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Body size and pubertal development explain ethnic differences in structural geometry at the femur in Asian, Hispanic, and white early adolescent girls living in the U.S.

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Abstract

Variation in structural geometry is present in adulthood, but when this variation arises and what influences this variation prior to adulthood remains poorly understood. Ethnicity is commonly the focus of research of skeletal integrity and appears to explain some of the variation in quantification of bone tissue. However, why ethnicity explains variation in skeletal integrity is unclear.

Methods—Here we examine predictors of bone cross sectional area (CSA) and section modulus (Z), measured using dual-energy X-ray absorptiometry (DXA) and the Advanced Hip Analysis (AHA) program at the narrow neck of the femur in adolescent (9–14 yr) girls ($n=479$) living in the United States who were classified as Asian, Hispanic, or white if the subject was 75% of a given group based on parental reported ethnicity. Protocols for measuring height and weight follow standardized procedures. Total body lean mass (LM) and total body fat mass (FM) were quantified in kilograms using DXA. Total dietary and total dairy calcium intakes from the previous month were estimated by the use of an electronic semi-quantitative food frequency questionnaire (eFFQ). Physical activity was estimated for the previous year by a validated self-administered modifiable activity questionnaire for adolescents with energy expenditure calculated from the metabolic equivalent (MET) values from the Compendium of Physical Activities. Multiple regression models were developed to predict CSA and Z.

Results—Age, time from menarche, total body lean mass (LM), total body fat mass (FM), height, total calcium, and total dairy calcium all shared a significant ($p<0.05$), positive relationship

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with CSA. Age, time from menarche, LM, FM, and height shared significant ($p < 0.05$), positive relationships with Z. For both CSA and Z, LM was the most important covariate. Physical activity was not a significant predictor of geometry at the femoral neck ($p = 0.339$), even after removing LM as a covariate. After adjusting for covariates, ethnicity was not a significant predictor in regression models for CSA and Z.

Conclusion—Variability in bone geometry at the narrow neck of the femur is best explained by body size and pubertal maturation. After controlling for these covariates there were no differences in bone geometry between ethnic groups.

Keywords

Bone; Lean Mass; Fat Mass; Menarche; Ethnicity; Race

1. Introduction

Bone growth during adolescence is particularly important to the prevention of bone fragility during senescence. Growth during adolescence is at its maximum velocity; the rate and pattern of growth during this life stage are regulated by genes and hormones [1–3] as well as the environment [4–6]. Understanding the variation in bone growth during adolescence will better equip researchers to develop interventions targeted at preventing osteoporosis.

Some indicators of bone growth, such as bone mineral content (BMC) and bone mineral density (BMD), have been well documented [4–12]. However, less is known about changes in structural geometry during adolescence. Structural geometry describes the surface-specific nature of bone loss and acquisition [13]. The surface, or envelope, on which bone is deposited determines how large the bone is and also its shape. These features describe its bending strength or rigidity, with bending strength increasing more with bone deposition at the periosteal surface [14].

Structural geometry is influenced by physical activity, nutrition, body size and composition, pubertal development, and ethnicity [7,15–23]. Greater activity levels benefit bone acquisition and maintenance, but this is dependent on the activity type, duration, intensity of exertion, and substrate [14,24]. Lean muscle mass has a strong effect on structural geometry, but also shares a strong association with activity [25,26]. It is possible that loads induced by muscles pulling on bone contribute sufficient loading to impact geometry, but partitioning the effects of activity and lean mass (LM) is difficult at best. Peak lean tissue mass accrual precedes peak growth in bone cross sectional area and section modulus, supporting an osteogenic response to increased muscle mass and associated hormonal changes that accompany growth in lean tissue [27]. This relationship varies by sex, as girls have on average a greater proportion of bone to muscle mass than do boys [28]. In adolescent girls, bone responds positively to the interaction between estrogen (E2) levels and physical activity [15]. Ruff found that growth velocity in femoral strength was very poorly correlated with growth velocity in thigh muscle mass and strongly correlated with growth velocity in body weight $\times$ femur length, suggesting perhaps a greater role for body mass in development of femoral strength [29]. But growth in fat mass independent of muscle mass appears to have a negative impact on periosteal expansion and thus section modulus [30–32]. However, precisely when these factors influence growth in structural geometry and to what degree remains largely unanswered. While more is known about ethnic variation in structural geometry of adults [33–36], relatively little has been done to examine ethnic variation in structural geometry during late childhood and early adolescence. Further, a better understanding of the cause(s) of variation in structural geometry between ethnic groups is needed to better assess future risk of bone fragility and intervention.

