Published in final edited form as:

J Bone Miner Metab. 2010 July ; 28(4): 424–432. doi:10.1007/s00774-009-0143-x.

Adiposity and genetic admixture, but not race/ethnicity, influence bone mineral content in peripubertal children

Krista Casazza,

Department of Nutrition Sciences, Clinical Nutrition Research Center, University of Alabama at Birmingham, Webb 415, 1530 3rd Ave S, Birmingham, AL 35294-3360, USA

Olivia Thomas,

Department of Epidemiology, Clinical Nutrition Research Center, University of Alabama at Birmingham, Webb 415, 1530 3rd Ave S, Birmingham, AL 35294-3360, USA

Akilah Dulin-Keita, and

Department of Nutrition Sciences, Clinical Nutrition Research Center, University of Alabama at Birmingham, Webb 415, 1530 3rd Ave S, Birmingham, AL 35294-3360, USA

Jose R. Fernandez

Department of Nutrition Sciences, Clinical Nutrition Research Center, University of Alabama at Birmingham, Webb 415, 1530 3rd Ave S, Birmingham, AL 35294-3360, USA

Krista Casazza: kristac@uab.edu

Abstract

The effect of fat mass on bone mineral content (BMC) in children is not clear, particularly when considering a diverse population. Ancestral genetic admixture may be an approach to accurately identify population differences in BMC. Our objective was to evaluate the relationships between self-reported race/ethnicity, genetic admixture, and fat mass on BMC in a multiethnic sample of children ($n = 270$), taking into account dietary and physical activity variables. Ancestral genetic admixture was estimated using 140 ancestry informative markers, body composition by dual-energy X-ray absorptiometry, diet by 24-h recall, and physical activity by accelerometry. Multiple linear regression examined the relationships between race/ethnicity or genetic admixture and percent fat on BMC. Additional analyses were conducted to examine the relationship between race/ethnicity or genetic admixture and BMC stratified by body fat percentage cutpoints. In regression models, there was no association between race/ethnicity and BMC. In contrast, African admixture (AFADM) was positively associated with BMC, American Indian admixture (AMINADM) was inversely associated with BMC, and there was no association between European admixture (EUADM) and BMC. When stratified by percent fat group, high body fat percentage was inversely associated with BMC with EUADM and AMINADM ($P = 0.03$ and $P = 0.02$, respectively) and positively associated with AFADM ($P < 0.001$). Diet and physical activity were not related to BMC in this sample. Our findings suggest that genetic admixture and percent body fat, but not race/ethnicity, diet, or physical activity, influence BMC in our sample of peripubertal children. Further, there is a differential impact of percent fat on BMC that may be mediated by genetic admixture.

Correspondence to: Krista Casazza, kristac@uab.edu.

Conflict of interest statement There are not any potential, perceived, or real conflicts of interests, especially any financial arrangements, to be disclosed by any of the authors of this manuscript.

Keywords

Bone mineral content; Genetics; Admixture; Puberty; Race/ethnicity

Introduction

The drastic rise in pediatric obesity combined with recent evidence suggesting that excessive fat accumulation may be associated with increased fractures in childhood indicates a need to understand the interplay between body composition components [i.e., fat mass and bone mineral content (BMC)]. Previous studies have been equivocal, with some suggesting a beneficial effect of weight on BMC mediated by lean mass [1], and others reporting a detrimental effect of weight, purportedly leading to fat infiltration within bone compartments [2]. The underlying reasons for the variable results have not been elucidated but may be related to genetic architecture contributing to the partitioning of body tissue. As estimates suggest that more than 50% of the variation in adult bone mass is inherited, genetic makeup plays a major role in bone mass accrual [3,4]. However, the role of genetic expression is complicated by a range of physiological states in response to various environmental conditions (i.e., obesity status, diet, physical activity). This relationship is particularly salient in childhood because the majority of bone acquisition takes place during puberty [5]. As such, identification of factors that influence BMC during the pubertal transition is essential.

Bone mass of an individual in later life, although highly heritable [3], depends largely on peak bone mass attained during skeletal growth during the pubertal transition [6,7]. Population studies have demonstrated some inconsistencies in characteristics of bone mineralization, which may be accounted for by ancestral genetic background. Genetic admixture, an estimation of ancestral genetic background using ancestry informative biallelic markers, known to differ in frequency among European, African, and Amer-indian populations, can be used to quantify genetic differences related to bone mass among groups. For example, a positive association between African admixture (AFADM) and BMC [8], as well as an inverse association between European genetic admixture (EUADM) and bone mineralization [9], have been demonstrated in adult women. Although persons of European descent are more susceptible to osteoporosis, risk may increase in other groups if peak bone mass is not attained in childhood for biological, behavioral, or environmental reasons.

