Published in final edited form as:

Calcif Tissue Int. 2017 November ; 101(5): 479–488. doi:10.1007/s00223-017-0303-2.

Obese Versus Normal-Weight Late-Adolescent Females have Inferior Trabecular Bone Microarchitecture: A Pilot Case-Control Study

Joseph M. Kindler¹, Norman K. Pollock², Hannah L. Ross¹, Christopher M. Modlesky³, Harshvardhan Singh⁴, Emma M. Laing¹, and Richard D. Lewis¹

¹Department of Foods and Nutrition, The University of Georgia, 279 Dawson Hall, 305 Sanford Drive, Athens, GA, USA

²Department of Pediatrics, Augusta University, HS-1640 Health Sciences Campus, Augusta, GA, USA

³Department of Kinesiology, The University of Georgia, Ramsey Center, 330 River Rd, Athens, GA, USA

⁴Department of Kinesiology & Applied Physiology, University of Delaware, 201 N STAR Health Sciences Complex, Newark, DE, USA

Abstract

Though still a topic of debate, the position that skeletal health is compromised with obesity has received support in the pediatric and adult literature. The limited data relating specifically to trabecular bone microarchitecture, however, have been relatively inconsistent. The aim of this pilot cross-sectional case-control study was to compare trabecular bone microarchitecture between obese (OB) and normal-weight (NW) late-adolescent females. A secondary aim was to compare diaphyseal cortical bone outcomes between these two groups. Twenty-four non-Hispanic white females, ages 18–19 years, were recruited into OB ($n = 12$) or NW ($n = 12$) groups based on pre-specified criteria for percent body fat (>32 vs. <30 , respectively), body mass index (>90 th vs. 20 th– 79 th, respectively), and waist circumference (>90 th vs. 25 th– 75 th, respectively). Participants were also individually matched on age, height, and oral contraceptive use. Using magnetic resonance imaging, trabecular bone microarchitecture was assessed at the distal radius and proximal tibia metaphysis, and cortical bone architecture was assessed at the mid-radius and mid-tibia diaphysis. OB versus NW had lower apparent trabecular thickness (radius and tibia), higher apparent trabecular separation (radius), and lower apparent bone volume to total volume (radius; all $P < 0.050$). Some differences in radius and tibia trabecular bone microarchitecture were retained after adjusting for insulin resistance or age at menarche. Mid-radius and mid-tibia cortical bone volume and estimated strength were lower in the OB compared to NW after adjusting for fat-

Correspondence to: Richard D. Lewis.

Compliance with Ethical Standards

Conflict of interest: The authors have nothing to disclose.

Human and Animal Rights and Informed Consent: This study was approved by the Institutional Review Board for Human Subjects at The University of Georgia. Informed consent was obtained from all participants included in the study.

free soft tissue mass (all $P<0.050$). These trabecular and cortical bone deficits might contribute to the increased fracture risk in obese youth.

Keywords

Obesity; Trabecular bone; Magnetic resonance imaging; Insulin resistance

Introduction

The adolescent years are characterized by rapid bone mineral accrual, increases in cortical bone size, density and strength, and improvements in trabecular bone microarchitecture [1–3]. Although conflicting data have been reported, there is considerable evidence supporting the position that bone development is compromised in obese youth. This may help explain, at least in part, the greater risk for skeletal fracture in obese children and adolescents [4, 5]. Whereas this elevated fracture risk may also be attributed to deficits in physical function and greater subsequent risk for falling [6], in vitro mechanisms implicating adipose tissue as a negative determinant of adolescent bone structure and strength have been shown in vivo [7–9].

Few studies have considered measures of trabecular bone microarchitecture while examining the obesity–bone relationship in children and adolescents. The recent availability of high-resolution imaging techniques, however, has bolstered research endeavors aimed at examining these skeletal endpoints; measures that were otherwise unattainable through traditional imaging modalities. Studies in adults support the notion that obesity per se is beneficial to trabecular bone microarchitecture [10, 11]; yet, the limited data in younger cohorts have been less clear. For example, in two separate studies, children with greater adiposity had lower trabecular thickness, but greater trabecular number and lower trabecular separation [12, 13]. Whereas the findings relating to trabecular thickness can be perceived as detrimental to trabecular bone quality, those pertaining to number and separation are considered advantageous. Data from Hoy et al. [14] suggest that these inconsistent relationships in younger children foreshadow suboptimal trabecular bone microarchitectural outcomes in obese individuals during later adolescence and young adulthood (ages 15–21 years). However, no strictly designed case-control studies have been performed to examine differences in trabecular bone microarchitecture between obese versus normal-weight adolescents.

In contrast to the limited evidence specific to trabecular bone microarchitecture, data on cortical bone outcomes are more abundant in the pediatric literature. Fat-free soft tissue mass (FFST) is one factor explaining why obesity might be advantageous to cortical bone, as FFST tends to be greater in obese individuals and is a strong positive predictor of cortical bone [8, 15–17]. However, despite overt differences in lean body mass, various studies have shown similar cortical bone size and strength between obese versus normal-weight children [7, 8, 12]. Considering that the majority of fractures sustained by obese youth occur at cortical bone regions, these outcomes warrant further examination within the context of the obesity–bone relationship [4, 5, 18].

The primary aim of this pilot cross-sectional case-control study was to compare radius and tibia trabecular bone microarchitecture between obese (OB) and normal-weight (NW) non-Hispanic white late-adolescent females. We hypothesized that trabecular bone microarchitecture would be inferior in the OB versus NW. As a secondary aim, we compared mid-radius and mid-tibia cortical bone architecture and strength between our two groups.