To this end we analyzed structural geometry at the femoral neck in a sample of adolescent girls ($n=479$) living in the U.S. Subjects identified as one of three ethnic groups: Asian, Hispanic, or white. In our analysis of structural geometry we had three goals: 1. consider the relationship between lean muscle mass and fat mass and bone geometry; 2. evaluate the relationship between physical activity, total calcium intake and total dairy intake, and bone geometry; and 3. determine if ethnic differences in bone geometry develop by late childhood/early adolescence after controlling for covariates, with Asians presenting smaller measures of bending rigidity than Hispanics or whites; and whites presenting the greatest measures of bending rigidity.

2. Materials and Methods

2.1. Research Design

This cross-sectional analysis used baseline data from the Adequate Calcium Today (ACT) project, a school-randomized educational intervention conducted at sites in six states (Arizona, California, Hawaii, Indiana, Nevada, and Ohio). The baseline data from the multi-site ACT study have been used to investigate the effects of body size and ethnicity on BMC [12], perceived lactose intolerance and BMC [37], ethnic differences in the android/gynoid fat ratio [38], development and reliability testing of psychosocial measurement of calcium intake [39], assessment of breast density using DXA [40], development and analysis of a calcium testing tool [41], the effects of race, acculturation, and SES on obesity, and the relationship between tanning and bone mass and structure [42]. Subjects used in this analysis were girls in the sixth grade, with age at baseline measurement ranging from 9–14 years. Middle schools within 1-hour driving time of one of the designated DXA measurement sites were eligible for participation in ACT if their student populations had a higher proportion of Asian or Hispanic students than the state average. Sixth-grade girls within participating schools were recruited as volunteers via presentations, mailings, and flyers. Recruitment goals for the total number of Asian, Hispanic, and non-Hispanic white girls within a school were established based on goals for the overall ACT project and enrollment distributions in each school.

Subjects were classified as Asian, Hispanic, or white (Table 1). Inclusion into a particular ethnic group required that a subject be 75% of a given group based on parental or guardian reported percentage ethnicity of each biological parent [38]. Ethnicity choices given to the parents were American Indian or Alaska Native, Asian or Asian American, black or African-American, Hispanic or Latino, Native Hawaiian or Pacific Islander, white, or other. Many parents reported multiple ethnic backgrounds, so each child was assumed to be one half of each of the ethnicities of each parent. These percentages were summed and had to total 75% of the recruited ethnic group (Asian, Hispanic, or white). For example, a child might get 50% of their Asian ancestry from one parent and 25% from the other parent that was one-half Asian and one-half white. Letters requesting consent were sent to parents of the interested students and girls were enrolled into the program subsequent to completion of required informed consent and assent documentation. The study was approved by local Institutional Review Boards.

2.2. Bone and Body Composition Measurements

Dual energy x-ray absorptiometry (DXA; Lunar Prodigy, software version 6.5 and 6.7 GE Medical Instruments, Madison, WI) was used to derive measures of body composition. Matching instruments and software were used at each site. Training of DXA staff was conducted by the same individual at each geographic site, and measurements followed standardized procedures. Within laboratory and between-laboratory variance in DXA measurements were determined by measuring total-body, spine, forearm, and femur

phantoms (Hologic, Bedford, MA) at each geographic site. Each phantom was scanned ten times, with repositioning of the phantom between each scan, so positioning error is included in the results. The phantom measurements were analyzed and reviewed for accuracy errors by an independent group at the University of California-San Francisco. The means and standard deviations were calculated for each scanned phantom including BMD and BMC for total-body, spine, femur, and forearm as well as whole body lean mass (LM) and whole body fat mass (FM). Results indicated that no study site adjustments for DXA measurements were needed. The coefficient of variation (percent) for BMD ranged from 0.59 (spine) to 5.36 (total forearm 800 density); for BMC from 1.18 (spine) to 1.93 (total-body); for area from 1.19 (spine) to 4.06 (neck). The coefficients of variation for LM and FM were 0.72 and 1.10, respectively. A single DXA operator performed all scans at each site, except in California and Indiana, where two operators were used. The reliability between operators was 0.96–0.99 for California and 0.97–0.99 for Indiana. [12]. Subjects provided a urine sample for use in a pregnancy testing prior to the DXA scan. If the test was negative, then a subject could proceed with scanning.

The Advanced Hip Analysis (AHA) program provided measures of structural geometry at the femoral neck. The AHA software calculated several measures of structural geometry using data derived from the DXA curves calculated from proximal femur scans [16,43,44]. From this information the following measures of geometry were included in this analysis:

1. Cross sectional moment of inertia (CSMI, mm^4) was measured at the section of minimum CSMI within the region of interest (ROI) on the femoral neck. This measure reflects the distribution of material around the neck axis and is necessary in order to calculate a bone's resistance to bending stress:

$$CSMI = \frac{k(dx)}{\rho} \left[\sum PBMx^2 - \left(\frac{\sum PBMx}{PBM} \right)^2 \right] \text{mm}^4$$

where PBM is a pseudo-bone mineral value based on the X-ray absorption data, k is a PBM to BMC conversion factor, ρ is the average physical density of bone (1.85 g/cm^3), and dx is the distance between scan lines. This variable was not a unit of analysis, rather its calculation was necessary to quantify section modulus.

