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Does Fat Fuel the Fire: Independent and Interactive Effects of Genetic, Physiological, and Environmental Factors on Variations in Fat Deposition and Distribution across Populations

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Abstract

Markers of inflammation (MOI) have been reported to influence bone health in adults, with reports of inverse associations. Adipose has also been linked to bone. In children, the interrelationships are unclear. The objective of this study was to evaluate the relationship between MOI (i.e. CRP, TNFR2, IL-6) and bone mineral content (BMC) and determine the contribution of fat deposition/distribution in children. Forty-nine children (59% male) 7–12 y participated. Body composition was evaluated by DXA, and MOI and insulin sensitivity (S_I) were obtained during an IVGTT. Multiple linear regression was used for analyses. TNFR2 was inversely associated with BMC. In boys, TNFR2 was inversely associated with BMC, and in girls IL-6 was inversely associated with BMC, and total and percent fat influenced the relationships. Our results suggest a potential inhibitory role of inflammation on bone as well as a negative impact of adiposity. Future investigations are warranted to further investigate these relationships.

INTRODUCTION

The drastic rise in pediatric obesity, combined with recent evidence suggesting that excessive fat accumulation may be associated with increased fractures in childhood^{1–3}, indicates a need to understand the interplay between bone and fat compartments. This is particularly salient during puberty, as suboptimal bone growth coupled with excessive fat accumulation during this period has been postulated to increase osteoporosis and obesity risk later in life^{3–5}. Previous research has primarily focused on dietary intake and engagement in daily physical activity as the major contributors to bone health and body fat trajectories. However, diet and physical activity have not consistently been shown to be sole contributors to either the increased prevalence of obesity^{6,7} or the increased incidence of fracture risk in the pediatric population^{5,8}. As such, other factors are important to explore.

A growing body of evidence suggests that excessive fat accumulation leads to abnormal activation of pro-inflammatory pathways^{9–11}. This is of particular interest in the growing child, as the growth process itself is associated with some degree of inflammation¹². The

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CONFLICTS OF INTEREST

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additional ‘insult’ of increased inflammation due to obesity may compromise other aspects of growth (i.e. bone mass accrual). Accordingly, obesity may exacerbate inflammatory pathways and inhibit bone mineralization, thereby leading to increased incidence of skeletal fractures in childhood. Adipose tissue is a well-established source of various markers of inflammation (MOI) such as interleukin-6 (IL-6) and tumor necrosis factor (TNF), and increased adiposity has been shown to be associated with both systemic inflammation and bone integrity in adults^{5,10,11,13,14}. Further, elevated inflammation stimulates osteoclastic activity¹⁵ and is associated with lower bone mineral density in adults^{9,16}. Indeed, many inflammatory markers have catabolic properties, and a reduction in osteoblast differentiation due to increased inflammation has been reported *in vitro*¹³. We¹⁷ and others^{12,13,18,19} have also reported a positive relationship between adiposity and circulating MOI in children. However, the relation between inflammation and bone mass in children has not been thoroughly defined.

The dynamic growth and development occurring throughout the pubertal transition is met with crucial interactions of physiologic and behavioral underlying factors. These factors may act independently or synergistically to affect body tissue partitioning (i.e. increased fat mass at the expense of bone mass). There is limited research regarding the secretion of MOI as mediators in the context of the interplay between fat and bone, particularly in the pediatric population. The complexity of these interactions is augmented when considering diverse populations, as population-based differences have been consistently documented in fat mass and distribution as well as bone mineral content (BMC)^{20,21,21–23}. Taken together, further investigation on the mediation of the relationship between fat and bone by MOI is warranted, particularly in the pediatric population. The main objective of the study was to evaluate the relationship among circulating MOI (i.e. CRP, TNF receptor 2/TNFR2, IL-6) and BMC and to determine if the relationship between MOI and BMC is moderated by fat deposition/distribution. We also wanted to determine if these relationships differed by race/ethnicity and/or sex. Because both fat deposition and bone mineralization are affected by metabolic parameters (e.g. glucose/insulin homeostasis), a secondary objective was to evaluate if insulin sensitivity mediates the relationship between MOI and BMC.