Subjects and Methods

Study Design and Participant Characteristics

Participants were non-Hispanic white late-adolescent females (ages 18–19 years) who were recruited and individually matched based on a priori criteria for our NW ($n = 12$) and OB ($n = 12$) groups. A telephone pre-screening questionnaire was used to determine initial participation eligibility. Potential participants were excluded if they had not yet reached menarche, had an irregular menses, reported significant weight loss or gain in the past 6 months ($\pm 10\%$ initial body weight), had a previous diagnosis of an eating disorder or chronic disease, participated in Division-I collegiate athletics, or reported the use of medications/herbal supplements known to effect bone metabolism.

If self-reported inclusion criteria were met, participants attended a second screening session that included anthropometric measurements [height, weight, and waist circumference (WC)] and dual-energy X-ray absorptiometry (DXA)-assessed percent body fat (%BF). Body mass index (BMI)-for-age percentile for individuals 2–19 years of age was calculated (available at: <https://nccd.cdc.gov/dnpabmi/calculator.aspx>) and WC-for-age percentile cutoffs were determined [19]. The a priori criteria for inclusion in the OB or NW groups are as follows: participants in the OB group were required to have %BF ≥ 32 , BMI-for-age > 90 th percentile, and WC-for-age ≥ 90 th percentile and participants in the NW group were required to have %BF < 30 , BMI-for-age between the 20th and 79th percentiles, and WC-for-age between the 25th and 75th percentiles. Participants in the NW and OB groups were also individually matched on age (± 6 months), height (± 1 inch), as well as oral contraceptive use (yes or no) and duration (± 6 months). Using a health history questionnaire, all participants provided a retrospective self-report of the age at which menstruation commenced. All procedures were approved by the Institutional Review Board for Human Subjects at The University of Georgia, and all participants provided written consent.

Anthropometrics

Height, weight, and WC were measured in light indoor clothing and without shoes by one trained researcher. All measures were collected in duplicate and averaged. If the two measures differed by 1.0 cm or 1.0 kg, a third measure was obtained. Height was measured with the use of a wall-mounted stadiometer (Novel Products Inc., Rockton, IL) and rounded to the nearest 0.1 cm. Body weight was measured with an electronic scale (Seca Bella 840, Columbia, MD), and rounded to the nearest 0.1 kg. WC was measured with anthropometric tape (Rosscraft, Inc., Surrey, Canada) and rounded to the nearest 0.1 mm. WC was determined by placing the anthropometric tape at the uppermost boarder of the ilium and the measure was recorded following a normal expiration [20]. In females 18–24 years of age (n

= 12), intraclass correlation coefficients (ICCs) were calculated in our laboratory for weight, height, and WC (0.92–0.99) [21].

Physical Activity

As an estimate of lifetime participation in physical activities most beneficial to skeletal growth, bone-loading scores were calculated through the use of the bone-specific physical activity questionnaire (BPAQ) [22]. A more detailed description of this retrospective physical activity recall instrument was published previously [21].

Biochemistries

Serum samples were obtained following an overnight fast by a trained phlebotomist and held at -80°C . Serum glucose and insulin were measured at The University of Alabama at Birmingham Metabolism Core Laboratory. Glucose was assayed using a Stanbio Sirtus analyzer (Stanbio Laboratory, Boerne, TX) through the glucose oxidase method. Mean inter- and intra-assay coefficients of variation (CVs) were 2.56 and 1.28%, respectively. Insulin was assayed using a TOSOH AIA-600 II analyzer through a two-site immunoenzymometric assay method. Mean inter- and intra-assay CVs were 4.42 and 1.49%, respectively. As a function of fasting insulin and glucose concentrations, the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated [23]. IGF-1 (ng/mL) was measured in duplicate using a quantitative sandwich immunoassay technique (R&D Systems). Mean interassay CVs were 5.6–8.7%.

Dual-Energy X-ray Absorptiometry

Total body FFST, fat mass, and %BF were assessed by DXA (Discovery A; Hologic Inc., Waltham, MA). The same technician performed and analyzed all scans using APEX software, version 3.3. Calibration against a three-step soft tissue wedge (Hologic, Inc.) was carried out in order to ensure quality assurance for these measures. For determination of measurement reproducibility, ICCs were calculated in college-aged females (18–24 years of age; $n = 12$) who were scanned twice in our laboratory over a two-week period for FFST, fat mass, and %BF (all ICCs > 0.96).

Magnetic Resonance Imaging

Magnetic resonance images of the non-dominant radius and tibia were acquired at The University of Georgia Bio-Imaging Research Center using a General Electric 16-channel fixed-site Signa HDx 3.0 Tesla magnet as reported previously [21]. Trabecular bone scan sites included the distal radius and proximal tibia metaphysis. Scans at the radius were performed approximately 7 mm below the radial plateau while using an 8-channel wrist coil (Invivo, Inc.). Fifty contiguous images of the distal radius were 0.5 mm thick and were acquired using a 3-D Fast Gradient Echo pulse sequence (7.3 ms echo time; 19.5 ms repetition time; 40 degree flip angle; 61 kHz bandwidth; 8 cm field of view; 512 phase; 512 frequency; 1 excitation) and an imaging matrix of $156 \times 156 \times 500 \mu\text{m}^3$. Scan time was 8 min and 20 s. A single-channel phase array knee coil (GE) was placed around the proximal tibia for trabecular bone scan acquisition. Forty contiguous images of 1 mm thickness were acquired using a 3-D Fast Gradient Echo pulse sequence (6.2 ms echo time; 17.0 ms

