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Suboptimal bone microarchitecure in adolescent girls with obesity compared to normal-weight controls and girls with anorexia nervosa

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ABSTRACT

Background: Despite their higher areal bone mineral density (aBMD), adolescents with obesity (OB) have an increase in fracture risk, particularly of the extremities, compared with normal-weight controls. Whereas bone parameters that increase fracture risk are well characterized in anorexia nervosa (AN), the other end of nutritional spectrum, these data are lacking in adolescents with obesity.

Objective: Our objective was to compare bone parameters in adolescent girls across the nutritional spectrum, to determine whether suboptimal bone adaptation to increased body weight may explain the increased fracture risk in OB.

Methods: We assessed bone endpoints in 153 adolescent girls 14–21 years old: 50 OB, 48 controls and 55 AN. We used (i) DXA to assess aBMD at the lumbar spine, proximal femur and whole body, and body composition, (ii) high resolution peripheral quantitative CT (HRpQCT) to assess bone geometry, microarchitecture and volumetric BMD (vBMD), and (iii) finite element analysis to assess failure load (a strength estimate) at the distal radius and tibia. All aBMD, microarchitecture and FEA analyses were controlled for age and race.

Results: Groups did not differ for age or height. Areal BMD *Z*-scores at all sites were highest in OB, intermediate in controls and lowest in AN ($p < 0.0001$). At the **radius**, cortical area and thickness were higher in OB compared to AN and control groups ($p = 0.001$) while trabecular area did not differ across groups. Compared to controls, OB had higher cortical porosity ($p = 0.003$), higher trabecular thickness ($p = 0.024$), and higher total, cortical and trabecular vBMD and rod BV/TV ($p < 0.04$). Plate BV/TV did not differ in OB vs. controls, but was higher than in AN ($p = 0.001$). At the **tibia,** total, cortical, and trabecular area and cortical thickness were higher in OB vs. controls and AN ($p < 0.005$). OB also had higher cortical porosity ($p < 0.007$) and lower trabecular thickness ($p < 0.02$) than the other two groups. Trabecular number, total and trabecular vBMD, and rod BV/TV were higher in OB vs. controls and AN ($p < 0.02$), while cortical vBMD and plate BV/TV did not differ in OB vs. the other two groups. Finally, **failure load** (a strength estimate) was higher in OB at the radius and tibia compared to controls and AN ($p < 0.004$ for all). However, after adjusting for body weight, failure load was lower in OB vs. controls at both sites ($p < 0.05$), and lower than in AN at the distal tibia.

Conclusion: Not all bone parameters demonstrate appropriate adaptation to higher body weight. Cortical porosity and plate BV/TV at the radius and tibia, and cortical vBMD and trabecular thickness at the tibia are particularly at risk. These effects may contribute to the higher risk for fracture reported in OB vs. controls.

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1. Introduction

Although low-weight conditions such as anorexia nervosa (AN) have typically been associated with an increased risk for fractures [1], recent data indicate that obesity, at the other end of the weight spectrum, may also be associated with increased fracture risk in both children and adults [2–5]. While the metabolic complications of obesity have been studied extensively, the impact of obesity on bone health remains understudied. Further, although factors contributing to increased fracture risk in AN have been well characterized in both adolescents and adults, these data are currently lacking for obesity, particularly in adolescents and young adults with obesity. Adolescence is a time characterized by high rates of bone accrual, with > 90% of peak bone mass (a determinant of future fracture risk) being attained by 18 years. With the prevalence of severe obesity increasing in adolescents [6], it is important to determine its impact on bone outcomes, given potential implications for peak bone mass, and both immediate and future fracture risk.

In AN, at the other end of the weight spectrum, increased fracture risk is attributable to lower bone mineral content (BMC) and areal BMD (aBMD) [measured by dual energy x-ray absorptiometry (DXA)] than seen in an age-matched healthy population [7]. Additionally, assessment using high-resolution peripheral quantitative computed tomography (HRpQCT) has revealed that adolescent girls with AN have lower cortical area, cortical and trabecular thickness, trabecular and total volumetric BMD, and higher cortical porosity than normal-weight controls [8], which contribute to reduced strength estimates and increased fracture risk. In contrast, children and adolescents who are overweight or have obesity have higher BMC and aBMD compared to normal-weight children and adolescents, which is likely adaptive to the greater mechanical loading that results from their higher body weight [9]. However, reports of increased fracture risk in those with obesity suggest impaired bone strength, despite their higher BMC and aBMD. Potential explanations include the following: (i) despite higher BMC and aBMD, individuals with obesity have impaired bone geometry or microarchitecture compared to controls, which in turn contribute to reduced bone strength, and (ii) higher BMC or aBMD does not fully compensate for the higher impact (resulting from higher body weight) during a fall in individuals with obesity. It is important to determine whether these possibilities apply to adolescents with obesity.

