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Bone mineral content as a driver of energy expenditure in prepubertal boys

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Abstract

Objective—To examine the associations of bone and bone-secreted factors with measures of energy metabolism in prepubertal boys.

Study design—Participants in this cross-sectional, observational study included 37 (69% black, 31% white) boys, 7–12y (Tanner stage<3). DXA was used to measure bone mineral content (BMC) and percent body fat. Indirect calorimetry was used to assess resting energy expenditure (REE). Fasting blood measures of osteocalcin (OCN), fibroblast growth factor-23 (FGF23), insulin, glucose, pre-cursor product of type-1 collagen (N-terminal pro-peptide; PINP) and type-I collagen, C-terminal cross-linked telopeptide (CTX) were obtained. Pearson correlations were performed to evaluate relationships among BMC, OCN, FGF23, fasting insulin and glucose, and REE. Multiple linear regression models were used to test associations between OCN and BMC (independent variables) with fasting insulin and glucose, and REE, adjusting for bone turnover markers, and further adjusted for percent body fat.

Results—BMC was correlated with REE and insulin. OCN was correlated with glucose in blacks only ($r=0.45$, $P<0.05$). FGF23 was not correlated with any markers of energy metabolism. BMC was associated with insulin in blacks ($\beta=0.95$, $P=0.001$); which was attenuated by percent body fat ($\beta=0.47$, $P=0.081$). BMC was associated with REE in whites ($\beta=0.496.7$, $P<0.05$) and blacks ($\beta=619.5$, $P<0.0001$); but accounting for percent body fat attenuated the association in whites ($\beta=413.2$, $P=0.078$).

Conclusions—Our findings suggest that BMC is a determinant of fasting insulin and REE, and the contribution of body fat appears to be race-specific. An endocrine effect of FGF23 and OCN on energy metabolism was not apparent.

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Trial registration ClinicalTrials.gov: NCT02040740, NCT02040727, and NCT01410643.

The authors declare no conflicts of interest.

Keywords

bone mineral content; biochemical markers of bone turnover; insulin; resting energy expenditure; FGF23; pre-/early pubertal children

Bone modeling (new bone deposition) and bone remodeling (concurrent bone formation and resorption) are highly active during prepuberty when relatively rapid increases in height and bone mineral accrual occur (1–3). These dynamic processes influence and are influenced by the metabolic milieu of this developmental period and are reliant upon readily available fuel (1, 4). As evidenced by the synthesis and release of locally-derived factors shown to influence both energy availability and insulin responsiveness in mice (5), the role of the skeleton extends beyond that of a mineral reservoir which simply responds to mineral and energy availability. Rather, a paracrine and an endocrine role of the skeleton have been speculated to regulate energy metabolism as well as promoting aspects of its own development (6). However, the extent of skeletal participation in governing whole-organism physiology (overall energy requirements and circulating glucose and insulin, in particular) in humans is not well understood.

Collectively, markers of bone modeling (e.g. pre-cursor product of type-I collagen, N-terminal pro-peptide; P1NP) and remodeling (degradation product of type-I collagen, C-terminal cross-linked telopeptide; CTX) increase in appearance in circulation with the onset and early progression of puberty (4, 7) and reflect an estimate bone turnover (8). While intuitively linked with bone turnover, bone composition can also be characterized quantitatively, as encompassed by the DXA-derived variable bone mineral content (BMC). In addition, endocrine-acting bone-secreted factors, including osteocalcin (OCN) and fibroblast growth factor 23 (FGF23), have been identified and described. A role for OCN in peripheral insulin resistance, energy metabolism and reproductive hormone biosynthesis in animal models has been well-described (5, 9–12). Although human studies remain controversial, positive correlations between serum osteocalcin levels and established indices of metabolic health have been reported (13–17). Similarly, an association between FGF23 and measures of glucose metabolism in adults and children has been reported, yet human data are even more lacking (18–20). While OCN and FGF23 may influence fuel availability and glycemia, elucidation of these relationships while accounting for markers specific to bone turnover, such as P1NP and CTX, may help clarify the independent role of these bone-derived endocrine factors in prepubertal children.