repetition time; 40 degree flip angle; 61 kHz bandwidth; 10 cm field of view; 512 phase; 512 frequency; 2 excitations) and an imaging matrix of $195 \times 195 \times 1000 \mu\text{m}^3$. Scan time was 12 min and 40 s. Fifteen images representing the distal radius and proximal tibia were analyzed using custom semi-automated software created with Interactive Data Language (IDL; Research Systems, Inc., Boulder, CO) [24]. This procedure is outlined in Fig. 1. To eliminate inhomogeneity, a low-pass filter-based correction was applied to all images and image signal intensity was inverted to facilitate visualization. For each image, regions of interest containing trabecular bone and marrow were manually identified. Samples of cortical bone were taken from the cortical rim of each image and used for calibration during the separation of pixels into bone and marrow phases. Trabecular bone microarchitecture measures, including apparent trabecular bone volume to total volume (appBV/TV), as well as trabecular number (appTb.N; 1/mm), thickness (appTb.Th; mm), and separation (appTb.Sp; mm) were calculated for each of the 15 binarized images using a procedure similar to that described previously [25]. The average values for the 15 images are reported. The CV for test–retest reliability of appBV/TV, appTb.N, appTb.Th, and appTb.Sp in the proximal tibia was reported as 4.0, 3.3, 1.4, and 4.6%, respectively [26].

The same single-channel phase array knee coil described previously was used to collect images of the radius and tibia diaphysis. Forearm length was measured from the ulnar styloid process to the olecranon and tibia bone length was measured from the distal edge of the medial malleolus to the tibial plateau. The coil was first placed around the forearm and 25 images of the mid-radius were collected (spin echo, 0.6 mm thickness, 0.6 cm spacing, 10 ms echo time; 750 ms repetition time; 90 degree flip angle; 61 kHz bandwidth; 10 cm field of view; 512 phase; 256 frequency; 2 excitations). Scan time was 6 min 30 s. The coil was then placed around the leg and 25 images of the mid-tibia were collected (spin echo, 0.6 mm thickness, 0.6 cm spacing, 9 ms echo time; 750 ms repetition time; 90 degree flip angle; 61 kHz bandwidth; 16 cm field of view; 512 phase; 256 frequency; 2 excitations). Images representing the middle third of the radius and tibia were analyzed using custom automated software created with IDL [27, 28]. For each image, a gradient image was created using Sobel operators while the optimal segmentation threshold was determined by maximizing the correlation between the original image and the gradient image. Images were then median-filtered and segmented with a fuzzy C-means clustering algorithm [29]. Pixels representing cortical bone and the medullary cavity were identified and summed to determine their cross-sectional areas. Cortical bone width (cm) of the anterior, posterior, medial, and lateral portions were determined, and then averaged. Volume was determined by accounting for image thickness and the spacing between each image. Cross-sectional moment of inertia (CSMI) was determined in the anterior–posterior and medial–lateral directions using the parallel-axis theorem and the average value was reported [30]. Polar moment of inertia was calculated by summing CSMI in the two directions and section modulus was calculated by dividing CSMI by the furthest distance from the neutral axis.

Statistical Analyses

Histograms of all variables were inspected for outliers and non-normal distributions. Radius CSMI (square root), radius polar moment of inertia (square root), and FFST (log) were transformed due to non-normal distributions. The results of the between-group comparisons

using the transformed and untransformed values were similar. Thus, for ease of interpretation, the untransformed data are presented in Tables 1 and 3. Unadjusted between-group comparisons of descriptive participant characteristics as well as radius and tibia trabecular and cortical bone outcomes were performed using independent samples two-tailed *t* tests or Mann–Whitney *U* tests. Analysis of covariance was performed to examine between-group differences in cortical and trabecular bone measures while controlling for FFST, HOMA-IR, or age at menses. The level of statistical significance was set at $P < 0.05$. All data were analyzed using SPSS version 23 (SPSS Inc).

Sample Size Calculations

Sample size calculations using SPSS (Sample Power, version 2.0, Chicago IL) were estimated from peripheral quantitative computed tomography (pQCT)- and MRI-derived adiposity and bone data previously collected in our laboratory [7, 8, 26]. Based on these preliminary findings, we estimated that 7–12 subjects in each adiposity group would provide 80–85% power ($\alpha = 5\%$) to detect an approximate 10% difference in radius appBV/TV, appTb.N, appTb.Th, and appTb.Sp, thus we included 12 subjects per group.

Results

Participant characteristics are presented in Table 1. There were no significant between-group differences in age, height, BPAQ score, glucose, or IGF-I. However, per our study design, BMI, BMI-for-age percentile, WC, and %BF were greater in the OB versus NW (all $P < 0.001$). Weight, fat mass, FFST, serum insulin, and HOMA-IR were also greater in the OB compared to NW (all $P = 0.001$). In addition, age at menarche was approximately 1.5 years earlier in the OB versus NW ($P < 0.050$).

Distal radius appBV/TV and appTb.Th were lower, and appTb.Sp was higher, in OB versus NW (all $P < 0.050$; Table 2). Proximal tibia appTb.Th was lower in the OB as compared to the NW ($P < 0.050$). Following adjustment for FFST, distal radius appBV/TV (0.386 ± 0.005 vs. 0.412 ± 0.005 , $P = 0.006$), and appTb.Th (0.244 ± 0.005 vs. 0.270 ± 0.005 , $P = 0.004$) were lower, and proximal tibia appTb.N (1.641 ± 0.012 vs. 1.587 ± 0.012 , $P = 0.012$) and distal radius appTb.N (1.579 ± 0.016 vs. 1.524 ± 0.016 , $P = 0.049$) were greater, in the OB compared to NW. After adjusting for HOMA-IR, OB versus NW had lower distal radius appBV/TV (0.388 ± 0.005 vs. 0.409 ± 0.005 , $P = 0.016$). Additionally, distal radius appBV/TV (0.390 ± 0.004 vs. 0.408 ± 0.004 , $P = 0.012$) remained lower and appTb.Sp (0.396 ± 0.004 vs. 0.381 ± 0.004 , $P = 0.013$) remained higher in the OB versus NW after adjusting for age at menses.

