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Variation in childhood skeletal robustness is an important determinant of cortical area in young adults

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Abstract

A better understanding of bone growth will benefit efforts to reduce fracture incidence, because variation in elderly bone traits are determined primarily by adulthood. The natural variation in robustness was used as a model to understand how variable growth patterns define adult bone morphology. Longitudinally acquired hand radiographs of 29 boys and 30 girls were obtained from the Bolton-Brush study for 6 time points spanning 8 to 18 years of age. Segregating individuals into tertiles based on robustness revealed that the biological activity underlying bone growth varied significantly with the natural variation in robustness. For boys, slender metacarpals used an osteoblast-dependent growth pattern to establish function, whereas robust metacarpals used an osteoclast-dependent growth pattern. In contrast, differences in biological activity between girls with slender and robust metacarpals were largely based on the age at which the marrow surface changed from expansion to infilling. Importantly, cortical area for slender metacarpals was as much as 19.7% and 32.2% lower than robust metacarpals for boys and girls, respectively, indicating that robustness was a major determinant of adult cortical area. Finally, after accounting for robustness and body weight effects, we found that the inter-individual variation in cortical area was established as early as 8 years of age. While variation in the amount of bone acquired during growth has primarily been attributed to factors like nutrition, exercise, and genetic background, we showed that the natural variation in robustness was also a major determinant of cortical area, which is an important determinant of bone mass. This predictable relationship between robustness and cortical area should be incorporated into clinical diagnostic measures and experimental studies.

INTRODUCTION

Identifying skeletal trait variants that predict the risk for future fractures is a major goal for developing advanced diagnostics that will aid in reducing fracture incidence. Studies aimed

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at solving this goal will benefit from a better understanding of how variable bone growth patterns define adult traits, because the majority of the variation in bone traits among the elderly is established by adulthood [24, 43]. Current methods used to assess skeletal health during development provide clinically useful measures of bone mass (e.g., BMD) and bone age (e.g., Greulich & Pyle, Tanner-Whitehouse 2), but do not provide quantitative structural measures that differentiate growth patterns among individuals within a population. Growth patterns have been well studied in terms of sub-periosteal and endocortical surface movements [9, 25], as well as the development of bone mineral density [12] and strength-related traits like section modulus [27, 32, 33, 37]. Although these studies provided fundamental information about typical growth patterns for a population, one problem with studying only the population average traits during growth is that individuals at risk of fracturing have narrow or wide bones compared to the population mean. Individuals with slender bones (narrow relative to length) are at increased risk of fracturing throughout life [2, 8, 19, 26, 39], whereas individuals with robust bones (wide relative to length) combined with thin cortices are at increased risk of fracturing later in life [18, 31]. Although bone size is known to vary widely among individuals and to contribute to fracture risk, the manner in which bone growth patterns vary relative to external size has not been studied.

In the current study, we used a systems-biomechanics approach to study how growth patterns are adjusted to accommodate the wide, inter-individual variation in bone robustness. Long bone robustness, which is established by approximately 2 years of age [29] and determined largely by genetic background [38], is a biologically relevant trait reflecting the relationship between transverse growth and longitudinal growth. All skeletal structures, whether slender or robust, must be functionally adapted to support physiological loads otherwise they will have compromised strength and be at increased risk of fracturing. Because small changes in external size are associated with large changes in strength, slender and robust structures exhibit significantly different compensatory responses that can be readily measured as variable growth patterns from birth to 8 years of age [29]. Importantly, the inter-individual variation in growth patterns results in the acquisition of sets of traits that are predictable based on robustness. Individuals with slender bones have a proportionally greater relative cortical area compared to individuals with robust bones. These studies demonstrated that the functional adaptation process operating within the skeletal system results in a situation where variation in cortical area is superimposed on the variation in robustness. This situation where 'variation in one trait is superimposed on variation in another trait' poses scientific-challenges for identifying the biological factors regulating the functional adaptation process, as well as translational-challenges for identifying traits that have practical clinical value as advanced diagnostic measures of future fracture risk. Understanding how cortical area and robustness covary is important because the combination of these two traits defines in large part adult bone mass and whole bone mechanical properties [22].

The first goal of this study was to determine if growth patterns and the associated biological processes continue to vary with robustness through adolescence. The second goal was to study variation in the compensatory process that is superimposed on the variation in robustness during adolescence by testing whether individuals with slender bones have the same cortical area as individuals with robust bones. Further, we tested whether the variation in cortical area relative to robustness tracks over time across a population. Growth patterns that deviate in a consistent way from the population average throughout growth are expected to result in adult trait sets that have greater (or less) cortical area relative to bone robustness and body size.

METHODS

Sample population

Digitized postero-anterior radiographs of the non-dominant hand were selected from the Bolton-Brush collection, which contains longitudinally collected radiographs of Caucasian boys and girls growing up in Cleveland, Ohio circa 1930. We examined 346 hand radiographs for 29 boys and 30 girls at six time points ranging from 8 to 18 years of age to study growth during adolescence. The starting and ending ages were chosen arbitrary and were based on acquiring a cohort of boys and girls from this collection with longitudinal data that spanned puberty. Pubertal status was not assessed for this study population. Degraded or missing radiographs were replaced by an adjacent time-point if available.

Metacarpal morphology

Morphological traits of metacarpal diaphyses were quantified using methods similar to those described previously [29]. Briefly, outer diameter, marrow diameter, and cortical thickness were measured at multiple locations along the shaft length using LabVIEW Vision Builder (National Instruments Inc., Austin, TX, USA). We report data measured at the minimum shaft thickness (~45–50% of shaft length) for both the second and third metacarpals. The average coefficient of variation for manual point-to-point measurements of the outer and inner bone widths was 1.6%. The only methodological difference relative to our prior study involved the measurement of metacarpal length. Because the growth plate fuses after puberty and is less visible radiographically, metacarpal length (Le) was measured from the proximal end of the metacarpal to the distal end of the secondary center of ossification. Growth plate closure occurred in 96% of girls by 16 years and in 93% of boys by 17–18 years of age. Measuring length relative to the distal end of the secondary center of ossification resulted in a ~20% increase in total metacarpal length and a ~17% decrease in robustness values in the current study compared to our prior study [29].