The interdependence of skeletal maintenance, energy requirements and nutrient delivery are relevant across the lifespan. However, the convergence during prepuberty is more apparent. This dynamic exchange is reflected by the characteristic transient state of insulin resistance which promotes fuel delivery to the peripheral tissues including the skeleton (21), as well as increased energy need for linear growth. In the context of growth, it is important to note race-related differences in rate of bone turnover (22, 23), resting energy requirements (24), pubertal insulin dynamics (25–27), and developmental timing and tempo (28, 29). The primary objective of this study was to examine the associations of bone mineral composition and bone-secreted endocrine factors with measures of energy metabolism [i.e., fasting

insulin and glucose, and resting energy expenditure (REE)]. We hypothesize that greater BMC (i.e., greater bone surface area) will be independently associated with greater values of energy metabolism indices given the high cost of bone (re)modeling which would coincide with greater fuel mobilization (insulin-mediated glucose delivery and calorie needs at rest). The bone-secreted hormones, osteocalcin and FGF23, are hypothesized to be inversely associated with insulin and glucose concentrations. Further, we hypothesize that these associations will be independent from markers of bone turnover and body fat. This investigation was limited to healthy white and black prepubertal boys to limit potential confounding due to race and sexual-dimorphism, as well as variation by maturation status.

Methods

Thirty-seven prepubertal healthy boys aged 7–12 years (Tanner stage < 3) were included. Data on boys were obtained via their participation in one of two research studies – a cross-sectional study (ClinicalTrials.gov: NCT02040740) or baseline measures from a longitudinal exercise intervention (ClinicalTrials.gov: NCT02040727). Data describing protocol and methods for these studies have been published (30). The study physician conducted an overall health assessment for each participant. Exclusion criteria for both studies included medical diagnoses (eg, diabetes; impaired fasting glucose, hypertension) and/or current use of medications known to affect body composition, or lipid or glucose metabolism [e.g., use of thyroid medication, diuretics, beta-blockers, insulin sensitizers; an allergy to lidocaine, which was used for topical anesthesia prior to venipuncture; and history of eating disorder(s)]. The research protocols were approved by the Institutional Review Board for human subjects at the University of Alabama at Birmingham (UAB). Subjects were enrolled after obtaining informed assent and consent. Race was self-reported by parents during telephone screening.

Weight was measured using a digital scale (Scale-tronix 6702W; Scale-tronix, Carol Stream, IL, USA) to the nearest 0.1 kg (converted to pounds in statistical analysis) and height was measured to the nearest 0.1 inches with a stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, WA, USA), each assessed with subject in minimal clothing without shoes. BMI percentile was calculated using age- and sex-specific growth charts reference (31).

Each enrolled subject was evaluated by a single study pediatrician to determine general health and pubertal stage. Assessment of pubertal stage was done by the study pediatrician according to the criteria of Marshall and Tanner (32). A composite number was assigned for Tanner staging, representing the higher of the two values defined by both testicular volume and pubic hair reference (33).

Whole-body dual energy x-ray absorptiometry (DXA) scanning was used to assess BMC and total percent body fat using an iDXA instrument (GE-Lunar, Madison, WI). Subjects were scanned in light clothing, while lying flat on their backs with arms at their sides. DXA scans were performed and analyzed using pediatric software (encore 2002 Version 6.10.029). The total body CV's for repeated measures for this instrument is 1.0% for BMC

1.0%, 1.5% for fat mass, and 1.0% for lean mass (34, 35). This variation is reduced by having same individual perform the scans, as per the protocol for these studies.

Indirect calorimetry was performed using a computerized open-circuit system with a ventilated canopy (Delta Trac II; Sensor Medics, Yorba Linda, California). Testing was performed with participant lying supine on a bed, and head enclosed in a plexiglass canopy. Subjects were instructed not to sleep and remain quiet and still, breathing normally. One minute average intervals of oxygen uptake and carbon dioxide production were measured continuously for 30 minutes, and the last twenty minutes were averaged to determine REE.