2. Section modulus (Z , mm^4) was derived using CSMI. Section modulus is a ratio of CSMI to the distance of the outer most point on the periosteum. Section modulus evaluates the size and shape of the cross section in a given ROI while controlling for distance from the neutral axis (y) to the periosteal diameter:

$$Z = \frac{CSMI}{y}$$

where y is the distance from the neutral axis to the periosteal margin.

3. Cross sectional area (CSA, mm^2) was used to measure the amount of cortical bone at the section of minimum CSMI within the ROI on the femoral neck.

Structural geometry was calculated for both the left and right femoral necks. These values were not significantly different from one another, so the average value of the left and right sides were used in this analysis.

2.3. Anthropometry and Sexual Maturity

Weight was measured with subjects wearing laboratory issued scrubs and without shoes on an electronic scale or beam balance and recorded to the nearest 0.1 kg. Sets of three measurements were taken until two measures were within a 0.2 kg agreement and the median was used for analysis. Height was measured with the subject standing barefoot against a wall with her head in the Frankfurt horizontal plane. A stadiometer was used to measure height and measurements were recorded to the nearest 0.1 cm. Sets of three measurements were taken until two measures were within a 0.2 cm agreement and the median was used for analysis. Protocols for measuring height and weight follow standardized procedures [45]. Total body lean mass (LM) and total body fat mass (FM) were quantified in kilograms using DXA. Within-laboratory and between laboratory variance in DXA measurements were determined following the same procedures outlined for bone above using a whole body phantom (Hologic, Bedford, MA). The coefficients of variation for lean mass and fat mass were 0.72 and 1.10, respectively.

To assess sexual maturity, subjects were asked about menarche status. Because we have 2 years of follow up data on the subjects analyzed here, we were able to quantify time to menarche measured as months away from menarche either pre- or post-menarche. Girls who were measured/scanned prior to menarche were assigned negative values in months prior to menarche ($n = 241$; $\bar{x} = -9.45$; $\sigma = 5.34$) at the time of data collection, while girls who had attained menarche were assigned a positive value denoting the amount of time between menarche ($n = 256$; $\bar{x} = 11.06$; $\sigma = 10.06$) and data collection.

2.4. Lifestyle

Dietary calcium intakes from the previous month were estimated by the use of an electronic semi-quantitative food frequency questionnaire (eFFQ) based on a FFQ developed for and evaluated with Asian, Hispanic, and non-Hispanic white youth [46]. The eFFQ was administered through an interactive multi-media computer program that provided images of a food or food grouping with a text message of the serving size(s). Respondents were instructed to indicate how often a food was consumed (e.g., never or less than once per month, 1–3 servings per month, 1 serving per week, 2–4 servings per week, 5 or more servings per week). Approximately 81 food items containing calcium and foods that may interfere with calcium consumption (e.g., soft drinks) were included in the eFFQ. Calcium intakes from food were categorized in five ways: (1) calcium exclusively from dairy foods (e.g., milk); (2) calcium from non-dairy foods (e.g., broccoli); (3) calcium from mixed-foods that include calcium from both dairy and non-dairy sources (e.g., pizza); (4) total dairy calcium determined from the sum of both calcium from dairy foods and calcium from mixed-foods; and (5) total calcium from food calculated for the entire diet. In this analysis total calcium and total dairy calcium are used to quantify calcium intake.

An estimate of daily food calcium intake was computed based on a calcium value assigned to each food item on the eFFQ using mean methods for assigning nutrient values [47] and multiplying by the corresponding frequency. Estimated daily food calcium intakes that were <100 mg/day or >2500 mg/day were considered improbable and individuals with such values were excluded from analyses using food calcium intakes.

Physical activity was estimated for the previous year by a validated self-administered modifiable activity questionnaire for adolescents [48,49]. Energy expenditure was calculated from the metabolic equivalent (MET) values from the Compendium of Physical Activities [50]. The MET values were expressed as hours of energy expenditure/week for all activities controlled for body weight. The sum of all MET values was used as a proxy for physical activity in the past year. Physical activity questionnaires were entered and checked for

accuracy at the Coordinating Center (Purdue University). Inconsistent or incomplete questionnaires were returned to the appropriate site for further clarification from the subject.