Radius and tibia diaphyseal cortical bone size and estimated strength parameters did not differ significantly between the two groups (Table 3). However, following adjustment for FFST, radius and tibia cortical bone volume (radius: 6.030 ± 0.269 vs. 7.479 ± 0.269 , $P = 0.004$; tibia: 28.025 ± 1.817 vs. 36.428 ± 1.817 , $P = 0.011$), CSMI (radius: 0.066 ± 0.006 vs. 0.098 ± 0.006 , $P = 0.004$; tibia: 1.072 ± 0.111 vs. 1.572 ± 0.111 , $P = 0.012$), polar moment of inertia (radius: 0.132 ± 0.012 vs. 0.197 ± 0.012 , $P = 0.004$; tibia: 2.144 ± 0.221 vs. 3.144 ± 0.221 , $P = 0.012$), and section modulus (radius: 0.110 ± 0.007 vs. 0.151 ± 0.007 , $P = 0.001$; tibia: 0.894 ± 0.065 vs. 1.198 ± 0.065 , $P = 0.010$) were lower in the OB versus NW.

After adjusting for HOMA-IR or age at menses, radius and tibia cortical bone measures did not differ between the two groups.

Discussion

The primary aim of this pilot cross-sectional case-control study was to compare trabecular bone microarchitecture at the radius and tibia between OB and NW late-adolescent females. Considering the high costs and labor intensity of MRI scan acquisition, we optimized our study design by employing strict classification criteria for OB and NW groups. Supporting our hypothesis, we found inferior trabecular bone microarchitecture at both the radius and tibia in the OB versus NW. Some trabecular bone deficits at the radius remained even after adjusting for insulin resistance and age at menarche. Despite the OB having ~20% greater FFST than the NW, unadjusted cortical bone outcomes at both the radius and tibia did not differ between the two groups. However, after adjusting for differences in FFST, cortical bone volume and estimated strength were upwards of 40% lower in the OB group.

The primary finding from this study was that OB had inferior trabecular bone microarchitecture in comparison to NW. Our observed differences in distal radius and proximal tibia appTb.Th are consistent with findings from two separate studies [12, 13], such that children with greater adiposity had thinner trabeculae. However, contradicting the notion that excess adiposity is detrimental to trabecular bone, these previous findings involving Tb.Th were paralleled by a greater number of trabeculae that were less separated. Evans and colleagues [10] found similar results, reporting a greater trabecular number and lower trabecular separation, but similar trabecular thickness, at both the radius and tibia in favor of obese versus normal-weight young adults (ages 24–45 years). Considering the cross-sectional nature and conflicting results of these earlier studies, we cannot deduce whether these mixed relationships depict a positive or negative influence of adiposity on trabecular bone microarchitecture. Our study is also limited by its cross-sectional design; however, given our strict group inclusion criteria based on WC, %BF, and BMI, it is likely that the girls in our OB group had been obese throughout adolescence, since body composition tracks closely throughout maturation [1]. Albeit indirectly, the nearly 1.5 years earlier onset of menses in the OB versus NW supports this position considering that excess adiposity is associated with an earlier sexual maturation in females [31]. Whereas earlier maturation is suspected to benefit skeletal development, trabecular bone parameters were consistently inferior in these participants. In fact, differences in radius and tibia appTb.Th, but not radius appBV/TV and appTb.Sp, were attenuated after adjusting for age at menarche. One possible explanation for this discrepancy is that during later adolescence, the adverse influence of excess adiposity on bone health mitigates the benefit of earlier maturation onset. Nevertheless, perhaps obesity-related trabecular bone inadequacies commence during childhood and become most evident at the latter stages of development. In adolescent and young adult males (ages 15–21 years), Hoy and colleagues showed a negative relationship between total body fat mass and Tb.Th and BV/TV at both the ultradistal radius and tibia [14]. We showed similar results, but rather through a case-control design, such that our OB late-adolescents had lower appTb.Th, higher appTb.Sp, and subsequently lower appBV/TV compared to their NW peers.

Considering that trabecular bone microarchitecture was of primary interest, our power to detect significant differences in unadjusted cortical bone architecture and strength might not have been sufficient. Unlike results showing greater radius and tibia cortical bone geometry and estimated bending strength in overweight versus normal-weight subjects [32], our data corroborate reports in younger children showing that cortical bone did not differ between OB and NW despite OB having nearly 10 kg greater FFST [12]. Lean body mass is among the strongest determinants of cortical bone development during the adolescent years, and is often greater in obese versus normal-weight individuals [8, 12, 15, 16]. Our group has shown upwards to 13% greater cortical bone measures acquired via pQCT in children with normal versus higher %BF after controlling for lean mass [7, 8]. In the current study, the unadjusted differences in mid-radius cortical bone strength were of a similar magnitude. However, after adjusting for FFST, these differences were amplified, being as high as nearly 40% in favor of the NW. These findings support the understanding that deviations in cortical bone between obese and lean children are highly dependent upon lean mass, and that skeletal tissue adapts more so to lean as opposed to adipose tissue. Consistent with our results involving trabecular bone microarchitecture, cortical bone differences were more pronounced at the radius versus the tibia. This is likely attributed to the extra weight associated with obesity, which may help partially attenuate the adverse effect of excess adiposity on bone [33]. We were not sufficiently powered to explore fracture risk in the current study; however, obese children have a greater propensity for fracture in comparison to their normal-weight peers [4, 5]. Our data show that an increased risk for fracture might be attributed to a negative influence of adiposity on bone size and subsequently bending strength, thus rendering the bone vulnerable to the excess load associated with a fall.