The dynamic growth and development occurring throughout the pubertal transition is met with crucial interactions of hormonal, environmental, and behavioral factors that may affect bone mass accrual. Dietary intake (e.g., calcium, vitamin D) and engagement in daily physical activity may play a role in bone development. Clinical studies have provided significant evidence that increasing vitamin D and calcium intake reduces fractures and osteoporosis risk [3,10,11]. Several reports have also shown physical activity to have a positive effect on bone metabolism among adolescents [3,12–16]. Recent studies suggest that fat mass accrual may also impact peak bone mineral acquisition, although the extent and direction of the relationship have not been clearly established [17–19]. It is evident that during the pubertal transition, when the trajectory of growth and development patterns plays a key role in current and future health, the interplay of body composition compartments warrants further study.

The relationships between biological, genetic, and environmental factors influencing BMC have been relatively unexplored in children, and to our knowledge no study has evaluated the extent to which AFADM, EUADM, and American Indian admixture (AMINADM) mediate the effect of adiposity on BMC. The objective of this study was to evaluate if

variations in BMC could be explained by ancestral background and to evaluate the independent/interactive effects of genetic admixture and fat mass on BMC in a multiethnic cohort of children self-identified as African-, European-, or Hispanic-American, taking into account dietary intake of calcium and vitamin D and engagement in regular daily physical activity.

Methods

Participants

Participants were 270 (53% male) children aged 7–12 years recruited as a part of a cross-sectional study that aimed to identify racial/ethnic differences in insulin-related outcomes among healthy children. Children were categorized into racial/ethnic groups according to parental self-report as African American (AA; $n = 94$), European American (EA; $n = 116$), or Hispanic American (HA; $n = 60$). The children were pubertal stage ≤ 3 as assessed by a pediatrician according to the criteria of Marshall and Tanner [20,21]. Exclusion criteria were medical diagnoses and/or taking any medications contraindicated for study participation (i.e., medications known to affect body composition, metabolism, cardiac function, etc.). Before participating in the study, the nature, purpose, and possible risks of the study were carefully explained to the parents and children. The children and parents provided informed assent and consent, respectively. The protocol was approved by the Institutional Review Board for human subjects at the University of Alabama at Birmingham (UAB). All measurements were performed at the General Clinical Research Center (GCRC) and the Department of Nutrition Sciences at UAB between 2004 and 2008.

Protocol

Participants completed two testing sessions. In the first session, anthropometric measurements, pubertal status, and body composition were assessed and a 24-h dietary recall was obtained. In the second session (an overnight visit), a second 24-h dietary recall was obtained. Participants were admitted to the GCRC in the late afternoon for the overnight visit. All participants were offered the same meal and snack foods. After 2000 (8:00 p.m.), only water and/or noncaloric decaffeinated beverages were permitted until after the morning testing. Upon completion of the overnight fast, blood samples were obtained for markers of glucose/insulin homeostasis and DNA genotyping analysis.

Anthropometric measures

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

Pubertal status

The Tanner stages have been demonstrated as reliable indicators of pubertal development. Direct observation for the assessment of pubertal stage by a pediatrician, the “gold standard” for differentiating among the five stages of maturity [22,23], was utilized. The staging based on the criteria of Marshall and Tanner [20,21] is according to both breast and pubic hair development in girls and genitalia and pubic hair development in boys. One composite number is assigned for Tanner staging, representing the higher of the two values defined by breast/genitalia and pubic hair [24].

Assessment of body composition

Whole-body BMC was measured by dual-energy X-ray absorptiometry (DXA) using a GE Lunar Prodigy densitometer (GE LUNAR Radiation, Madison, WI, USA). DXA scan data were also used to assess lean body mass and total and percentage fat mass. Participants were scanned in light clothing, while lying flat on their backs with arms at their sides. Body composition analysis by DXA has been found to be highly reliable in children [25].

Admixture analysis

Genotyping of the ancestry informative markers (AIMs) for the measurement of genetic admixture was performed at Prevention Genetics (<http://www.preventiongenetics.com>) using the Chemicon Amplifluor SNPs Genotyping System [26] coupled with ArrayTape technology (<http://www.global-array.com>). A panel of 140 AIMs was used to estimate the genetic admixture proportion of each subject. Molecular techniques for the allelic identification and methodology for genetic admixture application have been described elsewhere [27], 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 designation PSUANTH. The information from the AIMs was translated into estimates of AFADM, EUADM, and AMINADM for each subject using maximum-likelihood (ML) estimation based on the ML algorithm described by Hanis et al. [28]. 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 value of admixture based on the observed genotypes.