2.5 Statistical Analysis

Prior to analysis, outliers (± 3 standard deviations from mean) were removed from the total sample ($n=748$). If an individual was an outlier for any of the variables they were excluded from analyses, resulting in a reduced sample size ($n=678$). Subsequently, only subjects whose time to menarche was known were included in this analysis ($n=497$). Ethnicity was dummy coded in all regression models. Total calcium intake was log transformed to account for skewness in this measure, while total dairy calcium intake was transformed using the square root to correct for skewness. Physical activity data were also log transformed to account for skewness. Baseline data were summarized using means and standard deviations. ANOVA was used to test for differences in age, anthropometry, physical activity and calcium intake between ethnic groups. Bonferonni correction was used for post-hoc comparisons of individual groups. Multiple regression was used to examine ethnic differences in the two bone outcome variables at the proximal femur. Factors known to predict skeletal integrity (age, time to menarche, weight, height, lean muscle mass, physical activity, total calcium, total dairy, ethnicity) were entered in sequential models to evaluate improvement in predictive power and significance of predictors. Chronological age and time to menarche—an indicator of maturation—were both included in regression models; the former provides a reference of physical development and the latter indicates how far from maturity subjects are at the time of measurement. A series of statistics and plots were performed to check the assumptions underlying regression. The Durbin-Watson statistic was used to test for autocorrelation in regression models, while tolerance was used to evaluate the likelihood of multicollinearity. Standardized residuals were plotted against standardized predicted values to evaluate the assumptions of homoscedasticity, while predicted outcomes were plotted against their actual, respective values to evaluate linearity. The Levene's test statistic tested for homogeneity of variances. Statistics were performed using SPSS Version 19 [51].

3. Results

3.1. Subject characteristics

In this sample, ethnic groups differed significantly in age, CSA, Z, and anthropometry (Table 1). There were no differences in time to menarche. In post-hoc comparisons of age, CSA, Z, height, and LM Asians were significantly smaller than Hispanics and whites, and Hispanics were significantly smaller than whites ($P=0.02$). Asians weighed significantly less than Hispanics and whites ($P<0.001$), but there were no differences in weight between Hispanics and whites. Asians had significantly less FM than Hispanics and whites ($P<0.001$), but Hispanics and whites did not differ in FM. White girls were significantly more active than Hispanics ($P<0.001$), but not Asians and there were no differences between Hispanics and Asians. Hispanics consumed significantly more total calcium than whites ($P<0.03$) and Asians ($P<0.001$), but total calcium consumption in Asians and whites were not different. Total dairy calcium consumption was also significantly higher in Hispanics than Asians ($P<0.04$), but this was the only significant difference in total dairy calcium. To evaluate the effects of physical activity on CSA and Z models were analyzed without the variable LM. The results of these models were not different from those that included LM and thus only models including physical activity and LM are discussed.

3.2. Evaluating predictors of cross sectional area and section modulus

All models met the assumptions underlying regression. In the baseline regression models predicting CSA and Z, weight, height, LM and FM shared low tolerance values and likely

share variation explained in outcome measures. Total body fat and lean mass as well as height were used as anthropometric indicators in subsequent models as they directly relate to one of our research questions, while weight provides a proxy of body composition and body size. While including measures of body size are important for understanding variation in 2-dimensional DXA scans, LM, FM, height and weight are strongly correlated with one another and if included in the same regression model would increase the likelihood of collinearity. Because results affected by collinearity can be tenuous, only height was included with LM and FM in regression models. Total calcium and total dairy calcium were used as indicators of calcium intake in separate regression models as they are not independent of one another and thus would result in problems with collinearity. Subsequent to these modifications there were no issues with tolerance, suggesting collinearity was not a problem in successive models.

3.3. Cross sectional area

Results from regression models to predict CSA at the hip are shown in Table 2 and Figure 1. In general, age, time to menarche, FM, LM, height, total calcium, and total dairy calcium were significant predictors of CSA. Ethnicity and physical activity failed to reach significance in any of the models. The standardized coefficients (β) provided a useful means of analyzing the relative importance of predictors in each model and LM, time to menarche, and height produced the highest β values across all models. In the final model, age and FM were relatively equivalent to one another in terms of importance to predicting CSA. Predictive power of the models did not differ substantially between the three models, although the final model produced the highest R^2 value.

3.4. Section modulus

Multiple regression results for Z are also included in Table 2 and Figure 1. Age, time to menarche, LM, FM, and height were significant predictors across all models. Ethnicity, physical activity, total calcium, and total dairy calcium failed to reach significance in all models. The β values for LM suggest that this variable is a very important predictor of Z in all models, followed by height, time to menarche, and age. The R^2 value varies little in each model and ethnicity does not add any explanatory power to the model.

