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Racial Differences in the Association of Subcutaneous and Visceral Fat on Bone Mineral Content in Prepubertal Children

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Abstract. Total fat mass plays a significant role in determining bone mass, but the specific role of central adiposity independent of total fat mass has not been widely studied. Prepubertal (Tanner 1) children ($n = 181$; 65 boys, 116 girls, 7.8 ± 1.5 years), including 99 Caucasians and 82 African Americans from Birmingham, Alabama, participated in this study. Body composition, including total body and trunk fat mass, and bone mineral content (BMC) were measured using dual-energy X-ray absorptiometry. Subcutaneous abdominal adipose tissue (SAAT) and intra-abdominal adipose tissue (IAAT) were determined by single-slice computed tomography (CT). After adjusting for gender, age, height, total fat, and lean mass, trunk weight was inversely correlated with BMC in Caucasians ($r = -0.56$, $P < 0.0001$) and in African Americans ($r = -0.37$, $P < 0.05$). In Caucasians, independent of gender, age, height, total fat, and lean mass, there was an inverse correlation between SAAT and BMC ($r = -0.58$, $P < 0.0001$) but no significant correlation between IAAT and BMC; in addition, SAAT explained 6% of the variance in BMC. In contrast, in African Americans, SAAT and BMC were not significantly correlated. However, while adjusting for gender, age, height, SAAT, total fat, and lean mass, an inverse association between IAAT and BMC was observed in African Americans ($r = -0.50$, $P < 0.01$); IAAT also explained 3% of the variance in BMC. These findings suggest that, in general, total abdominal weight is negatively associated with bone mass, but there appear to be racial differences with regard to the contributions of subcutaneous and visceral fat to BMC in prepubertal children.

Key words: Android — Bone mass — Computed tomographic scan — Ethnicity — Fat distribution

The important link between android body fat distribution and several diseases (hypertension, diabetes, stroke, myocardial infarction) has been described previously [1, 2]. Metabolic disorders such as insulin resistance, impaired glucose tolerance, and diabetes have been previously linked to deficits in bone mass [3–6]; but the possible relationship between abdominal adipose tissue and osteopenia independent of general obesity has not been carefully examined. Ethnic disparities in the relationship between central adiposity and bone mass may be especially complex because of racial differences in body size [7], body composition [8], regional fat accumulation [9, 10], insulin levels and insulin resistance [11–13], hip geometry [14], and rates of skeletal loss [15].

Limited research in adults addressing these potential racial differences has found conflicting results. Tarquini et al. [16] concluded that Caucasian women with android-like obesity are protected from osteoporosis. Similarly, Heiss et al. [17] and Stewart et al. [18] showed that abdominal fat weight and waist-to-hip ratio (WHR) were significant positive predictors of bone mineral density (BMD) in postmenopausal women and men. In contrast, Jankowska et al. [19] found that visceral adiposity as assessed by WHR was significantly related to reduced bone mass in healthy Caucasian men. Likewise, Huang et al. [20] reported an inverse association between visceral abdominal fat and lumbar spine BMD in human immunodeficiency virus-infected men. Methodological differences (body mass index [BMI], WHR, computed tomography [CT], magnetic resonance imaging [MRI], dual-energy X-ray absorptiometry [DXA], peripheral quantitative CT [pQCT]) across studies make it difficult to compare results and draw definitive conclusions regarding the matter. In addition, because adults have higher amounts of visceral and subcutaneous fat than children, the physiological contribution of central fat to bone mass in adults may not be measured as accurately as in children. Also, studies in adults may be confounded by differences in hormones, diet, smoking, and alcohol intake. Thus, studies of racial differences in prepubertal children may be particularly useful because results are less likely to be confounded by these factors. Although previous pediatric studies [21–23] have examined the relationship between fat mass and bone mineral content (BMC), the specific contributions

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of subcutaneous and visceral fat to BMC have not been studied or reported in a population of children.

We studied the role of abdominal weight independent of total fat and lean mass in a group of prepubertal children. We hypothesized that independent of total fat and lean mass there would be an inverse correlation between abdominal weight and bone mass.