Fasting plasma c-terminal FGF23 was measured in duplicate using a second generation ELISA kit (Immutopics, San Clemente, CA) with a minimum sensitivity of 18 RU/ml, an inter-assay CV of 4.57% and an intra-assay CV of 3.33%. OCN was measured in fasting serum with an ELISA kit (ImmunoDiagnosticSystems, Fountain Hills, AZ) with a minimum sensitivity is 4.2 ng/ml, an inter-assay CV of 3.20% and an intra-assay CV of 3.62%.

Glucose was measured in 3 μ l sera with the glucose oxidase method using a SIRRUS analyzer (inter-assay CV 2.56%). Insulin was analyzed using a TOSOH AIA-600 II Automated Immunoassay Analyzer (TOSOH Bioscience, South San Francisco, CA, USA). Minimum assay sensitivity was 0.5uU/ml, mean intra-assay CV was 4.69%, and inter-assay CV was 6.0%.

CTX (intra-assay CV 9.14, inter-assay CV 6.29, sensitivity 0.178 ng/ml), a marker of bone resorption (8) (36), and P1NP (intra-assay CV, 3.06, inter-assay CV 3.98, sensitivity 5.6 ug/l), a marker of bone deposition (37), were measured by RIA and ELISA (ImmunoDiagnosticSystems, Fountain Hills, AZ), respectively.

Statistical analyses

Descriptive statistics (mean $\pm$ standard error) were generated for the overall sample and stratified according to race and Tanner stage. The Kolmogorov-Smirnov test and graphical inspections of data were used for evaluating distribution for normality. OCN, FGF23, CTX, nor insulin were normally distributed thus median and interquartile ranges are presented. In addition, log transformation of these variables was performed and used in analyses. Differences in descriptive statistics, metabolite concentrations, and DXA measures were evaluated using the Student's t-test for normally distributed data and non-parametric Mann-Whitney test for non-normally distributed variables. Pearson correlations were performed to analyze the relationships among skeletal characteristics (BMC and bone secretory factors, OCN, and FGF23), fasting insulin and glucose, and REE. Pearson partial correlations were also performed to evaluate these relationships while accounting for percent body fat. Although FGF23 was not correlated with any markers of energy metabolism after performing these initial analyses, it was not included in subsequent analyses. Aligning our objective to gain further insight on the skeletal role in energy metabolism, OCN and BMC were represented as independent variables for subsequent analyses. This rationale is centered on the unique nature of bone, a true homeostatic endocrine organ, which coordinates both anabolic and catabolic processes, collectively impacting fuel metabolism. To evaluate our capacity to observe an effect, power calculations were performed to

determine adequate sample size allowing for inclusion of three ($n=36$) -to-four ($n=39$) predictor variables to detect an effect size (r^2) of 0.35, with 80% power at $P<0.05$ in multiple linear regression (MLR). Based on these estimates, variable selection and our sample size ($n=37$), we performed two separate MLR models to analyze the association of the main independent variables (i.e., OCN and BMC) with indices relevant to energy utilization (i.e., fasting insulin, fasting glucose, and REE; dependent variables). The first model was adjusted for the bone turnover markers, CTX and P1NP (Model 1). We sequentially adjusted Model 1 for percent body fat (Model 2), which should be interpreted with caution as we did not reach the targeted sample size for four predictor variables. Based on established racial differences in pubertal- and insulin-related dynamics (27–29), models were stratified by race. Of note, this stratification further limited our power. Inclusion of Tanner stage in the models did not modify the strength or significance of any of the reported results (data not shown), thus was not included in the final models. All statistical analyses were performed using two-sided tests and assumed a 5% significance level using SAS software (version 9.3, SAS Institute Inc., Cary NC).

Complete data were available on all subjects with the exception of CTX concentration in one participant in which the blood specimen was insufficient for full testing and another who did not have a valid REE measurement; however, the subjects' available data points were included in all other analyses.

