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Relationship of total body fat mass to weight-bearing bone volumetric density, geometry, and strength in young girls

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Abstract

Understanding the influence of total body fat mass (TBFM) on bone during the peri-pubertal years is critical for the development of future interventions aimed at improving bone strength and reducing fracture risk. Thus, we evaluated the relationship of TBFM to volumetric bone mineral density (vBMD), geometry, and strength at metaphyseal and diaphyseal sites of the femur and tibia of young girls. Data from 396 girls aged 8–13 years from the “Jump-In: Building Better Bones” study were analyzed. Bone parameters were assessed using peripheral quantitative computed tomography (pQCT) at the 4% and 20% distal femur and 4% and 66% distal tibia of the non-dominant leg. Bone parameters at the 4% sites included trabecular vBMD, periosteal circumference, and bone strength index (BSI), while at the 20% femur and 66% tibia, parameters included cortical vBMD, periosteal circumference, and strength-strain index (SSI). Multiple linear regression analyses were used to assess associations between bone parameters and TBFM, controlling for muscle cross-sectional area (MCSA). Regression analyses were then repeated with maturity, bone length, physical activity, and ethnicity as additional covariates. Analysis of covariance (ANCOVA) was used to compare bone parameters among tertiles of TBFM. In regression models with TBFM and MCSA, associations between TBFM and bone parameters at all sites were not significant. TBFM explained very little variance in all bone parameters (0.2–2.3%). In contrast, MCSA was strongly related ($p < 0.001$) to all bone parameters, except cortical vBMD. The addition of maturity, bone length, physical activity, and ethnicity did not alter the relationship between TBFM and bone parameters. With bone parameters expressed relative to total body mass, ANCOVA showed that all outcomes were significantly ($p < 0.001$) greater in the lowest compared to the middle and highest tertiles of TBFM. Although TBFM is correlated with femur and tibia vBMD, periosteal circumference, and strength in young girls, this relationship is significantly attenuated after adjustment for MCSA. Nevertheless, girls with higher TBFM relative to body mass have markedly diminished vBMD, geometry, and bone strength at metaphyseal and diaphyseal sites of the femur and tibia.

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Keywords

Adiposity; Girls; Bone strength; Volumetric bone mineral density (vBMD); Peripheral quantitative computed tomography (pQCT)

Introduction

Recent studies have shown fracture incidence increases from 16% in normal weight children to 33% in overweight and obese children [1]. Given that obesity prevalence in children and adolescents has more than doubled over the past three decades [2], it is more important than ever to understand the influence of total body fat mass (TBFM) on the growing skeleton. To date, studies of adiposity and bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm²) in youth have given conflicting results [3–17]; studies have suggested that TBFM is positively [3–9], negatively [10–14], or not related [15–17] with bone outcomes. Much of the confusion may be due to reliance on dual-energy x-ray absorptiometry (DXA), which has inherent limitations in youth, because BMC and aBMD measured by DXA are influenced by size [18] and the rapid changes that occur during growth confound interpretation. The relatively recent development of low radiation dose, 3-dimensional imaging techniques, such as peripheral quantitative computed tomography (pQCT), has made it possible to safely estimate volumetric BMD (vBMD, mg/cm³) and geometric parameters in children without the confounding of growth. Application of these techniques should help to clarify the relationship between soft tissue composition and bone parameters.

Thus far, only four studies have used 3-dimensional imaging techniques to examine the effect of TBFM on bone parameters in children, adolescents, and young adults. Using computed tomography (CT), Janicka et al. [19] found that TBFM was not associated with cross-sectional and cortical bone areas at the lumbar spine and femoral midshaft of young adults, while Pollock et al. [20], using pQCT, found that young adult females with high-percent fat ($\geq 32\%$) compared with normal-percent fat ($< 32\%$) had significantly lower cortical bone area (CBA), cross-sectional area (CSA), and strength-strain index (SSI) at the 20% radius and lower CBA and SSI at the 20% distal tibia. In contrast, in a cohort of 1068 men ~19 yrs of age, Lorentzon et al. [21] reported that TBFM was an independent positive predictor of diaphyseal bone size of the tibia, suggesting that increased TBFM may act to enhance weight-bearing bone geometry. The conflicting results may be due to differences among studies in the adjustment for confounding factors. For example, studies that have adjusted for skeletal size (i.e. height) have been criticized because adiposity may directly stimulate skeletal growth [22]. Thus, adjusting for skeletal size is not necessarily appropriate. Also, adipose tissue may have both negative and positive effects on bone mediated via endocrine pathways (see [22] for review).

The relationship between TBFM and the growing skeleton is not well understood and to our knowledge, only one study in children has used 3-dimensional imaging techniques. In that study, Wetzsteon and colleagues [23] used pQCT and found that an at-risk for overweight group (BMI ≥ 85 th percentile) of girls and boys (aged 9–11 yrs) had greater absolute indices of tibial strength compared to a normal (BMI ≤ 75 th percentile) weight group and that bone strength was adapted to greater muscle area. Since obese children and adolescents have greater muscle mass for height [7], it is possible that the relationship between TBFM and bone is by association with increased muscle. Studies demonstrating that dynamic loads resulting from muscle forces play a dominant role in stimulating bone formation support this view [24]. In children and adolescents, maximum force production is strongly related with muscle cross-sectional area (MCSA) [25], an important predictor of bone strength [24]. Thus, after adjustment for MCSA, TBFM may not be an important predictor of weight-bearing bone strength. Conversely, some investigators have hypothesized that TBFM may stimulate weight-

bearing bone formation through its direct action of increased mechanical load on the skeleton [26], whereas others have suggested that higher TBFM may be more problematic than beneficial during growth because children with higher adiposity do not commensurately increase bone mineral accrual and enlarge their bones appropriately [11]. Furthermore, greater body mass increases the skeletal load engendered by a fall [22]. Contrary to findings in adults, fractures incidence has been shown to be higher in overweight children and adolescents [1, 27–30] and children with higher adiposity have lower BMC and aBMD for a given body mass [10,11].

The extent of the relationship between TBFM and bone after adjustment for muscle and whether girls with higher TBFM have reduced vBMD and geometric parameters for a given body mass remains unclear. Thus, we sought to determine the relationship of TBFM to vBMD, geometry, and strength of the femur and tibia at diaphyseal and metaphyseal sites in young girls after adjustment for MCSA using a 3-dimensional imaging method (pQCT) that is appropriate for growing children. Furthermore, since increased body mass resulting from greater adiposity increases the force of impact during a fall, we also examined how bone parameters vary in proportion to total body mass across tertiles of TBFM.

