample have a long history of above average fat gain comes from a 7-yr longitudinal study in children (10–13 yr at baseline) in which 79% of children in the highest quartile of FM at baseline remained at that level after 7 yr (34). If the longitudinal effect of greater FM gain persists over time, then cortical thickness relative to periosteal circumference should be too small to provide adequate strength. Thus, it is possible that the cross-sectional finding of reduced bone size is the result of an adaptive response to maintain bone strength in response to long-term gain in FM, which could be accomplished by a reduction in periosteal expansion and an increase in endosteal apposition (Fig. 3, based on male data). Support for this explanation comes from a longitudinal study in which DXA was used to follow changes at the proximal femoral shaft in young adult females (17–22 yr) who over 6 yr were consistent weight gainer or had stable weight (20). Females in the weight gain group had a stable LM over the 6 yr, and weight change was due to increased FM; this group had contraction of endosteal diameter and increase in cortical thickness compared with stable-weight females. If an adaptation occurs, it may be incomplete because FM had a negative cross-sectional association with the bone strength measure pSSI in males and older females. The mechanostat theory provides a mechanism for a LM effect on bone that involves a muscle-bone unit and skeletal adaptation to mechanical loads (35, 36). Mechanisms for FM effects on bone include both direct local and indirect systemic pathways (37), and there is evidence for a number of possible mechanisms, including leptin-mediated changes and interactions with sex hormones, that could mediate a negative influence of FM on bone (18 for review).

The lack of pubertal status for males was a limitation to our study. To some extent, age-dependent changes in bone include some influence of puberty. There is evidence that the association of FM and bone outcomes in boys does not interact with pubertal status (7); however, the interaction may depend on the particular bone measure. Thus, we cannot rule out interactions or confounding with puberty. Another limitation is that incomplete data due to missing measurements may introduce bias. However, missing data was largely due to incomplete follow-up, and age-specific sample size and baseline mean FM was not different between missing and nonmissing data. Finally, assigning a causal relationship to the association between within-individual FM changes and bone changes is difficult because
all longitudinal changes are occurring over the same period. Nonetheless, longitudinal associations provide unique information that is different from that provided by the cross-sectional associations. We speculate that cross-sectional associations represent the influence of long-term effects of increasing FM but are unable to precisely quantify long term, except that it is greater than 18–36 months. The current study has additional follow-up measurements planned that may allow a better evaluation of this process. The longitudinal design was a strength of the study, as was the relative homogeneity of the Hutterite population. This minimizes the influence of variation in genetic background and lifestyle factors such as socioeconomic status, although this may affect generalizability of results.

In conclusion, we confirmed negative effects of FM on bone outcomes that have been based on cross-sectional study designs and additionally find individual FM gain is negatively associated with bone outcomes in adolescents. The longitudinal associations present, at least in the radius, a pattern that is distinct from cross-sectional associations. We speculate that the longitudinal associations represent short-term influences of fat on bone that over a longer period of time in a high-fat environment would lead to an adaptive response that is represented by the cross-sectional associations.

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