Further, factors that determine bone density and structure in adolescents and young adults with obesity are unclear. One study in adults 25–64 years old suggested that high body fat percentage increases the risk of low aBMD and non-spine fractures across the weight spectrum [10]. However, the impact of body composition on bone outcomes in younger individuals with obesity is yet to be explored. Of note, estrogen and insulin-like growth factor-1 (IGF-1) increase markedly during adolescence, and both hormones are critical to the significant increase in bone accrual that characterizes puberty. Rising levels of estrogen in early puberty result in rising levels of growth hormone (GH) and IGF-1. IGF-1 is an important bone trophic hormone [11,12]that is also impacted by nutritional status [13]. In AN, IGF-1 levels are low despite elevated GH levels because of a nutritionally acquired GH resistance [14]. In obesity, total IGF-1 levels are usually normal despite decreased GH secretion [15]. While the impact of low IGF-1 levels on bone outcomes has been extensively studied in adults and adolescents with AN, associations of IGF-1 with bone outcomes in adolescents with obesity remain to be explored.

In this study, we examined bone density, geometry, microarchitecture and strength estimates using DXA, HRpQCT, and microfinite element analysis (FEA) in adolescent and young adult women with moderate to severe obesity compared to normal-weight females and those with anorexia nervosa between 14 and 21 years old to determine differences as well as similarities across the weight spectrum that may explain the reported increase in fracture risk in obesity. We hypothesized that (i) despite higher aBMD (assessed by DXA) than in the other two groups, females with obesity would demonstrate specific alterations in bone geometry and microarchitecture that would approximate findings in AN; (ii) strength estimates in the group with obesity would be attenuated after controlling for body size (total body weight); and (iii) body composition, menstrual status and IGF-1 would predict bone outcomes in obesity.

2. Participants and methods

2.1. Participants

We assessed bone endpoints in 153 adolescent girls and young women 14–21 years old: 50 with moderate to severe obesity (OB), 48 normal-weight controls, and 55 with AN. Data for OB, control and AN groups were obtained between 2010 and 2018 from baseline visits of three ongoing studies assessing bone outcomes in our institution: an observational study in obesity (R01DK103946), the observational component of a study that included normal-weight controls (K24HD071843), and an interventional study in AN (R01DK062249). Although some bone data for a subset of these participants have been previously reported [16,17], a comprehensive comparison of bone parameters across the weight spectrum has not been previously performed or reported. Of note, all studies used the same DXA and HRpQCT instruments. The Partners HealthCare Institutional Review Board approved this study. Informed consent was obtained from all participants ≥18 and parents of participants < 18 years old. Assent was obtained from participants < 18 years old.

Participants with obesity had a body mass index (BMI) > 35 kg/m^2, and did not have other conditions that would affect bone health, other than those related directly to obesity (such as polycystic ovary syndrome (PCOS), impaired glucose tolerance, or diabetes). We did not exclude the latter because we wanted our sample to be representative of the general population of youth with obesity, but did factor in presence of dysglycemia in our analysis given its known impact on bone health [18,19]. Specifically, four OB participants had a HbA1C ≥ 7%, and we ran the analysis both including and excluding these participants from the analysis [19,20]. We included those with moderate to severe obesity to allow assessment of a sample at greater risk of metabolic complications of obesity. For those with AN, the diagnosis was confirmed by the study psychologist/psychiatrist per DSM-IV-TR or DSM-5 (depending on timing of recruitment relative to the 2013 publication of DSM-5) by conducting a clinical interview. All controls were required to have a BMI between the 10th–90th percentiles for age and at least 9 menstrual cycles in the preceding year.

Participants on medications that affect bone metabolism [other than oral contraceptive pills (OCPs), calcium and vitamin D supplements] were excluded from study participation. We did not exclude participants on OCPs, calcium and vitamin D supplements given their very frequent use in these conditions and for concern that excluding them would result in a non-representative sample. However, we did factor in OCP use in our analysis. Lifetime fracture history was determined per self/parent-report using published strategies [21,22]. Hours of physical activity were assessed using the Paffenberger questionnaire [23].

2.2. Experimental protocol

A detailed history was obtained, with particular attention to the use of medications and/or conditions that affect bone health, menarchal age, and menstrual history. Participants were weighed on a calibrated electronic scale in the Clinical Research Center wearing a hospital gown, and height was measured in triplicate on a wall-mounted stadiometer. CDC tables were used to calculate the height z-score, BMI z-score, and percent median BMI [(BMI of participant/50th percentile BMI for age and sex)*100] [24]. Blood samples were drawn for assessment of IGF-1 levels by mass spectrometry (Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA; sensitivity 15.6 ng/mL;

intraassay CV 3.5%–15%). IGF-1 z-scores (SD scores for age) were also provided by Quest Diagnostics and are reported. We report available clinical data for intact parathyroid hormone (PTH) levels using a radioimmunoassay (range 10–60 pg/mL) in a subset of 16 OB participants who had these levels drawn for clinical purposes.

2.3. Assessment of bone and body composition endpoints

Areal BMD *Z*-scores of the lumbar spine, proximal femur, and whole body were determined using DXA (Hologic 4500 A, Waltham, MA), and are adjusted for age and race. All participants were scanned on the same machine and using the same software regardless of their parent study. Fat mass, lean mass, visceral and subcutaneous fat were also assessed using DXA.