METHODS

Subjects

Participants were 54 healthy children aged 7–12 y (59% boys and 69% African Americans) recruited as part of a larger cross-sectional study⁶. All subjects were pubertal stage ≤ 3 , as determined by a pediatrician according to the method of Marshall and Tanner (breast/genital development and pubic hair)²⁴. Participants were recruited during 2005 via newspaper advertisements, flyers, and word-of-mouth. Participants were excluded if they had a current diagnosis of diabetes, genetic disorders known to affect body composition or fat distribution, glucose or lipid metabolism disturbances, and/or use of medications known to affect body composition or physical activity. This study was approved by the University of Alabama at Birmingham (UAB) Institutional Review Board for Human Use. Consent and assent were obtained from a parent and the child, respectively, prior to study initiation.

Protocol

All data were collected during two visits. During the first visit, anthropometric measurements were obtained and a physical examination was conducted. For the second visit, participants were admitted to the General Clinical Research Center for an overnight stay. Upon admission, participants had a computed tomography (CT) scan. All participants were given the same meal and snack foods. After 2000h, only water and/or non-caloric decaffeinated beverages were permitted until after the morning testing session. Upon

completion of the overnight fast, an intravenous glucose tolerance test (IVGTT) was performed. This was followed by a dual-energy X-ray absorptiometry (DXA) scan in the Metabolism Core Laboratory of the UAB Clinical Nutrition Research Center.

Anthropometric measures

The same registered dietitian obtained anthropometric measurements on all children. Participants were weighed to the nearest 0.1 kg in minimal clothing without shoes (Scaletronix 6702W; Scaletronix, Carol Stream, IL). Height was recorded without shoes using a digital stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, WA). Height- and weight-for-age percentiles, along with BMI percentile, was calculated using CDC growth charts (<http://apps.nccd.cdc.gov/dnpabmi/>).

Pubertal Status

Direct observation for the assessment of pubertal stage by a pediatrician was utilized based on the criteria of Marshall and Tanner^{25,26}. One composite number is assigned for Tanner staging, representing the higher of the two values defined by breast/genitalia and pubic hair²⁷.

Body composition and fat distribution

Body composition measures were determined by DXA (Lunar Prodigy; software v 6.10.029; GE-Lunar Corp, Madison, WI). Participants were scanned in light clothing without shoes laying flat on their back with their hands placed at their sides. Total abdominal adipose tissue (TAAT), intra-abdominal adipose tissue (IAAT), and subcutaneous abdominal adipose tissue (SAAT) were determined via CT with a GE HiLight/Advantage scanner (General Electric, Milwaukee, WI). Participants were scanned in the supine position with their arms stretched above the head. A 5-mm single slice scan was taken at the level of the umbilicus, and a cross-sectional area analysis of adipose tissue (cm²) was performed by using a density contour computer program²⁸.

Intravenous glucose tolerance test (IVGTT)

An IVGTT was performed as previously described²⁰. In brief, flexible catheters were placed in the antecubital vein of both arms. Two blood samples were drawn for determination of basal glucose and insulin, and MOI. At time "0", glucose (50% dextrose, 300 mg/kg) was administered intravenously. Insulin (0.02 U/kg) was infused over a 5-min period from 20–25 min post glucose injection. Subsequent blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 15, 19, 20, 21, 22, 24, 26, 28, 30, 35, 40, 50, 60, 70, 180, and 240 min post glucose injection. Sera were stored at –85°C until analysis for glucose, insulin and MOI. The insulin sensitivity index (S_I) was determined using the Millennium version of the Bergman minimal model; MINMOD Millennium 5.18, 2000²⁹.

Glucose and insulin

Glucose was measured in 10 µL sera with a SIRRUS analyzer (Stanbio Laboratory, Boerne, TX). The mean intra assay CV for this analysis was 1.2%, and the mean inter assay CV was 1.9%. Insulin was assayed by using a double-antibody radioimmunoassay with 100-µL serum aliquots in duplicate (Linco Research Inc, St. Charles, MO). The mean intra- and inter assay CVs for the insulin assay were 3.7% and 6.5%, respectively, and the mean assay sensitivity was 3.35 µIU/mL.