Results

All measurements were performed at the Clinical Research Unit and the Department of Nutrition Sciences at UAB between March and November 2013. Boys were prepubertal (51% and 49% Tanner 1 and 2, respectively), 10.1 ± 1.7 y of age. The black category included one subject who self-reported as biracial, however by US definition he would be considered black and was categorized accordingly (www.census.gov/prod/cen2010/briefs/c2010br-06.pdf). There were no statistical difference by race in age or Tanner stage; however, blacks were taller and weighed more ($P < 0.05$). There were also no race differences in glucose, FGF23 or OCN levels. However, blacks had greater median fasting insulin (5.2 vs. 7.8 uU/ml), median CTX (1.8 vs. 2.7 ng/ml), and mean P1NP (610.5 vs 890.7mg/l) (all $P = 0.01$). A numerically higher REE in blacks did not reach statistical significance (1,271.8 vs. 1,468.2 kcal/d, $P = 0.07$). Similarly, greater BMC (1.7 vs 1.4 kg, $P < 0.10$) among subjects classified as Tanner stage 2 relative to Tanner stage 1 did not reach statistical significance, with no other apparent differences by pubertal stage.

BMC was positively associated with fasting insulin ($r=0.62$, $P < 0.0001$) and REE ($r=0.82$; $P < 0.0001$) in the overall sample. When stratified by race, relationships remained in both whites (insulin: $r=0.67$; REE: $r=0.79$, both $P < 0.05$) and blacks (insulin: $r=0.53$, $P < 0.05$; REE: $r=0.82$, $P < 0.0001$). After controlling for total percent body fat, BMC was correlated with insulin ($r=0.51$, $P < 0.01$) and REE ($r=0.82$, $P < 0.0001$). Stratifying by race revealed retained relationships in blacks (insulin: $r=0.40$, $P < 0.05$; REE: $r=0.82$, $P < 0.0001$), but only the relationship between BMC and REE remained in whites (insulin: $r=0.55$, $p=0.10$; REE: $r=0.70$, $P < 0.05$). OCN was significantly correlated with fasting glucose in blacks

only ($r=0.45$, $P < 0.05$), which remained after adjustment for percent body fat. FGF23 was not correlated with any markers of energy metabolism.

Regression analyses revealed an inverse association, though non-statistically significant, of OCN with fasting insulin in whites only (Model 1, controlling for bone turnover markers; $\beta = -1.93$, $p=0.098$; Model 2, controlling for bone turnover markers and total percent body fat: $\beta = -1.7$, $p=0.058$). BMC was associated with fasting insulin in blacks in Model 1 ($\beta = 0.95$, $p=0.001$); however, in Model 2, inclusion of percent body fat attenuated this significant association ($\beta = 0.47$, $p=0.08$). This association was non-statistically significant in whites in both models (Model 1: $\beta = 1.88$, $P=0.06$; Model 2: $\beta = 1.46$, $p=0.09$). Also among whites, BMC and glucose were non-statistically significantly associated in Model 1 ($\beta = 12.05$, $p=0.10$). BMC was also associated with REE in both whites and blacks in all models, though significance was not reached among whites when accounting for percent body fat ($\beta = 413.19$, $p=0.08$).

Discussion

We investigated the relationships of BMC and bone-secreted factors with energy metabolism in prepubertal white and black boys. We expand on our previous reports regarding the positive relationship between BMC and REE in children 4–12y (38) (39) and BMC and insulin in animal models (40). Here we report a positive association of BMC with REE among both black and white boys and fasting insulin apparent only in black boys. While we provide some evidence of proximity to the linear growth spurt as reflected by the marginal difference in BMC by Tanner stage, these associations were independent of circulating markers of bone turnover. In addition, associations between BMC and fasting insulin in blacks and between BMC and REE in whites were attenuated by the inclusion of body fat in the statistical models. Contrary to the seminal work identifying bone as an endocrine organ with integral involvement of OCN in animal models (6), OCN was not significantly associated with energy-related markers of metabolism in children. Racial differences were identified, such that higher fasting circulating levels of CTX, P1NP, and insulin, and greater BMC, were observed in blacks. Taken together, we provide support for interplay of bone and energy metabolism and a potential body fat-dependent association of bone mineral accrual with energy metabolism which appears to vary by race.

