Bone Mineral Content as a Driver of Energy Expenditure in Prepubertal and Early Pubertal Boys

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

Study design Participants in this cross-sectional, observational study included 37 (69% black, 31% white) boys, aged 7-12 years (Tanner stage <III). Dual-energy X-ray absorptiometry was used to measure bone mineral content (BMC) and percent body fat. Indirect calorimetry was used to assess resting energy expenditure (REE). Fasting blood levels of osteocalcin (OCN), fibroblast growth factor 23 (FGF23), insulin, glucose, precursor product of type I collagen (N-terminal pro-peptide) and type I collagen, and C-terminal cross-linked telopeptide were measured. 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 with 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 ($r = 0.45$, $P < .05$). FGF23 was not correlated with any markers of energy metabolism. BMC was associated with insulin level in blacks ($\beta = 0.95$, $P = .001$), which was attenuated by percent body fat ($\beta = 0.47$, $P = .081$). BMC was associated with REE in whites ($\beta = 0.496.7$, $P < .05$) and blacks ($\beta = 619.5$, $P < .0001$); but accounting for percent body fat attenuated the association in whites ($\beta = 413.2$, $P = .078$).

Conclusion Our findings suggest that BMC is a determinant of fasting insulin and REE, and that the contribution of body fat appears to be race-specific. Endocrine effects of FGF23 and OCN on energy metabolism were not apparent. *(J Pediatr 2015;166:1397-403)*.

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

Bone modeling (ie, new bone deposition) and bone remodeling (ie, concurrent bone formation and resorption) are highly active processes 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 on 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 that simply responds to mineral and energy availability. Rather, paracrine and endocrine roles of the skeleton have been speculated to regulate energy metabolism as well as to promote aspects of its own development.[6] 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, levels of markers of bone modeling (eg, precursor 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 the circulation with the onset and early progression of puberty[4,7] and reflect estimated bone turnover.[8] Although intuitively linked with bone turnover, bone composition also can be characterized quantitatively, as encompassed by the dual-energy X-ray absorptiometry (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

BMC	Bone mineral content
CTX	C-terminal cross-linked telopeptide
CV	Coefficient of variation
DXA	Dual-energy X-ray absorptiometry
ELISA	Enzyme-linked immunoassay
FGF23	Fibroblast growth factor 23
OCN	Osteocalcin
P1NP	N-terminal pro-peptide
REE	Resting energy expenditure

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biosynthesis in animal models has been well described.[5,9-12] Although human studies remain controversial, positive correlations between serum OCN level 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] Although 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 and early pubertal children.

The interdependence of skeletal maintenance, energy requirements, and nutrient delivery are relevant across the lifespan; however, the convergence is most apparent during prepuberty. 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 the 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 the present study was to examine the associations of bone mineral composition and bone-secreted endocrine factors with measures of energy metabolism (ie, fasting insulin and glucose, and resting energy expenditure [REE]). We hypothesized that greater BMC (ie, greater bone surface area) would 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 (ie, insulin-mediated glucose delivery and calorie needs at rest). The bone-secreted hormones OCN and FGF23 are hypothesized to be inversely associated with insulin and glucose concentrations. Furthermore, we hypothesized that these associations would be independent of markers of bone turnover and body fat. This investigation was limited to healthy white and black prepubertal and early pubertal boys, to limit potential confounding owing to race and sexual dimorphism, as well as variation by maturation status.

Methods

Thirty-seven healthy prepubertal and early pubertal boys aged 7-12 years (Tanner stage <III) were included in this study. Data on the boys were obtained via their participation in 1 of 2 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 previously.[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 (eg, use of thyroid medication, diuretics, beta-blockers, insulin sensitizers; allergy to lidocaine, which was used for topical anesthesia before venipuncture; history of eating disorder). The research protocols were approved by the Institutional Review Board for human subjects at the University of Alabama at Birmingham. 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 (model 6702W; Scale-Tronix, Carol Stream, Illinois) to the nearest 0.1 kg (converted to pounds in statistical analysis), and height was measured to the nearest 0.1 inch with a stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, Washington), each assessed with subject in minimal clothing without shoes. Body mass index percentile was calculated using age- and sex-specific reference growth charts.[31]

Each enrolled subject was evaluated by a single-study pediatrician to determine general health and pubertal stage. Pubertal stage was assessed 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 2 values defined by both testicular volume and pubic hair reference.[33]

Whole-body DXA scanning was used to assess BMC and total percent body fat using an iDXA instrument (GE Lunar, Madison, Wisconsin). Subjects were scanned in light clothing while lying supine with arms at sides. DXA scans were performed and analyzed using pediatric software (enCORE 2002, version 6.10.029; GE). The total body coefficient of variation (CV) for repeated measures for this instrument was 1.0% for BMC 1.0%, 1.5% for fat mass, and 1.0% for lean mass.[34,35] This variation was reduced by having the same individual perform the scans, in accordance with 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 the subject lying supine on a bed with the head enclosed in a plexiglass canopy. The subject was 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 values for the last 20 minutes were averaged to determine REE.

Fasting plasma c-terminal FGF23 was measured in duplicate using a second-generation enzyme-linked immunoassay (ELISA) kit (Immutopics International, San Clemente, California) with a minimum sensitivity of 18 RU/mL, an interassay CV of 4.57%, and an intra-assay CV of 3.33%. OCN was measured in fasting serum using an ELISA kit (Immunodiagnostic Systems, Fountain Hills, Arizona) with a minimum sensitivity of 4.2 ng/mL, an interassay CV of 3.20%, and an intra-assay CV of 3.62%.

Glucose was measured in 3 μL of serum with the glucose oxidase method using a SIRRUS analyzer (Stanbio Laboratory, Boerne, Texas; interassay CV, 2.56%). Insulin was analyzed using a TOSOH AIA-600 II Automated Immunoassay Analyzer (TOSOH Bioscience, South San Francisco, California). The minimum assay sensitivity was 0.5 μU/mL, mean intra-assay CV was 4.69%, and mean interassay CV was 6.0%.