Whereas we do not present data on specific pathogenic fat depots, one criterion for adipose group classification in this study was WC, which is a surrogate measure of visceral adiposity. Cohen et al. [34] showed lower trabecular BV/TV, number, thickness, and stiffness in women with higher versus lower visceral fat. Greater visceral adiposity was also accompanied by higher values of insulin resistance, which may have in turn influenced bone metabolism [34, 35]. That the obesity-related trabecular bone deficits were consistently attenuated following adjustment for HOMA-IR supports an intermediary role of insulin resistance in the fat-bone connection [36]. However, the manner in which insulin resistance influences skeletal tissue is relatively uncertain. One possible explanation involves the bone-augmenting hormone insulin-like growth factor I (IGF-I), which plays a pivotal role in musculoskeletal development [37, 38]. IGF-I signals upon target cells, specifically the bone-forming osteoblasts, through a similar downstream signaling pathway to that of insulin [39, 40]. Thus, children who are insulin resistant may be at risk of suboptimal IGF-I-dependent muscle and bone accrual [41, 42]. Although IGF-I is most known for its role in promoting cortical bone areal expansion [43, 44], Kirmani and colleagues found that boys with a greater IGF-I concentration had trabeculae that were less separated than those with lower IGF-I [45]. Therefore, the above-mentioned between-group differences in trabecular bone microarchitecture may be attributed to differences in IGF-I action secondary to insulin resistance. This hypothesis is supported by the similar IGF-I concentrations between the two groups, perhaps indicating a disconnect in IGF-I-dependent skeletal development in obese adolescents. Further, as suggested by Dimitri and colleagues [12], the adipose-derived leptin

might contribute to obesity-related trabecular bone inadequacies in obese youth. Unfortunately, data on leptin were not available in these participants, but this endocrine factor warrants consideration in future studies.

Limitations of this study include the outwardly small sample size and the cross-sectional design, as well as our inclusion of only non-Hispanic white females. Thus, it would be inappropriate to make any causal claims based on our results or translate our findings to other racial groups and/or males. The central strength of this study was the case-control design comparing OB and NW girls after carefully matching on age, height, and OC use. It is uncertain how the definition of obesity influences the presence, directionality, and/or magnitude of the fat–bone relationship. In the current study, we employed strict criteria for adipose group classification based on BMI-for-age percentile, WC, and %BF; whereas, cutoffs based on BMI are often used as the lone criterion. These differences in study design could explain the conflicting results between this study and others relating obesity and bone.

Conclusions

This pilot study is the first to compare both trabecular bone microarchitecture and cortical bone architecture and estimated strength in OB versus NW late-adolescent females through a strict case-control study design. Overall, our data show that OB versus NW had suboptimal unadjusted trabecular bone microarchitecture, and after accounting for differences in lean body mass, presented with inferior cortical bone strength. Some obesity-related trabecular bone deficits remained even after adjusting for insulin resistance and age at menarche. Because the distal radius is a bone site associated with a high incidence of fractures in adolescents [46], the differences in cortical bone volume and estimated bending strength, coupled with the inferior trabecular bone microarchitecture in the OB versus NW, may help explain the increased risk of fracture in obese youth. That our findings involving trabecular and cortical bone were more pronounced at the distal radius versus the proximal tibia supports the position that the greater body weight associated with obesity helps preserve skeletal integrity at habitually loaded regions. Future studies should explore the skeletal consequences of long-term obesity and obesity-related health complications throughout adolescence, including assessment of specific pathogenic fat depots, cardiometabolic health outcomes, and endocrine mediators involved in pediatric musculoskeletal development.

Acknowledgments

The authors would like to acknowledge the staff and students of the Bone and Body Composition Laboratory at the University of Georgia for their assistance in conducting this study. We also thank the participants for their time and commitment to this research.

Funding: This work was supported by Grant HL 87923-03S1 from the National Institutes of Health and the USDA, CSRS, National Institute of Food and Agriculture Hatch Projects GEO00797 and GEO00647.

References

1. Cheng S, Volgyi E, Tylavsky FA, Lyytikäinen A, Tormakangas T, Xu L, Cheng SM, Kroger H, Alen M, Kujala UM. Trait-specific tracking and determinants of body composition: a 7-year follow-up study of pubertal growth in girls. *BMC Med.* 2009; 7:5. [PubMed: 19171028]

2. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C. Peak Bone Mass. *Osteoporos Int.* 2000; 11:985–1009. [PubMed: 11256898]
3. Nishiyama KK, Macdonald HM, Moore SA, Fung T, Boyd SK, McKay HA. Cortical porosity is higher in boys compared with girls at the distal radius and distal tibia during pubertal growth: an HR-pQCT study. *J Bone Miner Res.* 2012; 27(2):273–282. [PubMed: 22028110]
4. Kessler J, Koebnick C, Smith N, Adams A. Childhood obesity is associated with increased risk of most lower extremity fractures. *Clin Orthop Relat Res.* 2013; 471(4):1199–1207. [PubMed: 23054515]
5. Taylor ED, Theim KR, Mirch MC, Ghorbani S, Tanofsky-Kraff M, Adler-Wailes DC, Brady S, Reynolds JC, Calis KA, Yanovski JA. Orthopedic complications of overweight in children and adolescents. *Pediatrics.* 2006; 117(6):2167–2174. [PubMed: 16740861]
6. Duncan MJ, Stanley M. Functional movement is negatively associated with weight status and positively associated with physical activity in british primary school children. *J Obes.* 2012; 2012:697563. [PubMed: 22545208]
7. 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(5):1530–1538. [PubMed: 17991669]
8. Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. *Osteoporos Int.* 2011; 22(2):655–665. [PubMed: 20449571]
9. Wey HE, Binkley TL, Beare TM, Wey CL, Specker BL. Cross-sectional versus longitudinal associations of lean and fat mass with pQCT bone outcomes in children. *J Clin Endocrinol Metab.* 2011; 96(1):106–114. [PubMed: 20926531]
10. Evans AL, Paggiosi MA, Eastell R, Walsh JS. Bone density, microstructure and strength in obese and normal weight men and women in younger and older adulthood. *J Bone Miner Res.* 2015; 30(5):920–928. [PubMed: 25400253]
11. Sornay-Rendu E, Boutroy S, Vilayphiou N, Claustrat B, Chapurlat RD. In obese postmenopausal women, bone microarchitecture and strength are not commensurate to greater body weight: the Os des Femmes de Lyon (OFELY) study. *J Bone Miner Res.* 2013; 28(7):1679–1687. [PubMed: 23371055]
12. Dimitri P, Jacques RM, Paggiosi M, King D, Walsh J, Taylor ZA, Frangi AF, Bishop N, Eastell R. Leptin may play a role in bone microstructural alterations in obese children. *J Clin Endocrinol Metab.* 2015; 100(2):594–602. [PubMed: 25412414]
13. Farr JN, Amin S, LeBrasseur NK, Atkinson EJ, Achenbach SJ, McCready LK, Joseph Melton L III, Khosla S. Body composition during childhood and adolescence: relations to bone strength and microstructure. *J Clin Endocrinol Metab.* 2014; 99(12):4641–4648. [PubMed: 25243571]
14. Hoy CL, Macdonald HM, McKay HA. How does bone quality differ between healthy-weight and overweight adolescents and young adults? *Clin Orthop Relat Res.* 2013; 471(4):1214–1225. [PubMed: 23001501]
15. Jackowski SA, Faulkner RA, Farthing JP, Kontulainen SA, Beck TJ, Baxter-Jones AD. Peak lean tissue mass accrual precedes changes in bone strength indices at the proximal femur during the pubertal growth spurt. *Bone.* 2009; 44(6):1186–1190. [PubMed: 19236963]
16. Kindler JM, Lewis RD, Hamrick MW. Skeletal muscle and pediatric bone development. *Curr Opin Endocrinol Diabetes Obes.* 2015; 22(6):467–474. [PubMed: 26414082]
17. Leonard MB, Zemel BS, Wrotniak BH, Klieger SB, Shults J, Stallings VA, Stettler N. Tibia and radius bone geometry and volumetric density in obese compared to non-obese adolescents. *Bone.* 2015; 73:69–76. [PubMed: 25497572]
18. Adams AL, Kessler JI, Deramerian K, Smith N, Black MH, Porter AH, Jacobsen SJ, Koebnick C. Associations between childhood obesity and upper and lower extremity injuries. *Inj Prev.* 2013; 19(3):191–197. [PubMed: 22789612]
19. Fernandez JR, Redden DT, Pietrobello A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr.* 2004; 145(4):439–444. [PubMed: 15480363]