Changes in markers of bone turnover, energy availability and the endocrine role of the skeleton across the pubertal transition intuitively reflect the dynamic physiologic stage of development with greatest activity early in the maturation process (41). Paralleling peak height velocity, levels of bone turnover markers are expected to peak during mid-puberty (Tanner stages II and III). Due to greater height gains early in puberty in blacks compared with whites, conceivably markers of turnover would be higher in blacks; however, lower circulating concentrations in blacks compared with whites have been reported (42). In a longitudinal study investigating bone markers in children ages 6–14, relative to whites, blacks had 50% lower post-intervention concentrations of OCN and tartrate-resistant acid phosphatase, a marker of bone resorption (albeit the relationship was evaluated in the context of a calcium supplementation intervention (22)). We found no difference in OCN between whites and blacks, although OCN concentrations were numerically lower in blacks,

a finding similar to that observed in a study including a narrower age-range of children aged 11–14y (14). A race difference has also been reported in adults, reporting serum OCN and CTX to be lower in black men compared with white men (42, 43). In contrast to findings of others (22, 44), we observed CTX, a marker of resorption and PINP, a marker of formation, were higher in blacks than whites. Pratt et al (44) found that bone resorption markers were similar or slightly lower in blacks relative to white children aged 6–15y. These studies represented children across the maturation continuum, which may explain the differences in findings from this study including only prepubertal boys, in which anabolic hormones initiating the growth spurt may have greater contribution to the relationships.

As an anabolic growth-related hormone, insulin promotes periosteal apposition, geometrical remodeling and cortical widening (8). The consistent positive association of BMC with fasting insulin supports this for black boys. In addition, both BMC and fasting insulin were higher in blacks. Prior investigators have speculated that greater insulin secretion in blacks in pre-puberty may account for their greater bone size and growth (23, 45, 46). Racial divergence in bone mineral accrual has been examined in terms of mineral metabolism (47, 48), PTH sensitivity (49) and growth factors (50, 51). One study reported that blacks reach greater peak bone mass due to more rapid bone accrual during prepuberty, whereas whites have a more progressive accretion of bone mass throughout pubertal maturation (23). Intuitively, this more rapid growth could be associated with higher REE, although one report observed otherwise (24). We found that REE was greater among blacks relative to whites. Reasons for this finding might include known accelerated pubertal timing and tempo among blacks relative to whites and/or potentially greater energy requirements for initiation of pubertal growth. While there were no differences in body fat observed between blacks and whites, racial variation in the effect of body fat on the relationship of BMC and fasting insulin as well as REE was detected, such that body fat attenuated the positive association between BMC and REE in whites and between BMC and insulin in blacks. While the anabolic effects of insulin on bone mass are well-established, body fat accumulation (or consequent metabolic effects) may interfere with the salutary contribution of insulin in black boys. Further, it has been speculated that there are differences in the tissue/organ metabolic activity in blacks and white adults (52), a hypothesis worthy of exploration in future studies, particularly during this critical period.

OCN has been speculated to participate in the bone-fat-energy axis. Among adults and children, total OCN has been inversely associated with measures of glycemia (11, 15–17), and the association has been noted to be race-specific (14). Correlation between OCN and glycemic measures among white school children aged 11–14y was shown, but a potential lack of power to detect a relationship among other racial groups, including blacks, was noted (14). In our cohort, an independent inverse relationship between OCN and fasting insulin, though marginal, was apparent in whites. An inverse correlation of OCN with peripheral insulin resistance has been reported in obese Caucasian children (13). While OCN may simply serve as a marker for bone metabolism, to dissociate specific effects of OCN from more general effects of bone turnover/formation, we accounted for contribution of bone turnover markers in the analysis. OCN also positively correlated with fasting glucose in blacks in our cohort, however, the correlation was not apparent when bone turnover was taken into account. Another factor which may explain discordance in findings is variation by

circulating isoforms of OCN. In both normoglycemic and pre-diabetic overweight prepubertal children (43% female, 46% black, 84% obese), a positive association between the uncarboxylated fraction of OCN (which has been viewed as the ‘metabolically active’ fraction) and β -cell function was observed in the pre-diabetic group only (11). However, in both groups the carboxylated fraction and total OCN were positively associated with insulin sensitivity. Significant correlations between undercarboxylated and total OCN has been reported (14). Whether the circulating isoforms of OCN differ in contribution to energy metabolism and their extent of involvement in prepubertal boys warrants further consideration.