4. Discussion

The results of this study are unique in that structural geometry at the neck of femur was analyzed in a large, multi-ethnic, multi-site sample of adolescent girls living in the US. Our results suggest that ethnic differences in CSA and Z at adolescence are best explained by covariates. These results are consistent with our observations of BMC in this sample (12), but are contrary to some of the literature on ethnic differences in bone geometry [7,19,32,33,36].

By this stage of development, age, time to menarche, FM, LM, and height are all significant predictors of CSA and Z. Total calcium and total dairy calcium were significant predictors of CSA, but not Z. It is possible that increases in calcium and protein contribute to the amount of bone present in a given section, but do not contribute to the bone's shape as it pertains to bending stress. Physical activity was not a significant predictor of either outcome. After controlling for body size, ethnicity was not a significant predictor of CSA or Z. LM appears to be a very important predictor of structural geometry which is not surprising given its role in loading of bone and its association with body size, consistent with previous research in this area [25,26,28]. Age was significantly different across ethnic groups, but multiple regression allowed for the effects of this covariate to be included in subsequent

statistical models as physiological processes associated with age during this period are clearly associated with physical growth.

When multiple predictors were included in regression models to predict CSA and Z, ethnicity was not significant. These findings are consistent with prior observations in this age group for BMC [12]. There is evidence, however, that ethnic differences are present in bone geometry [7,19,32,33,36]. Ethnicity's significance as a predictor in some studies may support an underlying genetic basis to differences in skeletal integrity. This assumption is tacit when ethnicity or race are used to group individuals, although relatively few have actually employed genetic analyses to confirm this. There is a clear genetic basis to femoral neck geometry [53–56] and this variation interacts with the environment, but not all bones respond to environmental stimuli to the same degree [29,57]. Even with this knowledge we still do not know how these genes vary across ethnic groups. The efficiency with which early adolescent African American girls [58] and Chinese girls [59] maintain calcium homeostasis relative to other groups and the observation that Asian young women maintain greater trabecular bone thickness than do white young women [60] also supports use of ethnicity or race as a proxy for some underlying biological basis to variability in skeletal health. But pooling of subjects by ethnicity or race may also miss important variation. For example, Finklestein et al., found significant differences in unadjusted bone mineral apparent density (BMAD) at the lumbar spine and femur between Chinese and Japanese women, a difference that would have gone unnoticed had subjects been pooled as Asian [61]. The ability to define meaningful categories, however, may not be possible in many situations. The degree to which these differences reflect genetic or epigenetic influence remains unknown.

Variables that captured aspects of growth and development, age and time to menarche, were also significant in every model predicting CSA or Z. These results support previous findings suggesting that growth and pubertal development are important contributors to skeletal integrity [15,27,62,63]. Fluctuations in hormone levels responsible for this are in turn affected by genes and energy availability [64,65]. This relationship likely affects bone growth and development.

Height was important for predicting CSA and Z. These results correspond with our previous findings for BMC and body size in this sample [12] and support other observations regarding the relationship between bone geometry and body size in general [17,30,52]. If indicators of body size are important covariates of bone geometry, then the effects of body composition must also be considered to investigate potential differences in LM and FM, each of which correspond to body size.

LM was a significant predictor of CSA and Z in all models and explained most of the variance in these outcomes. Although, in this sample, levels of physical activity were not significantly different across ethnic groups; these results do not reflect long term loading history or the intensity, duration, and substrate on which loading has occurred. There is some evidence that load bearing bones are less sensitive to physical activity in pre- and early pubertal girls than in boys [66], which may also factor into our findings. Further, LM may share variance with physical activity, with the former explaining more of the variation in CSA and Z and thus masking the effects of the latter. LM also shares a strong positive relationship with body size. Anthropometric indicators of body size have been previously noted as important predictors of bone health outcome [7,12,29], so some of the variation in CSA and Z explained by LM may be owed in part to differences in body size. Body size is relevant in terms of functional loading of the axial and lower appendicular skeleton during locomotion and thus affects skeletal integrity. Differences in LM and body size across the lifespan would certainly have an effect on variability in bone quantity and quality. This

relationship is certainly complex in that variability in type of movement, substrate, and frequency of activity would interact to produce variability in loading regimes. Some have argued that muscle and bone should be viewed as a functional unit with muscle development driving bone development [25]. Indeed peak LM growth velocity occurs prior to peak growth in bone, perhaps suggesting that loads imposed by muscle are largely responsible for the timing and pattern of bone growth [25,28,67]. However, the data presented here are cross sectional and relate to early adolescence, so it is not possible to determine if our data support these findings.