MOI

Concentrations of serum CRP, IL-6, and soluble TNFR2 were measured from fasting blood samples obtained during the IVGTT. High-sensitivity enzyme-linked immunosorbent assays

were used to determine CRP (ALPCO Diagnostics, Windham, NH) and IL-6 concentrations (R&D Systems, Minneapolis, MN). The mean inter- and intra assay CVs for CRP and IL-6 were 10.2%, 10.0%, 15.9%, and 10.1%, respectively. An enzyme-amplified sensitivity immunoassay (EASIA) was used to determine TNF-R2 concentrations (BioSource Europe, Nivelles, Belgium). The mean inter- and intra assay CVs for TNF-R2 were 8.7% and 6.5%, respectively.

Genetic Admixture

Genotyping of the ancestry informative markers (AIM) for the measurement of genetic admixture was performed at Prevention Genetics (www.preventiongenetics.com) using the Chemicon Amplifluor SNPs Genotyping System (Genome Research 11:163–169, 2001) coupled with ArrayTape technology (www.global-array.com). A panel of 140 AIM was used to estimate the genetic admixture proportion of each participant. Molecular techniques for the allelic identification and methodology for genetic admixture application have been described elsewhere³⁰, and information regarding marker sequences, experimental details, and parental population allele frequencies has been submitted to dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>) under the handle PSU-ANTH. The information from the AIM was translated into estimates of ancestral genetic admixture for each subject using maximum likelihood (ML) estimation based on the ML algorithm described by Hanis *et al.*³¹. In brief, the ML method estimates the proportion of genetic ancestry for an individual, using a range of proportions from 0 to 1 and identifies the most probable estimate of admixture based on the observed genotypes. An estimate of European genetic admixture (more widely represented among this sample) was used in the models as genomic control.

Calcium and Vitamin D Intake

Reported calcium and vitamin D intakes were estimated by averaging two 24 h recalls obtained using the multiple pass method. Recalls were based on participant report and were always performed in the presence of at least one parent. One recall was performed at each visit. A trained dietitian coded and analyzed dietary intake data using Nutrition Data System for Research Software version 2006, (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN), a dietary analysis program designed for the collection and analyses of dietary recalls.

Physical Activity

The MTI Actigraph accelerometer (Actigraph GT1M –Standard Model 198-0100-02, ActiGraph LLC, Pensacola, FL and accompanying software) was used to measure physical activity levels and patterns for seven days. Epoch length was set at one minute and data was expressed as counts per minute (counts min⁻¹). Daily and total counts per minute were summed and averaged as determined by the software accompanying the device.

Statistical Analyses

Descriptive statistics were analyzed using ANOVA. The relationships between BMC, MOI, measures of fat deposition/distribution, and insulin sensitivity were initially evaluated using bivariate correlation analysis. Measures of adiposity included in the models were total fat and percent fat, TAAT, IAAT and SAAT.

Multiple linear regression analysis was used to evaluate the relationship between MOI and BMC in models independently controlling for total fat, percent fat, TAAT, IAAT, and SAAT, respectively. Models were also controlled for height, Tanner, sex, and European admixture. The contribution of dietary as well as physical activity variables were investigated; however, no relationship was detected. As such, these variables were removed

from final analyses. As sex- and ethnic specific changes in body composition are apparent during growth and development, the overall models were stratified individually by sex and race/ethnicity. Data were also stratified by S_1 , using median sample S_1 as the dividing point. Those in the top 50th percentile were characterized as having “high S_1 ” and those below the top 50th percentile were characterized as having “low S_1 .” Stratified models were controlled for sex (where appropriate), pubertal stage, height and European admixture. European admixture was included since it is the admixture with the widest variation among our sample. Sex was coded: males=0 and females=1. Race/ethnicity was coded with European Americans =0 and African Americans=1.

RESULTS

Descriptive Statistics

Descriptive statistics are shown in Table 1. There were no sex or racial/ethnic differences in age, height percentile, weight percentile, BMI percentile or BMC. However, females had higher percent fat than males, and EA had higher percent fat than AA. AA were reproductively more mature than EA, reported lower calcium intake, were less active, and had lower European admixture. Mean concentration of MOI are presented in Table 2. There were no differences between sex or race/ethnicity in MOI levels; however, males had marginally higher IL-6 ($p=0.07$).