Bone geometry, microarchitecture, and volumetric BMD (vBMD) were assessed at the distal radius and tibia using HRpQCT (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland) at the non-dominant wrist and leg unless there was an acute fracture at those sites in which case the non-fractured side was assessed. All participants were scanned on the same machine regardless of their parent study. 2D scout views were obtained and used to locate the distal CT slice at 9.5 mm and 22.5 mm from the radius and tibia endplates, respectively. Automated analysis provided information regarding (i) bone geometry, including cross-sectional area of the cortical and trabecular bone compartments, total cross-sectional area, and cortical thickness, (ii) volumetric BMD of total, cortical, and trabecular bone, and (iii) trabecular microarchitecture, including trabecular number and thickness. Individual trabecula segmentation was used to further characterize rod-plate trabecular morphology [25–27]. Trabeculae can be plate-like or rod-like, and plate-like trabeculae are known to confer greater strength to bone than rod-like trabeculae. Therefore, their relative proportions can have an impact on bone strength, and were assessed in this study [28,29]. Extended cortical analysis was used to assess cortical porosity (a measure of cortical microarchitecture), which can contribute to fracture risk independent of other bone measures [30].

FEA was performed to estimate biomechanical properties of bone in the setting of simulated axial compression. Of note, strength estimates from FEA correlate strongly with bone strength assessed using cadaveric bone [31]. Failure load was estimated by scaling the resultant load from a 1% apparent compressive strain until 2% of all elements reached an effective strain > 7000 μstrain, similar to published methods [32,33]. All HRpQCT and FEA analyses were controlled for age and race. Same-day reproducibility for repeated measurements is 0.2 to 1.4% for density values, 0.3 to 8.6% for trabecular microarchitecture parameters, 0.6 to 2.4% for cortical microarchitecture parameters, 7.3 to 20.2% for cortical porosity measurements, and 2.1 to 3.0% for FEA measures.

2.4. Statistical analysis

Data analysis was performed using JMP Statistical Discovery Software. We used analysis of variance or the Kruskal-Wallis test to compare clinical characteristics of the three groups (depending on distribution), followed by pairwise comparisons if the *p*-value for the overall ANOVA or Kruskal Wallis test was < 0.10. Fisher's Exact test was used to compare proportions. For areal BMD using DXA, we report age and race adjusted *Z*-scores per DXA output (Hologic database). For HRpQCT and FEA parameters, we used multivariate analysis to control for race and age (because bone geometry, microarchitecture, vBMD, and estimated strength change with increasing age during adolescence), and only adjusted measures are reported. For FEA-derived failure load, in addition to race and age, we also controlled for body weight to determine the extent of adaptation to weight loading. Specifically, we determined whether failure load remains higher in the OB group vs. controls and AN after controlling for body weight. Our premise was that a lower failure load in OB vs. controls, after controlling for body weight, would indicate that the otherwise higher (unadjusted for weight) failure load in this group was insufficient to counter the impact resulting from the higher body weight during a fall. For comparison of bone parameters, we used the Dunnett test to determine differences between groups (OB vs. controls and OB vs. AN), while controlling for multiple comparisons. AN vs. control comparisons are not included because these have been previously reported in many studies [17,34].

Pearson correlations were used to determine associations of weight, body composition (fat and lean mass), gynecologic and menarchal age, current OCP use, 25(OH) vitamin D [25(OH)D] levels and IGF-1 z-scores with bone endpoints in the OB group. We assessed associations of weight rather than BMI, given that total body weight may be a better indicator of the total bone loading than BMI. Finally, multivariate analysis was used to assess associations of a covariate (e.g. IGF-1 z-scores) with bone endpoints while controlling for a second covariate (e.g. OCP use). Data are reported as mean ± SEM. A *p*-value of < 0.05 was used to denote significance.

3. Results

3.1. Clinical characteristics

Clinical characteristics of the three groups are shown in Table 1. Groups did not differ for age or height. Per design, weight and BMI were highest in OB (class III obesity) and lowest in AN. Per study design, weight, BMI, fat and lean mass were lowest in AN and highest in OB ($p < 0.002$ for all comparisons). Activity levels were higher in AN vs. the other two groups ($p < 0.04$). Race differed among groups and reflected the demographics of AN and obesity. Mean 25(OH)D levels were between 20 and 30 ng/mL in OB and control groups, with a similar proportion of subjects in both groups being vitamin D deficient [25(OH)D < 20 ng/mL]. Moreover, we assessed PTH levels in a subset of 16 subjects with obesity, and 82% had levels in the normal range (6–60 pg/mL), with the highest level being 76 pg/mL. This suggests that our cohort did not exhibit significant biochemical secondary hyperparathyroidism.

Menarchal age was younger in OB vs. the other two groups, but gynecological age (duration since menarche) did not differ across groups. The duration since last menses did not differ between AN and OB groups, but was higher in both groups compared to controls. The proportion of participants using OCPs was higher in OB vs. AN and control groups, and higher in AN vs. controls. Proportion of participants experiencing more than three months of amenorrhea preceding the study was higher in OB and AN groups vs. controls, but did not differ in OB vs. AN groups. IGF-1 z-scores were lower in the OB group vs. the other two groups.

The proportion of individuals with one or more fractures (stress and non-stress) showed a trend for being highest in AN, followed by OB, and was lowest in controls; $p = 0.07$. Stress fractures were reported in 9.1% of AN vs. 0% of controls and 2% of OB participants.

3.2. DXA measures of areal bone mineral density

Areal BMD *Z*-scores at all sites were highest in OB, intermediate in controls, and lowest in AN ($p < 0.0001$ for all comparisons of OB vs. AN or controls) (Table 2A**).** After controlling for weight, differences between OB vs. controls, and OB vs. AN were no longer significant (data not shown).