In this sample, FM was also a significant, positively associated predictor of CSA and Z, although clearly not as important a predictor as LM. There is equivocal evidence that FM can influence bone growth. There is evidence that increases in FM during late adolescence/early adulthood limits periosteal expansion among girls and thus decreases bone strength relative to the load imposed on it [31]. However, there is also evidence that during adolescence FM in girls is positively associated with periosteal circumference and thus increased bending strength, similar to our results [67]. It may be that body composition is interacting with physical activity; more active, heavier individuals with greater LM impose larger loads, inducing skeletal growth to accommodate these loads.

In terms of lifestyle, total calcium and total dairy calcium independent of one another were significant predictors of CSA, but not Z. Physical activity failed to achieve significance. Research using calcium “intakes” or “intake of dairy foods” as a predictor of geometry has been equivocal, with some studies finding significant results [22,68] and others not [20,69,70]. Dairy foods contribute protein and calcium and may be the more appropriate predictor of bone geometry [22]. The importance of physical activity as a determinant of structural geometry is well documented [17,18,20,21,28,66,71], with some evidence that these effects last into early adulthood [72,73], so failure of physical activity to predict bone geometry here is at odds with most of the published literature. However, our findings support the observations of Macdonald et al., who found no significant increase in bone strength in pre- and early adolescent girls exposed to a jumping intervention compared to controls [21]. Bone growth and maintenance are influenced by intensity and duration of activity, with greater loading at irregular intervals producing the strongest effects [14,24]. Because MET values are quantified as hours of energy expenditure/week for all activities controlled for body weight, we cannot comment on variability in osteogenic effect by activity type or intensity. Additionally, because LM and physical activity are not independent of one another it is possible that the former is accounting for the variation in geometry explained by physical activity. However, tolerance values for these variables remain high (0.72) suggesting that the amount of the variance in geometry shared by LM and physical activity is insufficient to produce collinearity. Additionally, to see if physical activity had an effect on bone geometry regression models including all covariates except LM were evaluated, but physical activity still failed to reach significance (results not shown).

After controlling for covariates, however, these differences disappear. Covariates indicative of body size—LM, height, FM—explained most of the variation in CSA and Z, suggesting structural geometry scales positively with body size. If ethnic differences in bone geometry are explained by body size, then our understanding of when and why these differences arise may best be framed within an intergenerational perspective. There is a strong possibility that health and lifestyle across generations can influence skeletal integrity, particularly given the strong association between body size and measures of bone health outcome. Growth insult is dependent on severity of environment, duration and timing. For instance, a sample of adults whose mothers experienced and survived the Dutch Winter Famine (winter of 1944–1945) presented with different patterns of methylation on the IGF2 gene, with those exposed to

stresses earlier in development deviating the most from unexposed same sex siblings [74]. Because IGF2 plays a major role in promoting intrauterine growth, modifications to expression may influence skeletal integrity later in life. Migration studies examining changes across generations have consistently demonstrated that increasing body size is associated with improvements in health and nutrition [75,76]. Secular trends in increasing body size also associated with improvements in health and nutrition seem to corroborate this as well. Genetic potential limits body size attainment and thus ultimately height would plateau following generations of life in a healthy environment. Therefore, this type of change may not completely buffer against poor bone health and the infrastructure to insure optimal health and nutrition alone is likely impossible to attain. However, awareness of these issues will contribute greater understanding of factors influencing bone health and inform future intervention.

There are limitations to this study worthy of discussion. For instance, we only measured girls and thus our results are not generalizable to both sexes. Although our sample was large, ethnically diverse, and representative of cities in six states, it may not be representative of the population of girls since not all ethnicities were represented in the sample. Additionally, all subjects lived and attended school in the United States, so results may not be generalizable to other cultures. In terms of methods, the physical activity questionnaire used in this study was validated on adolescents aged 15–18 years; our sample ranged in age from 9–14 years so this may have influenced our results for physical activity. Variation in AHA precision across sites has not been quantified with a phantom to date, but %CV for BMC across sites suggested that no adjustments were needed to control for scanner/operator differences. Additionally, scanners were consistently calibrated using a daily calibration phantom. Finally, these results are limited in that they are cross sectional, although a longitudinal analysis of geometric change in this sample is currently underway.

5. Conclusion

Our results suggest that CSA and Z in early adolescence are associated with LM and FM, but LM explains most of the variance in all regression models. This further supports the relationship between muscle and bone as a functional unit. In terms of lifestyle, physical activity and calcium and dairy intake were not significant predictors of bone geometry. There were no significant differences between ethnic groups after controlling for body size. These results contribute to a growing body of literature on variation in structural geometry at adolescence.

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Highlights

- Bone geometry in a sample of multi-ethnic early adolescent girls is assessed
- We use anthropometry, lifestyle, menarche status and ethnicity to predict bone
- Height, lean and fat mass are significant positive predictors of bone geometry
- Dietary calcium and dairy predict bone area, but not bending strength
- Ethnic effects on bone geometry are explained by covariates

Figure 1.
Comparison of actual versus predicted (A) cross sectional area and (B) section modulus at the femoral neck for multiple regression models V. 95% confidence intervals are indicated adjacent to the line of best fit.