Overall Models

Bivariate correlations evaluated using the entire sample revealed positive associations between BMC and fat deposition (total fat and percent fat; $p<0.0001$ and 0.01 , respectively), BMC and fat distribution (TAAT, IAAT, SAAT; $p=0.001$, 0.001 and 0.01 , respectively), as well as marginal significance with BMC and CRP ($p=0.07$) (Table 3). As previously published in this sample¹⁷, all measures of fat deposition and distribution were positively correlated with both CRP and TNFR2. In overall regression models (Tables 4–6), only TNFR2 showed independent, inverse associations with BMC. Statistical significance in the inverse relationship between BMC and TNFR2 remained after adjustment for each measure of adiposity, with the exception of IAAT ($p=0.087$).

Stratification by Sex

Sexual dimorphism was apparent in the relationships of adiposity and BMC, MOI and measures of adiposity and MOI and BMC. There were positive correlations between BMC and all parameters of adiposity in males ($p<0.05$); however, in females there was a positive correlation between BMC and total fat ($p<0.01$), and a trend towards significance with TAAT and SAAT (both $p<0.10$). CRP was positively correlated with all measures of adiposity in males and females ($p<0.01$). In males TNFR2 was positively associated with all measures of adiposity ($p<0.05$), and IL-6 was similar for measures of fat deposition, but there was a trend only for those reflecting fat distribution ($p<0.10$). In females, TNFR2 was not related to any measure of adiposity. There were positive correlations between IL-6 and percent fat, and a trend with SAAT.

In regression models, our results also indicated differential relationships of MOI with BMC between the sexes as well as variation in the contribution of fat deposition and distribution between girls and boys. Among males, in the models controlling for total fat and percent fat, TNFR2 was inversely associated with BMC (Table 5). In girls, there were no significant relationships observed between TNFR2 and BMC. In boys, there was no significant relationship between IL-6 and BMC irrespective of fat parameter included as a covariate; however, in girls, there was a significant inverse relationship in the models controlling for total fat and percent fat, but not measures of fat distribution ($p<0.05$; Table 6).

Stratification by Race/Ethnicity

Among AA, there was a strong correlation between CRP and fat deposition and distribution but not with TNFR2 or IL-6 (<0.0001). Among EA, percent fat was positively correlated with CRP, and TNFR2 was positively correlated with all measures of adiposity ($p<0.01$). There was a positive correlation between IL-6 and all measures of adiposity ($p<0.05$) with the exception of IAAT ($p=0.5$). There were also racial/ethnic differences in the associations between BMC and adiposity parameters. Whereas among AA, there were positive correlations between BMC and all adiposity parameters ($p<0.01$), in EA there were no significant correlations between BMC and adiposity measures. After stratifying by race/ethnicity, among EA a trend towards a positive correlation between BMC and TNFR2 was identified ($p=0.07$), but no statistically significant association with CRP or IL6 was observed. In AA, however, BMC and CRP were positively correlated ($p=0.03$).

For regression models, we also observed a differential relationship of MOI with BMC between EA and AA. There was a positive relationship between TNFR2 and BMC in EA independent of total fat and percent fat, but not measures of fat distribution (Table 5). Conversely, there was a significant inverse relationship between TNFR2 and BMC among AA in the models controlling for each measure of adiposity and fat distribution. There was an inverse relationship between IL-6 and BMC in EA after accounting for total fat mass, but no significant relationship was observed between IL-6 and BMC among AA (Table 6). Among AA there was a positive relationship between CRP and BMC only in the model controlling for IAAT.

Stratification by Insulin Sensitivity

Among subjects with low S_I , TNFR2 was significantly, inversely associated with BMC in the models adjusting for total fat, percent fat, TAAT, and SAAT (Table 7). This relationship showed a trend in the model including IAAT ($p=0.06$). There was a positive relationship between CRP and BMC among those with low S_I in the model including IAAT but no other relationships among parameters were observed. Relationships between MOI and BMC were not statistically significant in subjects with high S_I .