Total cross-sectional area (Tt.Ar), cortical area (Ct.Ar), marrow area (Ma.Ar), and polar moment of inertia (Jo) were calculated using a circular approximation [3, 9, 21, 28]. Cortical thickness (Ct.Th) was calculated by averaging medial and lateral cortical thicknesses. Relative cortical area (RCA), a measure of the relative amount of bone, assesses the relationship between sub-periosteal expansion and endocortical expansion or contraction and was calculated as $Ct.Ar/Tt.Ar$.

Means and standard deviations were calculated for the 8, 10, 12, 14, 16, and 18 year old age groups. Age and sex-specific differences in traits were determined for the second and third metacarpals separately using a repeated measures two-way ANOVA. Because hand radiographs were obtained for only 26 individuals (11 girls, 15 boys) in the 18 year old group, the two-way ANOVA was conducted for the 8, 10, 12, 14, and 16 year old age groups only. Individual comparisons were made by post-hoc t-tests and corrected for multiple comparisons ($p < 0.008$). Post-hoc repeated measures (one-way) ANOVAs were conducted to test for changes in a specific trait over time for a single bone and sex.

Changes in robustness during adolescence

Robustness ($Tt.Ar/Le$) reflects the relationship between biological processes defining transverse growth and longitudinal growth. Longitudinal growth is measured directly by changes in bone length (Le) over time. Changes in Tt.Ar over time were used to measure transverse growth, because the amount of tissue deposited during sub-periosteal expansion increases by the square of bone width (i.e., area). We plotted Tt.Ar against Le and robustness against age to assess how the relationship between growth in width and growth in length changes during adolescence.

Variation in growth patterns relative to robustness

To understand how compensation varies among individuals, we first tested how growth patterns varied relative to robustness. Compensation is described here in the context of functional adaptation. Slender bones are associated with proportionally greater relative cortical area, whereas robust bones are associated with proportionally less cortical area. This relationship can be regarded as compensation in the context that the amount of bone (cortical area) must be maximized in slender bones to maximize stiffness, whereas the amount of bone (cortical area) must be minimized in robust bones to avoid developing a bulky structure. Boys and girls were segregated into tertiles based on robustness of the third metacarpal measured at 10 years of age. Tertiles were determined from data measured at 10 years of age because there were a few hand radiographs missing from the 8 year old cohort which precluded assigning all individuals into tertiles. This did not affect the tertile assignment since robustness measured at 8 years of age correlated significantly with robustness measured at 10 years ($R^2=0.65-0.92$, $p<0.0001$). Using methods similar to Wang et al [42], we tested whether the variation in robustness at 16–18 years of age was established prior to puberty. First, the percentage of individuals that remained in the tertile at 10 years was assessed at 12, 14, and 16 years of age. An insufficient number radiographs at 18 years precluded this age group from being included in the analysis. Second, individuals were ranked according to robustness measured at each age, and the rank at 10 years was regressed against the ranks measured at 12, 14, and 16 years of age. The slopes and correlation coefficients were determined using linear regression analysis.

Body size and morphological traits were calculated for each tertile at 8, 10, 12, 14, and 16 years of age. Growth patterns and the associated underlying biological activities were assessed by comparing the changes in total area, marrow area, and cortical area over time among the tertiles using a repeated measures two-way ANOVA. This was done only for those individuals that tracked within their tertile from 8 to 16 years of age. Data for males and females were analyzed separately. Post-hoc analyses included testing for changes in trait values with age for each tertile using a repeated measures ANOVA. Further, differences in trait values among tertiles were determined at each age using a one-way ANOVA. Individual comparisons were made by post-hoc t-tests and corrected for multiple comparisons ($p<0.008$).

Tracking of cortical area relative to robustness

To determine when variation in cortical area becomes apparent during growth, we corrected cortical area for robustness and body size, and tested whether those that tended to compensate well (or poorly) early in life also tended to compensate well (or poorly) at 16–18 years of age. Because factors like nutrition and exercise can affect bone development, we suspect there should be variation in cortical area for individuals that have slender bones just like there should be variation in cortical area at the other extreme for individuals with robust bones. Our ability to identify genetic and lifestyle factors affecting bone strength will be advanced by better understanding when this variation in cortical area arises during growth. Because cortical area varies predictably with robustness, we had to adjust for robustness to test whether individuals with low cortical area for their robustness at 8–10 years of age retain this variation at 16 years of age. Likewise, we wanted to know if individuals with high cortical area for their robustness at 8–10 years of age also retain this greater cortical area at 16 years of age.

To accomplish this, we developed a multivariate model to quantify the degree to which an individual's growth pattern deviated from what would be considered functionally adapted. Because there are no normative data that characterize functionally adapted growth patterns relative to robustness, we quantified deviations in cortical area by comparing an individual's

growth pattern to the average growth pattern for the current population. This comparison assumes the population average traits lead to a functionally adapted structure, which is reasonable given that only fit children from economically stable homes were enrolled into the Bolton-Brush study. Regardless of whether this assumption is correct, the population average traits provide a way to track an individual over time relative to others in the study population.