Although emerging data in humans have suggested a role of FGF23 in bone turnover and glycemic measures (19, 20, 53), circulating level of FGF23 was not associated with glycemic- or energy-related markers in this cohort. Normative data and/or reference ranges of FGF23 in children, particularly salient during developmental stages, are lacking but would be helpful for comparisons across the life course.

Strengths of this study include representation of an under-studied group (prepubertal children), as well as a robust measure of body composition (i.e., DXA). However, we were limited by the cross-sectional nature of the study, precluding causal inference. We recognize that a multitude of factors even in children with overall good health impact the relationships studied herein (e.g., nutritional status, genetics, renal function), therefore necessitating further study. Furthermore, the relatively small sample size precludes the identification of statistically significant associations of modest effect size, while reinforces the strength in those that have been clearly identified – particularly pertaining to the influence by BMC to fasting insulin and REE. Inclusion of only boys reduced the potential for confounding due to variation in sex-specific pubertal changes in body composition and metabolism, but also allow for elimination of confounding due to sexual dimorphism. We also recognize that the stage of maturation of the subjects is characterized by rapid skeletal modeling, which while providing uniqueness, also highlights lack of generalizability to other age/maturational stage groups.

Our findings provide support for a relationship of BMC with insulin concentration and REE, with race-specific influence of percent body fat on these relationships. Whereas in whites, the association between BMC and REE was body fat-dependent and no association between BMC and fasting insulin was apparent, among blacks, the association between BMC and fasting insulin was body fat-dependent. Although we hypothesized that these associations were in physiologic response to increased demands of bone turnover prior to the linear growth spurt, the exact nature of the interplay cannot be clearly disentangled by this study. Notwithstanding, this study serves as a platform for further investigation of race-specificity in bone turnover markers and metabolic regulation during prepuberty, and expansion on our findings may yield insight into racial differences in metabolic and skeletal outcomes.

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List of Abbreviations

BMC	Bone mineral content
P1NP	N-terminal pro-peptide
CTX	C-terminal cross-linked telopeptide
OCN	osteocalcin
FGF23	fibroblast growth factor 23
REE	resting energy expenditure

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Table 1

Subject descriptive and metabolic characteristics.

Variable	Overall (n=37)	Whites (n=11)	Blacks (n=26)	Tanner 1 (n=19)	Tanner 2 (n=18)
Age (y)	10.1 ± 1.7	9.5 ± 1.7	10.3 ± 1.7	9.6 ± 1.6	10.6 ± 1.6 ^c
Tanner 1 (%)	51	54.5	50	N/A	N/A
Tanner 2 (%)	49	45.5	50	N/A	N/A
Race (% Black)	69	N/A	N/A	45	55
Height (in.)	56.7 ± 5.1	54.5 ± 4.4	57.6 ± 5.1	55.0 ± 4.5	58.5 ± 5.1^b
Weight (lbs.)	109.9 ± 48.1	82.5 ± 23.5	121.6 ± 51.3^b	102.5 ± 49.2	117.8 ± 47.0
BMI (%)	80.0 ± 25.7	68.4 ± 30.0	84.9 ± 22.5	79.4 ± 26.4	80.7 ± 25.7
Total Fat (%)	32.2 ± 10.7	30.1 ± 9.2	33.2 ± 11.4	32.5 ± 11.8	32.0 ± 9.8
FGF23 (ng/ml)	82.5 (60.1, 88.2)	83.6 (66.4, 107.7)	73.5 (57.9, 87.5)	73.5 (63.3, 88.2)	77.9 (58.7, 90.6)
OCN (ng/ml)	77.3 (52.5, 104.2)	79.8 (57.7, 102.1)	67.9 (50.9, 124.6)	78.5 (53.2, 105.8)	73.3 (52.2, 102.1)
CTX (ng/ml)	2.6 (2.0, 3.0)	1.8 (1.7, 2.2)	2.7 (2.3, 3.1)^a	2.3 (1.9, 3.0)	2.6 (2.0, 3.0)
P1NP (mg/l)	807.4 ± 256.4	610.5 ± 165.2	890.7 ± 243.9^a	745.1 ± 184.5	873.2 ± 307.0
BMC (kg)	1.6 ± 0.5	1.3 ± 0.3	1.7 ± 0.5^b	1.4 ± 0.4	1.7 ± 0.4
Fasting Insulin (uU/ml)	6.4 (3.9, 8.9)	5.2 (1.6, 7.1)	7.8 (4.4, 12.1)^a	7.4 ± 5.5	8.5
Fasting Glucose (mg/dl)	88.7 ± 5.6	89.1 ± 4.8	88.1 ± 5.9	88.1 ± 5.1	89.2 ± 6.2
REE (kcal/d)	1,408.2 ± 305.0	1,271.8 ± 248.6	1,468.2 ± 312.4	1,364.8 ± 279.6	1456.8 ± 332.7