DISCUSSION

Increased adiposity is known to affect circulating levels of inflammatory markers in adults^{10,11} and children¹⁷. Research has also shown independent relationships between both adiposity and inflammation with bone health in adults. It is unclear whether reported associations between adiposity, inflammation and bone in adults apply to the pediatric population. Further, the extent to which adiposity and MOI may act in concert to influence bone mass accrual has not been clearly elucidated, particularly during growth and development. This study shows, for the first time in a pediatric population, a potential mediating effect of adiposity on the relationship of MOI with bone. Results indicated that fat deposition and distribution mediated overall inverse relationships between MOI and BMC, particularly for TNFR2. In addition, these relationships were divergent between race/sex.

Fat mass has consistently been shown to influence measures of bone health; however, the direction of the relationship is unclear^{5,13,14}. The understanding of the impact fat mass imparts on bone health is growing, with recent findings indicating adipose region-specific effects on bone in both animals and humans^{13,32–35}. Whereas SAAT has generally been reported to associate positively with bone density, equivocal results have been reported regarding the relationship between IAAT and bone^{13,36}. A pediatric study investigated such relationships and reported inverse associations between intra-abdominal adiposity and measures of bone density¹³. However, another study has also reported positive associations

of waist circumference values (used as a surrogate for IAAT) and bone density³⁷. Our findings suggest inconsistencies may not reside in the relationship among variables but rather the population/variable studied.

In our population, BMC was positively associated with all measures of adiposity and fat distribution in males, whereas BMC was only associated with total fat in females. Further, BMC was positively associated with all adiposity measures in AA, whereas the relationships were null in EA. The positive relationship observed between adiposity and BMC seems contradictory to the hypothesis that adiposity secretes pro-inflammatory cytokines, which in turn may inhibit bone mineralization. However, DXA-derived BMC does not necessarily reflect bone micro-architecture, thereby limiting the assessment of true bone health. Thus, whether total and/or central adiposity is beneficial or harmful for bone remains unclear. Nonetheless, it is likely that there is a differential contribution of adiposity based on inherent differences in physiology between sexes and/or racial/ethnic population.

The role of inflammatory markers in bone metabolism is unclear, but there is evidence suggesting inflammation promotes bone resorption through negative effects exerted on osteoblast differentiation^{16,38}, inhibition of osteoclast apoptosis¹³ and/or increasing the pool size of preosteoclasts in bone marrow¹⁵. In our sample, the most compelling relationship with BMC was that of TNFR2. In overall models, there were significant inverse correlations between TNFR2 and BMC, suggesting a catabolic effect of TNFR2 on BMC. Although the role of TNFR2 has not been well-characterized, it has been hypothesized to be a marker of TNF α activity. TNF α is known to directly activate osteoclast differentiation and activation and inhibit osteoclast apoptosis¹³. Elevated TNFR2 may thus result in increased bone resorption during the critical stage of bone mass accrual representative of our sample. It has also been hypothesized that TNFR2 slowly releases TNF α into circulation³⁶. Plausibly this “slow release” may mediate long-term adverse effects on bone mass accrual particularly during this period of rapid bone mass acquisition.

The relationship between TNFR2 and BMC was independent of all measures of adiposity except IAAT, suggesting IAAT could largely be accountable for the relationship. Indeed, the significance between TNFR2 and BMC no longer existed including this parameter of adiposity, providing further evidence to previous findings that IAAT is a metabolically detrimental fat compartment³⁹ and has been shown to secrete more pro-inflammatory cytokines than other compartments¹⁹. Contrary to this hypothesis, Cartier *et al.* observed that both SAAT and IAAT play a role in the degree to which MOI increases inflammatory status in adults¹⁰, and we previously observed that the relationship between MOI and central adiposity was not independent of general adiposity in children¹⁷, thereby suggesting no site-specific contribution of adiposity to MOI. As such, the extent to which the compartments influence inflammatory markers and their respective effects on BMC, particularly in children, remains unclear and deserves further exploration.