A population average growth pattern for bones exhibiting a wide range in robustness was generated based on our prior work which showed a negative correlation between robustness and relative cortical area ($RCA = Ct.Ar/Tt.Ar$) [29]. The negative correlation can be explained mathematically because individuals used relatively similar amounts of bone ($Ct.Ar$) to construct their metacarpals during growth. A similar phenomenon was observed in the femoral neck [43]. Thus, deviations in $Ct.Ar$ relative to robustness and body weight can be used as a measure of the degree of covariation among individuals. We used multivariate regression analysis to generate equations for $Ct.Ar_{predicted}$ as a function of robustness, body weight, and age for both metacarpals and sexes separately. Multiple regressions were calculated using data for all ages. The residuals calculated from these regressions ($Residual = Ct.Ar_{actual} - Ct.Ar_{predicted}$) were used as a measure of whether a person's $Ct.Ar$ was smaller (negative residual) or larger (positive residual) for their bone size and body size. The residuals calculated at 8 years of age were compared to residuals calculated at 16 years to determine whether individuals with reduced (or greater) cortical area relative to bone size and body size track over this age-range.

RESULTS

Sex-specific differences in morphological traits during growth

Boys and girls were compared to determine if the changes in the population average traits over time were consistent with a sexually dimorphic growth pattern. The number of radiographs available to calculate trait means at each particular time point is shown in Table 1. Body weight and height increased significantly with age ($p < 0.0001$; repeated measures 2-way ANOVA), as expected (Table 1). The change in body weight and height slowed appreciably after 14 years of age for girls, but not until after 16 years for boys resulting in significant body size differences between sexes at 16 and 18 years of age ($p < 0.008$, post-hoc t-test). Body mass index (BMI) did not differ between boys and girls at any age. All morphological traits examined showed a significant age-effect ($p < 0.0001$ – 0.0004 ; Repeated measures 2-way ANOVA) (Tables 2 and 3). The second and third metacarpals showed similar age- and sex-specific differences for Le , $Tt.Ar$, Jo , but not for $Ct.Ar$, $Ma.Ar$, and RCA . Boys had a significantly greater $Tt.Ar$ and Jo at all time points compared to girls, resulting in boys showing greater robustness ($Tt.Ar/Le$) at all ages ($p < 0.008$, post-hoc t-test). Since Le was not different between boys and girls for most ages, the difference in robustness between sexes was due primarily to differences in transverse expansion rather than longitudinal growth. Examination of the average traits revealed greater increases in $Tt.Ar$ over time for boys compared to girls, and an age-related increase in $Ma.Ar$ for boys (expansion) and a decrease in $Ma.Ar$ for girls (infilling). These changes in the average traits over time were consistent with expectations of the development of a sexually dimorphic diaphysis.

Changes in robustness during adolescence

Changes in the relationship between transverse expansion and longitudinal growth during adolescence were assessed by determining how these traits change relative to each other over time. Although $Tt.Ar$ and Le increased nonlinearly with age (Tables 2 and 3) and despite differences in units between $Tt.Ar$ (mm^2) and Le (mm), $Tt.Ar$ increased in a linear

manner relative to Le from 8 to 18 years of age. This relationship was well fitted by a linear regression (average $R^2=0.95$; $p<0.0001$). Robustness (Tt.Ar/Le), which was normally distributed at all ages for both metacarpals ($p > 0.10$, Kolmogorov-Smirnov test), varied widely among individuals as evidenced by an average coefficient of variation of 14.7% for girls and 14.3% for boys. Robustness increased with age for both metacarpals and sexes (Figure 1a,b), indicating that transverse expansion outpaced longitudinal growth during adolescence.

The slopes of the linear regression between robustness and age were on average 67% and 78% greater for the second metacarpal compared to the third metacarpal for boys and girls ($p<0.00001$, paired t-test), respectively. This age-difference resulted in a $27.7 \pm 9.7\%$ increase in robustness from 8 to 16 years for the second metacarpal of girls, but only a $13.4 \pm 10.7\%$ increase for the third metacarpal ($p<0.0001$, paired t-test). Likewise, for boys, robustness increased $35.0 \pm 15.0\%$ for the second metacarpal from 8 to 16 years of age but only $17.4 \pm 10.6\%$ for the third metacarpal ($p<0.0001$, paired t-test). The significantly lower age-changes in robustness of the third metacarpal modestly affected how individuals tracked within robustness tertiles from 8 – 16 years of age. For boys and girls, 64 – 71% and 77 – 78% of individuals remained in their tertile through 16 years of age for the second and third metacarpal, respectively. The ranks established at 8–10 years of age correlated significantly with ranks established at 16 years of age for both metacarpals ($R^2=0.64-0.82$, $p<0.0001$). Further, robustness at 8 years of age was a significant predictor of robustness at 16 years for both the second (boys: $R^2=0.42$, $p<0.0014$, girls: $R^2=0.77$, $p<0.0001$) and third (boys: $R^2=0.62$, $p<0.0014$, girls: $R^2=0.70$, $p<0.0001$) metacarpals. These analyses indicated that the relationship between transverse expansion and longitudinal growth was established early and tracked with tremendous consistency during adolescence. The significant differences in growth rates between the second and third metacarpals indicated that growth patterns of the second and third metacarpals should be examined separately.

Variation in growth patterns relative to robustness

Growth patterns were assessed for those individuals that remained in their tertile from 8 to 16 years of age. We imposed this limitation on the analysis because individuals that jumped tertiles were expected to affect the average trait values for a given tertile and to obscure robustness-specific growth patterns. Body weight, height, BMI and metacarpal length increased with age ($p<0.00001$, 2-way repeated measures ANOVA), but did not differ significantly among tertiles at any age ($p<0.4$, 2-way repeated measures ANOVA). This was true for both metacarpals and sexes indicating that segregation into robustness tertiles occurred independently of body size and that the variation in robustness within each sex resulted from differences in transverse expansion and not longitudinal growth.