Table 1

Characteristics, bone geometry, and lifestyle among Asian, Hispanic, and white early adolescent girls (10–13 years)

Variable	Asian (<i>n</i> = 124)	Hispanic (<i>n</i> = 152)	White (<i>n</i> = 221)	Total (<i>n</i> = 497)
	Mean ± SD (range)			
Age (yr)	11.1 ± 0.5 (10–13)	11.3 ± 0.6 (10–14)	11.6 ± 0.6 (9–13)	11.4 ± 0.6 (9–14)
Time to Menarche (mo)	−0.7 ± 10.9 (−17–36.5)	2.4 ± 13.6 (−20.3–38.8)	1.3 ± 13.8 (−23.8–53.3)	1.1 ± 13.1 (−23.8–53.3)
Height (cm) *	147.3 ± 7.1 (129.8–170.3)	151.2 ± 6.4 (131.9–166.3)	155.5 ± 7.1 (139.4–175.1)	152.1 ± 7.7 (129.8–175.1)
Weight (kg) †	43.1 ± 9.7 (27.4–71.1)	51.5 ± 12.7 (28.1–88.7)	52.4 ± 14.2 (29.7–109.2)	49.8 ± 13.4 (27.4–109.2)
Total-body fat mass (kg) †	11.5±6.2 (2.6–28.5)	17.8.7±8.6 (4.1–38.8)	16.7±9.7 (4.1–52.1)	15.8±8.9 (2.6–52.1)
Total-body lean mass (kg) *	29.2±6.2 (20.1–44.5)	31.1±4.9 (20.4–47.9)	33.3±5.4 (22.2–57.1)	31.6±5.3 (20.1–57.1)
CSA (mm ²) *	106.5 ± 18.5 (63.5–159.4)	117.6 ± 21.7 (64.2–176.7)	124.8 ± 22.7 (77.9–188.1)	118.1 ± 22.6 (63.5–188.1)
Z (mm ⁴) *	363.9 ± 87.3 (184.8–602.4)	414.1 ± 101.9 (192.7–781.2)	459.1 ± 111.9 (214.1–900.8)	421.7 ± 109.9 (184.8–900.8)
Calcium (mg) *	1118.8 ± 733.7 (108.7–3878.1)	1536.8 ± 916.6 (108.7–3980.6)	1206.2 ± 707.4 (110.4–3980.6)	1285.0 ± 799.8 (108.7–3980.6)
Dairy (mg) ††	675.7 ± 456.7 (38.5–105.2)	835.3 ± 582.4 (39.3–270.8)	766.2 ± 502.2 (51.2–609.5)	764.9 ± 519.9 (38.5–609.5)
Physical Activity (MET hrs/wk) ‡	41.7 ± 41.4 (0.0–235.7)	36.3 ± 45.4 (0.0–323.5)	54.6 ± 60.0 (0.0–443.9)	45.8 ± 52.1 (0.0–443.9)

CSA = Cross Sectional Area; Z = Section Modulus; MET = Metabolic Equivalent.

Bonferroni comparisons by ethnic groups:

* A<H<W (*P*<0.001);

† A<H, A<W (*P*<0.001);

†† A<H (*P*<0.001);

‡ H<W (*P*<0.001).

Table 2

Regression models describing the relationship between predictors and bone CSA and Z.