Interestingly, upon stratification based on race/ethnicity, the direction of the relationship between TNFR2 and BMC was positive in EA, whereas in AA the relationship remained negative, and was significant controlling for all measures of adiposity, including IAAT. It is not clear why differential relationships of TNFR2 with BMC would exist between race/ethnicity. These differences may reflect adaptations in paracrine/autocrine secretions among individuals of varying genetic background as a consequence of selective pressures based on ability to resist infectious disease (e.g. malaria²³, tuberculosis⁴⁰). Whereas metabolic needs of essential organs are similar across populations, groups inhabiting different geographic regions have been exposed to different disease loads. Accordingly, survival strategies relevant to inflammatory response, plausibly a target of selective pressures, may underlie

population-based variation in physiology and metabolism as well as inflammatory responses.

Stratification of data by sex modified the relationship of TNFR2 with BMC, remaining significant only among males and limited to models controlling for total and percent fat. In males, our findings suggest that the relationship was independent of general adiposity but may have been mediated by central adiposity. Findings among females suggest that fat deposition, but not necessarily distribution, may mediate the relationship between TNFR2 and BMC. The sexual dimorphism in the interrelationships between MOI, bone and fat could be explained by several mechanisms. Similar to our findings, Cartier reported differential impact of MOI in the relationship between fat compartments such that IAAT was associated with CRP in men and SAAT was associated with CRP in women¹⁰. It is plausible that fat distribution and sex hormone concentrations represent the most likely of these mechanistic differences; however, independent and interactive effects of in fuel oxidation and resource partitioning may contribute to these differences as well.

We observed an inverse relationship between BMC and TNFR2 only in those with low S_I . This is the first study, to our knowledge, to investigate the influence of insulin sensitivity status on the relationship between BMC and MOI. Several mechanisms may explain the observed differential relationship. Bone mass accrual is at least partially dependent on growth factors such as insulin⁴¹, and insulin is a known anti-inflammatory agent⁴². Furthermore, cross-talk between MOI and growth factors suggests an influence on regulation of insulin signaling⁴³. Finally, a higher S_I may be reflective of greater metabolic efficiency which may translate into a protective effect on bone through enhanced ability of nutrient handling, even in the presence of catabolic, pro-inflammatory markers.

The strengths of this study are inclusion of a bi-ethnic sample and use of robust measures of fat deposition/distribution and insulin homeostasis, as well as use of ancestral genetic admixture for genomic control. Limitations include the use of DXA to measure BMC as it may not accurately estimate BMC in growing children and does not provide information on bone micro-architecture. The possibility of other measures contributing to BMC (e.g. serum calcium, parathyroid hormone, vitamin D, leptin, IGF) cannot be ignored; however due to restrictions in amount of sera collected and/or funding, these measures were not available. Our sample size was also relatively small and cross-sectional in nature. The results then should not be used for inference of long-term or causal relationships and should serve as preliminary data to expand upon in larger cohorts. Although the interrelationships between BMC, MOI, and adiposity were marginal, these relationships warrant further investigation.

Together, our analyses provide insight into the interconnected parameters of body composition and the contribution of inflammatory status during growth and development. Evidence is provided for not only a potential inhibitory role of inflammation on bone mineralization, but also possible mediation by adiposity. Outcomes varied by sex, race/ethnicity, and degree of insulin sensitivity, such that inverse relationships were seen particularly in males, AA, and those with lower S_I . Interrelationships exemplified between BMC, fat deposition and distribution and MOI in a bi-ethnic sample during early puberty is suggestive of potential mechanisms related to the concomitant increases in obesity and fracture risk observed in this population over the past few decades. Future research on factors relating to inflammatory status, body habitus and hormones which influence bone mass accrual in children is warranted.