20. Anthropometry Procedures Manual. National Health and Nutrition Examination Survey (NHANES). 2007
21. Kindler JM, Ross HL, Laing EM, Modlesky CM, Pollock NK, Baile CA, Lewis RD. Load-specific physical activity scores are related to tibia bone architecture. *Int J Sport Nutr Exerc Metab.* 2015; 25(2):136–144. [PubMed: 25029040]
22. Weeks BK, Beck BR. The BPAQ: a bone-specific physical activity assessment instrument. *Osteoporos Int.* 2008; 19(11):1567–1577. [PubMed: 18414964]
23. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care.* 1998; 21(12):2191–2192. [PubMed: 9839117]
24. Modlesky CM, Subramanian P, Miller F. Underdeveloped trabecular bone microarchitecture is detected in children with cerebral palsy using high-resolution magnetic resonance imaging. *Osteoporos Int.* 2008; 19(2):169–176. [PubMed: 17962918]
25. Majumdar S, Genant HK, Grampp S, Newitt DC, Truong VH, Lin JC, Mathur A. Correlation of trabecular bone structure with age, bone mineral density, and osteoporotic status: in vivo studies in the distal radius using high resolution magnetic resonance imaging. *J Bone Miner Res.* 1997; 12(1):111–118. [PubMed: 9240733]
26. Modlesky CM, Majumdar S, Dudley GA. Trabecular bone microarchitecture in female collegiate gymnasts. *Osteoporos Int.* 2008; 19(7):1011–1018. [PubMed: 18074110]
27. Johnson DL, Miller F, Subramanian P, Modlesky CM. Adipose tissue infiltration of skeletal muscle in children with cerebral palsy. *J Pediatr.* 2009; 154(5):715–720. [PubMed: 19111321]
28. Modlesky CM, Kanoff SA, Johnson DL, Subramanian P, Miller F. Evaluation of the femoral midshaft in children with cerebral palsy using magnetic resonance imaging. *Osteoporos Int.* 2009; 20(4):609–615. [PubMed: 18763012]
29. Suckling J, Sigmundsson T, Greenwood K, Bullmore ET. A modified fuzzy clustering algorithm for operator independent brain tissue classification of dual echo MR images. *Magn Reson Imaging.* 1999; 17(7):1065–1076. [PubMed: 10463658]
30. Turner, CH., Burr, DB. Experimental techniques for bone mechanics. CRC Press; Boca Raton: 2001.
31. Glass NA, Torner JC, Letuchy EM, Burns TL, Janz KF, Eichenberger Gilmore JM, Schlechte JA, Levy SM. The relationship between greater prepubertal adiposity, subsequent age of maturation, and bone strength during adolescence. *J Bone Miner Res.* 2016; 31(7):1455–1465. [PubMed: 26861036]
32. Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. *Am J Clin Nutr.* 2009; 90(4):1104–1111. [PubMed: 19710192]
33. Farr JN, Dimitri P. The impact of fat and obesity on bone microarchitecture and strength in children. *Calcif Tissue Int.* 2017; 100(5):500–513. [PubMed: 28013362]
34. Cohen A, Dempster DW, Recker RR, Lappe JM, Zhou H, Zwahlen A, Muller R, Zhao B, Guo X, Lang T, Saeed I, Liu XS, Guo XE, Cremers S, Rosen CJ, Stein EM, Nickolas TL, McMahon DJ, Young P, Shane E. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. *J Clin Endocrinol Metab.* 2013; 98(6):2562–2572. [PubMed: 23515452]
35. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev.* 2013; 93(1):359–404. [PubMed: 23303913]
36. Sayers A, Lawlor DA, Sattar N, Tobias JH. The association between insulin levels and cortical bone: findings from a cross-sectional analysis of pQCT parameters in adolescents. *J Bone Miner Res.* 2012; 27(3):610–618. [PubMed: 22095452]
37. Breen ME, Laing EM, Hall DB, Hausman DB, Taylor RG, Isaacs CM, Ding KH, Pollock NK, Hamrick MW, Baile CA, Lewis RD. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. *J Clin Endocrinol Metab.* 2011; 96(1):E89–E98. [PubMed: 20962027]
38. Xu L, Wang Q, Wang Q, Lyytikainen A, Mikkola T, Volgyi E, Cheng S, Wiklund P, Munukka E, Nicholson P, Alen M, Cheng S. Concerted actions of insulin-like growth factor 1, testosterone, and

- estradiol on peripubertal bone growth: a 7-year longitudinal study. *J Bone Miner Res.* 2011; 26(9): 2204–2211. [PubMed: 21590732]
39. Entingh-Pearsall A, Kahn CR. Differential roles of the insulin and insulin-like growth factor-I (IGF-I) receptors in response to insulin and IGF-I. *J Biol Chem.* 2004; 279(36):38016–38024. [PubMed: 15247278]
40. Duan C, Ren H, Gao S. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen Comp Endocrinol.* 2010; 167(3): 344–351. [PubMed: 20403355]
41. Kindler JM, Pollock NK, Laing EM, Oshri A, Jenkins NT, Isales CM, Hamrick MW, Ding KH, Hausman DB, McCabe GP, Martin BR, Hill Gallant KM, Warden SJ, Weaver CM, Peacock M, Lewis RD. Insulin Resistance and the IGF-I-Cortical Bone Relationship in Children Ages 9–13 Years. *J Bone Miner Res.* 2017; doi: 10.1002/jbmr.3132
42. Kindler JM, Pollock NK, Laing EM, Jenkins NT, Oshri A, Isales C, Hamrick M, Lewis RD. Insulin resistance negatively influences the muscle-dependent IGF-I-bone mass relationship in pre-menarcheal girls. *J Clin Endocrinol Metab.* 2016; 101(1):199–205. [PubMed: 26574958]
43. Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nasser P, Courtland HW, Williams V, Boussein M, Rosen C, Jepsen KJ. Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. *J Bone Miner Res.* 2009; 24(8):1481–1492. [PubMed: 19257833]
44. Hamrick MW, McNeil PL, Patterson SL. Role of muscle-derived growth factors in bone formation. *J Musculoskelet Neuronal Interact.* 2010; 10(1):64–70. [PubMed: 20190381]
45. Kirmani S, Christen D, van Lenthe GH, Fischer PR, Boussein ML, McCready LK, Melton LJ 3rd, Riggs BL, Amin S, Muller R, Khosla S. Bone structure at the distal radius during adolescent growth. *J Bone Miner Res.* 2009; 24(6):1033–1042. [PubMed: 19113916]
46. Cooper C, Dennison EM, Leufkens HG, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. *J Bone Miner Res.* 2004; 19(12):1976–1981. [PubMed: 15537440]

Fig. 1.