Materials and Methods

Study Cohort and Subject Description

Children were recruited by newspaper and radio advertisements, presentations at local schools, mailings to university and hospital employees, and by word of mouth. Children were excluded if they had taken or were taking medications known to affect bone mass or body composition; were diagnosed with syndromes or diseases known to affect bone mass, body composition, or fat distribution (e.g., Cushing's, Down, type 1 or type 2 diabetes); or were diagnosed with any major illness since birth. Ethnicity (Caucasian or African American) was determined by self-report and defined by all four grandparents being of the same ethnic group as the child in the study. Participants were selected on the basis of (1) age 5–10 years and (2) Caucasian or African American ancestry. Data were collected between 1997 and 2001. Only children in Tanner stage 1 were included. The Institutional Review Board of the University of Alabama at Birmingham approved this study. Informed consent and assent were obtained from all parents and children, respectively.

Based on inverse correlation coefficients reported by Jankowska et al. [19] between WHR and BMC ($r = -0.34$) and by Huang et al. [20] between visceral fat from CT and BMD ($r = -0.47$), we expected an effect size in the range 0.34–0.47, which would require 27–53 participants. It is noteworthy that with a sample size of 99 Caucasians and 82 African Americans and observed r values in the range 0.45–0.65, we had over 99% power.

Protocol Design

Outpatient screening visit. Children arrived at the General Clinical Research Center (GCRC) at approximately 8:00 a.m. after an overnight fast. Weight and height were measured, followed by a detailed medical history and physical examination conducted by a board-certified pediatric endocrinologist. Physical examinations included assessment of Tanner stage based on breast stage and pubic hair development in girls [24] and genitalia development in boys [25]. Only children in Tanner stage 1 were included.

Inpatient visit. Children were admitted to the GCRC in the early afternoon and then completed tests for bone mass and body composition using DXA. Central fat distribution was measured by CT scanning.

Detailed Methodologies

Weight, height, and anthropometry. Height (by a wall-mounted stadiometer) and weight (by a balance beam medical scale) were recorded at each visit to the nearest 0.1 cm and 0.1 kg, respectively; and the average of the two measurements was used for analysis. BMI and weight and height Z scores for age and gender were determined based on established Centers for Disease Control and Prevention (CDC) normative curves using EpiInfo 2000, version 1.1. (CDC, Atlanta, GA). Waist girth was measured without clothing; hip girth was measured over light, single-thickness clothing. Both measurements were obtained with participants standing in an erect position with feet together. Waist girth was measured at the smallest circumference of the torso [26]. Hip girth was measured at the level of maximal extension of the buttocks posteriorly [26]. Values were recorded to the nearest 0.1 cm.

BMC, bone area, and body composition. A whole-body DXA scan was performed to determine total-body BMC, bone area, and body composition (trunk weight, trunk fat mass, trunk lean mass) using a Lunar (Madison, WI) DPX-L. The whole-body scan requires the subject to be placed supine with the arms and legs positioned according to the manufacturer's specifications. Quality control was performed daily using a phantom. The precision error (coefficient of variation for repeated measurements) for this technique is 0.46% for the whole body.

Fat distribution. Subcutaneous abdominal adipose tissue (SAAT) and intra-abdominal adipose tissue (IAAT) were measured by CT scanning with a HiLight/Advantage Scanner (General Electric, Milwaukee, WI), as described previously [27]. A single-slice scan (5 mm) of the abdomen was performed at the level of the umbilicus and analyzed for cross-sectional area of adipose tissue using a density contour program. CT data are presented as cross-sectional area of tissue (cm^2) with Hounsfield units for adipose tissue as −190 to −30. We have shown the test-retest reliability for IAAT to be 1.7% [28]. All scans were analyzed by the same investigator.

Exercise testing. A subsample ($n = 106$) of participants completed an all-out, progressive walking treadmill protocol appropriate for children, as described previously [29]. The children walked for 4 minutes at 0% grade and 4 km/hour, after which the treadmill grade was raised to 10%. Each ensuing work level lasted 2 minutes, during which the grade was increased by 2.5%. The speed remained constant until a 22.5% grade was reached, at which time the speed was increased by 0.6 km/hour until the subject reached exhaustion.

Oxygen consumption and carbon dioxide production were measured continuously and analyzed using a metabolic cart (model 2900; Sensormedics, Yorba Linda, CA). Heart rate was monitored by a Vantage XL heart rate monitor (model 61204; Polar Electro, Woodbury, NY). Three criteria were used to determine a successful maximal test: (1) a leveling or plateauing of VO_2 (defined as an increase of oxygen uptake < 2 mL/kg/minute); (2) heart rate > 195 beats per minute (bpm); and (3) respiratory exchange ratio > 1.0. VO_2max was defined by the attainment of at least two of the three criteria.