Models and R ²		Cross sectional area (CSA) (n=479)		Section modulus (Z) (n=479)	
Model I:					
Constant	B = -134.04	SE B = 28.88	B = -764.29	SE B = 136.05	
Age (yr)	B = 1.87	SE B = 0.93	β = 0.05	B = 10.57	SE B = 4.36
					β = 0.06 *
Time to Menarche (mo)	B = 0.32	SE B = 0.05	β = 0.19 **	B = 1.14	SE B = 0.22
					β = 0.14 **
Total body fat mass (kg)	B = 8.79	SE B = 3.00	β = 0.22 **	B = 27.36	SE B = 14.12
					β = 0.14
Total body lean mass (kg)	B = 0.00	SE B = 0.00	β = 0.59 **	B = 0.01	SE B = 0.01
					β = 0.53 **
Height (cm)	B = 0.54	SE B = 0.12	β = 0.19 **	B = 3.08	SE B = 0.57
					β = 0.22 **
Weight (kg)	B = -0.29	SE B = 0.19	β = -0.17 **	B = -2.55	SE B = 0.88
					β = -0.03
R ²	0.73		0.75		
Model II:					
Constant	B = -113.71	SE B = 19.69	B = -766.20	SE B = 93.57	
Age (yr)	B = 2.25	SE B = 0.92	β = 0.06 *	B = 11.88	SE B = 4.35
					β = 0.07 *
Time to Menarche (mo)	B = 0.33	SE B = 0.05	β = 0.20 **	B = 1.09	SE B = 0.21
					β = 0.13 **
Total body fat mass(kg)	B = 4.72	SE B = 1.19	β = 0.11 **	B = 22.28	SE B = 5.67
					β = 0.11 **
Total body lean mass (kg)	B = 0.00	SE B = 0.00	β = 0.52 **	B = 0.01	SE B = 0.00
					β = 0.53 **
Height (cm)	B = 0.54	SE B = 0.12	β = 0.19 **	B = 2.99	SE B = 0.57
					β = 0.21 **
Physical Activity(MET hrs/wk)	B = 0.47	SE B = 0.49	β = 0.02	B = 0.94	SE B = 2.33
					β = 0.01
Calcium (mg)	B = 1.68	SE B = 0.79	β = 0.05 *	B = 4.48	SE B = 3.77
					β = 0.03
R ²	0.74		0.76		
Model III:					
Constant	B = -106.70	SE B = 19.00	B = -751.35	SE B = 90.21	
Age (yr)	B = 2.33	SE B = 0.92	β = 0.06 *	B = 12.24	SE B = 4.35
					β = 0.07 *
Time to Menarche (mo)	B = 0.33	SE B = 0.05	β = 0.19 **	B = 1.08	SE B = 0.21
					β = 0.13 **
Total body fat mass(kg)	B = 4.32	SE B = 1.91	β = 0.11 **	B = 22.38	SE B = 5.65
					β = 0.10 **
Total body lean mass (kg)	B = 0.00	SE B = 0.00	β = 0.51 **	B = 0.01	SE B = 0.00
					β = 0.53 **

Models and R ²	Cross sectional area (CSA) (n=479)		Section modulus (Z) (n=479)	
	B	SE	B	SE
Height (cm)	B = 0.54	SE B = 0.12	β = 0.19**	SE B = 0.57
Physical Activity(MET hrs/wk)	B = 0.46	SE B = 0.49	β = 0.02	SE B = 2.33
Dairy (mg)	B = 0.13	SE B = 0.06	β = 0.05*	SE B = 0.27
R ²	0.75		0.76	
Model IV:				
Constant	B = -105.85	SE B = 22.34		SE B = 105.81
Age (yr)	B = 2.07	SE B = 0.94	β = 0.06*	SE B = 4.45
Time to Menarche (mo)	B = 0.33	SE B = 0.05	β = 0.19**	SE B = 0.22
Total body fat mass(kg)	B = 4.22	SE B = 1.24	β = 0.11*	SE B = 5.87
Total body lean mass (kg)	B = 0.00	SE B = 0.00	β = 0.52**	SE B = 0.00
Height (cm)	B = 0.51	SE B = 0.13	β = 0.17**	SE B = 0.60
Calcium (mg)	B = 1.67	SE B = 0.81	β = 0.05*	SE B = 3.83
†Ethnicity (<i>Asian</i>)	B = -1.26	SE B = 1.52	β = -0.02	SE B = 7.20
(<i>Hispanic</i>)	B = -11.33	SE B = 6.33	β = -0.05	SE B = 3.44
R ²	0.74		0.76	
Model V:				
Constant	B = -99.17	SE B = 21.56		SE B = 102.08
Age (yr)	B = 2.16	SE B = 0.94	β = 0.06*	SE B = 4.46
Time to Menarche (mo)	B = 0.33	SE B = 0.05	β = 0.19**	SE B = 0.22
Total body fat mass(kg)	B = 4.24	SE B = 1.24	β = 0.11*	SE B = 5.87
Total body lean mass (kg)	B = 0.00	SE B = 0.00	β = 0.52**	SE B = 0.00
Height (cm)	B = 0.51	SE B = 0.13	β = 0.18**	SE B = 0.60
Physical Activity(MET hrs/wk)	B = 0.45	SE B = 0.49	β = 0.02	SE B = 0.27
Dairy (mg)	B = 0.13	SE B = 0.06	β = 0.05*	SE B = 0.27
†Ethnicity (<i>Asian</i>)	B = -1.18	SE B = 1.52	β = -0.02	SE B = 7.20
(<i>Hispanic</i>)	B = -0.68	SE B = 1.33	β = -0.01	SE B = 6.28
R ²	0.75		0.76	

Unstandardized coefficients (B) with standard error and standardized coefficients (β) are provided.

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* $P < 0.05$;
** $P < 0.001$;
‡ Ethnic comparisons were in reference to white subjects.