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 (in person) 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, USA), a dietary analysis program designed for the collection and analyses of dietary recalls.

Physical activity by accelerometer

The MTI Actigraph accelerometer (Actigraph GT1M—Standard Model 198-0100-02; ActiGraph LLC, Pensacola, FL, USA, and accompanying software) was used to measure physical activity levels and patterns for 7 days. Epoch length was set at 1 min, and data were expressed as counts per minute (counts min⁻¹). Children were instructed to wear the monitor on an elastic belt at the waist above the right hip, removing only for sleeping, bathing, and swimming. Actigraph monitors have previously demonstrated a high degree of interinstrument reliability [27]. Daily and total counts per minute were summed and averaged as minutes spent in light, moderate, hard, or very hard activity, as determined by the software accompanying the device.

Socioeconomic status

Research suggests that socioeconomic status (SES) influences adiposity, diet, and physical activity patterns. To control for such differences, the variable SES was adjusted for in all statistical models. SES was measured with the Hollingshead 4-factor index of social class [29], which combines the educational attainment and occupational prestige for the number of working parents in the child's family. Scores ranged from 8 to 66 with the higher scores indicating higher theoretical social status.

Statistical analyses

Differences in descriptive statistics by sex and self-reported race/ethnicity were examined using analysis of variance (ANOVA) with Tukey's post hoc analysis. Multiple linear regression was used to test contributions of (1) self-reported race/ethnicity and percent fat to BMC and (2) genetic admixture and percent fat to BMC. Overall multiple regression models were adjusted for height, age, pubertal stage, sex, total fat and lean mass, and SES. Sex was coded as 0 for males and 1 for females. Because the independent variables "race/ethnicity" and "Tanner" are nominal variables, they were entered into models as orthogonally coded dummy variables. Interaction terms (i.e., sex by BMC, sex by admixture, sex by diet, sex by physical activity) were included in the exploratory statistical analyses. These interactions were not significant. However, because there is a sexual dimorphism in body composition changes throughout the maturation process, models were also evaluated stratified by sex and Tanner stage.

A second multivariate regression was used to evaluate bone mineral content stratified by cutpoints for percent fat with genetic admixture as a covariate. Although specific cutpoints do not exist, it has been suggested that the percent body fat in girls should not exceed 30 and that of boys should not exceed 25 [30]. We used these suggestions to divide the groups into high and normal body fat percent groups. Models stratified by percent fat cutpoints were adjusted for height, age, sex, Tanner stage, and SES.

To conform to the assumptions of linear regression, all statistical models were evaluated for residual normality and logarithmic transformations were performed when appropriate. All data were analyzed using SAS 9.1 software (SAS Institute, Cary, NC, USA).

Results

Participant characteristics are presented in Table 1. There were no sex differences in weight, height, SES, BMI, calcium and vitamin D intake, or total daily physical activity. There were no differences by (self-reported) race/ethnicity in terms of age, weight, or engagement in daily total or moderate to vigorous physical activity; however, there were racial/ethnic differences in BMI and body composition. Hispanic American children presented with greater adiposity as measured by BMI percentile ($P < 0.05$, for both), total fat mass ($P < 0.01$, for both), and percent fat ($P < 0.001$, for both) than European- and African Americans. African Americans had greater lean mass ($P < 0.05$, $P < 0.01$, respectively) and were reproductively more mature ($P < 0.001$) than both European- and Hispanic Americans. European Americans reported a higher SES than African Americans ($P < 0.001$), who in turn reported a higher SES than Hispanic Americans ($P < 0.001$). African American children presented greater BMC than European- or Hispanic Americans ($P < 0.01$, for both), yet reported lower dietary calcium ($P < 0.01$) and vitamin D intake ($P < 0.01$) relative to Hispanic Americans.

Table 2 illustrates the results of the multiple linear regression analysis evaluating relationships between BMC, percent fat, and race/ethnicity. There were no associations between race/ethnicity and BMC in the total sample or when stratified by percent fat. Percent fat was positively associated with BMC in the total sample, whereas calcium, vitamin D, and physical activity were not significant contributors.