Statistical Analysis

All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL), with a type I error set at $P < 0.05$. Analysis of variance was performed to determine whether there were ethnic differences in participant characteristics. Partial correlations were performed to determine the relationships between BMC, BMC/kg, bone area, and the independent variables. As determined by the Kolmogorov-Smirnov test of normality, BMC was not normally distributed and was log-transformed for the multiple linear regression analyses. Stepwise linear regression analysis was used to identify the significant covariates of BMC. A power analysis software program (nQuery Advisor, version 3; Statistical Solutions, Saugus, MA) was used for the power analysis results.

Results

Means and standard deviations (SDs) of each participant characteristic in each ethnic group are shown in

Table 1. Descriptive characteristics (mean ± SD)

Variable	Caucasian (n = 99)	African American (n = 82)
Gender		
Boys	44	21
Girls	55	61
Age (years)	7.7 ± 1.6	8.0 ± 1.4
Weight (kg)	31.9 ± 10.0	37.9 ± 14.2[a]
Weight Z score	0.84 ± 0.98	1.1 ± 1.1
Height (cm)	129.3 ± 11.3	129.8 ± 11.9
Height Z score	0.53 ± 0.97	0.38 ± 1.1
BMI (kg/m^2)	18.7 ± 3.7	22.0 ± 6.2[a]
Waist girth (cm)	18.7 ± 3.7	22.0 ± 6.2
Hip girth (cm)	62.1 ± 9.8	64.2 ± 11.7
Total fat mass (kg)	9.0 ± 5.6	10.6 ± 7.4
Percent fat (%)	27.7 ± 9.1	29.4 ± 10.2
Total lean mass (kg)	20.1 ± 4.3	21.1 ± 5.0
Trunk weight (kg)	12.6 ± 4.1	14.3 ± 5.7
Trunk fat mass (kg)	4.0 ± 3.0	5.3 ± 5.0
Trunk lean mass (kg)	10.7 ± 2.9	12.8 ± 4.7[a]
SAAT (cm^2)	81.5 ± 72.4	106.4 ± 100.1
IAAT (cm^2)	27.4 ± 17.7	33.7 ± 25.0
SAAT + IAAT (cm^2)	109.0 ± 88.2	140.2 ± 123.0[a]
VO_2max (mL/min)[b]	1.24 ± 0.31	1.25 ± 0.31
Total-body BMC (g)	1,170.1 ± 346.0	1,154.3 ± 298.4
Total-body bone area (cm^2)	1,297.7 ± 280.5	1,315.1 ± 238.1

[a] Significantly greater than Caucasians
[b] VO_2max values represent 106 subjects (64 Caucasians, 42 African Americans)

Table 1. African American children were heavier (body weight 37.9 vs. 31.9 kg), had greater trunk lean mass (12.8 ± 4.7 vs. 10.7 ± 2.9), and had greater abdominal fat (SAAT + IAAT) (140.2 ± 123.0 vs. 109.0 ± 88.2) than Caucasians. There were no significant differences in trunk fat, SAAT, and IAAT in Caucasians and African Americans. Means of total-body BMC were 1,170 and 1,154.3 g in Caucasians and African Americans, respectively. BMC and bone area were not significantly different between the two ethnic groups.

Table 2 shows partial correlations (adjusting for gender, age, height, total fat mass, and lean mass) between total trunk weight, trunk fat mass, trunk lean mass, SAAT, IAAT, and SAAT + IAAT with BMC, size-adjusted BMC (BMC divided by kilograms of body weight), and bone area in each ethnic group. Total trunk weight was significantly and inversely correlated with BMC in Caucasians ($r = -0.56$, $P < 0.0001$) and in African Americans ($r = -0.37$, $P < 0.05$). The correlation between total trunk weight and BMC/kg was $r = -0.57$ ($P < 0.0001$) in Caucasians but did not reach statistical significance in African Americans ($r = -0.30$, $P = 0.06$). Total trunk weight was inversely correlated with bone area in Caucasians ($r = -0.53$, $P < 0.0001$) and in African Americans ($r = -0.45$, $P < 0.01$). There were no significant associations between trunk fat mass and BMC, BMC/kg, or bone area in either Caucasians or African Americans. Trunk lean mass was not significantly correlated with BMC, BMC/kg, or bone area in Caucasians or African Americans. In Caucasians, SAAT was inversely correlated with BMC ($r = -0.58$, $P < 0.0001$), BMC/kg ($r = -0.53$, $P < 0.0001$), and bone area ($r = -0.49$, $P < 0.0001$). The partial correlations between SAAT and BMC, BMC/kg, or bone area in African Americans were not significant. In African Americans, IAAT was inversely correlated with BMC ($r = -0.50$, $P < 0.01$), BMC/kg ($r = -0.51$, $P < 0.01$), and bone area ($r = -0.43$, $P < 0.01$). The partial correlations between IAAT and BMC, BMC/kg, or bone area in Caucasians were not statistically significant. Abdominal fat mass from CT (SAAT + IAAT) was significantly and inversely correlated with BMC in Caucasians ($r = -0.61$, $P < 0.0001$) and in African Americans ($r = -0.34$, $P < 0.05$). SAAT + IAAT was also inversely correlated with BMC/kg ($r = -0.55$, $P < 0.0001$) and bone area ($r = -0.54$, $P < 0.0001$) in Caucasians and only with bone area ($r = -0.43$, $P < 0.01$) in African Americans.