Materials and Methods

Subjects

The sample included 396 healthy girls, aged 8–13 years, who were participants in the “Jump-In: Building Better Bones” study. The long-term goal of Jump-In is to assess prospectively the effects of high impact jumping exercises on bone macro-architecture in prepubescent and early pubescent girls. Girls were recruited from 14 elementary and 4 middle schools around Tucson, Arizona. Inclusion criteria were any female in school grade 4 or 6. Exclusion criteria included learning disabilities (identified by schools) that made it impossible to complete questionnaires or otherwise unable to comply with assessment protocols; medications, medical conditions, or a disability that limited participation in physical exercise [31]; and the inability to read and understand English. The study protocol was approved by the University of Arizona Human Subjects Protection Committee and it was conducted in accordance with the Helsinki Declaration. All guardians and participating girls provided written informed consent. After informed consent, guardians completed a health history questionnaire with questions on participant ethnicity and race.

Anthropometry

Anthropometric measures were obtained following standardized protocols [32]. Body mass was measured to the nearest 0.1 kg using a calibrated scale (Seca, Model 881, Hamburg, Germany) and height and sitting height were measured at full inhalation to the nearest mm using a stadiometer (Shorr Height Measuring Board, Olney, MD). Non-dominant femur length (nearest mm) was measured from the base of the patella to the inguinal crease. Non-dominant tibia length (nearest mm) was measured from the proximal end of the medial border of the tibial plateau to the distal edge of the medial malleolus. Coefficients of variation for femur and tibia lengths were 0.44% and 0.23%, respectively (n = 396). For each anthropometric variable, the mean of two measurements was taken. The two measurements were repeated if the first two differed by more than 4 mm for height, sitting height and bone lengths, and 0.3 kg for body mass. If repeat measures were required, the mean of the second set of measures was used.

Physical Maturation

Maturity assessment is necessary in the study of growing children because the range in maturation between individuals of the same chronological age is large, especially during the pubertal years [33]. In this study, maturity was assessed in two ways. The first method relied

on self-report (with assistance available) of breast development based on Tanner stages [34]. The questionnaire presents illustrations of stages of development, and has been validated [35] and shown to agree well with physician exam and grading. The questionnaire also inquired about menarcheal status. Although Tanner staging is common in developmental studies, its ability to accurately assess maturation has been shown to be limited [36]. Consequently, maturation was also assessed using the method of Mirwald et al. [33], who developed gender-specific algorithms to predict years from peak height velocity (PHV) based on data from a six-year longitudinal study in boys and girls [37]. These algorithms incorporate interactions between height, weight, sitting height, leg length, and chronological age to derive a maturity offset value. The following equation from Mirwald et al. [33] was used to estimate maturity offset in our sample of females: $\text{Maturity Offset} = -9.376 + 0.0001882 \cdot \text{Leg Length} + \text{Sitting Height interaction} + 0.0022 \cdot \text{Age} + \text{Leg Length interaction} + 0.005841 \cdot \text{Age} + \text{Sitting Height interaction} - 0.002658 \cdot \text{Age} + \text{Weight interaction} + 0.07693 \cdot \text{Weight by Height ratio}$. This equation explained 0.890 of the variance in years from PHV in girls [33].

Physical activity and diet questionnaires

Physical activity was assessed using the past year physical activity questionnaire (PYPAQ), which surveys all sport and leisure-time physical activity engaged in at least 10 times in the past year outside of physical education class. PYPAQ score was computed using a modified equation from Shedd et al. [38], which accounted for weight-bearing load, frequency, and duration of each activity. Similar to Shedd, we used a broader range of load values (0.5–3.0) based on peak strain scores reported by Groothausen et al. [39] to increase the contribution of weight-bearing activities to the overall score rather than just designating activities as weight-bearing or non-weight bearing. The Harvard youth/adolescent questionnaire (YAQ) is a self-administered (with assistance available) food-frequency questionnaire that has been validated in children and adolescents [40]. The YAQ was used to assess energy (kcal/day) and nutrient intakes (i.e. vitamin D (IU), Ca^{2+} (mg/day), and Mg^{2+} (mg/day)) over the past year.

Bone and body composition assessment

Bone geometry and vBMD were assessed at the distal 4% and 20% femur and 4% and 66% tibia sites of the non-dominant leg using pQCT (XCT 3000; STRATEC Medizintechnik GmbH, Pforzheim, Germany, Division of Orthometrix; White Plains, NY). Scout scans were performed to locate the distal growth plate of the femur and tibia, with the scanner programmed to subsequently find the sites of interest. pQCT scans were analyzed using Stratec software, Version 5.50. Further details on image processing, calculations (bone strength indices and muscle cross-sectional areas (MCSA)), and analysis, including descriptions of Contour, Peel, and Cort modes are published elsewhere [41]. At the 4% distal femur and tibia, we used Contour mode 3 at 169 mg/cm^3 to define the total bone. Because of the difficulties in interpreting metaphyseal pQCT bone density measurements from a single slice [42], we averaged 3 pQCT slices at both the femur and tibia 4% sites. At the 20% femur and 66% tibia sites, Contour mode 1 at 710 mg/cm^3 was used to measure total bone and Cort mode 2 at 710 mg/cm^3 was used for cortical bone analysis. Peel mode 2 at 710 mg/cm^3 was used to define the marrow area. Slice thicknesses were 2.2 mm and voxel sizes were set at 0.4 mm for all sites. Scanner speed was set at 30 mm/second. Bone strength index (BSI, mg^2/mm^4), calculated as described by Kontulainen [43], was assessed at the 4% femur and tibia sites, while strength-strain index (SSI, mm^3), calculated as described by Shedd [38], was assessed at the 20% femur and 66% tibia. BSI estimates the bone's ability to withstand compression at metaphyseal sites, while SSI is used to estimate the bone's ability to resist torsion at diaphyseal sites.

Total body mass, fat mass, lean soft tissue mass, and percent body fat were obtained from DXA whole body scans using GE Lunar Prodigy (software version 5.60.003) fan-beam densitometer

(GE Lunar Corp, Madison, WI, USA). Subjects were positioned using standard GE/Lunar protocols. Percent body fat was calculated as the ratio of fat mass to whole body mass.