Table 3 illustrates the results of the multiple linear regression analysis evaluating the relationship between genetic admixture and BMC stratified by sex and Tanner stage. EUADM was inversely associated with BMC in females and prepubertally (Tanner stage 1). AMINADM was inversely associated with BMC in males and with increasing reproductive maturation (Tanner stages 2 and 3), but only demonstrated a trend toward significance in

females ($P = 0.09$) and prepubertally (Tanner stage 1) ($P = 0.08$). Conversely, AFADM was positively associated with BMC in both sexes and across all Tanner stages ($P < 0.05$).

Figure 1 illustrates that among the entire sample there was a positive relationship between AFADM and BMC, an inverse relationship between AMINADM and BMC, and the influence of EUADM was not significant. Further, as illustrated in Fig. 2, the contribution of ancestral genetic admixture to BMC varied by percent fat. Among children with high body fat percent, EUADM and AMINADM were inversely related to BMC ($P = 0.03$, $P = 0.001$, respectively), whereas AFADM was positively associated with BMC ($P < 0.001$).

Discussion

The main objective of this study was to evaluate the effects of race/ethnicity, genetic admixture, and body fat on BMC among a sample of peripubertal children. There have been inconsistencies in results from studies investigating the influence of body fat on BMC among various populations. Some studies indicate that overweight children and adolescents have higher BMC compared with their normal-weight peers whereas others conclude that overweight is associated with lower BMC [19,31–33]. It is possible that the inconsistencies can be attributed to the population studied, the method used to assess body habitus (i.e., BMI versus DXA), or the confounding effect that body size, fat distribution, and body composition have on the evaluation of adiposity and bone mass [17]. In this study we show, in a multiethnic cohort of children, that percent fat assessed by DXA and ancestral genetic background (but not race/ethnicity) both contribute to BMC. Further, the relationship between admixture and BMC is fairly consistent in both sexes and across the maturation process. However, the impact of fat accumulation on BMC differs according to genetic admixture.

The degree of ancestry has been shown to be of clinical importance among adult women. Hill et al. [8] demonstrated that Tobagonian women with greater West African ancestry had 10–18 and 29% higher bone density compared to non-Hispanic black and white women in the United States, respectively. Others have demonstrated an inverse relationship between bone mass and European ancestry among women [9,34]. Although previous pediatric studies have examined the relationship between fat mass and BMC [17,19], the specific contributions of genetic markers and body fat to BMC have not been reported. Our study showed that ancestral genetic background was associated with BMC. Interestingly, the relationships were maintained, in general even after stratifying by sex or Tanner stage, suggesting a significant influence of genetic admixture irrespective of sexual dimorphism or body composition changes resulting from reproductive maturation.

The effect of ancestral genetic background may be mediated by fat distribution [17], insulin dynamics [35], adipokines [36], and/or hormones [37], all of which influence BMC. The racial/ethnic differences and interactions between fat deposit and BMC observed by Afghani and Goran underscore not only the complexity of body composition relationships in terms of physiology but also the importance of including genetic factors in the analysis [17]. The results of our study demonstrate the differential impact of genetic factors and adiposity on BMC that may not be identified using racial/ethnic category as the independent variable. The extent to which inherent physiological factors may also contribute to BMC is an avenue worth exploring.

Behavioral factors such as physical activity and dietary calcium and vitamin D have been consistently reported to impart an effect on BMC [38–40]; however, we did not detect a contribution of either in this sample. In our sample, racial/ethnic differences in these independent variables were shown; however, when included in regression models, there was

no relationship between the physical activity or diet with BMC. The lack of association suggests that, in our sample, racial/ethnic differences in BMC were not accounted for by disparate physical activity and/or diet levels. It is likely that the relatively small amount of physical activity, particularly in terms of moderate to vigorous activity, was not sufficient to explain the variance in this sample of healthy children. In addition, it is plausible that in this cohort the dietary calcium and vitamin D intakes were adequate to prevent deficiency but not high enough to produce a contribution in the presence of genetic and other environmental factors (high adiposity). Optimizing calcium and vitamin D intake has been shown to increase bone mineralization in children and adolescents, primarily in children with the lowest intake [38–40]. Nevertheless, throughout the pubertal transition, the interaction of genetic, biological, physiological, and environmental factors all play a role in the determination of not only bone mass accrual but also bone loss throughout life. Significant evidence indicates the full genetic potential is only attained if nutrition, physical activity, and other lifestyle factors are optimized. Our results suggest that efforts to improve dietary quality and engagement in physical activity in this population should be addressed.