Given there was no difference in body size or metacarpal length among tertiles, growth patterns could be examined simply by graphing the changes in Tt.Ar (sub-periosteal expansion) and Ma.Ar (endocortical expansion or infilling) over time. Significant differences in Tt.Ar were observed among the tertiles for all groups, as expected (Figure 2). Age-related increases (expansion) and decreases (infilling) in Ma.Ar (Figure 3) revealed whether a growth pattern relied on osteoclastic or osteoblastic activities, respectively, to establish function. Girls with robust metacarpals showed an initial endocortical expansion followed by infilling after 12 years of age. The middle and slender tertiles both showed infilling throughout growth. Boys with robust metacarpals showed the expected age-related increase in Ma.Ar indicating endocortical expansion. In contrast, the middle and slender tertiles showed early endocortical expansion but this was followed by infilling. The amount of infilling was particularly substantial for the third metacarpal where Ma.Ar of the slender metacarpals decreased by 39% between 14 and 16 years of age ($p<0.003$, paired t-test).

Effect of robustness-specific growth patterns on bone mass

The effect of variable growth patterns on the amount of bone acquired during growth was examined by plotting Ct.Ar over time (Figure 4). For both sexes and metacarpals, the growth patterns for individuals with slender bones lead to significantly lower diaphyseal Ct.Ar by 14–16 years of age compared to those with robust bones ($p < 0.005$). This difference in Ct.Ar was evident at 8 years of age for most groups. For females, growth patterns also lead to reduced Ct.Ar that was significant ($p < 0.008$) for the middle tertile of the third metacarpal. For males, growth patterns resulted in similar diaphyseal Ct.Ar for both metacarpals. Thus, ~2/3 of the boys had similar Ct.Ar independent of external bone size, whereas Ct.Ar was entirely dependent on external bone size for girls. A partial regression analysis, which was conducted to account for the effects of body size, showed that Ct.Ar correlated significantly with robustness for both metacarpals and sexes ($R^2 = 0.33 - 0.72$, $p < 0.001$ for all regressions) (Figure 5). This confirmed that individuals with slender metacarpals relative to body size had reduced Ct.Ar compared to individuals that had robust metacarpals relative to body size.

Tracking variation in cortical area relative to robustness over time

Residuals calculated from multivariate regressions were used to quantify how an individual's Ct.Ar deviated from the population average and whether this residual tracked over time. A multiple regression model was necessary because Ct.Ar varied with both robustness and body size. The multivariate regressions for Ct.Ar_{predicted} showed large adjusted R^2 values (Table 4), indicating that robustness, body weight, and age together accounted for 74 – 93% of the variation in Ct.Ar, as expected. The residuals calculated from multiple regressions that used data for all ages correlated significantly with the residuals calculated based on regressions that used data for individual ages (average $R^2 = 0.92$; range = 0.70 – 0.99, $p < 0.0001$). This indicated the longitudinal study design (i.e., repeated measures) did not affect the calculation of residuals. A linear regression analysis showed the residuals at 8 – 10 years of age correlated significantly with the residuals at 16 years of age for both sexes and metacarpals ($R^2 = 0.27 - 0.54$, $p < 0.005$). This indicated the variation in Ct.Ar tracked over time, such that individuals that had reduced (or greater) Ct.Ar relative to robustness and body weight at 8–10 years of age also tended to have reduced (or greater) Ct.Ar relative to robustness and body weight at 16 years of age. It was important to include robustness in the regressions to properly correct cortical area for the natural variation in robustness. Robustness is largely independent of body size, such that two individuals with the same height and weight can have very different bone robustness values (one slender and one robust). This analysis indicated that the amount of bone accrued during growth (i.e., cortical area) depended not only on body size, but also whether a person had slender bones or robust bones. This is an important finding because it means that the amount of bone a person acquires is not only dependent on body size and lifestyle factors but also on bone robustness.

DISCUSSION

The natural variation in robustness provided a systematic way to advance our understanding of the biology underlying skeletal growth. The variation in robustness must be compensated by coordinated changes in morphological and tissue-quality traits, otherwise slender structures will be weak and prone to fracturing, whereas robust structures will be bulky and thus metabolically expensive to maintain, energetically expensive to move through space, and prone to problems associated with sclerotic phenotypes. Weight-bearing mouse and human long bones show a consistent pattern in the way morphological and tissue-quality traits covary [11, 41], and a similar pattern among morphological traits develops between 3 months and 8 years of age in human metacarpals [29]. In the current study, we extended our

prior work to determine how robustness and morphological compensation changed during adolescence.

Although the biased enrollment of fit, healthy children from economically stable homes into the Bolton-Brush study does not represent the diversity of a general, modern population, we specifically chose this population because skeletal function (stiffness, strength) cannot be measured directly and the generally high fitness status of the participants was expected to reveal how variable growth patterns lead to functionally adapted structures. The age-changes in the population average traits were consistent with expectations of the development of a sexually dimorphic diaphysis during puberty, indicating that growth patterns for this cohort were consistent with modern populations [35]. The longitudinal database allowed us to study how bone growth patterns during adolescence differed relative to the natural variation in robustness that exists among individuals. Prior studies reported typical growth patterns for a population and did not segregate growth patterns according to the variation in external bone size [9], which we show here to be a major determinant of the biology underlying skeletal growth and the amount of bone acquired by young adulthood. Further, other longitudinal studies reported changes in strength-related morphological traits throughout growth, but did not report how the periosteal and endocortical surfaces change over time and thus did not address the biology underlying the development of bone strength [32].

Our results confirmed that robustness, which is an important morphological variant contributing to fracture risk in the elderly [2, 39], is established early during postnatal growth [17, 29]. Robustness increased from 8 to 18 years of age for both metacarpals indicating that transverse expansion exceeded longitudinal growth during adolescence. The significant correlation between robustness rankings assessed at 8 and 16 years of age combined with the observation that 70–80% of individuals remained within their robustness tertiles established at 8–10 years of age indicated that adolescent growth did not substantially modify how an individual's bone robustness compared to others. Given that tracking of morphological traits during adolescence has been consistently observed for axial and appendicular bones [24, 33, 42], children with slender (or robust) bones at 2 years of age [29] have a very high likelihood of having slender (or robust) bones as an adult.