3.3. HRpQCT parameters

Data from HRpQCT, including extended cortical analysis and individual trabecula segmentation, are shown in Table 2. At the **radius** (Table 2B**),** cortical area and thickness increased progressively from AN to controls to OB, while trabecular area did not differ across groups. Of note, cortical pore volume (which is associated with lower bone

Table 1
Clinical characteristics of adolescents and young women with anorexia nervosa, of normal-weight, and with obesity.

	Anorexia nervosa (AN) (n = 55)	Normal-weight controls (NW-C) (n = 48)	Obesity (OB) (n = 50)	ANOVA p-value	OB vs. NW-C p-value	AN vs OB p-value	AN vs. NW-C p-value
Clinical characteristics							
Age (y)	18.5 ± 0.2	18.7 ± 0.2	17.9 ± 0.3	0.105	–	–	–
Weight (kg)	50.2 ± 0.7	57.6 ± 1.0	121.7 ± 2.7	< 0.0001	< 0.0001	< 0.0001	0.002
Height (cm)	163.9 ± 0.8	163.1 ± 1.0	164.9 ± 1.0	0.425	–	–	–
% Median BMI	87.9 ± 1.2	101.9 ± 1.7	209.6 ± 5.5	< 0.0001	< 0.0001	< 0.0001	0.003
BMI (kg/m^2)	18.7 ± 0.2	21.7 ± 0.3	44.8 ± 0.9	< 0.0001	< 0.0001	< 0.0001	0.0003
BMI Z-score	−1.15 ± 1.02	0.34 ± 0.11	2.43 ± 0.03	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Activity (hours)	6.2 ± 0.9	3.0 ± 0.6	3.9 ± 0.7	0.0061	0.345	0.031	0.002
Total fat mass (kg)	13.0 ± 0.5	17.8 ± 0.6	61.5 ± 1.7	< 0.0001	< 0.0001	< 0.0001	0.002
Total lean mass (kg)	36.6 ± 0.5	40.4 ± 0.7	61.9 ± 1.2	< 0.0001	< 0.0001	< 0.0001	0.001
Percent body fat (%)	24.9 ± 0.7	30.0 ± 0.7	49.6 ± 0.5	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Percent lean mass (%)	71.7 ± 0.7	68.6 ± 0.7	50.3 ± 0.5	< 0.0001	< 0.0001	< 0.0001	0.004
Vitamin D (ng/mL)	32.5 ± 1.3	25.8 ± 1.6	23.7 ± 1.5	< 0.0001	0.327	< 0.0001	0.001
% with vitamin D deficiency (< 20 ng/mL)	7.27%	36.17%	38.30%	< 0.0001	1.000	0.0002	0.0004
Calcium (mg/dl)	9.5 ± 0.1	9.1 ± 0.1	9.2 ± 0.0	< 0.0001	0.296	0.0015	< 0.0001
Age at menarche (y)	12.8 ± 0.2	12.5 ± 0.2	11.8 ± 0.2	0.0003	0.003	< 0.0001	0.340
Gynecological age (y)	5.7 ± 0.3	6.2 ± 0.3	6.2 ± 0.3	0.524	–	–	–
Current OCP usage (%)	18.2%	0%	36%	< 0.0001	< 0.0001	0.048	0.002
Duration since last menses (months)	5.1 ± 9.0	0.4 ± 0.4	3.9 ± 7.8	0.004	0.002	0.52	0.0007
% with amenorrhea for at least 3 months preceding the study	26.9%	0%	18.8%	< 0.0001	0.003	0.353	< 0.0001
Race (White/Black/Other) %	89.1/1.8/9.1	54.2/6.3/39.6%	51.1/21.3/27.7	< 0.0001	0.084	< 0.0001	0.0004
IGF-1 z-scores	−0.55 ± 0.19	−0.14 ± 0.14	−1.22 ± 0.24	0.004	0.005	0.047	0.389
Fracture history							
% with a history of ≥1 fracture	45.5%	25.0%	30.0%	0.070	–	–	–
% with a history ≥2 fractures	14.6%	6.3%	8.0%	0.322	–	–	–
% with up ext/low ext/both extremity/other fractures	23.6/7.3/3.6/10.9%	12.5/6.3/2.1/2.1%	10.0/12.0/4.0/ 4.0%	0.208	–	–	–

OCP: oral contraceptive pill; Up: upper; Ext: extremity; Low: lower.

strength) was higher in OB vs. AN and control groups. OB had higher trabecular number and thickness, and higher total, cortical, and trabecular vBMD vs. the other two groups. Plate BV/TV did not differ in OB vs. controls, but was lower in AN; rod BV/TV was higher in OB vs. AN and controls. Controlling for vitamin D status or excluding four OB participants with HbA1C ≥ 7% from the analysis did not significantly impact these results (data not shown). After controlling for current OCP use, differences between OB and control groups mostly persisted, and lost significance only for trabecular number and cortical vBMD ($p > 0.05$).

At the **tibia** (Table 2C**),** total, cortical, and trabecular area and cortical thickness were higher in OB vs. AN and controls. Of note, OB had higher cortical porosity and lower trabecular thickness than the other two groups. Trabecular number, total and trabecular vBMD, and rod BV/TV were higher in OB vs. AN and controls, but cortical vBMD and plate BV/TV did not differ in OB vs. the other two groups. Controlling for vitamin D status or excluding four OB participants with HbA1C ≥ 7% from the analysis did not significantly impact these results. After controlling for current OCP use, differences between OB and control groups lost significance for cortical and trabecular thickness ($p > 0.05$), but other differences persisted.