Calibration and quality assurance of the pQCT and DXA instruments were performed daily to ensure the accuracy and precision of measurements. Operators were trained on pQCT scanning and software analyses following guidelines provided by Bone Diagnostics, Inc. (Fort Atkinson, WI). One operator performed all pQCT scans and one technician performed all scan analyses. DXA scan analyses were done by one certified technician following GE/Lunar guidelines. The within-subject ($n = 29$ per skeletal site) pQCT precision error (coefficient of variation: CV) for trabecular vBMD at the 4% femur and tibia were 0.5% and 0.8%, respectively, while CVs for cortical vBMD at the 20% femur and 66% tibia were 0.3% and 0.5%, respectively. pQCT CVs for femur and tibia periosteal circumference were 0.4% and 1.1%, respectively. DXA CVs and BMD precision in our laboratory have been previously reported [44].

Statistical analysis

Data were checked for outliers and normality using histograms and all variables were tested for skewness and kurtosis. Two of the bone variables (BSI and SSI) were modestly skewed. We ran both log transformed and untransformed analyses; all results were similar, thus we report the untransformed data for clarity. Descriptive statistics were calculated for the entire sample and for tertiles of total body fat mass (TBFM), and differences in descriptive characteristics among tertiles of TBFM were tested using ANOVA or the Chi Square test for proportions as appropriate. To identify relationships between bone parameters and potential regression covariates, bivariate correlations were computed using Pearson's r for continuous and Spearman's ρ for categorical variables. Multiple linear regression was used to regress bone parameters on TBFM, controlling for MCSA. Regression analyses were then repeated with maturity offset, bone length, physical activity, and ethnicity, along with MCSA, to determine whether the relationship between TBFM and bone parameters would be altered after adjustment for confounders established in correlation analyses. To protect against colinearity between MCSA and total body lean mass (femur: $r = 0.87$; tibia: $r = 0.90$), only MCSA was included in regression models. MCSA was chosen based on past work suggesting that bone strength in children should be interpreted relative to the loads (mainly skeletal muscle forces) typically imposed on the skeleton [45]. We repeated all regression analyses substituting maturity offset [33] with Tanner stage [34]. All results were similar, thus we only report analyses that included maturity offset because its relation to bone parameters was consistently stronger (Table 2). Prior to multiple linear regression analyses, all variables were checked for normality, linearity, and homoscedasticity using residual plots. Also, possible interactions between TBFM and predictor variables were explored in all models. R^2 values presented are the adjusted R^2 and represent the proportion of variability in the dependent variable explained by the statistical model. We also compared bone parameters among tertiles of TBFM using analysis of covariance (ANCOVA) adjusting for MCSA, maturity offset, bone length, physical activity score, and ethnicity. Since increased body mass resulting from greater TBFM increases the force of impact during a fall, we divided bone parameters by body mass to evaluate how bone parameters vary in proportion to total body mass. ANCOVA, with the same covariates, was used to compare the ratio of bone parameters to total body mass among tertiles of TBFM. In all figures, outcome values were normalized to the middle tertile, thus setting the middle tertile values to 1.0 and lower and higher values to <1.0 and >1.0 , respectively. Normalization of values was necessary because of differences in units among pQCT outcome variables. Bonferroni *post hoc* tests were used to adjust for multiple comparisons. A significance level of $p = 0.05$ was used in all tests. All analyses were performed using The Statistical Package for the Social Sciences for Windows, Version 18.0 (SPSS, Chicago, IL, USA).

Results

Descriptive characteristics

Descriptive characteristics are shown in Table 1 for the total sample ($n = 396$) and by tertiles of TBFM. Sample ethnicity was 22% Hispanic and 78% Non-Hispanic, while sample race was 87% white, 6% Asian, 4% black or African American, 0.5% Native American or Alaska Native, 0.5% Native Hawaiian or other Pacific Islander, and 2% other. Ranges of TBFM for the lowest, middle, and highest tertiles were 1.8–7.9 kg, 8.0–12.7 kg, and 12.8–42.4 kg, respectively. Age, Tanner stage, maturity offset, anthropometric measures (i.e., height, body mass, BMI, sitting height, leg length, and femur and tibia length), total body lean mass, and femur and tibia MCSA all increase across tertiles of TBFM (Table 1). Furthermore, average percent body fat increases from 20% in the lowest to 27% in the middle to 37% in the highest tertile. Tertiles of TBFM were similar in the proportion of Hispanic and non-Hispanic girls and in physical activity score and total energy intake, but the lowest tertile reported significantly greater intakes of Ca^{2+} , Mg^{2+} , and vitamin D than the highest tertile.

Association of body composition and other factors with pQCT bone parameters

TBFM was positively correlated with cortical and trabecular vBMD ($r = 0.02$ – 0.26) and periosteal circumference, BSI, and SSI ($r = 0.41$ – 0.55) (Table 2). At both femur and tibia diaphyseal (20% femur, 66% tibia) and distal metaphyseal (4% femur and tibia) sites, most pQCT bone parameters were positively correlated with bone length, MCSA, maturity offset, Tanner stage, physical activity score, and ethnicity (Table 2). Weak, non-significant correlations were found between bone parameters and dietary measures (dietary Ca^{2+} , Mg^{2+} , vitamin D and total energy intake) (data not shown).

Table 3 shows standardized regression coefficients (β) and p values for TBFM and MCSA, along with adjusted R^2 values for regression models. In models with TBFM and MCSA (Table 3, Model 1), associations between TBFM and bone parameters at all sites were not significant. TBFM explained very little variance in all bone parameters (0.2–2.3%). In contrast, MCSA was strongly related ($p < 0.001$) to all bone parameters, except cortical vBMD (Table 3). The addition of maturity offset, bone length, physical activity score, and ethnicity (Table 3, Model 2) increased the overall adjusted R^2 values of all bone parameters (Δ adjusted $R^2 = 0.01$ – 0.19), but did not alter the relationship between TBFM and bone parameters which remained non-significant ($p > 0.05$). Substitution of TBFM with percent body fat (%) or percent body fat category (normal body fat $<32\%$, high body fat $\geq 32\%$) gave similar results (data not shown) for all multiple linear regression analyses.

pQCT bone parameters across tertiles of TBFM

Adjusted means (95% CI) for vBMD, geometry, and strength are reported by tertiles of TBFM in Table 4. Periosteal circumference was significantly greater in the highest as compared to the lowest TBFM tertile at diaphyseal sites (20% femur, 66% tibia) and SSI at the 66% tibia was significantly greater in the highest compared to the middle TBFM tertile. Figure 1 illustrates adjusted means for vBMD, geometry, and strength for tertiles of TBFM after dividing bone parameters by total body mass. All resulting outcome values were significantly ($p < 0.001$) greater in the lowest compared to both the middle and highest TBFM tertiles. Thus, relative to their body mass, girls with higher TBFM had greatly reduced vBMD, geometry and bone strength at all sites.