Robustness can be viewed not only as a morphological trait but also as a biological trait representing the relationship between orthogonally directed growth processes. The similarity in bone length among robustness tertiles indicated that the variation in robustness among boys and girls resulted from differences in transverse growth, which is an appositional process facilitated by osteoblasts and osteoclasts, rather than longitudinal growth, which is an endochondral process involving chondrocyte proliferation, hypertrophy, and apoptosis. Finding that the variation in robustness is established by 2 years of age [29] and is not modified substantially during adolescence, as shown in the current study, narrows the window of factors defining the variation in adult bone width to the biological processes regulating the rate of periosteal expansion during postnatal growth. The lack of differences in body size parameters (weight, height, BMI) among robustness tertiles in the current study further suggested the biological processes regulating robustness are not easily related to those regulating body size. In fact, heritability estimates for metacarpal width are high, ranging from 64–72% after adjusting for cofactors such as sex, age, height, and weight [6, 15]. Prior work in inbred mouse strains identified quantitative trait loci (QTLs) harboring genes regulating bone length, periosteal circumference, and body weight individually, as well as QTLs harboring genes regulating the relationship among these traits [23]. Further studies identified QTLs harboring genes regulating robustness independently of body weight [10]. Given that robustness has a high heritability and is established by ~2 years of age, we suspect that robustness is largely genetically defined but can be modified by environmental factors to a certain degree during growth and adolescence.

Physical activity is an important environmental factor known to influence robustness. The asymmetry in external size of the dominant arm of tennis players indicates that periosteal expansion is adaptive to exercise, particularly if the increased loading associated with training begins early in life [13, 14, 34]. This adaptive response is generally observed for athletes, and it is unclear to what extent variation in the normal range of physical activity within the general population affects periosteal expansion during postnatal growth and thus would account for the variation in robustness among the Bolton-Brush participants. Moderate exercise regimens appear to promote cortical thickness in adolescents through changes in marrow expansion but not periosteal expansion [5, 30]. However, other studies show increased physical activity was associated with periosteal expansion and when combined with calcium resulted in greater cortical thickness [36]. Although boys and girls enrolled in the Bolton-Brush study were healthy and fit, we do not have measures of activity level and thus cannot determine whether variation in activity levels contributed to the variation in robustness. Further, we do not know to what extent the degree of adaptation depends on whether a person has genetically slender or robust bones. Clearly, further research is needed to better understand the environmental factors contributing to the variation in robustness, because these factors can be modified to enhance the development of skeletal strength.

Segregating the relative movements of the periosteal and endocortical surfaces over time into tertiles based on robustness provided new insight into the ontogenic origins of adult trait variation. Growth patterns varied with robustness in a highly predictable way for boys and girls, suggesting individuals with different genetic backgrounds and life histories share common biological controls regulating functional adaptation. The dependence of growth patterns on robustness has important clinical value for assessing the development of skeletal function and the associated biology directing bone growth on an individualized basis. Finding that growth patterns differed among robustness tertiles means the dimorphic growth patterns characterized for the population average do not predict the biology underlying growth for each individual within our study. Differences in how marrow area changed over time indicated that slender and robust metacarpals utilized fundamentally different biological processes to establish cortical area, which is a major determinant of bone mass. Cortical area is determined by the relative movements of the periosteal and endocortical surfaces over time. If the rate and amount of periosteal expansion is genetically prescribed early in life, then the amount of bone acquired by an individual depends mathematically on the adaptive biological factors regulating the movement of the endocortical surface. Although age-changes in marrow area do not specify the amount of osteoblastic and osteoclastic activities if transverse growth involves cortical drift or a non-uniform surface expansion, age-changes in marrow area nevertheless do provide critical information about the net biological behavior (i.e., the relative activities of osteoblasts and osteoclasts) that must be regulated to establish cortical area. An increase in marrow area over time reflects endocortical surface expansion and net osteoclastic activity, whereas a decrease in marrow area over time reflects endocortical infilling or net osteoblastic activity.

The changes in total area and marrow area over time for the traditional (i.e., population average) dimorphic growth pattern indicate that long bones expand periosteally and endocortically for boys throughout growth, and thus rely on osteoblastic activity for periosteal expansion and osteoclastic activity for marrow expansion. In the current study, metacarpals in the robust tertile for boys showed age-changes in marrow area that were consistent with this paradigm (Figure 6A). However, the population-average growth pattern did not capture the biology required to establish skeletal function for boys with metacarpals in the slender tertile. For the third metacarpals in the slender tertile, age-related reductions in marrow area began at approximately 12 years of age indicating that the amount of bone accrued in an osteoblast-dependent manner for boys during this time, which is different from

the osteoclast mediated marrow expansion predicted by the population average. For girls, all tertiles showed the expected age related periosteal expansion which slowed appreciably by 12–14 years of age (Figure 6B), as expected [9]. To establish function, the robust tertile showed marrow expansion (osteoclast-dependent growth) until 12 years of age which then shifted to marrow infilling (osteoblast-dependent growth). In contrast, the middle and slender tertiles showed marrow infilling (osteoblast-dependent growth) throughout adolescence. For girls, the primary difference among tertiles was the age at which endocortical infilling began. Thus, the biology underlying accrual of bone was entirely dependent on whether the child had slender or robust bones. In fact, robustness values overlapped for the slender tertile of boys and the robust tertile of girls, and the change in marrow area over time was similar for these two groups. Given that bone robustness played a crucial role in directing biological processes underlying the amount of bone accrued during growth, our results indicated that the timing and type of prophylactic treatment (anabolic versus anti-catabolic) aimed to increase bone mass during growth will benefit from knowledge of a child's robustness status.

An important outcome of the current study was finding that cortical area of the metacarpal correlated negatively with robustness, independent of body size. For girls, cortical area was entirely dependent on robustness tertile, whereas for boys only the slender tertile showed reduced cortical area compared to the robust tertile. We found a 9.6–19.7% and 24.9–32.2% difference in cortical area between slender and robust tertiles for boys and girls, respectively. These results indicated that robustness was a major determinant of cortical area in young-adults. Although the covariation between cortical area and external bone size has been reported previously for the tibia [40] and femoral neck [43], these studies did not test whether there were differences in cortical area relative to robustness.