3.4. Microfinite element analysis

Failure load (a strength estimate) adjusted for age and race increased from AN to controls to OB for the radius and tibia. However, after also adjusting for weight, failure load was lower in OB vs. controls at both sites ($p < 0.05$), did not differ in OB vs. AN at the radius, but was lower than in AN at the tibia. (Fig. 1).

3.5. Associations of bone parameters with covariates

At both the **distal radius and distal tibia**, weight and lean mass were most strongly associated with bone parameters; very few associations were observed between fat mass and bone parameters. Gynecologic age, which reflects the duration of estrogen exposure, was associated positively with cortical thickness and total and cortical vBMD at both sites, and negatively with radial trabecular area. Menarchal age demonstrated a negative association with total and trabecular vBMD of the tibia in the OB group ($r \leq -0.336$, $p \leq 0.028$), but no other associations were noted with bone parameters (data not shown). Current OCP use, duration of amenorrhea, proportion of participants with amenorrhea, 25(OH)D levels and IGF-1 z-scores were not associated with bone endpoints ($p > 0.05$, data not shown). We also assessd associations of IGF-1 z-scores with bone endpoints after controlling for OCP use (given that OCP use can reduced systemic IGF-1 z-scores), and still found no associations with HRpQCT and FEA measures ($p > 0.05$).

Both lean and fat mass were strongly associated with body weight ($r = 0.97$ and 0.99, $p < 0.0001$ for both) for the group as a whole. However, while there was a positive association of percent fat mass with body weight ($r = 0.90$, $p < 0.0001$), we found a negative association of percent lean mass with body weight ($r = -0.89$, $p < 00001$) (Table 3).

4. Discussion

Our study demonstrates that not all bone parameters demonstrate appropriate adaptation to higher body weight in young women with obesity, and that cortical porosity, cortical vBMD, plate BV/TV at the radius and tibia, and tibial trabecular thickness are particularly at risk. These and other differences could contribute to relatively weaker bones for body size in OB. Consistent with this, although failure load was higher in OB vs. the other two groups at both sites, after adjusting for body weight, failure load was lower in OB vs. controls at both the radius and the tibia, and lower than in AN at the tibia. These features may

Table 2
Age and race adjusted measures of areal BMD (using DXA) (A), bone geometry, microarchitecture, volumetric bone mineral density (using HRpQCT) and estimated strength (using FEA) at the distal radius (B) and distal tibia (C) in adolescent and young adult women with anorexia nervosa, of normal-weight, and with obesity.

	Anorexia nervosa (AN) (n = 55)	Normal-weight controls (NW-C) (n = 48)	Obesity (OB) (n = 50)	ANOVA p-value	OB vs. NW-C p-value	OB vs. AN p-value
Areal BMD *Z*-scores (DXA)						
Lumbar spine	−1.226 ± 0.157	−0.467 ± 0.147	1.300 ± 0.138	< 0.0001	< 0.0001	< 0.0001
Total hip	−0.784 ± 0.122	−0.045 ± 0.146	2.164 ± 0.172	< 0.0001	< 0.0001	< 0.0001
Femoral neck	−0.925 ± 0.137	−0.202 ± 0.149	1.977 ± 0.180	< 0.0001	< 0.0001	< 0.0001
Whole body	−1.266 ± 0.130	−0.819 ± 0.136	0.290 ± 0.158	< 0.0001	< 0.0001	< 0.0001

B. Distal radius	AN	NW-C	OB	ANOVA p-value	OB vs. NW-C *p*-value	OB vs. AN p-value
Bone geometry						
Total Area (mm^2)	261.8 ± 8.4	265.3 ± 7.7	274.1 ± 7.3	0.467	0.595	0.374
Ct. Area (mm^2)	45.3 ± 2.1	52.2 ± 2.0	61.5 ± 1.9	< **0.0001**	**0.001**	< **0.0001**
Tb. Area (mm^2)	212.7 ± 8.5	220.7 ± 7.8	207.4 ± 7.4	0.877	0.925	0.827
Ct. Thickness (mm)	0.68 ± 0.03	0.77 ± 0.03	0.91 ± 0.03	< **0.0001**	**0.002**	< **0.0001**
Microarchitecture						
Ct. Pore volume (mm^3)	4.79 ± 0.55	4.52 ± 0.51	7.59 ± 0.48	< **0.0001**	< **0.0001**	**0.0001**
Ct. Porosity (%)	1.13 ± 0.11	0.93 ± 0.10	1.35 ± 0.09	**0.006**	**0.003**	0.165
Tb. number (1/mm)	1.94 ± 0.05	2.04 ± 0.05	2.18 ± 0.05	**0.001**	0.056**	**0.0004**
Tb. thickness (mm)	0.070 ± 0.002	0.071 ± 0.002	0.077 ± 0.002	**0.005**	**0.024**	**0.004**
Vol. BMD (vBMD)						
Total vBMD ($mgHA/cm^3$)	290.6 ± 10.1	318.5 ± 9.4	363.1 ± 8.8	< **0.0001**	**0.0009**	< **0.0001**
Ct. vBMD ($mgHA/cm^3$)	806.5 ± 9.4	823.2 ± 8.8	851.1 ± 8.3	**0.0009**	**0.033***	**0.0004**
Tb. vBMD ($mgHA/cm^3$)	162.1 ± 6.3	175.0 ± 5.9	202.2 ± 5.5	< **0.0001**	**0.001**	< **0.0001**
Plate BV/TV	0.090 ± 0.008	0.111 ± 0.007	0.125 ± 0.007	**0.002**	0.264	**0.001**
Rod BV/TV	0.173 ± 0.006	0.176 ± 0.005	0.195 ± 0.005	**0.007**	**0.018**	**0.006**
Strenth estimate						
Failure load (kN)	3.60 ± 0.14	4.05 ± 0.13	4.61 ± 0.12	< **0.0001**	**0.003**	< **0.0001**