Discussion

In this sample of girls aged 8–13 years, we evaluated the influence of TBFM on vBMD, geometry, and indices of bone strength at metaphyseal and diaphyseal sites of the femur and

tibia. The results of correlation and regression analyses demonstrate that TBFM is correlated with vBMD, geometry, and indices of bone strength, although these relationships are no longer significant after adjustment for MCSA. Comparisons of adjusted ANCOVA models across tertiles of TBFM showed evidence of a positive association between TBFM and bone geometry and indices of strength at diaphyseal sites of the femur and tibia, which suggests a persistent, although weak association. These results are not necessarily conflicting, since the lack of significant association in the regression model may be due in part to a power issue ($n = 396$). Lorentzon et al. [21] reported a significant, although weak association between TBFM and diaphyseal bone size of the tibia in 1068 young men (aged ~19 yrs). However, across skeletal sites, TBFM explained only 0.2–2.3% of the variance in bone parameters in our sample of girls when MCSA was combined with TBFM in regression models as compared to 5.6% of the variance in tibial CSA in the study by Lorentzon et al. [21]. These findings suggest a weaker relationship between TBFM and weight-bearing vBMD, geometry, and indices of bone strength during growth as compared to young adulthood. In contrast, MCSA explained a much larger proportion of the variance in all bone parameters, independent of covariates, which is consistent with previous studies of children [17,23] and supports the proposed functional model of bone development which posits that forces from muscle contractions are the main mechanical challenges to which bones adapt [24].

Goulding has hypothesized that lower BMC and aBMD relative to body mass might make children and adolescents with higher adiposity vulnerable to fractures [46]. In support of this hypothesis, a number of studies have reported that overweight children and adolescents have low BMC and aBMD relative to their body mass [10,11]. However, no previous studies have evaluated the relationship of vBMD, geometry, or indices of bone strength relative to body mass. Our findings showed that when judged relative to total body mass, vBMD, geometry, and bone strength were markedly reduced in girls with higher TBFM. Across skeletal sites, these reductions averaged 10–14% in the highest compared to the middle tertile and 16–24% in the highest compared to the lowest tertile. These findings suggest that despite potentially earlier puberty than girls with less TBFM [36], girls with higher TBFM display a discrepancy between gains in body mass and appropriate adaptations in vBMD and geometric parameters during growth. This discrepancy, along with the increased force of impact during a fall, may help explain why overweight and obese girls have been reported to suffer more fractures than normal weight girls [1,30].

A number of potential mechanisms for the fat-bone relationship exist. For example, TBFM may have a direct effect on skeletal loading [26]. Furthermore, hormones secreted from adipocytes or pancreatic β cells may have both negative and positive effects on bone [22]. For example, leptin has been shown to have direct effects on bone tissue by stimulating proliferation and differentiation of osteoblasts [47] and inhibiting osteoclastogenesis [48] *in vitro*. However, in young men, increased leptin secondary to higher TBFM has been shown to be a negative independent predictor of diaphyseal bone size [21]. Furthermore, adipocytes are known to express aromatase enzymes that convert androgens into estrogens, which have been reported to stimulate [49] and suppress [50] periosteal expansion. Alternatively, TBFM may stimulate bone formation by association with increased secretion of bone active hormones from pancreatic β cells [22] or by the indirect effects of TBFM on the timing of puberty [36].

Previous studies in children and adolescents have examined the associations between TBFM and bone size, aBMD, and BMC [8,9,14,17]. Petit et al. (2005) [17] used hip structure analysis software to study the proximal femur of subjects who were at risk for overweight ($\text{BMI} \geq 85\text{th}$ percentile) or healthy weight ($\text{BMI} \leq 75\text{th}$ percentile) subjects and found that greater BMI did not have an independent effect on proximal femur bending strength. Clark et al. (2006) [8] and Goulding et al. (2008) [9] concluded that TBFM is associated with increases in total-body-less-head (TBLH) bone area independently of lean mass, while Wosje et al. (2009) [14] showed

that higher baseline TBFM was associated with greater TBLH bone area, but that increases over the next 3.5 years were smaller for children with higher baseline TBFM. Overall, studies using DXA in children and adolescents have reached contradictory conclusions regarding the relationship between fat and bone during growth, presumably because there has been no protocol to adequately address the limitations of DXA in growing children [18].

The use of pQCT in our study allows for the unique opportunity to compare our findings with previous studies that have used 3-dimensional imaging techniques to evaluate the relationship between fat and bone. Janicka et al. [19] used CT to study 300 late adolescent and young adult males and females (mean age = 17 yrs) and reported that TBFM was not related to bone geometry at the vertebrae and midshaft of the femur. Pollock et al. [20] used pQCT to study regions of the radius and tibia in 115 young adult females (mean age = 18 yrs). In that study, after adjustment for MCSA, the high-percent body fat ($\geq 32\%$) group had significantly lower CBA, CSA, and SSI at the 20% distal radius and lower CBA and SSI at the 20% distal tibia compared to the normal percent body fat ($< 32\%$) group. In contrast, no group differences were found at the 4% distal radius and tibia sites. Our findings at the 20% femur are consistent with those of Janicka et al. [19] at the 50% femur. Furthermore, our results at the 4% tibia are consistent with those of Pollock et al. [20], although we did not find significantly lower CBA and SSI at the diaphysis of the tibia. The different findings may be explained by differences in maturity (prepubescent and early pubescent vs. post-pubescent) since the more sexually mature females in the studies by Janicka et al. [19] and Pollock et al. [20] likely had higher estradiol levels, which in combination with greater aromatization of androgens to estrogens in females with greater fat mass, may have attenuated periosteal bone apposition [50]. Further studies in larger samples of females advancing through puberty will be necessary to clarify whether the relationship between TBFM and bone geometry is attenuated by puberty.