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TABLE 1

Descriptive statistics and adiposity measures in the total sample, by sex and by self-reported race/ethnicity (mean±SE)

	Total (n=49)	M (n=29)	F (n=20)	EA (n=17)	AA (n=32)
Age (yrs)	9.9±0.2	9.7±0.3	10.2±0.4	9.8±0.4	10.0±0.2
Height percentile	61.4±4.0	57.9±5.9	66.6±4.8	64.2±6.2	59.9±5.3
Weight percentile	66.3±4.1	64.7±5.7	68.7±6.0	68.4±7.2	65.3±5.1
BMI percentile	63.5±4.3	62.3±5.8	65.1±6.3	62.4±7.3	64.1±5.3
Percent fat	24.0±1.6	21.4±2.0*	27.8±2.3*	28.8±2.4*	21.5±1.9*
BMC (kg)	1.38±0.05	1.31±0.06	1.48±0.10	1.31±0.07	1.41±0.08
Tanner	1.45±0.10	1.38±0.12	1.55±0.17	1.18±0.10*	1.59±0.13*
Calcium intake (mg/d)	759.7±54.0	801.8±74.1	698.7±77.2	976.7±93.2*	644.5±57.3*
PA (min/d)	280.4±11.9	276.3±15.7	287.1±18.6	307.7±15.0*	258.2±16.1*
EUADM	0.43±0.06	0.38±0.07	0.51±0.09	0.95±0.02*	0.16±0.02*

* Superscript indicates significant differences between sex or self-identified racial/ethnic category, $p \leq 0.05$;

M=males, F=females, EA=European American, AA=African American, BMI=body mass index, BMC=bone mineral content, PA=physical activity, EUADM=European admixture

TABLE 2

Inflammatory marker levels in the total sample, by sex and by self-reported race/ethnicity (mean±SE)

	Total (n=49)	Males (n=29)	Females (n=20)	EA (n=17)	AA (n=32)
IL-6	0.94±0.10	1.09±0.13 ^ψ	0.72±0.13 ^ψ	1.2±0.23	0.85±0.09
CRP	0.47±0.11	0.42±0.134	0.55±0.18	0.65±0.23	0.38±0.11
TNFR2	4.35±0.10	4.324±0.14	4.39±0.16	4.55±0.18	4.24±0.13

^ψ
p=0.07;

EA=European American, AA=African American, IL-6=Interleukin 6, CRP=C-Reactive Protein, TNFR2=Tumor Necrosis Factor Receptor 2

TABLE 3

Bivariate correlations (r, p-value)

	BMC	CRP	IL-6	TNFR2	Total Fat	% Fat	TAAT	SAAT	IAAT
BMC		0.257, 0.075	-0.138, 0.343	-0.024, 0.871	0.606, <0.0001	0.351, 0.013	0.455, 0.001	0.452, 0.001	0.378, 0.008
CRP			0.376, 0.008	0.223, 0.123	0.658, <0.0001	0.704, <0.0001	0.642, <0.0001	0.626, <0.0001	0.612, <0.0001
IL-6				0.187, 0.200	0.239, 0.098	0.313, 0.028	0.270, 0.064	0.266, 0.067	0.250, 0.086
TNFR2					0.379, 0.007	0.418, 0.003	0.397, 0.005	0.393, 0.006	0.377, 0.008
Total Fat						0.948, <0.0001	0.937, <0.0001	0.950, <0.0001	0.769, <0.0001
% Fat							0.927, <0.0001	0.948, <0.0001	0.753, <0.0001
TAAT								0.989, <0.0001	0.897, <0.0001
SAAT									0.826, <0.0001

BMC=bone mineral content, IL-6=Interleukin 6, CRP=C-Reactive Protein, TNFR2=Tumor Necrosis Factor Receptor 2, TAAT=total abdominal adipose tissue, SAAT=subcutaneous abdominal adipose tissue, IAAT=intra-abdominal adipose tissue,

Bolded values p<0.05; *Italicized* values 0.10>p>0.05

Relationships between C-Reactive Protein (CRP) and bone mineral content, with models controlling individually for fat deposition and distribution parameters

TABLE 4

Covariate	Overall*		Males ψ		Females ψ		EA*		AA*	
	β	p	β	p	β	p	β	p	β	p
Total Fat	-0.002	0.879	0.008	0.681	-0.040	0.208	-0.030	0.219	0.017	0.469
% Fat	0.004	0.805	0.008	0.673	-0.019	0.616	-0.030	0.287	0.024	0.225
TAAT	0.005	0.715	0.019	0.333	-0.034	0.237	-0.036	0.072	0.035	0.141
IAAT	0.018	0.248	0.030	0.129	-0.016	0.636	-0.033	0.100	0.064	0.009
SAAT	.006	0.668	0.016	0.383	-0.020	0.475	-0.036	0.077	0.033	0.131