Growth patterns were made further complex because not only were there predictable differences in cortical area relative to robustness, but we also found there was variation in cortical area for any given robustness value, as expected. After accounting for robustness and body weight, we found that variation in cortical area among individuals in the 8–18 year old cohort was established by 8 years of age and did not change during adolescence. To determine whether the variation in cortical area may be apparent earlier than 8 years of age, we reanalyzed the skeletal traits of the second metacarpal reported in our prior study for an independent cohort of children who were 3 months to 8 years of age [29]. The prior study included 377 hand radiographs for 37 Caucasian girls and 32 Caucasian boys at 6 ages (3 months, 9 months, 2 years, 4 years, 6 years, and 8 years). Variation in compensation for the 0 to 8 year old cohort was determined using the same methods described for the 8 to 18 year old cohort. Residuals for Ct.Ar for the 3 month - 8 year old cohort were derived from multiple regressions for girls ($\text{Ct.Ar} = -3.0 + 0.120 \text{ Age} + 14.8 \text{ Robust} + 0.12 \text{ BW}$, $R^2=0.93$) and boys ($\text{Ct.Ar} = -6.5 + 0.077 \text{ Age} + 15.98 \text{ Robust} + 0.39 \text{ BW}$, $R^2=0.91$). Residuals calculated at 8 years of age correlated significantly with residuals calculated as early as 4 years of age for girls ($R^2=0.43$, $p<0.0001$) and boys ($R^2=0.38$, $p<0.0001$). This suggested that the variation in cortical area relative to robustness and body weight was observable early during childhood growth. Taking into consideration that we pieced together information from two independent longitudinal datasets, our analysis suggested that individuals with reduced (or greater) cortical area relative to robustness and body weight at 16–18 years of age likely had this variation as early as 4 years of age. This is an important outcome, and one that is worthwhile confirming in a larger dataset that spans birth to adulthood, because it means that variation in the amount of bone for young adults is established early in life, well before most interventions aimed at increasing bone mass begin. Because BMD is complexly related to bone geometry [22], it will be important to correct BMD for the natural variation in robustness to determine whether a child has high or low cortical area for their bone size and body size.

The reasons for the early determination of cortical area cannot be discerned from the Bolton-Brush study collection. We certainly expect that environmental factors such as nutrition and exercise play an important role and that individuals arrive at reduced (or greater) cortical area relative to robustness and body weight for different reasons. Prior work in inbred mouse strains identified QTLs harboring genes regulating robustness independently from those regulating cortical area [10] suggesting that the variation in cortical area for any given robustness value may have a genetic basis. This is consistent with prior work showing that familial resemblance of bone mass is established before puberty [7]. Our analyses suggest that cortical area values should be adjusted for robustness when seeking biological or genetic determinants of traits related to bone mass. Genetic analyses based on absolute values of cortical area or bone mass may find genes that are also related to external size, since we show that these morphological traits covary in a highly predictable and significant way.

Our small sample size did not affect whether we could detect differences in traits between boys and girls (Tables 2, 3). For those traits in Tables 2 and 3 where no difference was found (e.g., Ct.Th for second metacarpal at 16 years), we would need to triple the sample size to detect a difference (Minitab, Inc., State College, PA USA). At this point, we would then question whether the small differences were meaningful. Thus, our study design had adequate power for assessing differences between girls and boys. However, the limited sample size did affect the traits when we segregated the samples into robustness tertiles. Significant differences were found between the slender and robust tertiles for most traits and age-groups (Figures 2 – 4). A power analysis showed that we needed sample sizes of 11–15 to detect differences among all groups with 80% power. Thus, our small sample size did not affect the outcome indicating that traits like Ct.Ar and Ma.Ar changed over time differently for slender and robust bones, but did limit our ability to report whether the middle tertile was different from the other two groups.

In addition to the limitations already discussed, we could only assess compensation in terms of morphology and not tissue-level mechanical properties, which we know also play a critical role in whole bone function [40]. Variation in mineralization and porosity must be included in future studies as the associated changes in tissue-stiffness have large effects on whole bone stiffness, strength and toughness. Whether the variation in tissue-modulus is sufficient to fully compensate for the decreased width combined with the reduced cortical area to equilibrate stiffness and strength among robustness phenotypes is unknown, but has important clinical implications for assessing strength on an individualized basis. Although using hand radiographs allows access to large databases as well as limiting X-ray exposure to the participants, future prospective studies may consider using peripheral quantitative computed tomography (pQCT), which can offer true cross-sectional information as well as information on tissue-quality [4]. We estimated cross-sectional morphology using a circular approximation, which is the traditional approach for studying historical collections such as the Bolton-Brush. Work by others showed that using a circular approximation overestimates cross-sectional morphology for the metacarpal, but that the errors associated with this approximation were independent of bone size [20]. We included the analysis of the third metacarpal primarily to confirm the results found for the second metacarpal. Cross-sectional shape varies among the metacarpals [1], and these shape differences will certainly affect the accuracy of estimating cross-sectional morphology using a circular approximation. It is not possible to estimate errors on an individualized basis for the current study, and further studies would need to be conducted using modern, non-invasive imaging modalities such as pQCT to test for systematic errors within and between metacarpals. The longitudinal study design allowed us to look at changes in each trait over time, and to confirm that we quantified cross-sectional traits in a consistent manner. Nevertheless, the use of modern imaging modalities would be needed to confirm the results of this study hold if errors

associated with estimating morphology from hand radiographs were not random but varied systematically with external bone size and would account for a proportion of the robustness-specific differences in cortical area.