It was not possible to determine a causal relationship between TBFM and bone in our cross-sectional analysis. We found only one longitudinal study of bone structure and vBMD in children [23]. In that study, Wetzsteon and colleagues found that at-risk for overweight (BMI ≥ 85 th percentile) children had greater tibial bone strength than normal-weight (BMI ≤ 75 th percentile) children after adjustment for sex, ethnicity, Tanner stage, and leg length. However, results from models including MCSA suggested that the higher baseline bone strength and the changes in vBMD, geometry, and bone strength resulted from adaptations to the greater MCSA of overweight children [23]. Our findings at metaphyseal and diaphyseal sites of the tibia and femur are consistent with those findings. Although the study by Wetzsteon et al. [23] is strengthened by longitudinal data, the study was limited because the investigators compared overweight and normal weight children based on BMI. This ratio captures other components of body composition as well as fat, thus, a comparison based on BMI does not answer the question of whether fat has an independent effect on bone. Nevertheless, when bone outcomes were expressed relative to loads from fat and lean mass in the study by Wetzsteon et al. [23] and total body mass in our study, children who were overweight or in the highest tertile of TBFM had relatively lower vBMD, geometry, and bone strength at all sites.

The present study had several strengths. First, this study evaluated the relationship between TBFM and vBMD, geometry, and strength at skeletal sites (4% and 20% distal femur) that have not been previously reported using pQCT. The results at these sites are consistent with those at the 4% and 66% tibia, more commonly reported sites, suggesting that the effects of fat on weight-bearing bones are similar along various regions. In addition, our analyses utilized TBFM instead of ratios such as percent body fat or BMI. The latter capture other components of body composition and do not allow for the examination of the independent effects of fat on bone. Lastly, our study utilized an objective and practical solution for the measure of biological maturity or bone age assessment [33], an important factor in skeletal studies of children and adolescents given the large range of physical maturation among individuals of the same

chronological age. Previous studies of the relationship between fat and bone using 3-dimensional techniques in children and adolescents have either not measured maturation [20] or have relied on Tanner staging [19,23].

Limitations

We acknowledge several limitations of the study. First, the cross-sectional design does not allow determination of a causal relationship between fat mass and vBMD, geometry, and bone strength. Longitudinal data are needed to determine whether these findings track through puberty. Secondly, there are well known difficulties in assessing diet and physical activity through self-report questionnaires. While the physical activity questionnaire used in this study has not been validated in children, it has been shown to predict vBMD and bone geometric parameters in postmenopausal women [38] and in our sample of girls (data not shown). Thirdly, pQCT cannot be used to measure the axial skeleton (e.g. spine) and proximal femur (hip) sites, skeletal regions that are more susceptible to fractures later in life. Lastly, our pQCT measurements were limited to weight-bearing bones (femur and tibia). Future prospective studies of both weight-bearing and non-weight-bearing skeletal sites in girls will be necessary to confirm site-specific differences in the effects of fat on bone reported by Lorentzon et al. [21].

Conclusion

In conclusion, we demonstrated that MCSA is more strongly associated with vBMD, geometry, and indices of bone strength at metaphyseal and diaphyseal regions of the femur and tibia than fat mass, which has a persistent, although weak association with bone parameters. These findings suggest that girls who have a greater proportion of lean mass are more protected against fracture. Relative to their body mass, however, girls with higher TBFM have markedly reduced vBMD, geometry, and indices of bone strength at metaphyseal and diaphyseal regions of the femur and tibia. These findings suggest a mismatch between gains in body mass and acquisition of vBMD and geometry during growth that may help explain why obese girls suffer more fractures than normal weight girls [1,30].

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References

1. Goulding A, Grant AM, Williams SM. Bone and body composition of children and adolescents with repeated forearm fractures. *J Bone Miner Res* 2005;20:2090–2096. [PubMed: 16294262]
2. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA* 2006;295:1549–1555. [PubMed: 16595758]
3. Sabatier JP, Guaydier-Souquieres G, Benmalek A, Marcelli C. Evolution of lumbar bone mineral content during adolescence and adulthood: a longitudinal study in 395 healthy females 10–24 years of age and 206 premenopausal women. *Osteoporos Int* 1999;9:476–482. [PubMed: 10624453]
4. Pietrobelli A, Faith MS, Wang J, Brambilla P, Chiumello G, Heymsfield SB. Association of lean tissue and fat mass with bone mineral content in children and adolescents. *Obes Res* 2002;10:56–60. [PubMed: 11786602]
5. Ellis KJ, Shypailo RJ, Wong WW, Abrams SA. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol* 2003;40:S274–S277. [PubMed: 14618492]