The strengths of this study were the use of ancestral genetic admixture in addition to racial/ethnic classification to separate the biological and nonbiological aspects of race and robust measures of fat and lean mass in a multiethnic population with a wide range of body habitus. To the best of our knowledge, this was the first study to investigate the independent roles of genetic admixture and adiposity in the pediatric skeletal system. There are, however, limitations in using DXA as it may imprecisely estimate BMC, particularly in overweight individuals [41]. However, because our participants were early pubertal children with BMIs in the average range for children and well below the average range for adults, as well as the type of DXA used, we believe that the observed BMC findings in our study were accurate. We do not rule out the possibility that other measures [e.g., serum calcium, parathyroid hormone, vitamin D, leptin, insulin-like growth factor (IGF)] levels may also mediate the effect of ancestry on BMC; however, for reasons of limitations in amount of sera collected and funding, we do not have these measures available. In addition, the cross-sectional nature of the study design prevented the inference of long-term relationships over the pubertal transition; longitudinal data will be required to determine the long-term contribution of individual adiposity and genetic makeup on ultimate bone health. Further, the relatively small sample included only participants from a discrete geographic area, limiting generalizability. Nevertheless, although the interaction terms were not significant, it is not likely because of power, because power calculations indicated that we needed a sample of 55 to obtain a significant effect, and we had this sample size. Additional analyses examining sex-related differences across Tanner stages were evaluated. This analysis demonstrated a sex-related difference at Tanner 1 with an effect in girls but not in boys, whereas the effect reverses as puberty progresses. However, the separation of samples according to this stratification might bring bias to our results, preventing an accurate conclusion, partly because of the variation in reproductive maturation across groups (i.e., variance in admixture estimates would be limited by stratification). The evaluation of the role of genes in the etiology of racial/ethnic differences in physiological outcomes requires the understanding that genetic similarity cannot be inferred simply based on racial categories. When accounting for the genetic heterogeneity of the participants in our study, the genetic and biological factors influencing fat mass accumulation in African Americans in fact may be the same aspects decreasing susceptibility to osteoporosis in the adult population among this group. Conversely, excess body fat may worsen the influence of AMINADM on BMC. These results indicate population differences in body composition are at least in part the consequence of ancestral genetic background, suggesting the utility of inclusion of admixture for identifying susceptibility for osteoporosis and obesity. Future research on the evaluation of genes influencing bone mass accrual in children is warranted.

Acknowledgments

This work was funded by R01 DK067426-01, M01 RR00032.

References

1. Vicente-Rodriguez G. How does exercise affect bone development during growth? *Sports Med* 2006;36:561–569. [PubMed: 16796394]
2. Goulding A, Grant AM, Williams SM. Bone and body composition of children and adolescents with repeated forearm fractures. *J Bone Miner Res* 2005;20:2090–2096. [PubMed: 16294262]
3. Bachrach LK. Consensus and controversy regarding osteoporosis in the pediatric population. *Endocr Pract* 2007;13:513–520. [PubMed: 17872354]
4. Liu YJ, Shen H, Xiao P, Xiong DH, Li LH, Recker RR, Deng HW. Molecular genetic studies of gene identification for osteoporosis: a 2004 update. *J Bone Miner Res* 2006;21:1511–1535. [PubMed: 16995806]
5. Heaney RP, Abrams S, Lawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C. Peak bone mass. *Osteoporos Int* 2000;11:985–1009. [PubMed: 11256898]
6. Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. *J Clin Endocrinol Metab* 1991;73:555–563. [PubMed: 1874933]
7. Martin AD, Bailey DA, McKay HA, Whiting S. Bone mineral and calcium accretion during puberty. *Am J Clin Nutr* 1997;66:611–615. [PubMed: 9280181]
8. Hill DD, Cauley JA, Bunker CH, Baker CE, Patrick AL, Beckles GL, Wheeler VW, Zmuda JM. Correlates of bone mineral density among postmenopausal women of African Caribbean ancestry: Tobago women's health study. *Bone (NY)* 2008;43:156–161.
9. Bonilla C, Shriver MD, Parra EJ, Jones A, Fernandez JR. Ancestral proportions and their association with skin pigmentation and bone mineral density in Puerto Rican women from New York City. *Hum Genet* 2004;115:57–68. [PubMed: 15118905]
10. Aloia JF. African Americans, 25-hydroxyvitamin D, and osteoporosis: a paradox. *Am J Clin Nutr* 2008;88:545S–550S. [PubMed: 18689399]
11. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* 2005;293:2257–2264. [PubMed: 15886381]
12. Janz KF, Burns TL, Torner JC, Levy SM, Paulos R, Willing MC, Warren JJ. Physical activity and bone measures in young children: the Iowa bone development study. *Pediatrics* 2001;107:1387–1393. [PubMed: 11389262]
13. Petit MA, McKay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. *J Bone Miner Res* 2002;17:363–372. [PubMed: 11874228]
14. Sardinha LB, Baptista F, Ekelund U. Objectively measured physical activity and bone strength in 9-year-old boys and girls. *Pediatrics* 2008;122:e728–e736. [PubMed: 18762509]
15. Tobias JH, Steer CD, Mattocks CG, Riddoch C, Ness AR. Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: findings from a large population-based cohort. *J Bone Miner Res* 2007;22:101–109. [PubMed: 17014381]
16. Vicente-Rodriguez G, Ezquerro J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP, Casajus JA, Moreno LA. Independent and combined effect of nutrition and exercise on bone mass development. *J Bone Miner Metab* 2008;26:416–424. [PubMed: 18758899]
17. Afghani A, Goran MI. Racial differences in the association of subcutaneous and visceral fat on bone mineral content in prepubertal children. *Calcif Tissue Int* 2006;79:383–388. [PubMed: 17115240]
18. Di IN, Rosol M, Mittelman SD, Gilsanz V. Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults. *J Clin Endocrinol Metab* 2008;93:2281–2286. [PubMed: 18381577]

19. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 2007;86:1530–1538. [PubMed: 17991669]
20. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291–303. [PubMed: 5785179]
21. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13–23. [PubMed: 5440182]
22. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics* 1997;99:505–512. [PubMed: 9093289]
23. Coleman L, Coleman J. The measurement of puberty: a review. *J Adolesc* 2002;25:535–550. [PubMed: 12234559]
24. Malina, RM.; Bouchard, C. Growth, maturation, and physical activity. Human Kinetics Books; Champaign: 1991.
25. Huffman DM, Landy NM, Potter E, Nagy TR, Gower BA. Comparison of the Lunar DPX-L and Prodigy dual-energy X-ray absorptimeters for assessing total and regional body composition. *Int J Body Compos Res* 2005;48:25–30.
26. Myakishev MV, Khripin Y, Hu S, Hamer DH. High-throughput SNP genotyping by allele-specific PCR with universal energy transfer labeled primers. *Genome Res* 2001;11:163–169. [PubMed: 11156625]
27. Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, Forrester T, Allison DB, Deka R, Ferrell RE, Shriver MD. Estimating African American admixture proportions by use of population-specific alleles. *Am J Hum Genet* 1998;63:1839–1851. [PubMed: 9837836]
28. Hanis CL, Chakraborty R, Ferrell RE, Schull WJ. Individual admixture estimates: disease associations and individual risk of diabetes and gallbladder disease among Mexican-Americans in Starr County, Texas. *Am J Phys Anthropol* 1986;70:433–441. [PubMed: 3766713]
29. Cirino PT, Chin CE, Sevcik RA, Wolf M, Lovett M, Morris RD. Measuring socioeconomic status: reliability and preliminary validity for different approaches. *Assessment* 2002;9:145–155. [PubMed: 12066829]
30. Williams DP, Going SB, Lohman TG, Harsha DW, Srinivasan SR, Webber LS, Berenson GS. Body fatness and risk for elevated blood pressure, total cholesterol, and serum lipoprotein ratios in children and adolescents. *Am J Public Health* 1992;82:358–363. [PubMed: 1536350]
31. Weaver CM, McCabe LD, McCabe GP, Novotny R, Van LM, Going S, Matkovic V, Boushey C, Savaiano DA. Bone mineral and predictors of bone mass in white, Hispanic, and Asian early pubertal girls. *Calcif Tissue Int* 2007;81:352–363. [PubMed: 17989943]
32. Wosje KS, Khoury PR, Claytor RP, Copeland KA, Kalkwarf HJ, Daniels SR. Adiposity and TV viewing are related to less bone accrual in young children. *J Pediatr* 2009;154:79–85. [PubMed: 18692201]
33. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. *J Bone Miner Metab* 2008;26:73–78. [PubMed: 18095067]
34. Shaffer JR, Kammerer CM, Reich D, McDonald G, Patterson N, Goodpaster B, Bauer DC, Li J, Newman AB, Cauley JA, Harris TB, Tyavsky F, Ferrell RE, Zmuda JM. Genetic markers for ancestry are correlated with body composition traits in older African Americans. *Osteoporos Int* 2007;18:733–741. [PubMed: 17235662]
35. Gower BA, Fernandez JR, Beasley TM, Shriver MD, Goran MI. Using genetic admixture to explain racial differences in insulin-related phenotypes. *Diabetes* 2003;52:1047–1051. [PubMed: 12663479]
36. Lara-Castro C, Doud EC, Tapia PC, Munoz AJ, Fernandez JR, Hunter GR, Gower BA, Garvey WT. Adiponectin multimers and metabolic syndrome traits: relative adiponectin resistance in African Americans. *Obesity (Silver Spring)* 2008;16(12):2616–2623. [PubMed: 18820653]