Data illustrated as mean ± SD, median (interquartile range), or frequencies

^a $P < 0.01$ ^b $0.01 < P < 0.05$ ^c $0.05 < P < 0.10$; bolded values represent significant differences between groups

FGF23=fibroblast growth factor 23; OCN=osteocalcin; P1NP= N-terminal pro-peptide of type-1 collagen; CTX= C-terminal cross-linked telopeptide; BMC=bone mineral content; REE=resting energy expenditure

Simple and partial (adjusting for percent body fat) Pearson correlations (r) between markers of bone and energy metabolism

Table 2

Variables	Overall			Whites			Blacks		
	Insulin ^a	Glucose	REE	Insulin ^a	Glucose	REE	Insulin ^a	Glucose	REE
<i>Simple</i>									
FGF23 [^]	-0.09	-0.06	-0.01	0.04	-0.22	0.36	-0.07	-0.03	-0.09
OCN [^]	-0.17	0.30^c	0.05	-0.38	-0.29	0.03	-0.13	0.45^b	-0.03
BMC	0.62^a	0.13	0.82^a	0.67^b	0.44	0.79^b	0.53^b	0.03	0.82^a
<i>Partial</i>									
FGF23 [^]	-0.05	-0.06	0.01	-0.32	-0.28	-0.00	0.09	-0.06	0.04
OCN [^]	-0.17	0.29^c	0.06	- 0.68^b	-0.32	-0.9	-0.01	- 0.44^b	0.13
BMC	0.51^b	0.12	0.82^a	0.55^c	0.49	0.70^b	0.40^b	0.10	0.82^a

^a $P < 0.0001$ ^b $P < 0.05$ ^c $0.05 < P \leq 0.10$; bolded values represent significant correlations[^] log

FGF23=fibroblast growth factor 23; OCN=osteocalcin; BMC=bone mineral content; REE=resting energy expenditure

Table 3

Multiple linear regression analyses of associations between dependent variables, insulin, glucose and resting energy expenditure, and independent variables, OCN and BMC

<i>Independent variable</i>	<i>Insulin[^] (dependent variable)</i>			
	<i>Model 1</i>		<i>Model 2</i>	
	Whites	Blacks	Whites	Blacks
	β (P-value):	β (P-value):	β (P-value):	β (P-value):
OCN [^]	-1.93 (0.098)	-0.26 (0.442)	-1.70 (0.058)	-0.19 (0.419)
BMC	1.88 (0.063)	0.954 (0.001)	1.46 (0.087)	0.47 (0.081)
<i>Glucose (dependent variable)</i>				
OCN [^]	-7.87 (0.382)	3.63 (0.179)	-7.71 (0.440)	3.56 (0.196)
BMC	12.05 (0.101)	-1.27 (0.629)	12.5 (0.138)	-0.71 (0.830)
<i>Resting energy expenditure (dependent variable)</i>				
OCN [^]	-278.81 (0.386)	-62.59 (0.709)	-224.90 (0.439)	-31.45 (0.785)
BMC	496.66 (0.046)	619.54 (<0.0001)	413.19 (0.078)	467.09 (<0.0001)

OCN=Osteocalcin; BMC=bone mineral content; bolded values represent significant associations

[^]
log

Model 1 adjusted for bone turnover markers

Model 2 adjusted for bone turnover markers (N-terminal pro-peptide of type-1 collagen; C-terminal cross-linked telopeptide) and percent body fat