C. Distal tibia	AN	NW-C	OB	ANOVA p-value	OB vs. NW-C *p*-value	OB vs. AN p-value
Bone geometry						
Total area (mm^2)	634.2 ± 17.8	647.1 ± 16.5	741.9 ± 15.8	< **0.0001**	< **0.0001**	< **0.0001**
Ct. area (mm^2)	104.7 ± 3.4	120.3 ± 3.2	143.6 ± 3.1	< **0.0001**	< **0.0001**	< **0.0001**
Tb. area (mm^2)	528.5 ± 17.9	525.4 ± 16.7	592.8 ± 16.1	**0.003**	**0.005**	**0.006**
Ct. thickness (mm)	1.07 ± 0.04	1.22 ± 0.04	1.37 ± 0.03	< **0.0001**	**0.004***	< **0.0001**
Microarchitecture						
Ct. Pore Volume (mm^3)	18.30 ± 3.63	9.42 ± 2.99	41.25 ± 2.84	< **0.0001**	< **0.0001**	< **0.0001**
Ct. Porosity (%)	2.22 ± 0.29	1.1 ± 5 0.24	3.36 ± 0.22	< **0.0001**	< **0.0001**	**0.001**
Tb. Number (1/mm)	1.72 ± 0.06	1.93 ± 0.05	2.48 ± 0.05	< **0.0001**	< **0.0001**	< **0.0001**
Tb. Thickness (mm)	0.091 ± 0.002	0.085 ± 0.002	0.078 ± 0.002	**0.0001**	**0.043***	< **0.0001**
Vol. BMD (vBMD)						
Total vBMD ($mgHA/cm^3$)	305.6 ± 8.6	329.02 ± 8.0	359.3 ± 7.7	< **0.0001**	**0.009**	< **0.0001**
Ct. vBMD ($mgHA/cm^3$)	869.2 ± 5.6	885.3 ± 5.3	879.2 ± 5.1	0.053	0.583	0.238
Tb. vBMD ($mgHA/cm^3$)	189.1 ± 6.3	195.2 ± 5.9	228.2 ± 5.7	< **0.0001**	< **0.0001**	< **0.0001**
Plate BV/TV	0.170 ± 0.010	0.178 ± 0.009	0.161 ± 0.009	0.401	0.295	0.688
Rod BV/TV	0.123 ± 0.009	0.137 ± 0.008	0.201 ± 0.008	< **0.0001**	< **0.0001**	< **0.0001**
Strength estimate						
Failure load (kN)	10.47 ± 0.31	11.15 ± 0.29	13.26 ± 0.28	< **0.0001**	< **0.0001**	< **0.0001**

AN: anorexia nervosa; C: normal-weight controls; OB: participants with obesity; Ct: cortical; Tb: trabecular; BV/TV: bone trabecular volume.
* p between 0.05 and 0.1 after controlling for current OCP use.
** $p > 0.10$ after controlling for current OCP use.

contribute to the higher reported risk for fracture in OB than controls. This suboptimal adaptation to mechanical loading in OB may be the consequence of lower percent lean mass in this group than in controls.

Many studies have demonstrated a higher risk of fracture in adolescents and adults with AN than in normal-weight controls [1,21,35], and this has been attributed to not just lower areal BMD *Z*-scores, but also alterations in bone geometry, microarchitecture, vBMD, and strength estimates [21]. Important determinants of impaired bone endpoints include lower body weight, changes in body composition (particularly lower lean mass) than in controls, and a delay in menarchal age [36]. However, the increased risk of fracture in adolescents with obesity has been difficult to explain given their higher areal BMD *Z*-scores compared with controls [37,38].

Higher aBMD in the OB group compared to AN and controls is likely a result of the adaptive effect of increased loading of bones from increased body weight in this group compared to the other two groups. However, if this adaptive increase in aBMD is suboptimal, the higher aBMD in OB may not be sufficient to sustain the greater weight of the individual during a fall, resulting in fractures. In this study of adolescents spanning the weight spectrum, we found that after controlling for weight, aBMD *Z*-scores no longer differed in OB vs. controls or AN. However, these findings do not provide an explanation for the higher risk of fracture reported in OB in other studies [5,39]. It is possible that aBMD *Z*-scores do not accurately reflect bone changes in OB responsible