37. Higgins PB, Fernandez JR, Goran MI, Gower BA. Early ethnic difference in insulin-like growth factor-1 is associated with African genetic admixture. *Pediatr Res* 2005;58:850–854. [PubMed: 16183814]
38. Brannon PM, Yetley EA, Bailey RL, Picciano MF. Overview of the conference “Vitamin D and Health in the 21st Century: an Update”. *Am J Clin Nutr* 2008;88:483S–490S. [PubMed: 18689388]
39. Cranney A, Weiler HA, O'Donnell S, Puil L. Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. *Am J Clin Nutr* 2008;88:513S–519S. [PubMed: 18689393]
40. Salamoun MM, Kizirian AS, Tannous RI, Nabulsi MM, Choucair MK, Deeb ME, El-Hajj Fuleihan GA. Low calcium and vitamin D intake in healthy children and adolescents and their correlates. *Eur J Clin Nutr* 2005;59:177–184. [PubMed: 15483638]
41. Pocock NA, Noakes KA, Griffiths M, Bhalerao N, Sambrook PN, Eisman JA, Freund J. A comparison of longitudinal measurements in the spine and proximal femur using Lunar and Hologic instruments. *J Bone Miner Res* 1997;12:2113–2118. [PubMed: 9421245]

Fig. 1.
Relationship between European (EUADM), African (AFADM), and Amerindian (AMINADM) genetic admixture and bone mineral content (BMC) in the total sample

Fig. 2. Relationship between genetic admixture and bone mineral content (BMC) stratified by body fat percent cutpoints, adjusted by height, age, Tanner stage, sex, diet, physical activity, and socioeconomic status (SES). **a–c** Normal body fat; **d–f** high body fat. *EUADM*, European genetic admixture; *AFADM*, African genetic admixture; *AMINADM*, American Indian admixture

Table 1

Descriptive statistics (mean $\pm$ SE)