6. Arabi A, Tamim H, Nabulsi M, Maalouf J, Khalife H, Choucair M, Vieth R, El-Hajj Fuleihan G. Sex differences in the effect of body-composition variables on bone mass in healthy children and adolescents. *Am J Clin Nutr* 2004;80:1428–1435. [PubMed: 15531697]
7. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. *Am J Clin Nutr* 2004;80:514–523. [PubMed: 15277178]
8. Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab* 2006;91:2534–2541. [PubMed: 16621904]
9. Goulding A, Taylor RW, Grant AM, Murdoch L, Williams SM, Taylor BJ. Relationship of total body fat mass to bone area in New Zealand five-year-olds. *Calcif Tissue Int* 2008;82:293–299. [PubMed: 18404241]
10. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. *Bone* 2000;27:203–207. [PubMed: 10913912]
11. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. *Int J Obes Relat Metab Disord* 2000;24:627–632. [PubMed: 10849586]
12. Ackerman A, Thornton JC, Wang J, Pierson RN Jr, Horlick M. Sex difference in the effect of puberty on the relationship between fat mass and bone mass in 926 healthy subjects, 6 to 18 years old. *Obesity (Silver Spring)* 2006;14:819–825. [PubMed: 16855191]
13. Braillon PM, Serban A. Bone mineral content and body composition in overweight children and adolescents. *Pediatr Res* 2007;62:462–467. [PubMed: 17667858]
14. Wosje KS, Khoury PR, Claytor RP, Copeland KA, Kalkwarf HJ, Daniels SR. Adiposity and TV viewing are related to less bone accrual in young children. *J Pediatr* 2009;154:79–85. [PubMed: 18692201]
15. Manzoni P, Brambilla P, Pietrobelli A, Beccaria L, Bianchessi A, Mora S, Chiumello G. Influence of body composition on bone mineral content in children and adolescents. *Am J Clin Nutr* 1996;64:603–607. [PubMed: 8839506]
16. Lazcano-Ponce E, Tamayo J, Cruz-Valdez A, Diaz R, Hernandez B, Del Cueto R, Hernandez-Avila M. Peak bone mineral area density and determinants among females aged 9 to 24 years in Mexico. *Osteoporos Int* 2003;14:539–547. [PubMed: 12844213]
17. Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. *Bone* 2005;36:568–576. [PubMed: 15777684]
18. Bachrach LK. Osteoporosis in children: still a diagnostic challenge. *J Clin Endocrinol Metab* 2007;92:2030–2032. [PubMed: 17554053]
19. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, Gilsanz V. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 2007;92:143–147. [PubMed: 17047019]
20. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86:1530–1538. [PubMed: 17991669]
21. Lorentzon M, Landin K, Mellstrom D, Ohlsson C. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. *J Bone Miner Res* 2006;21:1871–1878. [PubMed: 17002557]
22. Reid IR. Relationships between fat and bone. *Osteoporos Int* 2008;19:595–606. [PubMed: 17965817]
23. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone Structure and Volumetric BMD in Overweight Children: A Longitudinal Study. *J Bone Miner Res* 2008;23:1946–1953. [PubMed: 18684083]
24. Robling AG. Is Bone's Response to Mechanical Signals Dominated by Muscle Forces? *Med Sci Sports Exerc*. 2009
25. Maughan RJ, Watson JS, Weir J. Relationships between muscle strength and muscle cross-sectional area in male sprinters and endurance runners. *Eur J Appl Physiol Occup Physiol* 1983;50:309–318. [PubMed: 6683155]

26. Reid IR. Relationships among body mass, its components, and bone. *Bone* 2002;31:547–555. [PubMed: 12477567]
27. Goulding A, Cannan R, Williams SM, Gold EJ, Taylor RW, Lewis-Barned NJ. Bone mineral density in girls with forearm fractures. *J Bone Miner Res* 1998;31:143–148. [PubMed: 9443800]
28. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. *J Bone Miner Res* 2000;15:2011–2018. [PubMed: 11028455]
29. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *J Pediatr* 2001;139:509–515. [PubMed: 11598596]
30. Skaggs DL, Loro ML, Pitukcheewanont P, Tolo V, Gilsanz V. Increased body weight and decreased radial cross-sectional dimensions in girls with forearm fractures. *J Bone Miner Res* 2001;16:1337–1342. [PubMed: 11450710]
31. American Academy of Pediatrics: Medical conditions affecting sports participation. *Pediatrics* 2001;107:1205–1209. [PubMed: 11331710]
32. Lohman, TG.; Roche, AF.; Martorell, R. *Anthropometric Standardization Reference Manual*. Champaign, Illinois: Human Kinetics; 1988. p. 3-16.
33. Mirwald RL, Baxter-Jones AD, Bailey DA, Beunen GP. An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc* 2002;34:689–694. [PubMed: 11932580]
34. Tanner, JM. *Foetus into Man: Physical Growth from Conception to Maturity*. Cambridge, MA, USA: Harvard Press; 1978.
35. Morris NM, Udry RJ. Validation of a Self-Administered Instrument to Assess Stage of Adolescent Development. *J Youth Adolesc* 1980;9:271–280.
36. Sherar LB, Baxter-Jones AD, Mirwald RL. Limitations to the use of secondary sex characteristics for gender comparisons. *Ann Hum Biol* 2004;31:586–593. [PubMed: 15739387]
37. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan Bone Mineral Accrual Study. *J Bone Miner Res* 1999;14:1672–1679. [PubMed: 10491214]
38. Shedd KM, Hanson KB, Alekel DL, Schiferl DJ, Hanson LN, Van Loan MD. Quantifying leisure physical activity and its relation to bone density and strength. *Med Sci Sports Exerc* 2007;39:2189–2198. [PubMed: 18046190]
39. Groothausen J, Siemer H, Kemper G, Twisk J, Welten D. Influence of peak strain on lumbar bone mineral density: an analysis of 15-year physical activity in young males and females. *Pediatr Exerc Sci* 1997;9:159–173.
40. Rockett HR, Breitenbach M, Frazier AL, Witschi J, Wolf AM, Field AE, Colditz GA. Validation of a youth/adolescent food frequency questionnaire. *Prev Med* 1997;26:808–816. [PubMed: 9388792]
41. Stratec. *Medizintechnik 2004 XCT 3000 manual*, software version 5.40 Pforzheim, Germany. In
42. Lee DC, Gilsanz V, Wren TA. Limitations of peripheral quantitative computed tomography metaphyseal bone density measurements. *J Clin Endocrinol Metab* 2007;92:4248–4253. [PubMed: 17684050]
43. Kontulainen SA, Johnston JD, Liu D, Leung C, Oxland TR, McKay HA. Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. *J Musculoskelet Neuronal Interact* 2008;8:401–409. [PubMed: 19147978]
44. Going S, Lohman T, Houtkooper L, Metcalfe L, Flint-Wagner H, Blew R, Stanford V, Cussler E, Martin J, Teixeira P, Harris M, Milliken L, Figueroa-Galvez A, Weber J. Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. *Osteoporos Int* 2003;14:637–643. [PubMed: 12844212]
45. Petit MA, Beck TJ, Kontulainen SA. Examining the developing bone: What do we measure and how do we do it? *J Musculoskelet Neuronal Interact* 2005;5:213–224. [PubMed: 16172512]
46. Goulding, A. Risk Factors for Fractures in Normally Active Children and Adolescents. In: Daly, RM.; Petit, MA., editors. *The Role of Physical Activity and Nutrition during Growth*. Karger Publishing; 2007. p. 102-120.

47. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 1999;140:1630–1638. [PubMed: 10098497]
48. Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, Gough TJ, Collier GR, Nicholson GC. Leptin inhibits osteoclast generation. *J Bone Miner Res* 2002;17:200–209. [PubMed: 11811550]
49. Bouillon R, Bex M, Vanderschueren D, Boonen S. Estrogens are essential for male pubertal periosteal bone expansion. *J Clin Endocrinol Metab* 2004;89:6025–6029. [PubMed: 15579754]
50. Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. *J Bone Miner Res* 2005;20:1334–1341. [PubMed: 16007330]

Figure 1a) Femur

Figure 1b) Tibia

FIGURE 1.

Adjusted femur (a) and tibia (b) pQCT bone parameter to body mass ratios for tertiles of TBFM normalized to the middle tertile after being divided by total body mass. Differences in outcome values among tertiles of TBFM were evaluated by ANCOVA using MCSA, maturity offset, bone length, physical activity score, and ethnicity as covariates. TrabBMD = trabecular volumetric bone mineral density (mg/cm^3); Pcir = periosteal circumference (mm); BSI = bone strength index (mg^2/mm^4); CortBMD = cortical volumetric bone mineral density (mg/cm^3); SSI = strength-strain index (mm^3).

^a Significantly ($p < 0.001$) different from lowest and highest tertiles.

^b Significantly ($p < 0.001$) different from lowest and middle tertiles.

Table 1

Descriptive characteristics of all girls aged 8–13 years and by tertiles of total body fat mass

	Tertiles of total body fat mass (TBFM)			All (n = 396)
	Lowest (n = 132)	Middle (n = 132)	Highest (n = 132)	
Fat mass (kg)	6.1 ± 1.4 ^{a,b}	10.2 ± 1.4 ^c	19.1 ± 6.0	11.5 ± 6.2
Age (yrs)	10.3 ± 1.0 ^{a,b}	10.9 ± 1.1	11.0 ± 1.1	10.7 ± 1.1
Tanner stage (1–5)	1.6 ± 0.8 ^{a,b}	2.2 ± 1.0 ^c	2.6 ± 1.0	2.1 ± 0.9
Maturity Offset	−1.8 ± 0.9 ^{a,b}	−0.9 ± 1.0 ^c	−0.5 ± 0.9	−1.1 ± 1.0
Ethnicity (%; Hispanic)	21	24	22	22
Height (cm)	138.9 ± 8.5 ^{a,b}	146.2 ± 9.5 ^c	149.3 ± 8.6	144.8 ± 9.9
Body mass (kg)	30.4 ± 4.6 ^{a,b}	38.5 ± 5.9 ^c	49.1 ± 9.3	39.4 ± 10.3
BMI (kg/m ²)	15.7 ± 1.2 ^{a,b}	19.2 ± 3.5 ^c	21.9 ± 2.9	18.5 ± 3.3
Sitting height (cm)	72.6 ± 3.9 ^{a,b}	76.4 ± 4.4 ^c	78.3 ± 4.1	75.7 ± 4.8
Leg length (cm)	66.3 ± 5.2 ^{a,b}	69.9 ± 5.9	71.0 ± 5.1	69.1 ± 5.8
Femur length (cm)	32.5 ± 2.8 ^{a,b}	34.8 ± 2.8	35.3 ± 2.7	34.2 ± 3.0
Tibia length (cm)	31.7 ± 2.6 ^{a,b}	33.7 ± 2.7	34.3 ± 2.5	33.2 ± 2.8
Fat mass (%)	19.9 ± 3.9 ^{a,b}	26.5 ± 3.8 ^c	36.9 ± 6.0	27.8 ± 8.3
Lean mass (kg)	23.4 ± 4.1 ^{a,b}	27.3 ± 4.9 ^c	30.3 ± 5.3	26.4 ± 5.4
Femur MCSA (mm ²)	3138.4 ± 548.5 ^{a,b}	3597.2 ± 640.5 ^c	4154.6 ± 724.4	3630.1 ± 763.5
Tibia MCSA (mm ²)	2834.8 ± 435.8 ^{a,b}	3255.5 ± 488.8 ^c	3682.9 ± 595.3	3257.7 ± 618.2
Calcium (mg/day)	1103.8 ± 435.18 ^a	942.3 ± 461.8	987.6 ± 466.94	1003.9 ± 455.8
Magnesium (mg/day)	265.2 ± 89.6 ^a	227.6 ± 100.5	241.9 ± 105.0	243.8 ± 99.0
Vitamin D (IU)	317.1 ± 190.1 ^a	254.2 ± 181.6	269.4 ± 177.9	278.9 ± 184.3
Total Energy (kcal/day)	1823.7 ± 619.4	1649.7 ± 676.5	1725.4 ± 832.0	1729.0 ± 710.2
Physical activity score	810.4 ± 968.8	1027.3 ± 1131.1	848.9 ± 984.7	895.5 ± 968.8

Presented as mean (SD) unless noted otherwise.

BMI = body mass index; MCSA = muscle cross-sectional area

^aIndicates significant ($P < 0.05$) difference between the lowest and middle tertile, ANOVA.^bIndicates significant ($P < 0.05$) difference between the lowest and highest tertile, ANOVA.^cIndicates significant ($P < 0.05$) difference between the middle and highest tertile, ANOVA.

Table 2

Bivariate relationships between pQCT bone outcomes and predictors of bone parameters

	TBFM	Femur length	Femur MCSA	Maturity offset	Tanner stage	Physical activity	Ethnicity
<i>Femur (4%)</i>							
TrabBMD	0.20 ^a	0.10	0.33 ^a	0.23 ^a	0.12	0.09	-0.03
Peir	0.46 ^a	0.69 ^a	0.69 ^a	0.77 ^a	0.53 ^a	0.12	-0.01
BSI	0.41 ^a	0.49 ^a	0.67 ^a	0.62 ^a	0.41 ^a	0.14 ^a	0.06
<i>Femur (20%)</i>							
CortBMD	0.02	0.09	0.06	0.17 ^a	0.18 ^a	-0.02	0.11
Peir	0.51 ^a	0.72 ^a	0.75 ^a	0.79 ^a	0.56 ^a	0.16 ^a	0.03
SSI	0.52 ^a	0.77 ^a	0.80 ^a	0.85 ^a	0.61 ^a	0.18 ^a	0.08
<i>Tibia (4%)</i>							
TrabBMD	0.26 ^a	0.14 ^a	0.36 ^a	0.23 ^a	0.18 ^a	0.12	-0.01
Peir	0.48 ^a	0.72 ^a	0.77 ^a	0.77 ^a	0.52 ^a	0.13 ^a	0.02
BSI	0.48 ^a	0.50 ^a	0.72 ^a	0.63 ^a	0.47 ^a	0.21 ^a	0.04
<i>Tibia (66%)</i>							
CortBMD	0.115 ^a	0.19 ^a	0.24 ^a	0.40 ^a	0.40 ^a	0.06	0.14 ^a
Peir	0.50 ^a	0.72 ^a	0.76 ^a	0.70 ^a	0.47 ^a	0.20 ^a	-0.04
SSI	0.55 ^a	0.73 ^a	0.82 ^a	0.81 ^a	0.57 ^a	0.19 ^a	0.06