Stepwise multiple linear regression analyses were used separately in each ethnic group to examine the independent association of gender, age, fat mass, lean mass, SAAT, IAAT, and VO_2max with BMC (Table 3). Table 3 shows that in Caucasians lean mass (76%), SAAT (6%), and fat mass (5%) explain a total of 87% of the variance in BMC, with no contribution by gender, age, IAAT, or VO_2max. In African Americans, lean mass (74%), IAAT (3%), age (3%), fat mass (2%), and gender (1%) explain a total of 83% of the variance in BMC, with no contribution by SAAT or VO_2max.

Discussion

The physiological basis for the relationship between body weight/fat distribution and bone mass remains uncertain. Although obesity-induced mechanical loading is probably the main factor contributing to variation in bone mass, factors other than skeletal loading also contribute since the correlations between fat mass and BMD in non-weight-bearing sites are comparable with those in weight-bearing sites [17, 30]. Furthermore, if the relationship between body weight and bone mass was simply a load-bearing phenomenon, the impact of fat mass to bone mass would be equal to the contribution of lean mass to bone mass; we [31–33] and others [30, 34] have found unequal contributions of lean and fat mass to bone mass. Therefore, mechanical loading is unlikely to be the complete explanation, and these relationships required further investigation.

In this study, we found that, in general, abdominal weight was negatively correlated with BMC in prepubertal children; this adverse association was independent of gender, age, height, total fat, and lean mass. In our preliminary analyses, we found gender differences in

Table 2. Partial correlations with BMC, BMC/kg, and bone area (BA; adjusting for gender, age, height, total fat mass, and lean mass)

	Caucasian (n = 99)			African American (n = 82)		
Variable	BMC	BMC/kg	BA	BMC	BMC/kg	BA
Trunk weight (kg)	−0.56*	−0.57*	−0.53*	−0.37***	−0.30	−0.45**
Trunk fat mass (kg)	0.09	0.16	0.09	−0.02	−0.06	−0.05
Trunk lean mass (kg)	0.18	0.20	0.04	−0.15	−0.17	−0.16
SAAT (cm^2)	−0.58*	−0.53*	−0.49*	−0.17	−0.12	−0.31
IAAT (cm^2)[a]	−0.16	−0.16	−0.12	−0.50**	−0.51**	−0.43***
SAAT + IAAT (cm^2)	−0.61*	−0.55*	−0.54*	−0.34***	−0.29	−0.43***

* $P < 0.0001$, ** $P < 0.01$, *** $P < 0.05$
[a] Additionally adjusted for SAAT

Table 3. Multiple linear regression model for log BMC

	Caucasian (n = 99)		African American (n = 82)	
	b ± SE	R^2	b ± SE	R^2
Intercept	5.95 ± 0.05		6.06 ± 0.10	
Gender	NS		−0.067 ± 0.029**	0.01
Age (years)	NS		0.046 ± 0.011*	0.03
Fat mass (kg)	0.00004 ± 0.00*	0.05	0.00002 ± 0.00*	0.02
Lean mass (kg)	0.00004 ± 0.00*	0.76	0.00004 ± 0.00*	0.74
SAAT (cm^2)	−0.002 ± 0.00*	0.06	NS	
IAAT (cm^2)	NS		−0.005 ± 0.001*	0.03
VO_2max (mL/min)	NS		NS	
Total R^2	0.87		0.83	

b, Multiple regression unstandardized coefficient; SE, standard error
* Significant at $P < 0.0001$, ** significant at $P < 0.05$; NS, nonsignificant

BMC (higher in boys than girls, data not shown), which is consistent with several previous studies in prepubertal children [35, 36]; for this reason, all partial correlations were additionally adjusted for gender. It was also important to remove puberty as a confounder by separating children from adolescents; thus, only Tanner 1 children were included in the analyses and age was also adjusted for.