	Total (n = 270)	Male (n = 143)	Female (n = 127)	EA (n = 116)	AA (n = 94)	HA (n = 60)
Age (years)	9.63 $\pm$ 0.1	9.82 $\pm$ 0.1 ^a	9.42 $\pm$ 0.1 ^b	9.69 $\pm$ 0.2	9.71 $\pm$ 0.1	9.38 $\pm$ 0.2
Height (cm)	140.3 $\pm$ 0.6	140.6 $\pm$ 1.0	139.9 $\pm$ 0.9	140.2 $\pm$ 1.0 ^{cd}	141.9 $\pm$ 1.0 ^c	137.8 $\pm$ 1.3 ^d
Weight (kg)	36.7 $\pm$ 0.6	36.7 $\pm$ 0.8	36.6 $\pm$ 0.8	35.6 $\pm$ 0.8	37.7 $\pm$ 1.0	37.2 $\pm$ 1.1
Tanner	2	1 ^a	2 ^b	1 ^c	2 ^d	1 ^c
BMI percentile	64.6 $\pm$ 1.6	63.8 $\pm$ 2.2	65.4 $\pm$ 2.4	59.7 $\pm$ 2.4 ^c	63.0 $\pm$ 2.7 ^c	77.2 $\pm$ 3.5 ^d
Total fat (kg)	8.8 $\pm$ 0.3	8.1 $\pm$ 0.5 ^a	9.5 $\pm$ 0.4 ^b	8.2 $\pm$ 0.5 ^c	8.2 $\pm$ 0.7 ^c	10.6 $\pm$ 0.7 ^d
Percent fat	23.0 $\pm$ 0.6	20.7 $\pm$ 0.8 ^a	25.7 $\pm$ 0.7 ^b	22.3 $\pm$ 0.8 ^c	20.5 $\pm$ 1.0 ^c	28.2 $\pm$ 1.1 ^d
Total lean (kg)	25.8 $\pm$ 0.3	26.7 $\pm$ 0.4 ^a	24.8 $\pm$ 0.4 ^b	25.4 $\pm$ 0.5 ^c	27.3 $\pm$ 0.6 ^d	24.3 $\pm$ 0.6 ^c
BMC (kg)	1.39 $\pm$ 0.02	1.31 $\pm$ 0.03	1.26 $\pm$ 0.03	1.24 $\pm$ 0.03 ^c	1.38 $\pm$ 38.5 ^d	1.22 $\pm$ 0.04 ^c
EUADM	0.54 $\pm$ 0.02	0.54 $\pm$ 0.04	0.55 $\pm$ 0.03	0.96 $\pm$ 0.01 ^c	0.15 $\pm$ 0.01 ^d	0.35 $\pm$ 0.02 ^e
AFADM	0.26 $\pm$ 0.03	0.32 $\pm$ 0.03	0.31 $\pm$ 0.03	0.01 $\pm$ 0.00 ^c	0.82 $\pm$ 0.01 ^d	0.09 $\pm$ 0.01 ^e
AMINADM	0.14 $\pm$ 0.02	0.14 $\pm$ 0.02	0.14 $\pm$ 0.02	0.03 $\pm$ 0.00 ^c	0.03 $\pm$ 0.00 ^c	0.56 $\pm$ 0.03 ^d
SES	40.0 $\pm$ 0.9	40.5 $\pm$ 1.2	39.4 $\pm$ 1.3	49.01 $\pm$ 1.0 ^c	38.02 $\pm$ 1.1 ^d	26.03 $\pm$ 1.6 ^e
Calcium (mg)	850.0 $\pm$ 20.7	874.2 $\pm$ 28.9	823.6 $\pm$ 29.7	876.0 $\pm$ 31.3 ^{cd}	734.4 $\pm$ 33.7 ^c	983.4 $\pm$ 40.0 ^d
Vitamin D (μ g)	4.8 $\pm$ 0.2	5.0 $\pm$ 0.2	4.5 $\pm$ 0.3	5.20 $\pm$ 0.30 ^{cd}	3.90 $\pm$ 0.31 ^c	5.46 $\pm$ 0.30 ^d
Total PA (min/day)	286.6 $\pm$ 3.8	287.4 $\pm$ 5.5	285.8 $\pm$ 5.1	289.3 $\pm$ 5.1	285.2 $\pm$ 7.3	283.3 $\pm$ 8.1
Mod/Vig PA (min/day)	25.7 $\pm$ 1.0	29.1 $\pm$ 1.5 ^a	20.8 $\pm$ 1.3 ^b	26.9 $\pm$ 1.6	25.2 $\pm$ 2.0	21.9 $\pm$ 1.7

EA European American, AA African American, HA Hispanic American, BMI/body mass index, BMC bone mineral content, EUADM European genetic admixture, AFADM African genetic admixture, AMINADM American Indian admixture, SES socioeconomic status, PA physical activity

^{a,b} Significant difference between sexes, $P < 0.05$

^{c-e} Difference based on self-identified racial/ethnic categorization, $P < 0.05$

Table 2

Multiple linear regression analysis for bone mineral content; all subjects combined, and by adiposity classification

Variable	β Coefficient	SE	P value
Total sample ^a			
Fat (%)	0.10	0.02	< 0.001
Ethnicity	0.01	0.01	0.47
Normal adiposity ^b			
Ethnicity	-0.01	0.01	0.31
High adiposity ^b			
Ethnicity	0.01	0.01	0.68

^aModel adjusted for sex, pubertal stage, age, height, total fat mass, total lean mass, SES, reported dietary calcium intake, reported dietary Vitamin D intake, and total daily physical activity

^bModel adjusted for sex, pubertal stage, age, height, total lean mass, SES, reported dietary calcium intake, reported dietary Vitamin D intake, and total daily physical activity

Table 3

Multiple linear regression analysis for bone mineral content stratified by sex and by Tanner stage

Independent variable	Group	β	P value
EUADM	Males	-0.034	0.273
	Females	-0.086	0.003
	Tanner 1	-0.056	0.019
	Tanner 2	-0.076	0.133
	Tanner 3	-0.087	0.240
AFADM	Males	0.073	0.009
	Females	0.092	0.001
	Tanner 1	0.060	0.008
	Tanner 2	0.115	0.008
	Tanner 3	0.121	0.050
AMINADM	Males	-0.139	0.004
	Females	-0.098	0.087
	Tanner 1	-0.143	0.082
	Tanner 2	-0.218	0.012
	Tanner 3	-0.434	0.024

Bolded values indicate significant relationship between dependent variable (BMC) and independent variable. All models adjusted for sex, pubertal stage, age, height, total fat mass, total lean mass, SES, reported dietary calcium intake, reported dietary Vitamin D intake, and total daily physical activity

EUADM European admixture, *AFADM* African admixture, *AMINADM* American Indian admixture