In conclusion, an analysis of changes in bone morphology of the metacarpal diaphysis during adolescence revealed that skeletal growth patterns vary predictably with bone robustness, a commonly expressed morphological variant established early during postnatal growth. The data indicated that slender bones are constructed with significantly less cortical area compared to robust bones, and that the inter-individual variation in cortical area may be evident as early as 4 years of age. Thus, we show that the natural variation in bone robustness is a major determinant of adult cortical area and that variation in adult trait sets is determined in large part during early childhood growth. The metacarpal diaphysis shows similar functional relationships between robustness and cortical area as the tibial diaphysis [40]. Insight would be gained from a comparative analysis of growth patterns among weight-bearing and non-weight bearing bones to determine whether functional interactions described here are a general phenomenon. Identifying fracture-risk traits in children and young adults rather than in the elderly is important, because early diagnosis of fracture risk will allow clinicians to modify skeletal traits during a time of net bone gain (i.e., growth, adulthood) rather than trying to rebuild resorbed structures during a period of net bone loss (i.e., aging) [16].

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List of Abbreviations

Tt.Ar	Total cross-sectional area
Ct.Ar	Cortical cross-sectional area
Ma.Ar	Marrow cross-sectional area
Le	Metacarpal length
Tt.Ar/Le	Robustness
Ct.Th	Cortical thickness
RCA	Relative cortical cross-sectional area (Ct.Ar/Tt.Ar)
B.Dm	Outer metacarpal diameter
Ma.Dm	Inner metacarpal diameter
R	Outer metacarpal radius
r	Inner metacarpal radius
Jo	Polar moment of inertia
BW	Body weight

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Research Highlights

1. The biological activity underlying bone growth varied significantly with the natural variation in robustness.
2. The prophylactic treatment aimed to increase bone mass during growth will benefit from knowledge of a child's robustness status.
3. Cortical area was significantly lower for slender compared to robust metacarpals, indicating robustness is a major determinant of bone mass.
4. Genetic analyses based on cortical area may find genes related to external size, since these traits covary in predictable ways.
5. Variation in cortical area relative to the natural variation in bone robustness is established largely by 4 years of age.

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Figure 1.

Age-changes in robustness (Tt.Ar/Le) of the a) second and b) third metacarpals for girls and of the c) second and d) third metacarpals for boys. Closed circles represent individual data points for each age and the lines indicate the regression for each boy and girl.

Figure 2.

A comparison of changes in total cross-sectional area (Tt.Ar) during adolescence among robustness tertiles established at 10 years of age for the a) second metacarpal of girls; b) third metacarpal of girls; c) second metacarpal of boys, and d) third metacarpal of boys. The symbols represent the average trait values for each time point, and the error bars are the standard deviations. Differences among groups were compared at each age based on t-tests corrected for multiple comparisons ($p < 0.008$). * indicates T1 versus T3; ** indicates T2 versus T3; *** indicates T1 versus T2.

Figure 3.

A comparison of changes in marrow area (Ma.Ar) during adolescence among robustness tertiles established at 10 years of age for the a) second metacarpal of girls; b) third metacarpal of girls; c) second metacarpal of boys, and d) third metacarpal of boys. The symbols represent the average trait values for each time point, and the error bars are the standard deviations. Differences among groups were compared at each age based on t-tests corrected for multiple comparisons ($p < 0.008$). * indicates T1 versus T3; ** indicates T2 versus T3; *** indicates T1 versus T2.

Figure 4.

A comparison of changes in cortical area (Ct.Ar) during adolescence among robustness tertiles established at 10 years of age for the a) second metacarpal of girls; b) third metacarpal of girls; c) second metacarpal of boys, and d) third metacarpal of boys. The symbols represent the average trait values for each time point, and the error bars are the standard deviations. Differences among groups were compared at each age based on t-tests corrected for multiple comparisons ($p < 0.008$). * indicates T1 versus T3; ** indicates T2 versus T3; *** indicates T1 versus T2.

Figure 5. Accounting for body weight (BW) effects by partial regression analysis showed significant correlations between cortical area (Ct.Ar) and robustness at 16 years of age for the a) second and b) third metacarpals.

Fig. 6a

Fig. 6b

Figure 6.

Schematic representation of growth patterns of the metacarpal diaphysis for a) boys and b) girls were developed based on the age-changes in Tt.Ar and Ma.Ar reported in Tables 2 and 3. Growth patterns were segregated relative to robustness tertiles to show differences in the biological activity underlying the development of bone function. Biological processes are indicated for the periosteal and endocortical surfaces by the line quality, where the solid line indicates apposition, the dashed line indicates resorption, and the dotted line indicates a balanced condition in which there is no net change in the bone surface movement over time.

Table 1

Body weight, height, BMI and sample number for females (F) males (M)

TRAIT	SEX	8 YEARS	10 YEARS	12 YEARS	14 YEARS	16 YEARS	18 YEARS
n	F	22	27	27	29	25	11
	M	22	28	27	26	28	15
WEIGHT (kg)	F	28.3±6.4	35.6±7.6	45.2±8.1	54.0±7.2	58.1±7.3	60.3±6.7
	M	29.1±2.8	35.0±5.5	44.6±7.0	55.1±10.0	65.4±8.5	68.4±6.9
HEIGHT (mm)	F	1304±49	1415±53	1548±60	1628±46	1646±41	1649±37
	M	1323±42	1417±52	1531±61	1665±79	1765±60	1767±40
BMI (kg/m ²)	F	16.5±3.0	17.7±3.1	18.8±2.5	20.3±2.1	21.4±2.1	22.1±1.7
	M	16.6±1.3	17.4±1.9	19.0±2.4	19.8±2.2	20.9±2.1	21.9±1.9

Bold text indicates p<0.008, adjusted for multiple comparisons, between males and females at each age (Student's t-test)

Data are shown as mean ± standard deviation

Morphology of the second metacarpal diaphysis for females (F) and males (M) from 8 years to 18 years of age.