In this study, we also found racial differences in the independent contributions of subcutaneous and visceral fat to BMC and bone area. In Caucasian children, there was a strong inverse correlation between subcutaneous fat and BMC but no significant correlation between visceral fat and BMC. In contrast, in African American children, visceral fat and BMC were inversely correlated; there was no significant correlation between subcutaneous fat and BMC. Similarly, results from multiple linear regression analyses revealed that BMC in Caucasians is explained by SAAT (6%) but not by IAAT. In contrast, BMC in African Americans was explained by IAAT (3%) and not by SAAT. We believe that there may be several explanations for these racial differences and that they relate to the complex interactions between ethnicity, body composition, body fat distribution, insulin resistance, and leptin.

Studies have shown that African Americans are more insulin-resistant than Caucasians [11, 37]. We have previously shown more insulin resistance [37] in these African American compared to Caucasian children. Studies have shown that insulin has a positive effect on bone mass [38, 39] and that bone mass is negatively correlated with leptin [40–43], the effect of which is influenced by insulin levels [40]. We therefore are in agreement with others who have hypothesized that leptin, a polypeptide hormone mainly produced by adipose tissue [44], possibly acts centrally through the sympathetic system and inhibits bone formation either directly or indirectly through suppression of insulin secretion [40]. We believe that in these African American children the predominant mechanism explaining the inverse correlation between IAAT and BMC may be insulin resistance. On the other hand, in Caucasian children, the adverse role of leptin on bone mass may be the principal mechanism that describes the inverse correlation between SAAT and BMC. The effect of leptin on bone mass and the interrelationships between leptin and insulin concentrations and resistance and their independent contributions to bone mass warrant further investigation in multiethnic children, adolescents, and adults.

To the best of our knowledge, this was the first study to investigate the independent role of abdominal weight on the pediatric skeletal system and to assess the relationships between subcutaneous fat, visceral fat, and bone mass in children. A racial difference in the influence of abdominal fat deposition on BMC and bone area has also not been previously reported. However, although we used only few inclusion/exclusion criteria in selecting our sample, generalizability should be interpreted with caution. A technical limitation of fan beam DXA may be that it would underestimate BMC and bone area in overweight individuals because of the fact that bones are elevated away from the scanner as a result of excess fat [45]. However, in light of the fact that our participants were prepubertal children with BMIs in the average range for children and well below the average range for adults, combined with the fact that we used a pencil beam DXA which is more accurate than a fan beam DXA, we believe that the observed BMC and bone area values in our study were accurate and were neither underestimated nor overestimated. Further, the use of a sophisticated technique such as CT scanning to accurately measure subcutaneous and visceral fat area is a strength of this study. Because of its high resolution, CT scans are able to distinguish between SAAT and IAAT and to identify even small deposits of visceral fat [10]. The use of this imaging technique in our study improves upon most previous studies, which have used WHR or waist circumference, an approach which is not able to separate subcutaneous abdominal fat from visceral fat. It is noteworthy that the correlation we observed between trunk fat mass from DXA and abdominal fat mass (SAAT + IAAT) from CT was not high ($r = 0.22$, $P = 0.004$); however, trunk weight from DXA and abdominal fat mass (SAAT + IAAT) from CT were highly correlated ($r = 0.92$, $P < 0.0001$). We think that these observations may be due to the fact that CT scans are especially accurate in detecting fat mass and are perhaps able to distinguish the different compartments of weight better than DXA can. At any rate, the inverse correlations obtained between abdominal fat mass (SAAT + IAAT) and those of BMC, BMC/kg, and bone area (Table 2) complement the significant inverse correlations observed between total trunk weight from DXA and the bone measures.

In conclusion, although general obesity, despite its severe burden on health, may have one beneficial effect, that of preventing osteoporosis, the findings of this study suggest that abdominal obesity (i.e., trunk weight) specifically and independently may adversely influence bone mass in children. The racial differences observed in the contributions of subcutaneous and visceral fat to bone mass underscore the complexity of these relationships in a sample of prepubertal children.

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