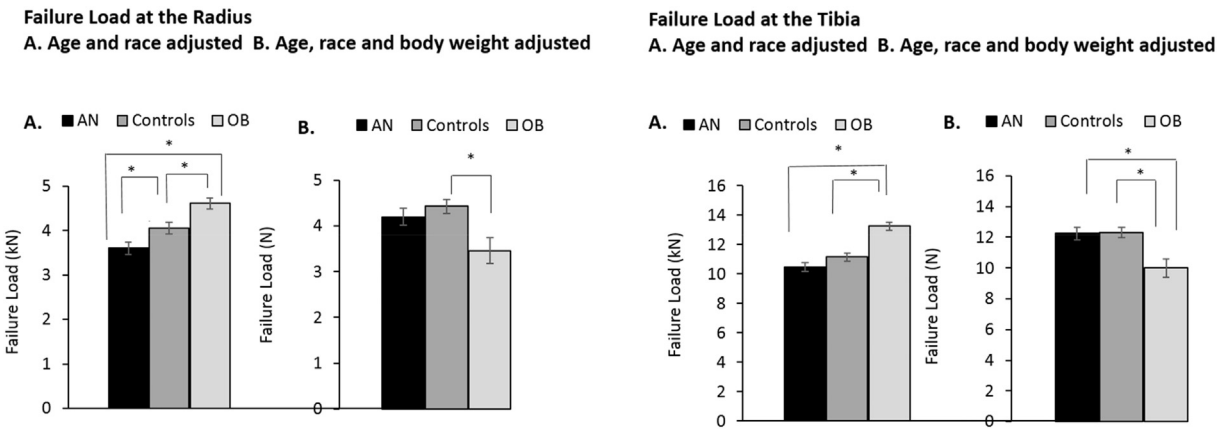

Fig. 1. Failure load at the distal radius and tibia, adjusted for age and race (A) and after adjusting for age, race, and body weight (B).
Before adjusting for weight, failure load increased progressively from anorexia nervosa to normal-weight controls to obesity at both sites. However, after adjusting for weight, failure load was significantly lower in the group with obesity compared with controls and the group with anorexia nervosa. *$p < 0.05$.

for this increased risk of fracture. We thus examined bone geometry, microarchitecture, and vBMD using HRpQCT, cortical porosity using extended cortical analysis, trabecular rod-plate morphology using individual trabecula segmentation, and strength estimates using FEA, to determine whether alterations in these measures may explain the higher risk of fracture reported in OB.

At both the distal radius and the distal tibia, many measures were higher in OB vs. controls and AN, as one would expect from higher mechanical loading from higher body weight, particularly for the weight-bearing tibia. However, certain measures did not demonstrate this adaptation. Particularly, cortical vBMD did not differ in OB compared to controls at the tibia, similar to a previous report using pQCT [40], and cortical porosity, which is known to reduce bone strength [41,42], was higher in OB than in AN and controls at both sites. Further, although trabecular vBMD was higher in OB vs. controls and AN, this was from a higher rod and not plate bone volume fraction (BV/TV), and the latter did not differ in OB vs. controls. This is relevant in that plate-like trabeculae are known to be stronger than rod-like trabeculae [43,44], and absence of higher plate BV/TV in OB may reflect suboptimal adaptation to this higher body weight in OB. Finally, at the distal tibia, the OB group had lower trabecular thickness than the other two groups. Consistent with these findings, after adjusting for body weight, failure load was lower in OB than in controls at both sites, and lower than in AN at the tibia.

Given that BMI, lean mass, menarchal and gynecological age have been reported to be key determinants of bone measures in AN and controls [36,45], we examined associations of these covariates with bone measures in OB. Weight and lean mass were strongly associated

Table 3
Associations of body composition measures and gynecological age with bone parameters in participants with obesity (OB) at the distal radius (3A) and tibia (3B).

A. Distal radius	Weight		Lean mass		Fat mass		Gynecological age	
	r	*p*-Value	r	p-Value	r	p-Value	r	p-Value
Total area	0.050	–	0.174	–	−0.037	–	−0.288	–
Ct. area	0.392	0.009	0.443	0.003	0.310	0.043	0.224	–
Tb. area	−0.020	–	0.089	–	−0.094	–	−0.329	0.031
Ct. thickness	0.281	–	0.264	–	0.264	–	0.352	0.021
Ct. pore vol.	0.373	0.013	0.327	0.033	0.376	0.013	−0.400	0.008
Tb. number	0.127	–	0.114	–	0.130	–	0.053	–
Tb. thickness	0.282	–	0.414	0.006	0.150	–	0.009	–
Total vBMD	0.266	–	0.284	–	0.237	–	0.351	0.021
Cort. vBMD	0.132	–	0.085	–	0.159	–	0.666	< 0.0001
Tb. vBMD	0.340	0.023	0.453	0.002	0.244	–	0.060	–
Failure load	0.389	0.009	0.588	< 0.0001	0.200	–	−0.072	–

B. Distal tibia	Weight		Lean mass		Fat mass		Gynecological age	
	r	p-Value	r	p-Value	r	p-Value	r	p-Value
Total area	0.327	0.03	0.412	0.006	−0.093	–	−0.183	–
Ct. area	0.418	0.005	0.487	0.001	−0.028	–	0.396	0.009
Tb. area	0.219	–	0.301	–	−0.094	–	−0.279	–
Ct. thickness	0.184	–	0.222	–	0.006	–	0.464	0.002
Ct. pore vol.	0.518	0.0004	0.516	0.0005	−0.121	–	0.035	–
Tb. number	0.412	0.006	0.420	0.006	0.093	–	0.095	–
Tb. thickness	−0.079	–	0.057	–	−0.062	–	0.016	–
Total vBMD	0.203	–	0.305	–	0.046	–	0.452	0.002
Ct. vBMD	−0.157	–	−0.169	–	0.071	–	0.467	0.002
Tb. vBMD	0.419	0.005	0.563	0.0001	0.020	–	0.170	–
Failure load	0.444	0.003	0.616	< 0.0001	−0.047	–	0.080	–