Table 2

TRAIT	SEX	8 YEARS	10 YEARS	12 YEARS	14 YEARS	16 YEARS	18 YEARS
Le (mm)	F	50.2±2.6	54.8±3.2	60.3±4.0	64.0±3.0	65.1±2.7	65.6±2.8
	M	50.9±2.6	54.9±3.0	59.7±3.5	66.6±4.5	71.4±3.6	71.7±2.7
Ct.Th (mm)	F	1.44±0.25	1.69±0.31	1.97±0.32	2.21±0.36	2.44±0.37	2.62±0.43
	M	1.42±0.20	1.59±0.26	1.71±0.30	1.96±0.30	2.27±0.28	2.40±0.36
Tl.Ar (mm ²)	F	26.8±4.8	32.4±6.1	38.1±6.6	42.8±7.4	45.6±7.9	47.4±7.6
	M	32.3±3.9	38.0±5.7	43.7±6.3	53.1±9.9	60.1±9.4	62.5±8.5
Ct.Ar (mm ²)	F	19.8±3.8	24.9±5.1	30.7±5.6	35.6±6.4	39.4±7.0	41.9±6.9
	M	22.1±2.8	26.5±4.2	30.6±5.0	38.3±6.8	45.8±6.2	48.9±7.6
Ma.Ar (mm ²)	F	7.0±2.8	7.4±3.1	7.4±3.2	7.2±3.6	6.3±3.5	5.5±4.0
	M	10.2±3.4	11.4±4.2	13.0±4.6	14.8±5.7	14.3±5.6	13.7±8.5
Jo (mm ⁴)	F	109.0±38.6	162.1±61.9	227.1±80.0	289.2±104.2	332.7±123.9	359.1±123.0
	M	150.5±32.7	210.8±57.3	279.1±77.4	423.6±152.4	551.4±161.1	600.0±162.2
RCA	F	0.74±0.09	0.77±0.08	0.81±0.07	0.83±0.08	0.86±0.07	0.89±0.07
	M	0.69±0.08	0.70±0.09	0.70±0.09	0.73±0.08	0.77±0.07	0.78±0.07
Tl.Ar/Le (mm)	F	0.53±0.08	0.59±0.10	0.63±0.10	0.67±0.10	0.70±0.11	0.72±0.09
	M	0.64±0.08	0.69±0.10	0.73±0.09	0.79±0.12	0.84±0.13	0.87±0.11

Bold text indicates p<0.008 between males and females at each age (Student's t-test)

Data are shown as mean ± standard deviation

Morphology of the third metacarpal diaphysis for females (F) and males (M) from 8 years to 18 years of age.

Table 3

TRAIT	SEX	8 YEARS	10 YEARS	12 YEARS	14 YEARS	16 YEARS	18 YEARS
Le (mm)	F	49.2+3.2	53.9+3.8	59.0+4.2	62.4+3.3	63.5+3.2	63.9+3.2
	M	49.1+2.9	52.8+4.1	57.9+3.3	64.2+4.5	68.9+3.1	69.2+2.6
Ct.Th (mm)	F	1.33+0.22	1.55+0.30	1.78+0.36	2.03+0.36	2.15+0.37	2.35+0.55
	M	1.54+0.26	1.62+0.25	1.68+0.26	2.09+0.30	2.43+0.33	2.31+0.34
Tl.Ar (mm ²)	F	29.2+4.8	34.5+5.5	39.2+6.6	42.4+6.3	44.2+7.0	46.7+6.4
	M	34.9+5.3	39.6+6.5	44.4+6.9	52.9+9.3	58.2+8.4	60.3+8.5
Ct.Ar (mm ²)	F	19.8+3.7	24.4+4.5	29.2+5.3	33.4+5.3	35.6+5.4	38.4+5.7
	M	24.7+4.6	27.7+4.6	30.6+5.1	39.9+7.2	46.5+5.4	46.1+5.1
Ma.Ar (mm ²)	F	9.4+2.7	10.0+4.1	10.0+4.7	9.0+4.7	8.6+4.5	8.2+5.5
	M	10.2+3.2	11.9+4.4	13.8+4.9	12.9+4.7	11.7+6.1	14.3+7.2
Jo (mm ⁴)	F	124.2+42.4	174.9+54.4	231.9+77.4	275.8+80.0	303.2+94.8	337.3+92.0
	M	180.0+54.6	230.5+68.8	287.2+81.9	428.3+150.1	522.5+138.1	537.3+141.4
RCA	F	0.68+0.08	0.71+0.10	0.75+0.10	0.79+0.10	0.81+0.09	0.83+0.11
	M	0.71+0.08	0.70+0.08	0.69+0.08	0.76+0.07	0.81+0.08	0.77+0.09
Tl.Ar/Le (mm)	F	0.59+0.08	0.64+0.09	0.66+0.10	0.68+0.09	0.70+0.10	0.73+0.07
	M	0.71+0.11	0.75+0.12	0.77+0.12	0.82+0.13	0.85+0.13	0.87+0.13

Bold text indicates p<0.008 between males and females at each age (Student's t-test)

Data are shown as mean ± standard deviation

Table 4Multiple regression analysis for cortical area, Ct.Ar_{predicted}

Sex	Metacarpal	Regression	R ² -Adj
8 – 18 years of age			
F	2nd	Ct.Ar = - 16.3 + 0.062 AGE + 44.8 ROBUST + 0.21 BW	0.86
F	3rd	Ct.Ar = - 9.4 + 0.075 AGE + 28.6 ROBUST + 0.19 BW	0.74
M	2nd	Ct.Ar = - 9.6 + 0.065 AGE + 22.1 ROBUST + 0.36 BW	0.89
M	3rd	Ct.Ar = - 12.3 + 0.097 AGE + 27.5 ROBUST + 0.23 BW	0.82

Ct.Ar (mm²), Robustness (mm), BW (kg), Age (months)