TBFM = total body fat mass (kg); MCSA = muscle cross-sectional area (mm²); TrabBMD = trabecular; volumetric bone mineral density (mg/cm³); Peir = periosteal circumference (mm); BSI = bone strength); index (mm²/mm⁴); CortBMD = cortical volumetric bone mineral density (mg/cm³); SSI = strength-strain); index (mm³).

^a $P < 0.001$; Pearson's r for continuous and Spearman's ρ for categorical variables.

Table 3

Multiple linear regression including total body fat mass and muscle cross-sectional area as independent predictors of bone parameters.

<i>pQCT</i>	Model 1 ^a						Model 2 ^b					
	TBFM			MCSA			Model ^a			TBFM		
	β	<i>p</i>	β	β	<i>p</i>	R^2	β	<i>p</i>	R^2	β	<i>p</i>	R^2
<i>4% Femur</i>												
TrabBMD	0.01	0.98	0.21	<0.001		0.11	-0.01	0.92		0.36	<0.001	0.12
Peir	0.06	0.18	0.46	<0.001		0.47	0.01	0.74		0.24	<0.001	0.63
BSI	0.01	0.91	0.42	<0.001		0.44	-0.03	0.60		0.47	<0.001	0.47
<i>20% Femur</i>												
CortBMD	-0.02	0.76	0.02	0.64		0.01	-0.09	0.16		-0.09	0.16	0.06
Peir	0.04	0.11	0.52	<0.001		0.56	0.06	0.12		0.32	<0.001	0.70
SSI	0.04	0.32	0.52	<0.001		0.65	-0.01	0.92		0.37	<0.001	0.81
<i>4% Tibia</i>												
TrabBMD	0.06	0.29	0.26	<0.001		0.12	0.08	0.18		0.43	<0.001	0.14
Peir	-0.01	0.77	0.48	<0.001		0.59	-0.02	0.66		0.45	<0.001	0.67
BSI	0.05	0.25	0.48	<0.001		0.51	0.06	0.18		0.55	<0.001	0.53
<i>66% Tibia</i>												
CortBMD	0.01	0.98	0.15	0.01		0.05	-0.04	0.45		-0.23	0.01	0.25
Peir	0.03	0.44	0.50	<0.001		0.58	0.05	0.16		0.53	<0.001	0.68
SSI	0.05	0.17	0.55	<0.001		0.68	0.05	0.11		0.45	<0.001	0.76

Standardized β coefficients and *p* values are presented for total body fat mass and muscle cross-sectional area.

Adjusted R^2 values are presented for both full models.

TBFM = total body fat mass (kg); MCSA = muscle cross-sectional area (mm²); TrabBMD = trabecular volumetric bone mineral density (mg/cm³); Peir = periosteal circumference (mm); BSI = bone strength index (mg²/mm⁴); CortBMD = cortical volumetric bone mineral density (mg/cm³); SSI = strength-strain index (mm³).

^aModel Covariates: Total body fat mass, muscle cross-sectional area.

^bModel Covariates: Total body fat mass, muscle cross-sectional area, maturity, bone length, physical activity, ethnicity.

Table 4

Adjusted vBMD, geometry, and strength by tertiles of total body fat mass

	Tertiles of total body fat mass (TBFM)		
	Lowest (n = 132)	Middle (n = 132)	Highest (n = 132)
<i>Femur (4%)</i>			
TrabBMD	238.4 (232.6, 244.2)	235.9 (230.6, 241.1)	235.9 (230.1, 241.7)
Pcir	123.9 (122.5, 125.3)	126.1 (124.8, 127.4)	126.5 (125.1, 127.9)
BSI	96.8 (92.8, 100.7)	95.6 (92.0, 99.1)	96.3 (92.4, 100.3)
<i>Femur (20%)</i>			
CortBMD	1046.3 (1042.1, 1050.5)	1045.6 (1041.8, 1049.5)	1046.0 (1041.8, 1050.2)
Pcir	76.7 (75.8, 77.6) ^a	77.7 (76.8, 78.5)	78.9 (77.8, 79.6)
SSI	1339.4 (1306.1, 1372.6)	1341.5 (1311.1, 1372.0)	1376.3 (1342.9, 1409.7)
<i>Tibia (4%)</i>			
TrabBMD	223.4 ± (218.6, 228.2)	218.7 (214.4, 223.1)	222.7 (217.9, 227.5)
Pcir	85.9 (85.0, 86.9)	86.4 (85.5, 87.2)	86.5 (85.6, 87.4)
BSI	52.1 ± (50.2, 54.0)	50.9 (49.2, 52.6)	53.1 (51.2, 55.0)
<i>Tibia (66%)</i>			
CortBMD	1030.5 (1025.2, 1035.8)	1027.9 (1023.1, 1032.7)	1030.5 (1025.2, 1035.8)
Pcir	70.8 (70.1, 71.5) ^b	71.7 (71.1, 72.4)	73.8 (72.1, 73.6)
SSI	1162.4 (1131.3, 1193.5)	1165.4 (1137.1, 1193.7) ^a	1218.5 (1187.4, 1249.6)

Presented as estimated marginal means (95% CIs) adjusted for ethnicity, maturity offset, physical activity score, bone length, muscle cross-sectional area (MCSA).

TrabBMD = trabecular volumetric bone mineral density (mg/cm^3); Pcir = periosteal circumference; (mm); BSI = bone strength index (mg^2/mm^4); CortBMD = cortical volumetric bone mineral density; (mg/cm^3); SSI = strength-strain index (mm^3).

^aSignificantly ($P < 0.05$) different from highest tertile, ANCOVA.

^bSignificantly ($P < 0.01$) different from highest tertile, ANCOVA.