EA=European American, AA=African American, TAAT=total abdominal adipose tissue, IAAT=intra-abdominal adipose tissue, SAAT=subcutaneous abdominal adipose tissue

* models controlled for height, tanner, sex and European admixture

ψ models controlled for height, tanner and European admixture

Bolded values p<0.05

Italicized values 0.10>p>0.05

TABLE 5

Relationships between Tumor Necrosis Factor Receptor 2 (TNFR2) and bone mineral content, with models controlling individually for fat deposition and distribution parameters

Covariate	Overall*		Males ψ		Females ψ		EA*		AA*	
	β	p	β	p	β	p	β	p	β	p
Total Fat	-0.23921	0.0210	-0.31300	0.0302	-0.18760	0.2917	0.36640	0.0197	-0.38293	0.0015
% Fat	-0.21841	0.0510	-0.29566	0.0469	-0.18361	0.3634	0.44182	0.0160	-0.41031	0.0022
TAAT	-0.23179	0.0321	-0.23060	0.1402	-0.26593	0.1255	0.27921	0.0198	-0.37817	0.0062
IAAT	-0.18337	0.0866	-0.12714	0.3663	-0.28268	0.1631	0.25199	0.0103	-0.32991	0.0205
SAAT	-0.23040	0.0311	-0.25462	0.0948	-0.23906	0.1820	0.30096	0.0148	-0.39119	0.0037

EA=European American, AA=African American, TAAT=total abdominal adipose tissue, IAAT=intra-abdominal adipose tissue, SAAT=subcutaneous abdominal adipose tissue

* models controlled for height, tanner, sex and euradm

ψ models controlled for height, tanner and euradm

Bolded values p<0.05

Italicized values 0.10> p>0.05

Relationships between Interleukin 6 (IL-6) and bone mineral content, with models controlling individually for fat deposition and distribution parameters

TABLE 6

Covariate	Overall*		Males ^ψ		Females ^ψ		EA*		AA*	
	β	p	β	p	β	p	β	p	β	p
Total Fat	-0.03631	0.1997	0.01135	0.7535	-0.12880	0.0102	-0.07702	0.0311	0.01949	0.6438
% Fat	-0.02899	0.3415	0.01673	0.6474	-0.13858	0.0275	-0.07265	0.0707	0.03145	0.4867
TAAT	-0.01736	0.5488	0.02047	0.5990	-0.08578	0.0836	-0.03710	0.0952	0.03788	0.4061
IAAT	0.00659	0.8276	0.03932	0.2683	-0.04371	0.4249	-0.04152	0.1716	0.06398	0.2020
SAAT	-0.01874	0.5145	0.01954	0.6032	-0.09015	0.0817	-0.06076	0.0542	0.03816	0.3905

EA=European American, AA=African American, TAAT=total abdominal adipose tissue, IAAT=intra-abdominal adipose tissue, SAAT=subcutaneous abdominal adipose tissue

* models controlled for height, tanner, sex and euradm

^ψ models controlled for height, tanner and euradm

Bolded values p<0.05

Italicized values 0.10> p>0.05

TABLE 7

Relationships between Tumor Necrosis Factor Receptor 2 (TNFR2) and bone mineral content stratified by low and high insulin sensitivity (S_I), with models controlling individually for fat deposition and distribution parameters

Covariate	Low S_I		High S_I	
	β	<i>p</i>	β	<i>p</i>
<i>Total Fat</i>	-0.35727	0.0343	-0.05945	0.6051
<i>% Fat</i>	-0.55561	0.0065	-0.00019664	0.9988
<i>TAAT</i>	-0.53632	0.0092	-0.03088	0.8060
<i>IAAT</i>	-0.48856	0.0634	-0.02312	0.8467
<i>SAAT</i>	-0.53420	0.0063	-0.02019	0.8746

TAAT=total abdominal adipose tissue, IAAT=intra-abdominal adipose tissue, SAAT=subcutaneous abdominal adipose tissue

* models controlled for height, tanner, sex and euradm

Bolded values $p < 0.05$

Italicized values $0.10 > p > 0.05$