Only *p*-values < 0.05 are shown.
Ct.: Cortical; Tb: Trabecular; vBMD: volumetric bone mineral density; Vol: volume.

with various bone measures in both OB; associations were generally stonger with lean mass than weight for most measures (other than cortical porosity). Of note, while lean mass was higher in OB vs. control and AN groups, the proportion of lean mass was lower in OB vs. the other two groups. Given that lean mass was more strongly associated than weight with several bone measures, a lower percent lean mass despite higher body weight may explain suboptimal bone adaptation in the OB group. Further studies are necessary to determine other factors that may account for this suboptimal adaptation.

In contrast to reported studies in AN [46,47], we found limited or no associations of menarchal age, current OCP use, duration of amenorrhea, and proportion of participants with more than three months of amenorrhea at study entry with bone parameters in the OB group. Only nine of the 50 OB participants had amenorrhea of more than three months in the six months preceding the study, which suggests that most subjects in the OB group did not have PCOS, although we cannot rule out eumenorrheic oligo-anovulation in our participants. Regardless, exposure to excess androgens in those with PCOS would be expected to result in higher BMD and bone strength and would not take away from our findings. Conversely, gynecological age (duration since menarche and a measure of cumulative estrogen exposure in normally menstruating females) was positively associated with cortical thickness, and total and cortical vBMD, and inversely with trabecular area. This likely reflects reduced endosteal bone resorption in those with longer durations of estrogen exposure [48,49].

Interestingly, IGF-1 z-scores were lowest in OB, and were not associated with bone geometry or microarchitecture in this group, although associations were noted in the other two groups with the expected directionality. Lower IGF-1 z-scores in those with moderate to severe obesity may reflect reduced GH secretion in OB, as previously reported [50,51], though at least one study noted that bioactive IGF-1 did not differ in obesity vs. a control sample [50]. It is unclear why IGF-1, a known determinant of pubertal bone accrual, did not show the expected associations with bone parameters in OB. This may be related to sample size, or less variability of IGF-1 in the OB group, which would limit our ability to observe associations. Further, bioactive IGF-1 (not measured in this study) may better predict bone parameters in obesity than total IGF-1. A larger proportion of OB than controls and AN were on OCPs, which are known to reduce IGF-1 levels [52]; however, even after controlling for OCP use, we found no associations of IGF-1 z-scores with bone endpoints. Moreover, we did not find any difference in the results after accounting for diabetes in the OB group which is likely due to the small sample size.

Limitations of this study include its cross-sectional nature, and the relatively small sample size. The latter precluded us from reliably assessing fracture rates (especially traumatic vs. non-traumatic fractures) across groups. A larger sample size may allow for better assessment of the impact of body weight on bone measures in the context of both estimated strength and the risk for fracture. We did, however, have sufficient participants to enable a comparison of strength estimates (a reliable and strong predictor of fracture risk) across groups, and after controlling for weight, failure load was markedly lower in OB than the other two groups. Another limitation is that we did not measure gonadal steroids, a known predictor of pubertal bone accrual. However, unlike the AN group, which was associated with a 26.9% prevalence of amenorrhea, only 18.8% of the OB group was amenorrheic. Unlike the hypoestrogenic state in AN, amenorrhea in OB is more likely to be due to PCOS, which is not a hypoestrogenic state (but is a hyperandrogenic state). Thus, it is unlikely that lower gonadal steroid levels will explain the higher fracture risk in obesity. Of note, we did assess associations of gynecological age, which reflects cumulative estrogen exposure, with various endpoints and higher gynecological age associated with several bone parameters in the OB group. This is consistent with estrogen inhibiting endosteal bone resorption, thus resulting in higher cortical thickness and lower trabecular area. Further, we do not have PTH levels in all our participants, and higher PTH levels in OB may contribute to higher cortical porosity in this group. However, in a subset with clinical PTH levels, 82% had PTH levels in the normal range. Finally, given the exploratory nature of this study, we did not adjust for multiple comparisons when assessing associations of covariates with bone endpoints; this will be necessary in a subsequent study using a larger cohort.

In conclusion, this is the first study to our knowledge that compares bone geometry, microarchitecture, and strength estimates in adolescents and young adults across the weight spectrum. We demonstrate possibly suboptimal adaptation to the higher body weight in obesity when it comes to several bone measures, particularly cortical porosity, cortical vBMD, trabecular thickness, and plate bone trabecular fraction, which together likely contribute to lower failure load, a strength estimate, in OB than in control and AN groups after controlling for body weight. Larger studies are necessary to determine whether these specific findings translate to an increased risk of documented fractures. Studies are also necessary to determine factors other than percent lean mass that may explain this suboptimal adaptation in obesity.

Conflicts of interest

The authors have no conflicts of interest to disclose relevant to this paper.

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