A visual description of the procedure used to determine measures of apparent trabecular bone microarchitecture in the distal radius and proximal tibia using magnetic resonance images. The procedure is depicted using images from the proximal tibia. Raw images (**a**) were filtered using a low-pass filter-based correction algorithm (**b**) and then reversed in *gray* scale to facilitate visualization (**c**). The region immediately inside the cortical shell was identified (large *black arrow*; **d**) and the trabecular bone region within the shell was masked manually. Twelve samples were taken from the cortical shell in each image (small *black arrow*; **e**). The three cortical bone samples with the highest signal intensity were then used to separate the region of interest into bone and marrow phases (binarized; **f**). Measures of trabecular bone microarchitecture were calculated for each binarized image as described by Majumdar et al. [25]

Table 1

Participant characteristics

	Normal-weight (<i>n</i> > 12)	Obese (<i>n</i> = 12)	<i>p</i> ^{<i>a</i>}
Age (years)	19.1 ± 0.4	19.0 ± 0.5	0.945
Age at menarche (years)	12.9 ± 1.2	11.5 ± 1.8	0.034
Height (cm)	165.7 ± 8.7	166.5 ± 8.2	0.821
Weight (kg) ^{<i>b</i>}	60.2 ± 6.0	88.9 ± 11.0	<0.001
BMI (kg/m ²)	22.0 ± 2.2	32.0 ± 2.5	<0.001
BMI-for-age percentile (%) ^{<i>b</i>}	50.6 ± 17.5	94.9 ± 1.6	<0.001
Waist circumference (cm)	75.2 ± 4.4	101.8 ± 6.0	<0.001
Fat mass (kg) ^{<i>b</i>}	15.8 ± 1.8	34.0 ± 6.6	<0.001
FFST (kg)	43.1 ± 5.2	53.9 ± 5.3	<0.001
Percent body fat (%)	25.9 ± 2.9	37.5 ± 3.5	<0.001
BPAQ score	38.8 ± 32.8	21.65 ± 18.2	0.128
Insulin (uU/mL) ^{<i>b</i>}	10.8 ± 4.6	24.7 ± 11.0	0.001
Glucose (mg/dL)	94.5 ± 6.5	98.4 ± 5.2	0.120
HOMA-IR	1.4 ± 0.6	3.2 ± 1.4	0.001
IGF-I (ng/mL)	172.1 ± 30.3	166.1 ± 39.0	0.681

Values are presented as mean ± SD

BMI, body mass index; *FFST*, fat-free soft tissue mass; *BPAQ*, bone-specific Physical activity recall questionnaire; *HOMA-IR*, homeostasis model assessment of insulin resistance; *IGF-I*, insulin-like growth factor I

^{*a*}Test of between-group significance based on independent samples *t* test

^{*b*}Test of between-group significance based on Mann–Whitney *U* test

Table 2

Comparison of radius and tibia trabecular bone microarchitectural outcomes in normal-weight versus obese late-adolescent females

	Normal-weight (<i>n</i> = 12)	Obese (<i>n</i> = 12)	<i>P</i> ^{<i>a</i>}	% diff. ^{<i>b</i>}
Radius (<i>distal metaphysis</i>)				
appBV/TV	0.409 ± 0.014	0.388 ± 0.015	0.002	−5.3
appTb.N (1/mm)	1.551 ± 0.064	1.551 ± 0.033	0.983	0.0
appTb.Th (mm)	0.264 ± 0.017	0.250 ± 0.012	0.030	−5.4
appTb.Sp (mm)	0.382 ± 0.013	0.395 ± 0.012	0.016	3.3
Tibia (<i>proximal metaphysis</i>)				
appBV/TV	0.368 ± 0.022	0.358 ± 0.018	0.212	−2.8
appTb.N (1/mm)	1.601 ± 0.040	1.627 ± 0.030	0.089	1.6
appTb.Th (mm)	0.230 ± 0.011	0.220 ± 0.010	0.027	−4.4
appTb.Sp (mm)	0.396 ± 0.021	0.395 ± 0.016	0.984	−0.3

Values are presented as mean ± SD

% diff., percent difference; *appBV/TV*, apparent bone volume-to-total volume ratio; *appTb.N*, apparent trabecular number; *appTb.Th*, apparent trabecular thickness; *appTb.Sp*, apparent trabecular separation

^{*a*}Test of between-group significance based on independent samples *t* test

^{*b*}% diff. = [NW − OB/(NW + OB)/2 × 100]

Table 3

Comparison of mid-radius and mid-tibia cortical bone outcomes between normal-weight versus obese late-adolescent females

	Normal-weight (<i>n</i> = 12)	Obese (<i>n</i> = 12)	<i>P</i> ^{<i>a</i>}	% diff. ^{<i>b</i>}
Radius (<i>mid-diaphysis</i>)				
Cortical bone width (cm)	0.284 ± 0.030	0.278 ± 0.041	0.672	−2.1
Cortical bone volume (cm ³)	6.863 ± 1.127	6.646 ± 0.723	0.580	−3.2
CSMI (cm ⁴)	0.087 ± 0.025	0.077 ± 0.013	0.204	−12.2
Polar moment of inertia (cm ⁴)	0.175 ± 0.049	0.154 ± 0.026	0.204	−12.9
Section modulus (cm ³)	0.139 ± 0.027	0.123 ± 0.015	0.082	−12.2
Tibia (<i>mid-diaphysis</i>)				
Cortical bone width (cm)	0.410 ± 0.069	0.396 ± 0.069	0.634	−3.5
Cortical bone volume (cm ³)	33.392 ± 5.949	31.061 ± 5.556	0.332	−7.2
CSMI (cm ⁴)	1.360 ± 0.433	1.284 ± 0.281	0.617	−5.7
Polar moment of inertia (cm ⁴)	2.719 ± 0.866	2.569 ± 0.561	0.617	−5.7
Section modulus (cm ³)	1.067 ± 0.253	1.024 ± 0.178	0.633	−4.1

Values are presented as mean ± SD

% diff, percent difference; *CSMI*, cross-sectional moment of inertia

^{*a*}Test of between-group significance based on independent samples *t* test

^{*b*}% diff. = [NW − OB/(NW + OB)/2 × 100]